MICROBIAL PHYSIOLOGY AND METABOLISM

M.Sc. MICROBIOLOGY

SEMESTER-II, PAPER-I

LESSON WRITERS

Prof. A. Amruthavalli
Professor
Department of Botany & Microbiology,
University College of Sciences,
Acharya Nagarjuna University

Prof. V. Umamaheswara Rao
Professor
Department of Botany & Microbiology,
University College of Sciences,
Acharya Nagarjuna University

Prof. K. Mallikarjuna
Professor
Department of Botany & Microbiology,
University College of Sciences,
Acharya Nagarjuna University

Dr. J. Madhavi
Assistant Professor
Department of Botany & Microbiology,
University College of Sciences,
Acharya Nagarjuna University

EDITOR

Prof. A. Amruthavalli
Professor
Department of Botany & Microbiology,
University College of Sciences,
Acharya Nagarjuna University

ACADEMIC ADVISOR

Prof. V. Umamaheswara Rao
Professor
Department of Botany & Microbiology,
University College of Sciences,
Acharya Nagarjuna University

DIRECTOR, I/c.

Prof. V. Venkateswarlu
M.A., M.P.S., M.S.W., M.Phil., Ph.D.
Professor
Centre for Distance Education
Acharya Nagarjuna University
Nagarjuna Nagar 522 510
Ph: 0863-2346222, 2346208
0863- 2346259 (Study Material)
Website www.anucde.info
E-mail: anucdedirector@gmail.com

M.Sc. MICROBIOLOGY: MICROBIAL PHYSIOLOGY AND METABOLISM

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com. courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
M.Tech., Ph.D.,
Vice-Chancellor I/c
Acharya Nagarjuna University.

M.Sc. MICROBIOLOGY

SEMESTER-II, PAPER-I

201MB24 - MICROBIAL PHYSIOLOGY AND METABOLISM

SYLLABUS

THEORY

UNIT-I

Concept of thermodynamic principles, entropy, enthalpy, concept of free energy. Oxidation – reduction potential, ATP structure, free energy change in oxidation/reductions, different types of phosphorylations, solute uptake - passive and active transport, Phosphotransferase system, Iron uptake, group translocation; Regulation of bacterial metabolism.

UNIT-II

Photosynthesis - Oxygenic (cyanobacteria) and anoxygenic (Rhodospirillaceae, Chromatiaceae, Chlorobiaceae, Chloroflexaceae); Photosynthetic pigments, Bacteriorhodopsin, Photochemistry of photosystems; Photosynthetic Carbon Reduction (Calvin Cycle, RTCA, Hydroxy propionate pathway, reductive acetyl COA pathway).

UNIT-III

Chemolithotrophy: Hydrogen (H_2), Carbon monoxide (Co), ammonia (NH_3), nitrite (NO^-_2), sulphur (S^0) and Iron (Fe^{2+}) Oxidizing Bacteria; bioluminescence.

Respiration - EMP, ED, HMP, Methyl glyoxylate. HMP pathways, TCA cycle, ETC in bacteria and mitochondria, ETC inhibitors. Anaplerotic sequences.

UNIT-IV

Anaerobic respiration (SO_4^{2-} and NO_3^-).

Fermentations - Mixed acid, propionate and Butyrate-Butanol fermentations. Syntrophy, anaerobic food chain, gluconeogenesis. Methanogenesis and its biological importance.

UNIT-V

Biosynthesis of aminoacid. Catabolism of amino acids (deamination, decarboxylation and transamination).

Protein degradation - exo and endo proteases.

Fatty acid synthesis (saturated and unsaturated), Fatty acid degradation (saturated and unsaturated).

Bacterial cell wall synthesis (+ve and -ve). Polyamine biosynthesis, Biochemistry of 'N_2' fixation.

SUGGESTED BOOKS:

1) Reddy and Reddy (2005). Microbial Physiology.
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th edition.
3) Nelson and Cox. 2000; Lehninger Principles of Biochemistry
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology
5) Caldwell, D.R.1995. Microbial Physiology and Metabolism
6) David White. 1995. The Physiology and Biochemistry of Prokaryotes
7) Gottschalk, G. Bacterial Metabolism
8) Hans G. Schlegel. General Microbiology
9) Lansing M. Prescott et. al. 2005. Microbiology.

(201MB24)

M.Sc. DEGREE EXAMINATION, MODEL QUESTION PAPER
MICROBIOLOGY - SECOND SEMESTER
MICROBIAL PHYSIOLOGY AND METABOLISM

Time: Three hours **Maximum: 70 marks**

Answer All Questions **5 × 14 = 70M**

UNIT-I

1) a) Give an account on different types of phosphorylations.

OR

b) Explain the mechanism of regulation of bacterial metabolism.

UNIT-II

2) a) Write an account on the oxygenic photosynthesis with suitable examples.

OR

b) Describe the Calvin cycle.

UNIT-III

3) a) Give an account on Sulphur and Iron oxidizing bacteria with suitable examples.

OR

b) Explain the EMP and HMP pathways of respiration in microbes.

UNIT-IV

4) a) Write an account on anaerobic respiration.

OR

b) Describe the methanogenesis and its biological importance.

UNIT-V

5) a) Explain the biosynthesis and catabolism of amino acids

OR

b) Write an account on cell wall synthesis in Gram positive and Gram-negative bacteria.

CONTENTS

LESSON-1

CONCEPT OF THERMODYNAMICS FREE ENERGY OXIDATION REDUCTION REACTIONS AND ATP

1.0 OBJECTIVE:

- Concepts of thermodynamics, free energy, oxidation-reduction reactions, and ATP is to understand the fundamental principles governing energy flow and transformation in biological systems.

STRUCTURE:

1.1. INTRODUCTION:

Thermodynamics governs life, with the **First Law** (energy conservation) meaning cells transform food energy (not create it), while the **Second Law** (entropy increases) shows life maintains order (low internal entropy) by taking in energy and releasing disordered heat/waste to the surroundings, increasing overall universal entropy. **Enthalpy (H)** tracks heat absorbed/released, and **Entropy (S)** measures molecular disorder; biological processes balance these via Gibbs Free Energy **(G = H - TS)**, where spontaneity ($G < 0$) is driven by enthalpy changes, entropy changes, or both, allowing complex organization like protein folding or cell growth despite the universe's drive toward disorder.

1.2. LAWAS OF THERMODYNAMICS:

First Law (Energy Conservation):

Energy isn't created or destroyed, just changed (e.g., light to chemical, chemical to heat). **In Biology:** Cells "parasitize" energy, taking in ordered energy (food) and converting it to work (growth, movement) and heat, keeping total energy constant.

Second Law (Entropy Increase):

Total entropy (disorder) of the universe always increases in spontaneous processes. Organisms are local pockets of *low* entropy (high order) but achieve this by increasing the entropy of their surroundings (releasing heat/waste), ensuring the *total* entropy rises.

Enthalpy (H):

Total heat content of a system (absorbed or released). Metabolism releases or consumes heat (exothermic/endothermic reactions); exothermic reactions release heat, contributing to spontaneity.

Entropy (S):

Measure of molecular disorder or energy dispersal. Building complex molecules (like proteins) decreases a *system's* entropy but releases heat and smaller molecules (increasing surrounding entropy), driving the overall reaction forward.

1.3. ENERGY TRANSDUCTION IN PROKARYOTES:

Life can be thought of as a process that transforms materials available from the environment into cellular components according to genetic information. Material transformation is also coupled to energy transduction. Energy is needed not only for growth and reproduction but also for the maintenance of viability in processes that include biosynthesis, transport, motility, and many others. Organisms use energy sources available in their environment. Light and chemical energies are converted into the biological energy for growth and maintenance of viability. Photosynthesis is the process where light energy is utilized, and chemical energy is used through fermentation and respiration. Organic compounds produced by photosynthesis are used by other organisms in fermentation and respiration. For this reason, photosynthesis is referred to as primary production. Reduced inorganic compounds are also used as energy sources in chemolithotrophs.

Figure 1.1 shows energy transduction processes in biological systems. In these processes, free energy is conserved in the exergonic (free energy producing) reactions and consumed in the endergonic (free energy consuming) reactions. To understand these biological reactions in terms of thermodynamics, the relationship between the biological reactions and the free energy change should be understood.

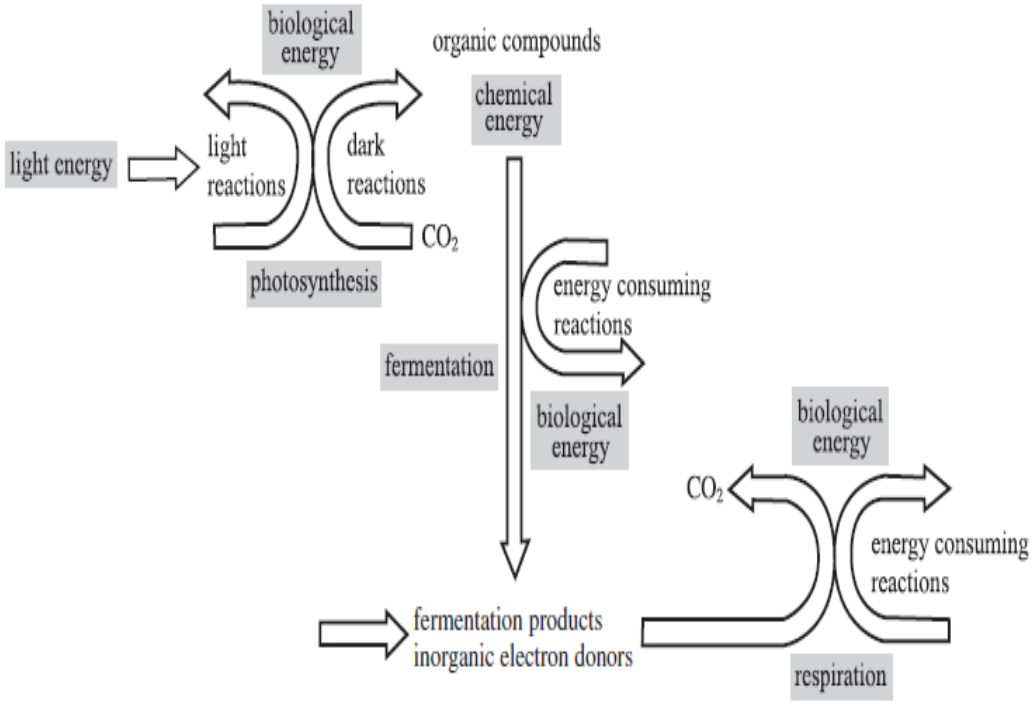

Fig: 1.1: Biological energy transduction processes. Photosynthesis: a process converting light energy into biological energy which in turn is consumed to fix CO_2 into organic compounds. Fermentation: a process converting chemical energy into biological energy without the external supply of electron acceptors. Respiration: a process converting chemical energy into biological energy by oxidizing organic and inorganic electron donors coupled with the reduction of externally supplied electron acceptors.

1.4. FREE ENERGY:

The free energy change in an exergonic reaction is expressed as a negative figure, and a positive figure is used to describe an endergonic reaction, since free energy leaves the system in an exergonic reaction while the system gains free energy in an endergonic reaction. The free energy change depends on the conditions of a given reaction. The standard condition is defined as the concentration of the reactants and products in one activity unit (when all are in active forms, a 1M concentration is the same as one activity unit) and at 25^0C for convenience.

The free energy change at standard conditions is expressed as ΔG^0, and $\Delta G^{0'}$ and ΔG are used to describe the free energy changes under standard conditions at pH-7, and at the given conditions, respectively. Since physiological pH is neutral, $\Delta G^{0'}$ is a frequently used term in biology. $\Delta G^{0'}$ can be calculated in various ways.

1.4.1. $\Delta G^{0'}$ From the Free Energy of Formation:

The free energy of formation ($\Delta G_f^{0'}$) of common compounds can be found in most chemical data handbooks. $\Delta G^{0'}$ is calculated from (ΔG_f^0) using the following equation:

$\Delta G^{0'}$ = Σ $\Delta G_f^{0'}$ of products - # Σ $\Delta G_f^{0'}$ of reactants for example, $\Delta G^{0'}$ is calculated in the reaction of glucose oxidation as:

$$C_6H_{12}O_6 + 6O_2 \; 6CO_2 + 6H_2O$$

Where the free energy of formation of each component is:

$$\Delta G_f^{0'} \text{ glucose} = - 917.22 \text{ kJ}$$

$$\Delta G_f^{0'} \quad O_2 = 0 \text{ kJ}$$

$$\Delta G_f^{0'} \quad H_2O = - 237.18 \text{ kJ}$$

$$\Delta G_f^{0'} \quad CO_2 = - 386.02 \text{ kJ}$$

$$\Delta G^{0'} [(- 237.18 \text{ X } 6)] + (- 386.02 \text{ X } 6)] – (-917.22)$$

$$= - 2821. 98 \text{ kJ/ mol glucose}$$

1.4.2. $\Delta G^{0'}$ from the Equilibrium Constant:

The equilibrium constant (K′ eq) of a reaction, A + B ⟵⟶C + D, is

expressed as:

K′ eq = ([C][D]) / ([A][B])

K′ eq shows in which direction the reaction takes place at pH-7.0

under standard conditions:

When K′ eq >1.0, $\Delta G^{0'}$ <0 and the reaction proceed in the right direction.

When K′ eq =1.0, $\Delta G^{0'}$ = 0 and the velocity is the same in both directions.

When K′ eq <1.0, $\Delta G^{0'}$ >0 and the reaction proceed in the reverse direction.

The equilibrium constant can be used to calculate $\Delta G^{0'}$ using the following equation:

$$\Delta G^{0'} = 2.303 \text{ RT log K′ eq}$$

R: gas constant (8.314 J/mol.K)

T: temperature in K (25ºC = 298 K).

1.4.3. ΔG from $\Delta G^{0'}$

ΔG^0 and $\Delta G^{0'}$ express free energy at reactant and product concentrations of one activity unit (=1 M). However, the concentrations inside a cell are much lower than that. ΔG at a given concentration of reactants and products can be calculated from $\Delta G^{0'}$ in a reaction of

$A + B \leftrightarrow C + D$ using:

$\Delta G = \Delta G^{0'} + 2{:}303\ RT \log([C][D]) / [A][B])$

Assuming that the concentration of ATP, ADP and Pi are 2.25, 0.25and 1.65mM, respectively, ΔG of ATP hydrolysis to ADP and Pi can be calculated from $\Delta G^{0'}$ = -30.5 kJ/mol ATP:

$\Delta G = -30.5 + (8.314 \times 298 \times 2.303)$

$\times \log [2.5 \times 10^{-4} \times 1.65 \times 10^{-3}] / [2.25 \times 10^{-3}]$

$= -51.8$ kJ / mol ATP

The figures used in the calculation are close to those values found in an actively growing bacterial culture. The free energy change of ATP hydrolysis under physiological conditions is referred to as the phosphorylation potential and is expressed as ΔGp

1.4.4. $\Delta G^{0'}$ from ΔG^0

ΔG^0 of a reaction where H^+ is involved is the free energy change at a H^+ concentration of 1M (pH= 0). Biologists are more interested in $\Delta G^{0'}$ than ΔG^0. $\Delta G^{0'}$ can be calculated from ΔG^0 using:

$$\Delta G^{0'} = \Delta G^0 - 2.303\ RT \times 7$$

1.5. OXIDATION/REDUCTION POTENTIAL:

Oxidation / reduction reaction Energy is generated from an oxidation/reduction reaction. Respiration is a series of oxidation/reduction reactions. The energy from respiratory oxidation/reduction reactions is conserved in biological systems.

The amount of energy generated from a reaction is proportional to the difference in the oxidation/reduction potential of the reductant and oxidant. Oxidation is defined as a reaction which loses electron(s) and reduction as a reaction that gains electron(s). Since an electron cannot ‘float’ in solution, oxidation and reduction reactions are coupled. A given compound can be an oxidant in one reaction and a reductant in another reaction. This property depends on the affinity of the compound for electrons, which is relative to that of other compounds. The affinity for electrons is referred to

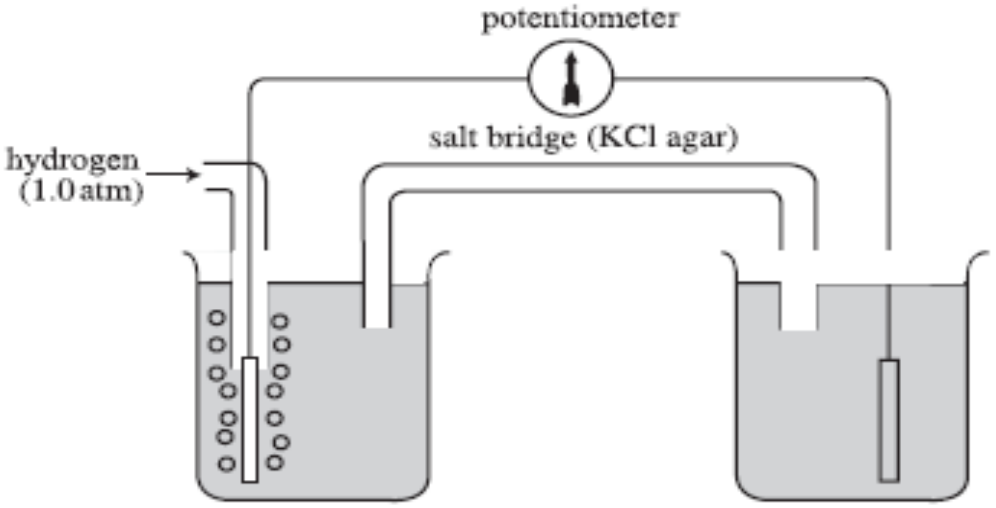

Fig 1.2: Determination of redox potential. A vessel is filled with a solution containing 1M of each of the reduced and oxidized forms of a compound of known redox potential, and the other vessel with1M of each of reduced and oxidized forms of the test compound. Platinum electrode is placed in each vessel. These two vessels relate to a KCl salt bridge, and the electrodes with a potentiometer. Due to the differences in the tendency to transfer electrons to the platinum electrode in each vessel, a potential developed. The potentiometer gives the difference in redox potential of the compounds. A solution of 1M H^+ (oxidized form) gassed with 1 atm H_2 (reduced form) is arbitrarily defined as a reaction that has a redox potential of 0 V.

as the oxidation/reduction (redox) potential. The higher the affinity for electrons, the higher the redox potential. Arbitrarily, the redox potential of the half reaction, $\frac{1}{2}H_2 \leftrightarrow H^+ + e–$, is defined as 0 V, and the relative values to this reaction are expressed as the redox potential of a given half reaction (Figure 1.2). The redox potential at standard conditions is expressed as E0 and that at standard conditions, pH 7.0, as $E^{0\prime}$. Biologists are often interested in $E^{0\prime}$., since physiological pH is neutral. $E^{0\prime}$. can be calculated from E^0 using the following equation:

$$E^{0\prime} = E^0 - 2{:}303(RT / nF) \times 7$$

R: gas constant (8.314 J/mol.K)

T: temperature in K (25^0C = 298 K)

n: number of electrons involved in the reaction

F: Faraday constant (96 487 J/V.mol).

$E^{0\prime}$ of the H^+/H_2 half reaction is calculated as:

$$E^{0\prime} - E0 - 2.303 \text{ x' } (8.314 \times 298/1 \times 96\,487) \times 7 = - 0.\,41V$$

When a temperature of 30^0C is used, the $E^{0'}$ is calculated as 0.42 V $E^{0'}$ values of some half reactions of biological interest are listed in Table 1.1

Table 1.1: Oxidation /Reduction Potentials of Compounds of Biological Interest

Electron carrier	$E^{0'}$(mV)	Electron donor and acceptor	$E^{0'}$ (mV)
Cytochrome f[a]	365	O_2/H_2O	812
Cytochrome a[a]	290	Fe^{3+}/Fe^{2+}	771
Cytochrome c[a]	254	NO_3^-/NO_2^-	421
Ubiquinone/ubiquinol	113	Crotonyl-CoA/butyryl-CoA	190
Cytochrome b[a]	77	Fumarate/succinate	31
Rubredoxin[a]	−57	Pyruvate/lactate	−185
$FMN/FMNH_2$	−190	Acetaldehyde/ethanol	−197
Cytochrome c_3[a]	−205	Acetoin/2,3-butanediol	−244
$FAD/FADH_2$	−219	Acetone/isopropanol	−286
Glutathione[a]	−230	CO_2/formate	−413
NAD(P)/NAD(P)H	−320	H^+/H_2	−414
Ferredoxin[a]	−413	Gluconate/glucose	−440
		CO_2/CO	−540
		Acetate/acetaldehyde	−581

Artificial electron carrier	$E^{0'}$(mV)	Artificial electron carrier	$E^{0'}$(mV)
Toluidine[a]	224	Janus green[a]	−225
DCPIP[a]	217	Neutral red[a]	−325
Phenazine methosulfate[a]	80	Benzyl viologen[a]	−359
Methylene blue[a]	11	Methyl viologen[a]	−446

[a] oxidized form/reduced form.
DCPIP, 2,6-dichlorophenolindophenol.

1.5.1. Free Energy From $\Delta E^{0'}$

Energy is generated from oxidation/reduction reactions, and the amount of energy is directly proportional to the redox potential difference between the reductant and the oxidant (ΔE00). Free energy from an oxidation/reduction reaction can be calculated using:

$$\Delta G^{0'} = - nF\Delta E^{0'}$$

n: number of electrons involved in the reaction

F: Faraday constant (96 487 J/V*mol)

$\Delta E^{0'}$: oxidation/reduction potential difference between the reductant and oxidant.

The $\Delta G^{0'}$ of succinate oxidation to fumarate with molecular oxygen can be calculated using this equation, as follows:

$$\text{succinate} + \tfrac{1}{2}\,O_2 \rightarrow \text{fumarate} + H_2O$$

$E^{0'}$ of fumarate/ succinate = + 0.03V

$E^{0'}$ of $\tfrac{1}{2}\,O_2 / H_2O$ = +0.82V

$\Delta G^{0'}$ = - 2 x 96487 x (0.82- 0.03) = - 152.45kJ/ mol succinate

Sum of free energy change in a series of reactions: Excessive heat would be fatal to the cell. Therefore, most biological reactions are catalysed in multiple steps. The sum of the free energy changes in each step is the same as that obtained in a one-step reaction. If a compound is metabolized through a different series of reactions, the free energy change is constant if the final product(s) are the same. For example, when glucose is metabolized to two pyruvates either through the EMP pathway or the ED pathway, the free energy change is the same.

1.6. ROLE OF ATP IN THE BIOLOGICAL ENERGY TRANSDUCTION PROCESS:

Biological reactions are divided into energy-generating catabolism and energy-consuming anabolism. Free energy generated from catabolism is conserved in the form of adenosine triphosphate (ATP) and the proton motive force which are consumed in catabolism (Figure 5.9). In this sense, it can be said that ATP and the proton motive force play a central role in biological metabolism linking catabolism and anabolism. ATP is used to supply energy for biosynthesis and transport by the ABC pathway (Section 3.4), while active transport, motility and reverse electron transport processes consume the proton motive force. ATP is well suited for this role since the energy needed for its synthesis and released from its hydrolysis is smaller than the energy available from most of the energy-generating catabolic reactions and bigger than most of the energy-consuming anabolic reactions In addition, ATP is a general intermediate for nucleic acid biosynthesis. As shown in Figure 5.10, ATP comprises adenosine with three phosphates bound to the 50-carbon of the ribose residue. The phosphate groups are termed and from the group nearest to ribose.

ATP is hydrolysed as shown below with the release of free energy. In most cases, ATP hydrolysis is coupled to energy-consuming reactions. However, pyrophosphate (PP_i) is hydrolysed without energy conservation in most cases by pyrophosphatase to pull the energy-consuming reactions coupled to ATP hydrolysis to AMP and PP_i. In some archaea, including *Thermoproteus tenax*, PP_i is used in place of ATP or ADP

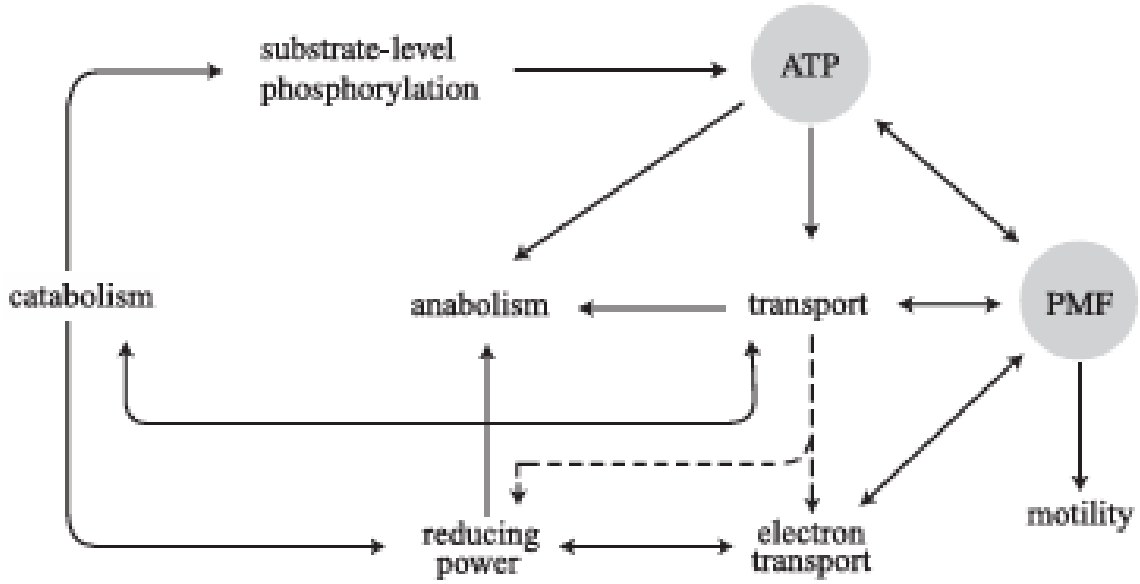

Fig 1.3: Biological energy transduction. Free energy generated from catabolism is conserved in the form of ATP and the Proton Motive Force (PMF) which provide the energy needed for anabolism.

$$ATP + H_2O \longrightarrow AMP + PP_i \ (\Delta G^{0'} = -32.2\ kJ/mol\ ATP)$$

$$ATP + H_2O \longrightarrow ADP + P_i \ (\Delta G^{0'} = -30.5\ kJ/mol\ ATP)$$

$$ADP + H_2O \longrightarrow AMP + P_i \ (\Delta G^{0'} = -30.5\ kJ/mol\ ADP)$$

$$PP_i + H_2O \longrightarrow 2P_i \ (\Delta G^{0'} = -28.8\ kJ/mol\ PP_i)$$

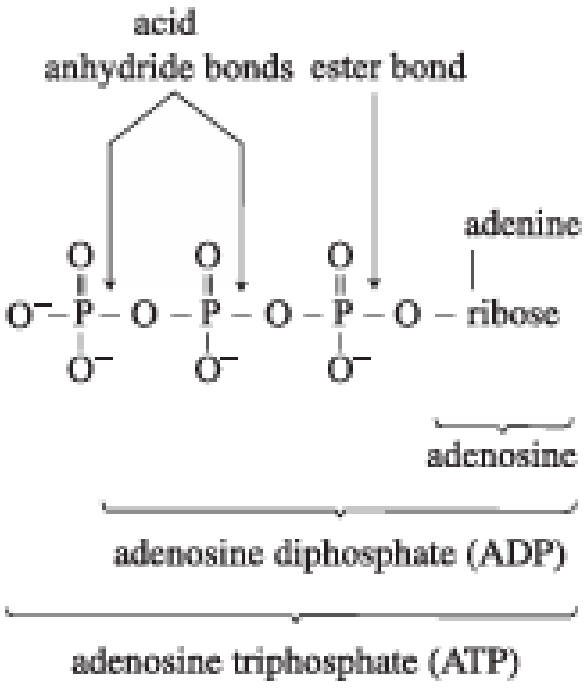

Fig 1.4: Structure of ATP High Energy Phosphate Bonds

It was mentioned previously that free energy is released on the hydrolytic removal of γ-phosphate from ATP and β-phosphate from ADP. For this reason, γ, β and β, α - phosphate linkages in ATP are called high energy bonds. There are several other metabolic intermediates with high energy bonds (Table 1.2).

Table 1.2: Metabolic Intermediates with High Energy Bonds

Intermediate	$\Delta G^{0'}$ (kJ/mol)
Phosphoenolpyruvate	−61.9
Carbamoyl phosphate	−51.5
1,3-diphosphoglycerate	−49.4
Acetyl phosphate	−47.3
Creatine phosphate	−43.1
Arginine phosphate	−38.1
Acetyl-CoA	−34.5

Phosphoenolpyruvate and 1,3-diphosphoglycerate are EMP pathway intermediates and are used to synthesize ATP from ADP in substrate-level phosphorylation (SLP). SLP processes are exergonic reactions because the free energy change ($\Delta G^{0'}$) from the reaction is bigger than that for ATP synthesis. Many ATP consuming anabolic reactions are exergonic. Since both catabolism and anabolism coupled to ATP synthesis and hydrolysis are exergonic reactions, ATP-mediated metabolic reactions are thermodynamically favourable.

The $\Delta G^{0'}$of ATP hydrolysis is –30.5 kJ/mol. ΔG is much larger than $\Delta G^{0'}$ under physiological conditions, where the ATP concentration is higher than that of ADP in the presence of Mg^{2+}. Mg^{2+} salts of ATP and ADP increase their free energy of hydrolysis. ATP is synthesized from ADP, and ATP is hydrolysed to AMP and PP_i in certain energy-consuming reactions. AMP is phosphorylated to ADP by adenylate kinase.

$$\text{ATP} + \text{AMP} \underset{\text{adenylate kinase}}{\rightleftharpoons} 2\ \text{ADP} \quad (\Delta G^{0'} = 0\ \text{kJ})$$

Since the $\Delta G^{0'}$ of this reaction is 0 kJ, the direction is determined by the concentration of cellular ATP, ADP and AMP, which reflects the energy status of the cell.

Adenylate Energy Charge:

A high ATP concentration means that the energy status is good, and when the energy supply cannot meet demand, the ADP and AMP concentration is high. Arbitrarily, the adenylate energy charge (EC) is a term used to describe the energy status of a cell. EC can be calculated using the following equation:

$$EC = ([ATP] + \tfrac{1}{2}[ADP])/([ATP] + [ADP] + [AMP])$$

The numerator of this equation is the sum of high energy phosphate bonds in the form of ATP, and the denominator the concentration of the total adenylate pool. An EC number of 1 means that the total adenylate is in the ATP form, and 0 in the AMP form.

Since the reaction catalysed by adenylate kinase is reversible with $\Delta G^{0'}$ of 0 kJ/mol, and enzyme activity is high in a cell, the relative concentrations of ATP, ADP and AMP are determined by the EC (Fig 1.5). Many catabolic reactions are repressed by ATP and activated by ADP and/or AMP: anabolic reactions are controlled in a reverse manner. The reactions are regulated not by the absolute concentration of each adenosine nucleotide, but by their ratio. Thus, overall metabolism is controlled by the EC value (Fig 1.6).

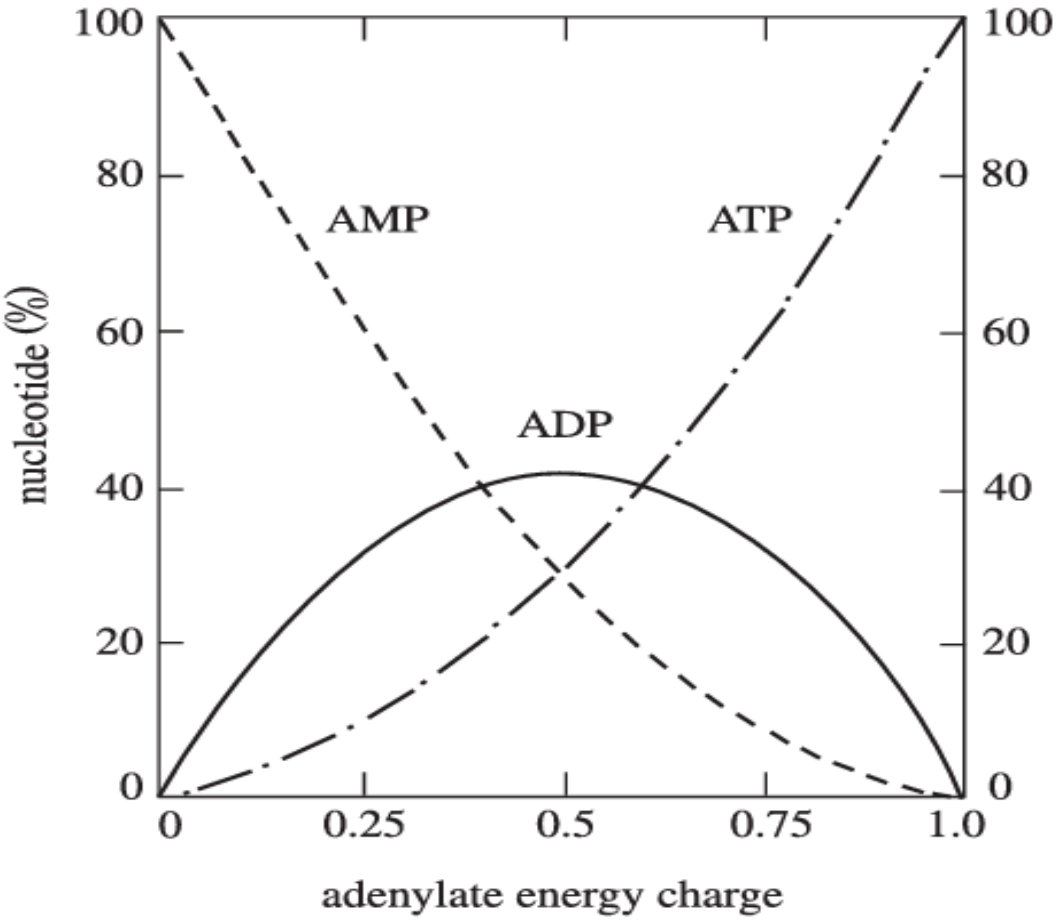

Fig 1.5: Relationship of adenylate energy charge (EC)and the relative concentration of ATP, ADP and AMP. (Dawes, E. A. 1986, Microbial Energetics, Figure 2.2. Blackie & Son, Glasgow)

Since $\Delta G^{0'}$ of the reaction catalysed by adenylate kinase is 0 kJ/mol, the direction of the reaction will be determined by the relative concentrations of ATP, ADP and AMP, which can be expressed by EC value.

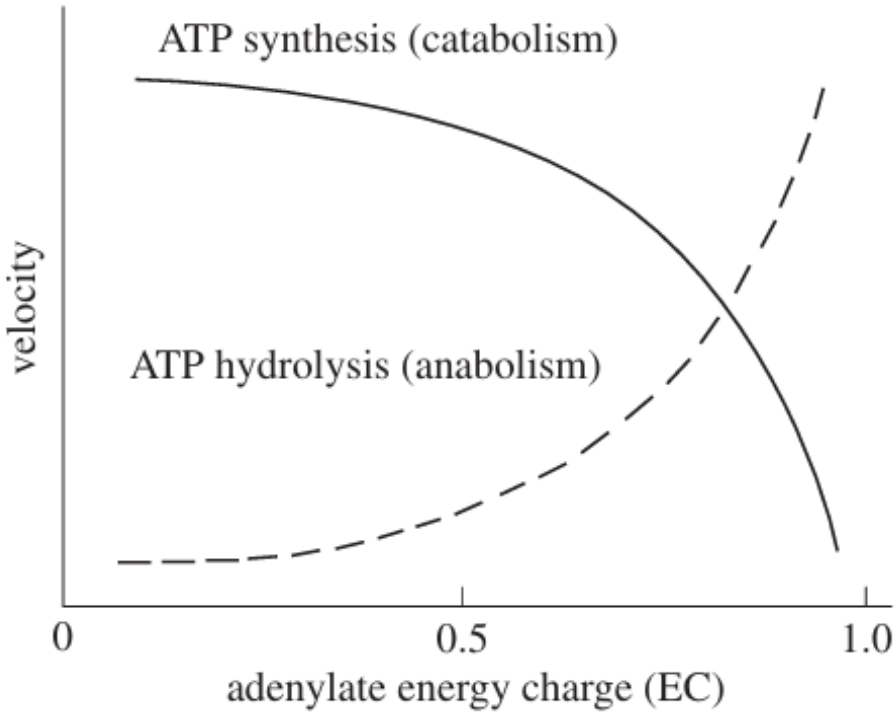

Fig 1.6: Regulation of catabolism and anabolism by the adenylate energy charge (EC). (Dawes, E. A. 1986, Microbial Energetics, Figure 2.3. Blackie &Son, Glasgow)

Adenylate energy charge controls the overall growth of microbes, regulating catabolism which synthesizes ATP, and anabolism which consumes it.

When a bacterium uses acetate as the sole carbon and energy source, acetate should be metabolized through the TCA cycle in catabolism and through the glyoxylate cycle in anabolism to supply carbon compounds for biosynthesis. It has been mentioned that AMP and ADP activate isocitrate dehydrogenase to metabolise the substrate through the TCA cycle. The activity is not controlled by AMP and ADP per se, but by the EC value, activated at EC<0.8 and inactivated at EC>0.8. An anabolic enzyme, aspartate kinase, is activated at a high EC value and repressed at a low EC value. When a bacterial culture is transferred from a rich medium to a poor medium, AMP is excreted or hydrolysed to adenosine or adenine to maintain a high EC value at a low rate of ATP synthesis. A growing bacterial culture maintains an EC value of 0.8–0.95, and the value gradually decreases to around 0.5 and rapidly thereafter when the culture starves. Bacterial cultures with EC value less than 0.5 cannot form colonies. This is not surprising because ATP is essential for viability. Since the EC value is a unitless figure, it does not give any information on the size of the adenylate pool, the concentration of each adenosine nucleotide or the turnover velocity. Since the EC value controls the overall metabolism that is observed during growth, similar EC values are expected in fast-growing and slow growing cultures, though a fast-growing culture has a bigger adenylate pool and a higher ATP turnover rate than the slow-growing culture.

1.7. SUMMARY:

In biological systems, thermodynamics governs energy flow, with Gibbs free energy (ΔG) determining the spontaneity of reactions. Energy is primarily harvested through oxidation-reduction (redox) reactions and stored in the molecule ATP, which acts as the universal energy currency for cellular work. Thermodynamics: The study of energy transformations. The first law states that energy is conserved (cannot be created or destroyed), only converted from one form to another. The second law states that the total entropy (disorder) of an isolated system always increases in a spontaneous process. Gibbs Free Energy (ΔG): This is the amount of energy in a system available to perform useful work at a constant temperature and pressure. A negative ΔG indicates an exergonic (energy-releasing) and spontaneous reaction. A positive ΔG indicates an endergonic (energy-requiring) and non-spontaneous reaction, which needs energy input to proceed. Oxidation-Reduction (Redox) Reactions: These reactions involve the transfer of electrons from one substance to another. Oxidation is the loss of electrons (and often hydrogen atoms). Reduction is the gain of electrons. Energy is released as electrons move from an electron donor to an acceptor, a process that can be quantified by the redox potential. ATP (Adenosine Triphosphate): A high-energy molecule that serves as the cell's main energy currency. The hydrolysis of ATP to ADP (adenosine diphosphate) and inorganic phosphate (Pi) is an exergonic reaction with a highly negative (ΔG)

1.8. SELF ASSESSMENT:

1) Explain Laws of Thermodynamics
2) Give an account on 3 Energy transductions in prokaryotes
3) Describe- Free energy
4) Explain the Oxidation/reduction potential
5) Explain the Role of ATP in the biological energy transduction process

1.9. REFERENCES:

1) Reddy and Reddy (2005). Microbial Physiology
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th Edition
3) Nelson and Cox.2000; Lehninger Principles of Biochemistry
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology
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7) Gottschalk, G. Bacterial Metabolism

Prof A. Amrutha Valli

LESSON-2

OXIDATIVE PHOSPHORYLATION AND SUBSTRATE LEVEL PHOSPHORYLATION

2.0 OBJECTIVE:

- Studying oxidative phosphorylation (OxPhos) and substrate-level phosphorylation (SLP) is to understand the distinct mechanisms, locations, and efficiency of these two cellular processes that generate ATP.

STRUCTURE:

2.1. INTRODUCTION:

Phosphorylation potential (ΔGp) Free energy needed for ATP synthesis or released by its hydrolysis in the cell is referred to as the phosphorylation potential (DGp) which is determined by the concentration of ATP, ADP and Pi as in the following equation.

$$\Delta Gp = \Delta G^{0'} + 2.303 \log([ADP][P_i]/[ATP])$$

In addition to the concentration of each adenosine nucleotide, DGp depends on the concentration of metal ions such as Mg2þwhich bind the nucleotide. Binding of metal ions increases the DGp value. The concentration of metal ions and Pi vary according to the growth conditions. DG00 for ATP hydrolysis is #30.5 kJ/mol, and DGp is around #51.8 kJ/mol ATP.

2.2. INTERCONVERSION OF ATP AND THE PROTON MOTIVEFORCE (Dp)

ATP and Dp link catabolism and anabolism, and that ATP is converted to Dp, and vice versa. The membrane-bound ATP synthase (ATPase) catalyzes this interconversion. When a microbe grows fermentatively generating ATP through substrate-level

phosphorylation, ATP is hydrolyzed to increase the Dp. In contrast, Dp is consumed to synthesize ATP when respiration is the main energy conservation process. ATP and Dp are therefore consumed for different purposes (Figure).

2.3. SUBSTRATE-LEVEL PHOSPHORYLATION (SLP):

ATP is synthesized at the cytoplasm as a result of the transfer of phosphate from metabolic intermediates with high energy phosphate bonds to ADP. 1,3-diphosphoglycerate, phosphoenolpyruvate and acyl-phosphate are the metabolic intermediates used to synthesize ATP through SLP. Succinyl-CoA conversion to succinate in the TCA cycle is another example of SLP and is catalyzed by succinyl-CoA synthetase:

2.4. ELECTRON TRANSPORT (OXIDATIVE) PHOSPHORYLATION:

Electron carriers such as NAD(P)þ, FAD and PQQ are reduced during glycolysis and the TCA cycle. Electrons from these carriers enter the electron transport chain at different levels. Electron carriers are oxidized, reducing molecular oxygen to water through ETP to conserve free energy as the proton motive force (Dp)

2.4.1. Chemiosmotic Theory:

It took many years to elucidate how the free energy generated fromETP is conserved as ATP. Compounds with high energy bonds are not involved in ETP as in substrate-level phosphorylation. ATP is synthesized only with an intact membrane or membrane vesicles, and ATP synthesis is inhibited in the presence of uncouplers or ionophores.

From these observations, a chemiosmotic mechanism was proposed. According to this, export of charged particles is coupled to oxidation–reduction reactions to form an electrochemical gradient which is used for ATP synthesis. Hþ are the charged particles exported, and the electrochemical gradient is the proton motive force, consisting of the Hþ gradient (DpH) across the membrane and the membrane

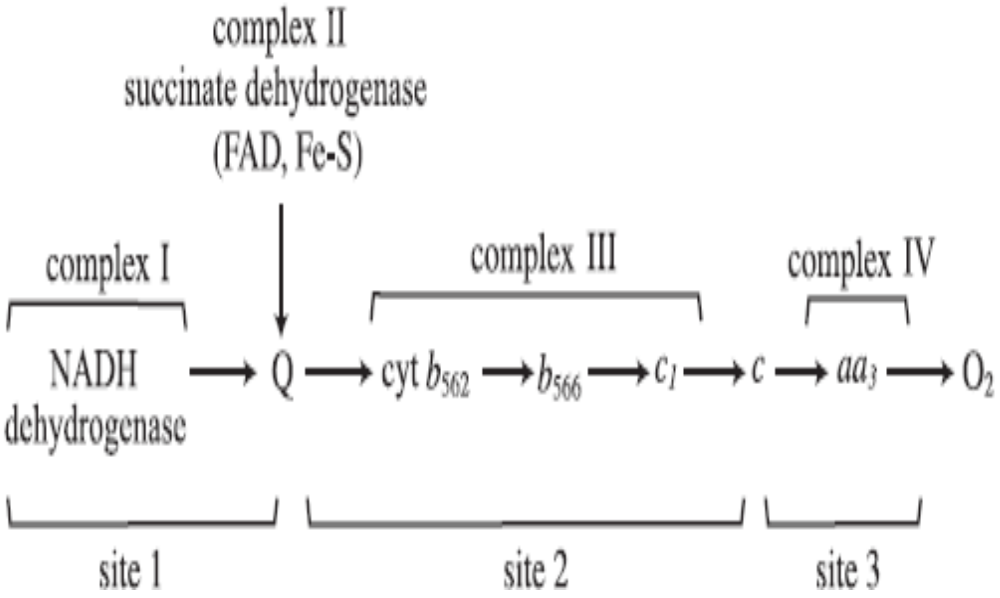
potential (Dy). The phospholipid membrane is impermeable to H+ and OH# and is suitable to maintain the proton gradient. Most of the electron carriers involved in ETP are arranged in the membrane, and the membrane-bound ATP synthase synthesizes ATP from ADP and Pi consuming the proton gradient.

2.4.2. Electron Carriers and the Electron Transport Chain:

Electron carriers involved in electron transport from NADH to molecular oxygen are localized in the mitochondrial inner membrane in eukaryotic cells and in the cytoplasmic membrane in prokaryotic cells. The mitochondrial electron transport chain is shown in Figure, and bacterial electron transport systems are shown in Figure 2.1. Bacterial systems are diverse depending on the species and strain as well as on the availability of electron acceptors.

2.4.3. Mitochondrial Electron Transport Chain:

Eukaryotic electron transport is discussed here as a model to compare with the process in prokaryotes. The mitochondrial electron transport chain consists of complex I, II, III and IV. The overall reaction can be summarized as dehydrogenases (complex I and II) and an oxidase (complex IV) connected by quinone (including complex III).



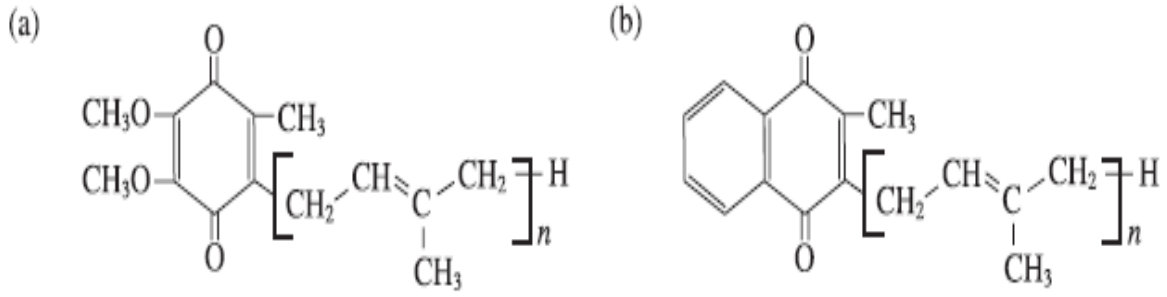
NADH dehydrogenase oxidizes NADH, reduced in various catabolic pathways, to NADþ. This enzyme contains FMN as a prosthetic group and forms with [Fe-S] proteins a complex known as complex I or NADH-ubiquinone reductase. FMN is reduced with the oxidation of NADH and the [Fe-S] proteins mediate electron and proton transfer from FMNH2 to coenzyme Q. This reaction generates enough free energy to synthesize ATP, and the electron transfer from NADH to ubiquinone (coenzyme Q) is referred to as site 1 of ETP. In a mitochondrion and in most bacteria, protons are translocated by this complex, but sodium ions are exported by the complex I of certain bacteria including Vibrio alginolyticus .

As a step in the TCA cycle, succinate dehydrogenase oxidizes succinate to fumarate, reducing its prosthetic group, FAD, before electrons are transferred to coenzyme Q. This enzyme forms complex II (or the succinate-ubiquinone reductase complex) of ETP with [Fe-S] proteins, cytochrome b558, and low molecular weight peptide. Other dehydrogenases containing FAD as a prosthetic group reduce coenzyme Q in a similar way. These include glycerol-3-phosphate dehydrogenase and acyl-CoA dehydrogenase.

Electrons from coenzyme Q are transferred to a series of reddish brown coloured proteins known as cytochromes. Cytochromes involved in mitochondrial electron transport are b562, b566, c1, c andaa3 as shown in Figure. Two separate protein complexes mediate electron transfer from coenzyme Q to molecular oxygen through the cytochromes. These are ubiquinol-cytochrome c reductase (complex III) and cytochrome oxidase (complex IV).

Complex III transfers electrons from coenzyme Qto cytochrome c. This complex consists of [Fe-S] protein, and cytochromes b562, b566 and c1. At this step, energy is conserved exporting protons (site 2). The cytochrome oxidase complex mediates electron

transfer from reduced cytochrome c to molecular oxygen. Energy is also conserved at this step (site 3). This terminal oxidase complex contains cytochromea and cytochrome a3.



Structure of (a) Ubiquinone and (b)

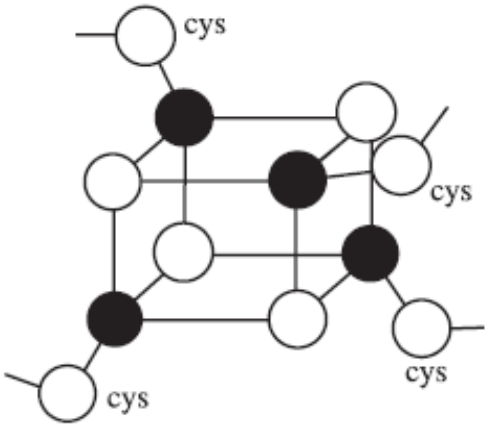


Fig 2.2: The structure of a[4Fe-4S] cluster. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Springer, New York)

2.5. ELECTRON CARRIERS:

Electron transport involves various electron carriers including flavoproteins, quinones, [Fe-S] proteins and cytochromes. Flavoproteins are proteins containing riboflavin (vitamin B2) derivatives as their prosthetic group. They are FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide). The redox potential of the flavoproteins varies not due to the flavin structure but due to the differences in the protein component.

Two structurally different quinones are involved in the electron transport process, ubiquinone and menaquinone, which serve ascoenzyme Q. Quinones are lipid electron carriers, highly hydrophobic and mobile in the semi-solid lipid phase of the membrane. As shown in Figure, quinones have a side chain of 6, 8 or 10isoprenoid units. These are named Q6, Q8 and Q10 according to the number of isoprenoid units. Ubiquinone is found in mitochondria, and bacteria have menaquinone (Figure). Both forms of quinones are found in Gram-negative facultative anaerobes.

The structure of coenzyme Q can be used as one characteristic for bacterial classification. Quinones can carry protons as well as electrons.

[Fe-S] proteins contain [Fe-S] cluster(s), usually [2Fe-2S] or [4Fe-4S]. The non-heme irons are attached to sulfide residues of the cysteines of the protein and acid-labile sulfur (Figure). The acid-labile sulfur is released as H2S at an acidic pH. [Fe-S] proteins participating in electron transport can carry protons as well as electrons. There are many different [Fe-S] proteins mediating not only the electron transport process in the membrane, but also various oxidation–reduction reactions in the cytoplasm. The redox potential of different [Fe-S] proteins spans from as low as #410mV (clostridial ferredoxin, Section 8.5) to þ350mV. Many enzymes catalyzing oxidation–reduction reactions are [Fe-S] proteins including hydrogenase, formate dehydrogenase, pyruvate: ferredoxin oxidoreductase and nitrogenase. Cytochromes are hemoproteins. They are classified according totheir prosthetic heme structures (Figure) and absorb light at550–650 nm. Cytochrome b562 refers to a cytochrome b with the maximum wavelength absorption at 562 nm. Heme is covalently bound to the proteins in cytochrome c, and hemes are non-covalently associated with the protein in other cytochromes. Since cytochromes

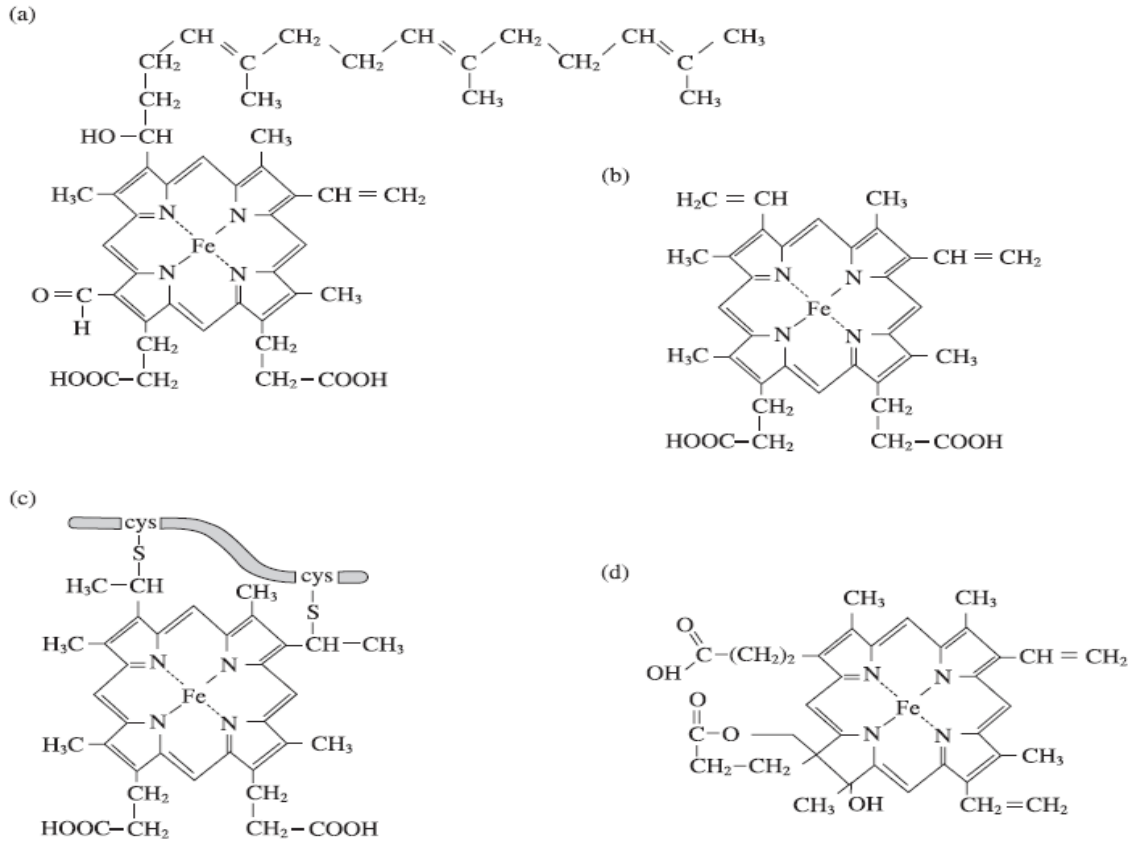


Fig 2.3: Structure of Hemes of Cytochromes. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 2.10. Springer, New York) (a)–(d) show the prosthetic hemes of Cytochromes a, b, c and d

2.6. SUMMARY:

Substrate-Level Phosphorylation (SLP) directly transfers a phosphate group from a substrate to ADP to make ATP, happening quickly in glycolysis & Krebs cycle, while Oxidative Phosphorylation (OxPhos) uses the electron transport chain (ETC) & ATP synthase to create a proton gradient, driving massive ATP production at the inner mitochondrial membrane (or plasma membrane in prokaryotes) using oxygen as the final electron acceptor. SLP is fast but produces little ATP; OxPhos is slower but generates most of the cell's ATP.

2.7. SELF ASSESSMENT:

1) Explain Interconversion of ATP and the Proton Motive Force (Dp)
2) Substrate-Level Phosphorylation (SLP)
3) Electron Transport (oxidative) Phosphorylation
4) Chemiosmotic Theory
5) Electron Carriers and the Electron Transport Chain
6) Mitochondrial Electron Transport Chain
7) Electron Carriers

2.8. REFERENCES:

1) Reddy and Reddy (2005). Microbial Physiology
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th Edition
3) Nelson and Cox.2000; Lehninger Principles of Biochemistry
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7) Gottschalk, G. Bacterial Metabolism

Prof A. Amrutha Valli

LESSON-3

MEMBRANE TRANSPORT-NUTRIENT TAKE AND PROTEIN EXCRETION

3.0 OBJECTIVE:

- Studying microbial membrane transport is to understand the fundamental molecular mechanisms by which microorganisms regulate the movement of substances across their cell boundaries.

STRUCTURE:

3.1. INTRODUCTION:

Microbes import the materials needed for growth and survival from their environment and export metabolites. the cytoplasm is separated from the environment by the hydrophobic cytoplasmic membrane, which is impermeable to hydrophilic solutes. Because of this permeability barrier exerted by the phospholipid component, almost all hydrophilic compounds can only pass through the membrane by means of integral membrane proteins. These are called carrier proteins, transporters or permeases

Solute transport can be classified as diffusion, active transport or group translocation according to the mechanisms involved. Diffusion does not require energy; energy is invested for active transport; and solutes transported by group translocation are chemically modified during this process. Some solutes are accumulated in the cell against a concentration gradient of several orders of magnitude, and energy needs to be invested for such accumulation.

3.2. IONOPHORES: MODELS OF CARRIER PROTEINS:

There are two models which explain solute transport mediated by carrier proteins: the mobile carrier model and the pore model. The solute binds the carrier at one side of the membrane and dissociates at the other side according to the mobile carrier model, while the pore model proposes that the carrier protein forms a pore across the membrane through which the solute passes. A certain group of antibiotics can make the membrane permeable to ions. These are called ionophores and are useful compounds to assist the study of membrane transport.

One ionophore, valinomycin, transports ions according to the carrier model, while gramicidin A, another ionophore, makes a pore across the membrane. Valinomycin is a circular molecule consisting of valine, lactate and hydroxy isovalerate (Figure 3.1).

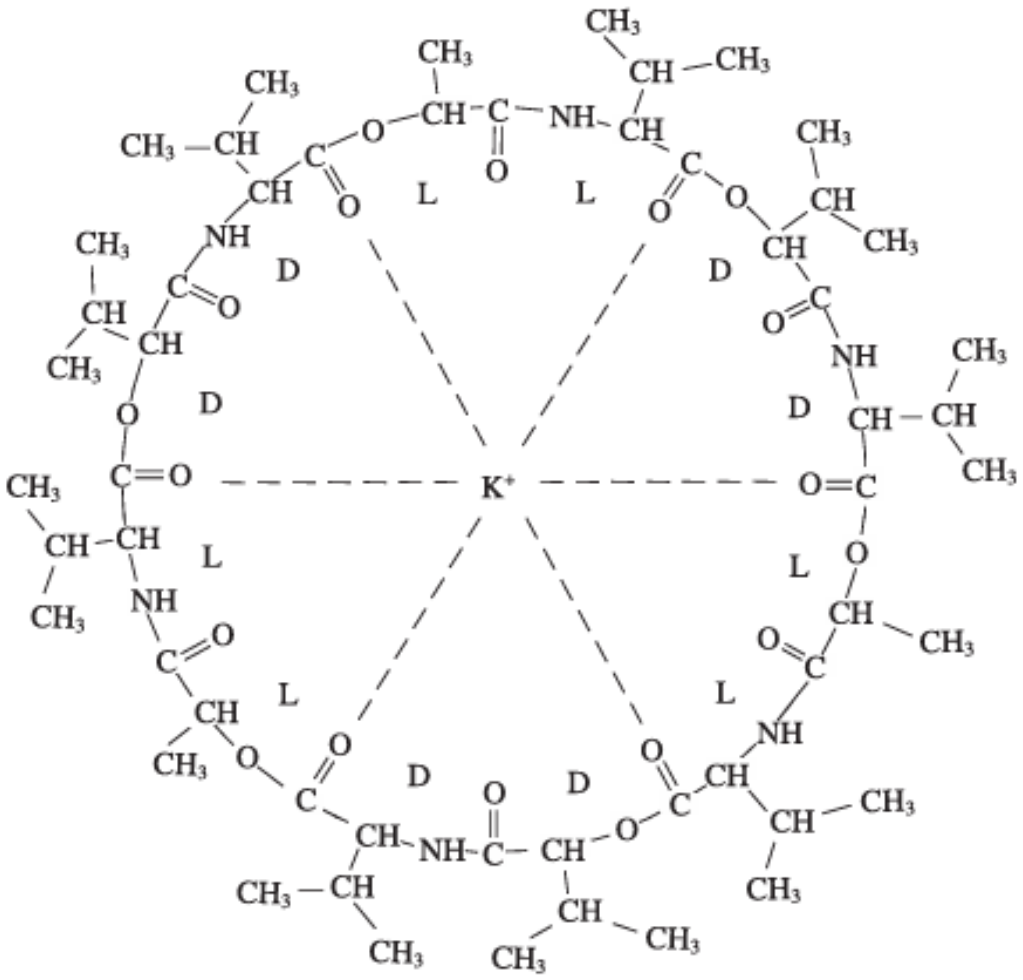
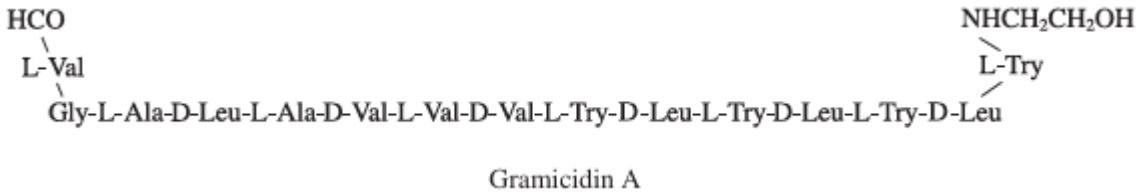


Fig 3.1: Structure of Valinomycin, a Model Mobile Carrier

[Cations bind to the hydrophilic interior of the molecule at the side of the membrane with the higher concentration of the cation, and the complex moves through the hydrophilic membrane to the other side where the cation concentration is lower. The cations dissociate to equilibrate with the low concentration.]



Gramicidin A

Fig 3.2: Gramicidin A, a model of a pore-forming carrier protein. Two molecules of this peptide with15 amino acid residues form a pore across the membrane through a hydrophobic interaction between the side chains of the amino acids and the membrane lipid. Solutes can move through the pore

Hydrophobic methyl and isopropyl groups form the surface of the circular molecule while hydrophilic carbonyl groups are arranged inside. Cations such as K^+ bind to the hydrophilic interior of the molecule. The complex moves through the hydrophilic membrane to the other side of the membrane where the cation dissociates. The efficiency of mobile carrier is temperature dependent and becomes less efficient at low temperature due to decreased membrane fluidity. Uncouplers are mobile carriers of H^+. Gramicidin A is a peptide consisting of 15 amino acid residues (Figure 3.2). These linear peptides (one hydrophilic side and one hydrophobic side. Two molecules of this compound form a hydrophilic pore across the membrane with the interaction between the hydrophobic side of the compound and the membrane lipid. Various cations can move through the pore thus created, the efficiency being temperature independent. It is believed that some carrier proteins are mobile carriers while others form pores.

3.3. DIFFUSION:

Some solutes enter cells by diffusion according to the concentration gradient without energy expenditure. Hydrophobic solutes diffuse through the lipid part of the membrane, and others diffuse through carrier proteins. The former is called simple diffusion and the latter facilitated diffusion. Facilitated diffusion shows different kinetics from that of simple diffusion. The initial diffusion rate is proportional to the concentration gradient in simple diffusion, while facilitated diffusion shows a relationship between the diffusion rate and the concentration of the solute like the Michaelis–Menten kinetics known in enzyme catalysis. Solutes transported through facilitated diffusion do not passively leak into the cell to any significant extent, and the rate of transport is directly proportional to the fraction of carrier proteins associated with them. When the carrier protein is fully saturated with the solute, the rate of transport reaches a maximum, and the rate does not increase further with any further increase in solute concentration. Charged solutes are not transported through diffusion, since the transport of charged solutes changes the membrane potential.

3.4. ACTIVE TRANSPORT AND ROLE OF ELECTROCHEMICAL GRADIENTS:

Solute transport coupled to energy transduction is divided into primary and secondary transport according to the energy source. Primary transport systems are driven by energy-generating metabolism. Primary transport includes proton export driven by electron transport in respiration and photosynthesis by ATP hydrolysis, and by light in halophilic archaea. Also included in primary transport are chloride ion import in halophilic archaea driven by light,

sodium ion export coupled to decarboxylation reactions, proton export coupled to fumarate reduction and fermentation product excretion and import of sugars through group translocation). These ion transport mechanisms are energy-conserving processes, except for sugar transport by group translocation, and will be discussed in the appropriate sections. This section is devoted to energy dependent transport of materials needed for growth and survival and includes the secondary transport and group translocation of sugars.

Energy needed for secondary transport is supplied as an electrochemical gradient (a proton motive or sodium motive force) or from high energy phosphate bonds, as the results of primary transport (the proton (acidic internal pH) gradient and membrane potential) are established. Since sodium ions are exported due to their coupling with some energy-yielding reactions such as decarboxylation and anaerobic respiration, a sodium gradient across the prokaryotic membrane is also established. Proton and sodium gradients are collectively termed electrochemical gradients, and they are used as energy for many secondary transport processes. All the carrier proteins studied have 12 helices spanning the membrane, some of which function as binding sites for solutes and others for protons (or sodium ions).

The proton motive force consists of a proton gradient (ΔpH) and membrane potential (ΔΨ). Depending on the nature of the solute, transport requires energy in the form of either ΔpH or ΔΨ, or both. According to the carrier proteins involved, electrochemical gradient-dependent solute transport can be classified as symport, antiport and uniport mechanisms (Figure 3.3).

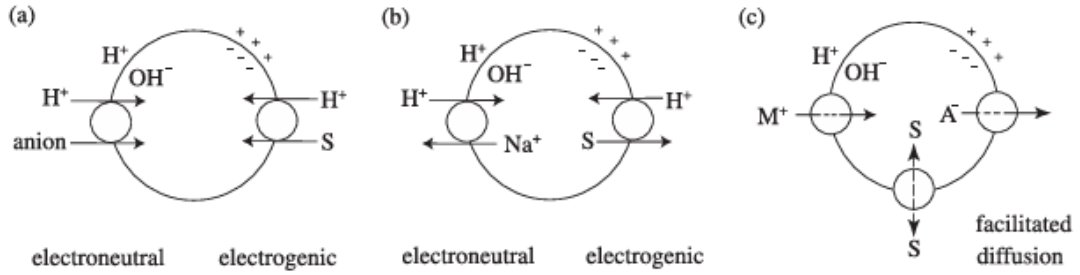


Fig 3.3: Electrochemical gradient dependent active transport. (Dawes, E. A. 1986, Microbial Energetics, Figure 6.1. Blackie &Son, Glasgow)

[Some solutes move across the membrane together with protons (or sodium ions) in the same direction (a, symport), while others move in opposite directions (antiport). Some ions cross the membrane along the electrochemical gradient (c, uniport). Transport involving the membrane potential is called electrogenic transport. Uniport and symport of uncharged solutes are electrogenic. When anions with a total negative charge of n are symporter with m protons, the transport becomes electroneutral in the case of n = m and electrogenic in the case of n # m. In antiport, where cations with a total positive charge of n are exchanged with m protons it becomes electroneutral in the case of n=m and electrogenic in the case of n #m.]

A solute cross the membrane in the same direction with protons (or sodium ions) in the symport mechanism but in the opposite direction in an antiport system. Uniporters transport ions along the electrochemical gradient without involving protons or sodium ions. Uniporters consume only the ΔΨ part of the proton motive (or sodium motive) force. This is

called electrogenic transport. When a monovalent anion is symported (cotransported) with a proton, the ΔpH is reduced without any change in the ΔΨ. This is called electroneutral transport. When an uncharged solute is symported with protons, the ΔpH as well as the ΔΨ supply the energy needed for the accumulation of the solute. Since ΔΨ is reduced this becomes an electrogenic transport system.

3.5. ATP-DEPENDENT TRANSPORT: ATP-BINDING CASSETTE (ABC) PATHWAY

Solute transport can be driven not only by the electrochemical gradient but also by ATP hydrolysis. An (ATPase)mutant of *Escherichia coli* was unable to take up maltose under conditions where a large protonmotive force was established, but the disaccharide was transported when the mutant was supplied with substrates that can produce ATP through substrate-level phosphorylation such as1,3-diphosphoglycerate and phosphoenolpyruvate. Maltose transport requires not only ATP but also a binding protein in the periplasm. Gram-negative bacteria have solute-binding proteins in the periplasm that are released when cells are subjected to osmotic shock with EDTA and $MgCl_2$ (cold osmotic shock). For this reason, solute-binding protein-dependent transport is called a shock sensitive

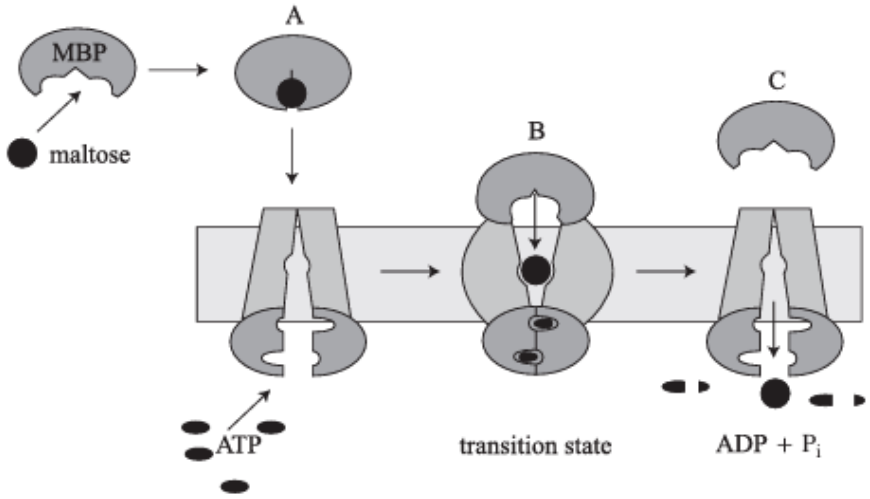


Fig 3.4: Maltose Transport Through the ATP-Binding Cassette (ABC) Transporter

(J. Bacteriol. 184:1225–1233, 2002) (A) Maltose-binding protein (MBP) binds maltose, undergoing a change from an open to a closed conformation, generating a high affinity sugar-binding site. In the closed conformation, MBP binds the maltose ATP-binding cassette (ABC) to initiate transport and ATP hydrolysis. (B) In the transition state for ATP hydrolysis, the MBP becomes tightly bound to the maltose ABC to transfer the sugar. (C) Maltose is transported, and MBP is released transport system. A variety of nutrients including sugars, amino acids and ions can be transported through the shock-sensitive transport system. Solutes cross the outer membrane through porins and bind specific binding proteins before being transported through the cytoplasmic membrane by a membrane-bound protein complex.

This protein complex is a member of a large super family of proteins that import nutrients or export cell surface constituents and extracellular proteins. They have an ATP-binding motif and hydrolyse ATP to supply energy for the transport. They are called ATP-binding cassette (ABC) transporters and are known in all organisms (Figure 3.4). Glutamate-binding protein is known in *Rhodobacter sphaeroides*, but glutamate transport is driven by the electrochemical gradient in this bacterium. This system is known as the TRAP (tripartite ATP independent periplasmic) transporter. TRAP transporters are shock insensitive. Through active transport, nutrients are accumulated in the cell with the expenditure of a large amount of energy in the form of the electrochemical gradient or ATP. Microbes can grow efficiently in environments with low nutrient concentrations due to active transport systems. Active transport can be summarized as follows:

1) Carrier proteins have solute specificity as in the enzyme–substrate relationship.
2) Energy is needed to change the affinity of the transporter for the transported solute at the other side of the membrane.
3) The transported solute can be accumulated against a concentration gradient.
4) The structure of the solute does not change during active transport

3.6. GROUP TRANSLOCATION:

Sugars are phosphorylated during their transport in many bacteria, especially in anaerobes. The phosphate donor in these transport systems is phosphoenolpyruvate (PEP), a glycolytic intermediate. Since the solute is phosphorylated, this transport is referred to as group translocation. This system is not known in eukaryotes. A group of proteins known as the phosphotransferase (PT) system transports and phosphorylates sugars (Figure 3.5). They are cytoplasmic enzyme I and HPr (histidine-containing protein), and membrane bound enzymes IIA, IIB and IIC. Enzyme I transfer phosphate from PEP to HPr. phosphorylated HPr transfers phosphate to enzyme IIA. The solute passes through the membrane-embedded enzyme IIC and enzyme IIB transfers phosphate from enzyme IIA to the solute. The cytoplasmic proteins HPr and enzyme I do not have any specificity towards the sugar and are common components of PT systems. On the other hand, the membrane-bound proteins are specific for each sugar transported. The specific enzymes are described as enzyme IIA Glu etc. As shown in Figure 3.5, there are variations in the proteins involved in the transport of different sugars. Enzyme IIA Glu is a soluble protein, and enzyme IIA and IIB involved in mannose transport are also soluble. In addition, the mannose PT system has an extra membrane protein, enzyme IID.

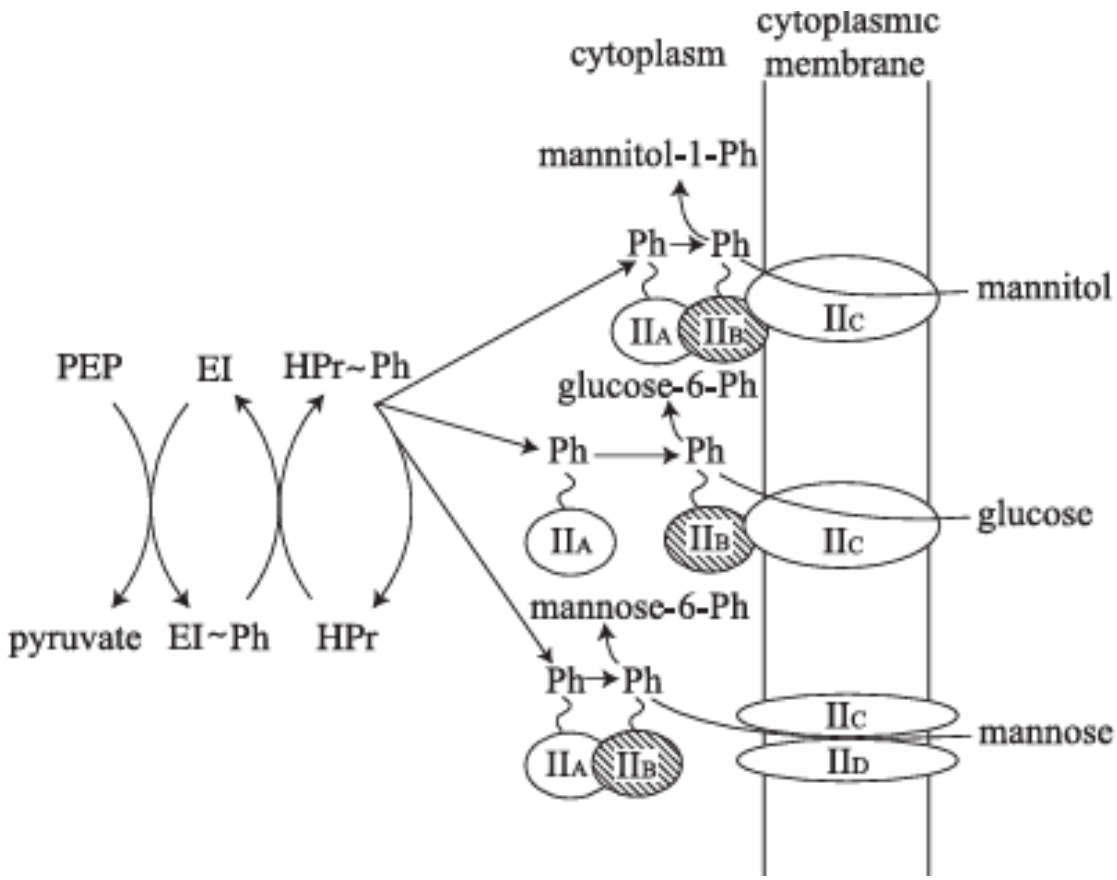


Fig 3.5: Group translocation of sugars mediated by the phosphotransferase system in anaerobic bacteria.

[(Microbiol. Rev. 57:543–594, 1993). Sugars are transported into the cell as phosphorylated forms mediated by the phosphotransferase (PT) system. Phosphoenolpyruvate (PEP) serves as the phosphate donor.]

3.7. PRECURSOR / PRODUCT ANTIPORT:

Some lactic acid bacteria utilize the potential energy developed by the accumulation of fermentation products in the cell to drive nutrient transport in a similar manner to antiport. Instead of H^+ or Na^+ a fermentation product is changed with its precursor and this system is therefore referred to as the precursor/product antiport (Figure 3.6).

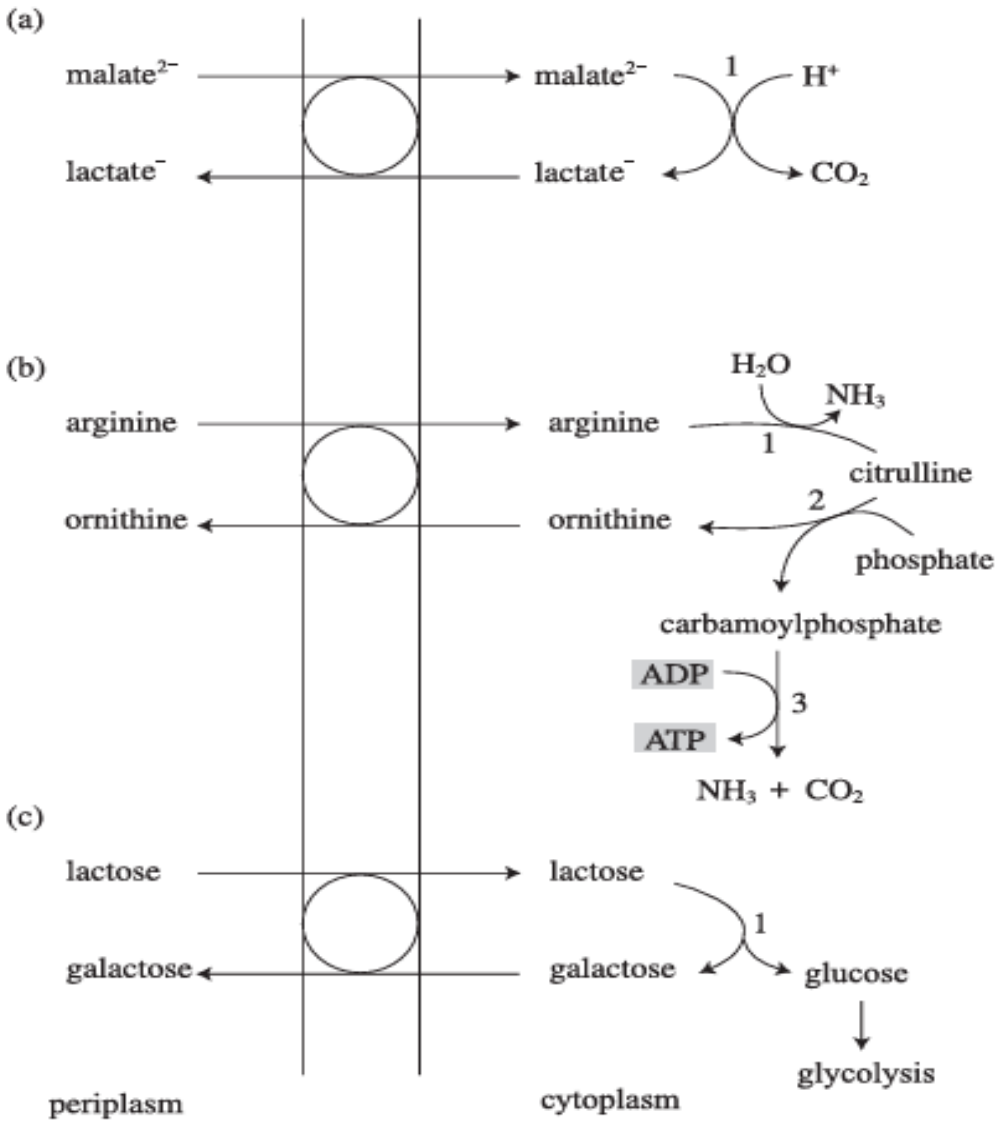


Fig 3.6: Nutrient Import Systems in Prokaryotes

[Precursor/product antiport systems of lactic acid bacteria. (Mol. Microbiol. 4:1629–1636, 1990) Some anaerobic fermentative bacteria, including lactic acid bacteria, utilize the potential energy of a high concentration of fermentation product inside the cell to import the precursor. (a) Malate/lactate antiport. 1, malolactic fermentation. (b) Arginine/ornithine antiport. 1, argininedeiminase;2, ornithine carbamoyl transferase; 3, carbamate kinase. (c) Lactose/galactoseantiport.1, -galactosidase]

A malate/lactate antiport system is known in species of *Lactobacillus, Streptococcus, Leuconostoc and Pediococcus.* They generate a proton motive force fermenting malate to lactate through the well-documented malolactic fermentation pathway.

Species of *Streptococcus* ferment arginine to ornithine through citrulline to produce ATP. Ornithine is exchanged with arginine in a 1:1ratio. Lactose is imported through a H^+- symport system in *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Lactose can be transported by a lactose/galactose antiport system in these bacteria when the lactose concentration is high. Lactose is hydrolysed to glucose and galactose by β-galactosidase. Glucose is metabolized through glycolysis and galactose is exchanged with lactose. Excreted galactose is then utilized after all the lactose is consumed.

In addition to lactic acid bacteria, precursor/product antiport systems are known in other anaerobic fermentative bacteria such as the oxalate / formate antiporter in *Oxalobacter formigenes* and the betaine/ N,N-dimethyl glycine antiporter in *Eubacterium limosum*. Figure 3.7 summarizes some of the nutrient uptake pathways known in prokaryotes.

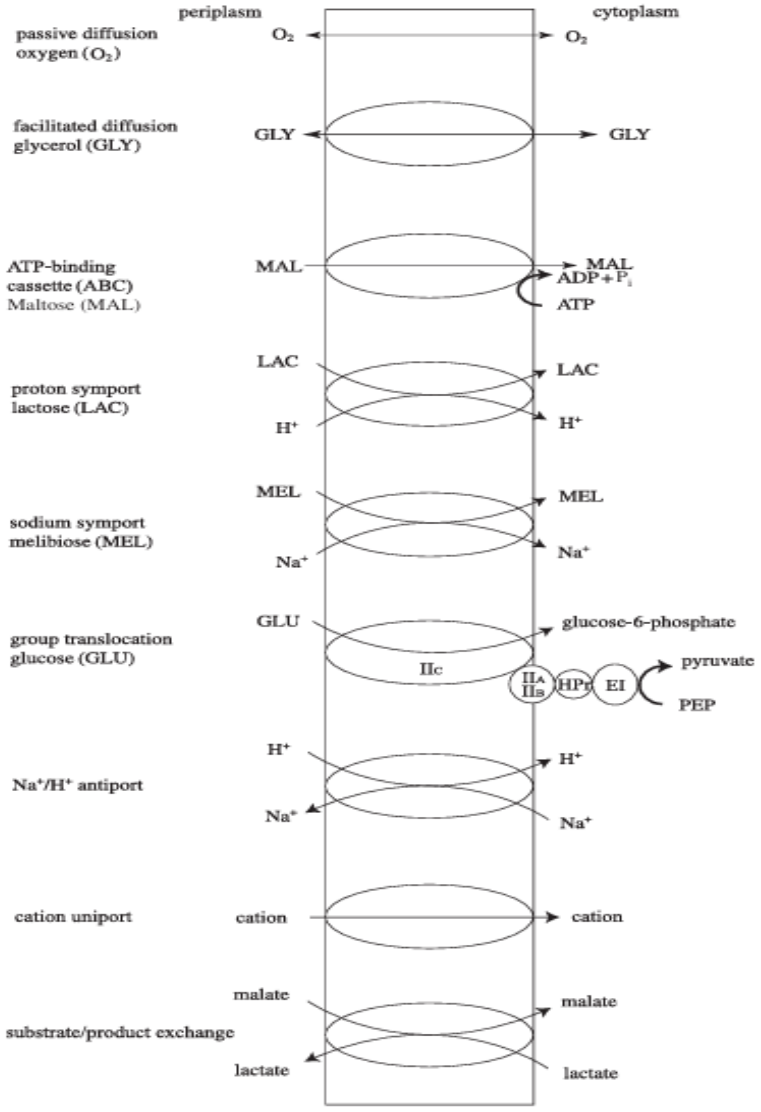


Fig 3.7: Nutrient Import Systems in Prokaryotes

3.8. FERRIC ION (Fe(III)) UPTAKE:

In natural aerobic ecosystems, almost all iron is present as the ferric ion (Fe(III)) since ferrous iron (Fe(II)) is auto-oxidized with molecular oxygen at neutral pH. Ferric iron is virtually insoluble in water with a solubility of around 10^{-20} M and this is much lower than the 10^{-6} M necessary to supply adequate iron for most microbes. To overcome this problem, many microbes synthesize and excrete low molecular weight ferric iron chelating compounds known as siderophores for the sequestration and uptake of iron. Siderophores form complexes with ferric iron that are imported into the cell by an ABC transport system. Siderophores are a collection of compounds with a variety of chemical structures. Two main structural classes of siderophores are the catechol amides and the hydroxamates (Figure 3.8). A given organism may produce siderophores of one or both classes.

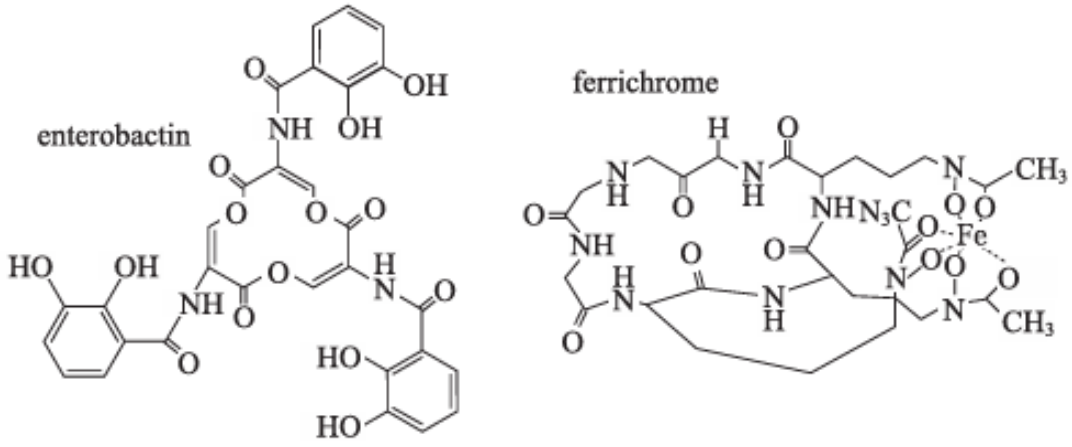


Fig 3.8: Structures of thesiderophores enterobactin(a catecholamide) andferrichrome (a hydroxamate).(FEMS Microbiol. Rev. 27:215–237,2003)

3.9. METABOLIC REGULATION:

Life processes transform materials available from the environment into cell components. Organic materials are converted to carbon skeletons for monomer and polymer synthesis, as well as being used to supply energy. Microbes synthesize monomers in the proportions needed for growth. This is possible through the regulation of the reactions of anabolism and catabolism. With a few exceptions, microbial ecosystems are oligotrophic with a limited availability of nutrients, the raw materials used for biosynthesis. Furthermore, nutrients are not usually found in balanced concentrations while the organisms have to compete for available nutrients.

Unlike animals and plants, unicellular microbial cells are more directly coupled to their environment, which changes continuously. Many of these changes are stressful so organisms have evolved to cope with this situation. They regulate their metabolism to adapt to the ever-changing environment. Since almost all biological reactions are catalysed by enzymes, metabolism is regulated by controlling the synthesis of enzymes and their activity (Table 3.1). Metabolic regulation through the dynamic interactions between DNA or RNA and the regulatory apparatus employed determine major characteristics of organisms.

different mechanisms of metabolic regulation are discussed in terms of enzyme synthesis through transcription and translation and enzyme activity modulation.

Table 3.1: Regulatory Mechanisms that Control the Synthesis and Activity of Enzymes

	Mechanism
Enzyme synthesis	
Transcription	promoter structure and sigma (σ) factor
	activator – positive control
	repressor – negative control
	termination – antitermination
Translation	attenuation
	autogenous translational repression
	mRNA stability
Enzyme activity	feedback inhibition
	feedforward activation
	chemical modification
	physical modification
	degradation

3.10. MECHANISMS REGULATING ENZYME SYNTHESIS:

The rate of biological reactions catalysed by enzymes is determined by the concentration and activity of the enzymes. Various mechanisms regulating the synthesis of individual enzymes are discussed here before multigene regulation is considered.

RNA polymerase synthesizes messenger RNA (mRNA) that is needed for protein synthesis according to the DNA template. RNA synthesis is initiated with the recognition of the promoter by the σ factor of the RNA polymerase. Several σ-factors have been found in all the bacteria tested. They are grouped in two main families: the σ-70 and σ-54 families. The latter is composed exclusively of σ- 54, while the σ-70 family includes all others. The σ-70 family is further divided into four groups (Table 12.2).

Table 3.2: RNA Polymerase-Factors in *Escherichia Coli*

σ-factor	Gene	Function	Promoter consensus sequence −35	Promoter consensus sequence −10	Anti-σ factor
σ−70 family					
Group 1					
σ^D (σ^{70})	*rpoD*	housekeeping	TTGACA	TATAAT	AsiA[a]
Group 2					
σ^S (σ^{38})	*rpoS, katF*	stringent response general stress response	–	CTATACT	RssB
Group 3					
σ^F (σ^{28})	*rpoF, fliA*	chemotaxis	TAAA	GCCGATAA	FlgM
σ^H (σ^{32})	*rpoH, htpR*	heat shock protein	CTTGAAA	CCCATnT	DnaK
Group 4					
σ^E (σ^{24})	*rpoE*	stress response	GAACTT	TCTRA	RseA
σ−54 family					
σ^N (σ^{54})	*rpoN, ntrA*	nitrogen metabolism	TGGCAC	TTGCW	

R: A or G; n: A, T, G, or C; W: A or T.
[a] Bacteriophage T4.

The group 1 σ^D is essential, and responsible for the transcription of most genes in exponentially growing cells. A group 2 σ-factor, σ^S, participates in the expression of stationary phase proteins. Group 3 σ-factors include σ- F, responsible for flagella synthesis, and σ- H, the heat shock response σ-factor in *Escherichia coli.* Sigma factors involved in spore formation in *Bacillus subtilis* belong to group 3. σ - E is a member of group 4 σ-factors. This group includes the extra cytoplasmic function σ-factor subfamily, the largest and most divergent group.

Each σ-factor recognizes a different promoter. The promoter region in *Escherichia coli* consists of six bases, each at -35 and -10 bases upstream of the transcription start site. Genes for proteins needed in large quantities have strong promoters with a high affinity for the σ-factors. For example, the housekeeping σ-^{D} (σ-^{70}) recognizes the promoter region consisting of TTGACA at -35 and TATAAT at - 10 most efficiently but is much less effective for those with substituted base(s) in *Escherichia coli.* It should be emphasized that promoter activity is regulated by other transcription factors such as a repressor and an activator as well as others that interact with DNA around the promoters, as discussed later, While the essential housekeeping σ^D functions in exponentially growing cells, other σ-factors are activated according to the growth conditions, including σ^E, σ^F, σ^H, σ^N and σ^S. The promoters recognized by σ^S (σ38) do not have a -35 region but have a longer -10 region known as the extended –10 region.

Sigma factors with similar functions have different names indifferent organisms. The housekeeping σ-factor is called σ^D in Gram-negative bacteria and σ^A in Gram-positive bacteria. HrdB is the housekeeping σ-factor in species of the genus *Streptomyces.* Consequently, σ-factors with the same name have different functions in different bacteria: σ^D is the housekeeping σ-factor in *Escherichia coli* but is the σ-factor for flagella formation in *Bacillus subtilis.* In a related terminological problem, the extra cytoplasmic function σ-factor, σ^E, should not be regarded as functioning extra cytoplasmically. Instead, σ^E participates in the expression of genes needed to repair denatured protein of extra cytoplasmic location. Since a σ-factor recognizes multiple promoters throughout the chromosome, a specific σ-factor

participates in the transcription of functionally unrelated genes. Regulation by σ-factor–promoter interaction is a type of global regulation system discussed later

Proteins known as anti-sigma (anti-σ) factors bind σ-factors and inhibit their activity. When *Escherichia coli* is infected with bacteriophageT4, a phage-originating anti-σ factor, AsiA, inhibits the activity of the bacterial housekeeping σ^D to produce more phage proteins. An anti-σ factor for σE, RseA, is a membrane protein. Under normal growth conditions σ^E binds RseA. When σ^E is required, either an anti-σ factor binds RseA releasing σ^E or RseA is degraded to release σ^E. In the case of σF responsible for flagellin synthesis, the anti-σ factor, FlgM, is exported into the environment through the flagellin export mechanism when more flagellin is needed. The stationary phase σ-factor is inactivated by the anti-σ factor RssB, before being hydrolysed by an ATP-dependent protease (ClpXP) when the σ^S is needed. Promoter activity is also regulated by the superhelix DNA structure and various general DNA-binding proteins such as H-NS, Fis, and StpA in addition to the specific activators and repressors.

3.11. INDUCTION OF ENZYMES:

Inducible and constitutive enzymes When *Escherichia coli* is transferred from a glucose medium to a lactose medium, the bacterium synthesizes "-galactosidase to hydrolyse the lactose into glucose and galactose (Figure 3.9). Enzymes synthesized in the presence of substrates are referred to as inducible enzymes and the substrate is termed the inducer. Constitutive enzymes are those enzymes that are produced under all growth conditions. Inducible enzymes are generally those used in the catabolism of carbohydrates such as polysaccharides (cellulose, starch, etc.), oligosaccharides (lactose, trehalose, raffinose, etc.) and minor sugars (arabinose and rhamnose), and aromatic compounds.

When a single inducer induces more than two enzymes, they are produced either simultaneously or sequentially. The former is referred to as coordinate induction, and the latter as sequential induction (Figure 3.10). Genes of coordinate induction are in the same operon, and genes from separate operons are induced sequentially.

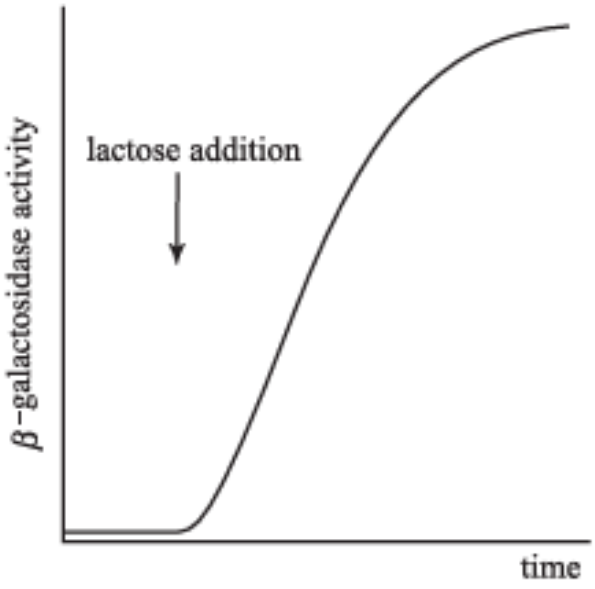


Fig 3.9: Induction of b-galactosidase by Lactose in *Escherichia Coli*

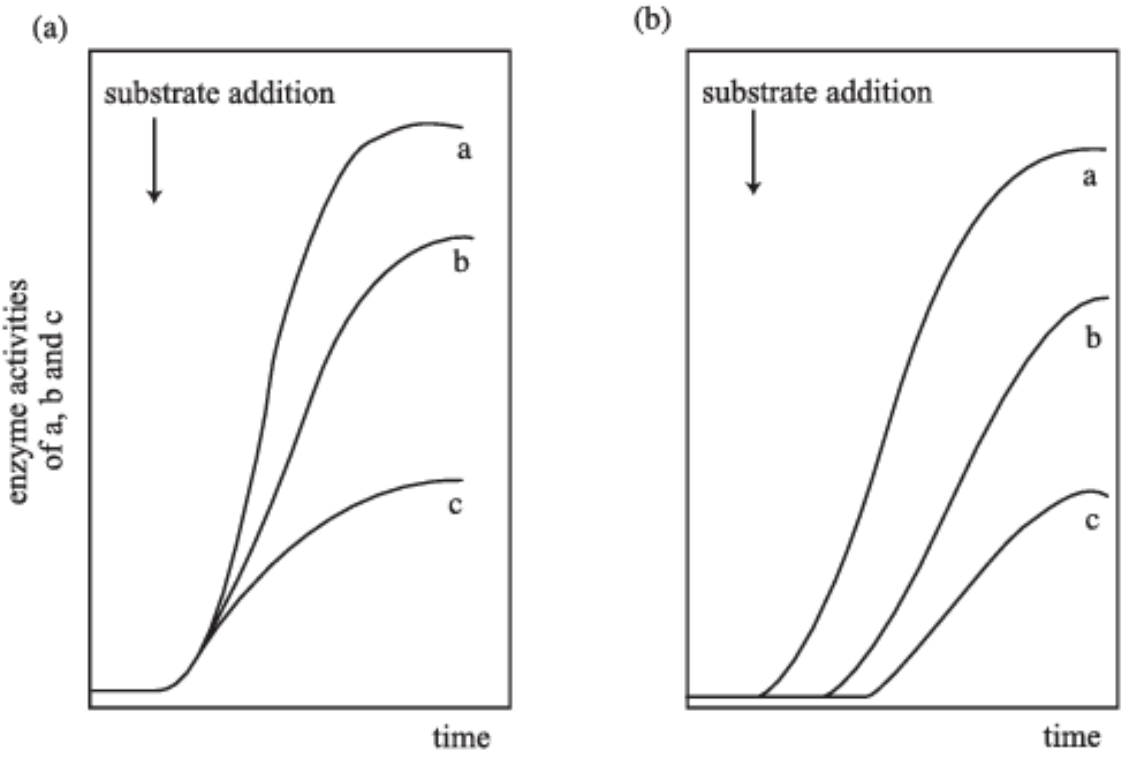


Fig 3.10: Coordinate induction and sequential induction of multiple enzymes by a single inducer.

(Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 7.2. Springer, New York) Enzymes from genes of the same operon are induced simultaneously in coordinate induction (a) and genes from different operons are induced sequentially (b). The product of the first enzyme reaction is the inducer of the second enzyme in sequential induction.

3.12. ENZYME INDUCTION:

Enzyme induction is regulated at the level of transcription. Lactose induces the production of β-galactosidase, permease and transacetylase. Their structural genes form an operon (lac operon) with a promoter and operator (Figure 3.11a). The regulatory gene (lacI) next to the 5' end of the operon is expressed constitutively with its own promotor. In the absence of the inducer, the repressor protein binds the operator region of the lac operon, inhibiting RNA polymerase from binding the promoter region. Consequently, the structural genes are not transcribed (Figure 3.11b1). On the other hand, when the inducer is available, it binds the repressor protein, removing it from the operator region (Figure 3.11b2). The repressor protein is not produced in a lacI mutant. This mutant transcribes the structural genes as constitutive enzymes in the absence of the inducer. In this sense, the regulation by repressor proteins is referred to as negative control.

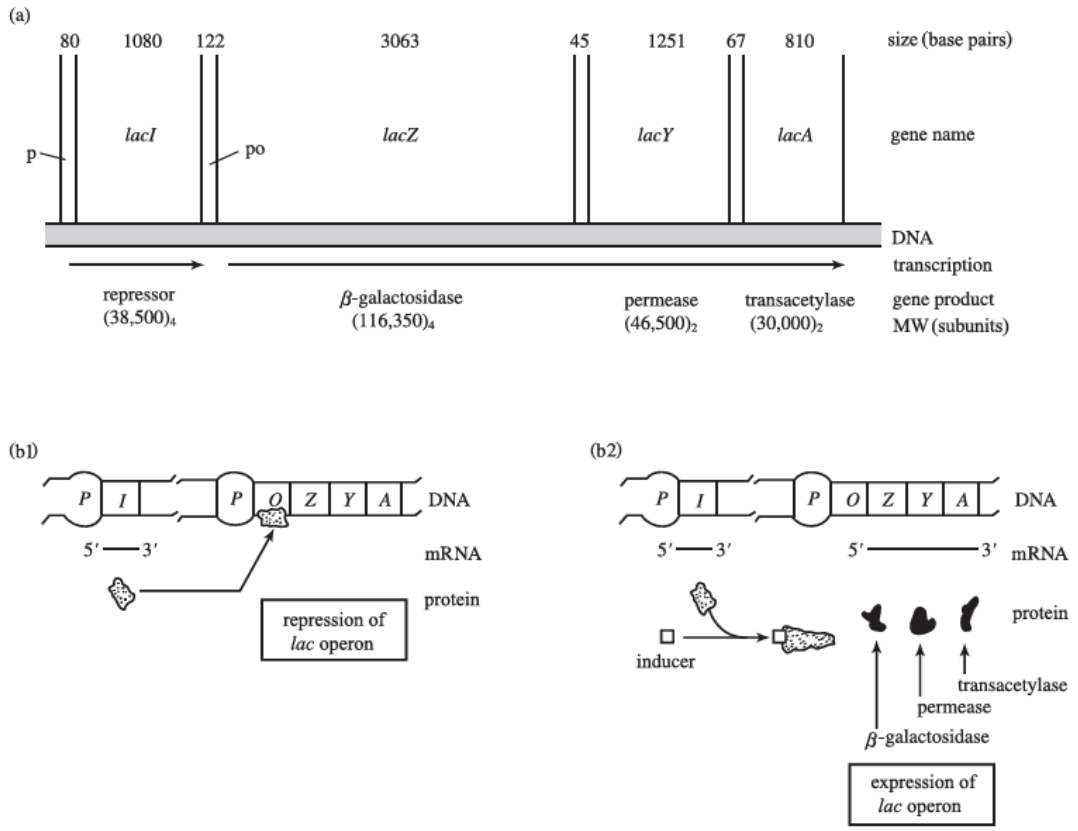


Fig 3.11: Induction mechanism of the lac operon in *Escherichia coli.*

(Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 7.4. Springer, New York)

[The lac operon consists of a promoter, operator and structural genes, and a repressor protein is produced from the lacI gene that has its own promoter (a). In the absence of inducer, the repressor protein binds the operator region of the operons, preventing transcription of the structural genes (b1). The inducer forms a complex with the repressor protein. The complex cannot bind the operator region, and the structural genes are transcribed (b2). The structural genes are transcribed as constitutive enzymes in the lacI mutant. The regulation by repressor proteins is referred to as negative control.]

3.13. POSITIVE AND NEGATIVE CONTROL:

As stated above, the lac operon is regulated by a negative control mechanism. Activator proteins are involved in the regulation of catabolic genes for arabinose, rhamnose, maltose and others. Genes for arabinose catabolism consist of *ara*A, B, C, D, E, and F; *ara*C is are gulatory gene encoding an activator protein. *ara*C mutants are unable to use arabinose, since an a *Ara*C complex with the inducer activates the transcription of the structural genes (Figure). araA, B, C and D form an operon, and araE and *ara*F occupy other parts of the chromosome. The term regulon is used to define genes of the same metabolism controlled by the same effectors scattered around the chromosome, such as *ara* genes. Regulation by an activator, as in the *ara* regulon, is referred to as positive control.

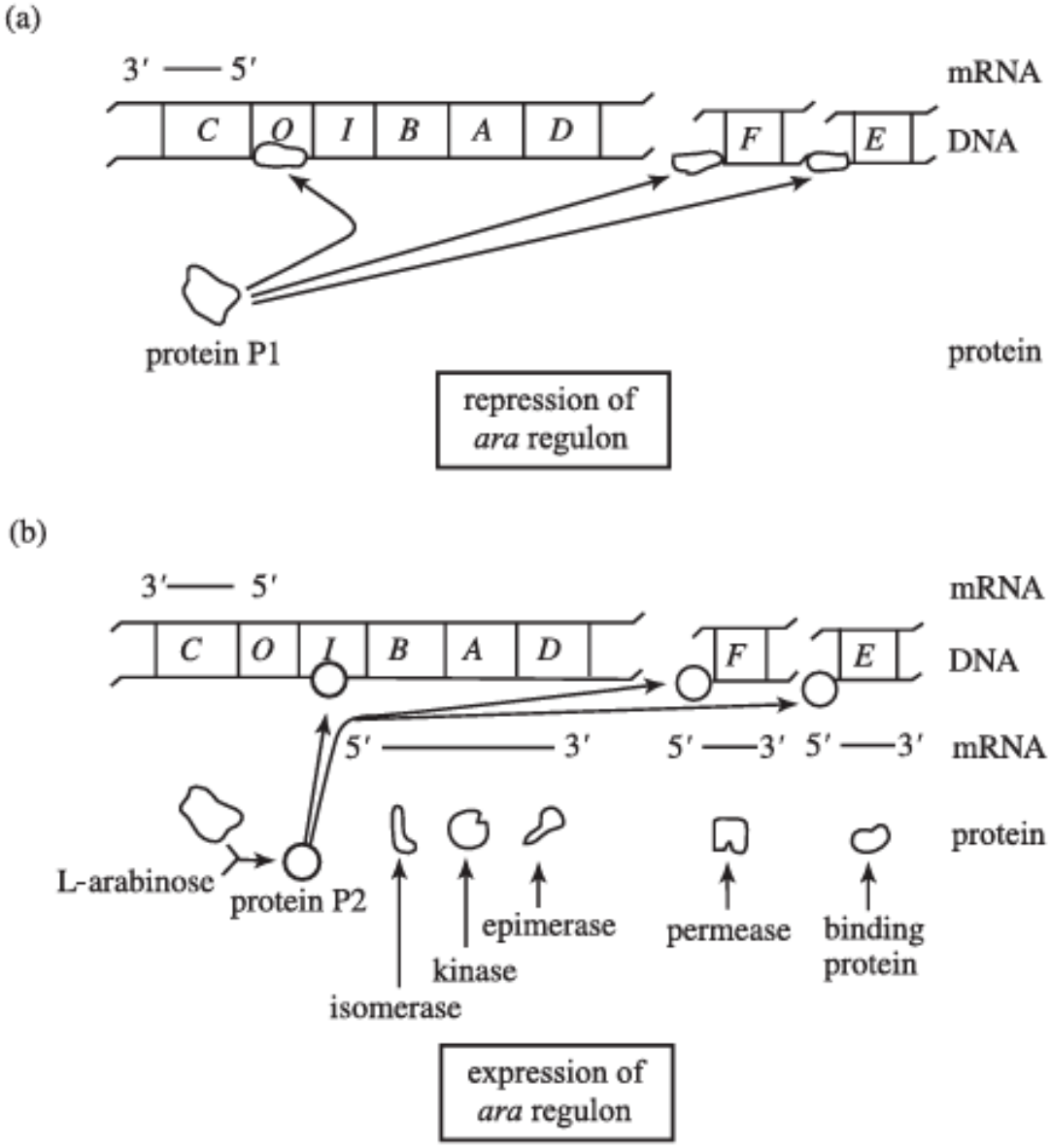


Fig 3.12: Induction of enzymes of arabinose metabolism in Escherichia coli.

(Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 7.5. Springer, New York)

[The regulator protein AraC is in the protein P1 form to bind the operator region, inhibiting transcription of the structural genes in the absence of arabinose (a). When the inducer arabinose binds, protein P1 is converted to protein P2. Protein P2 activates transcription of the structural genes. *ara*C mutants cannot use arabinose. The term positive control is used to describe metabolic regulation by activators.]

3.14. SUMMARY:

Microbial membrane transport is the essential process by which microorganisms regulate the movement of substances across their cell boundaries. This activity is critical for cellular function, allowing the intake of necessary nutrients for survival and the excretion of

waste products and proteins, such as enzymes or virulence factors. The cell membrane acts as a selective barrier, ensuring the cell can maintain homeostasis despite environmental changes.

3.15. Self-Assessment

1) The concept of ionophores and how they function as carrier proteins to facilitate ion transport across biological membranes.
2) Explain different models of carrier proteins and how ionophores differ from channel-forming transport systems.
3) Describe diffusion as a passive transport process driven by concentration gradients.
4) Factors affecting diffusion, including membrane permeability, molecule size, and concentration differences.
5) What is active transport mechanisms and their dependence on energy input. how electrochemical gradients influence the movement of ions and molecules across membranes.
6) Describe the ATP-binding cassette (ABC) transporters and their role in substrate transport using ATP hydrolysis, describe their structural components and biological significance.
7) Describe the group translocation as a transport mechanism in which substrates are chemically modified during transport and explain the phosphotransferase system (PTS) as an example.
8) Explain the mechanism of precursor/product antiport and how it supports metabolic efficiency by exchanging intracellular products with extracellular substrates.
9) What is the importance of iron uptake in microorganisms and can describe ferric ion transport mechanisms, including the role of siderophores.
10) Explain how metabolic pathways are regulated to maintain cellular efficiency.
11) Explain how enzyme synthesis is regulated at the transcriptional and translational levels, including repression and induction mechanisms.
12) Explain enzyme induction and how the presence of specific substrates stimulates enzyme synthesis.
13) Analyze Enzyme induction is a regulatory process that allows cells to adapt to environmental changes by producing specific enzymes when needed.
14) Differentiate between positive and negative control of gene expression and explain how activators and repressors influence enzyme synthesis.

3.16. REFERENCES:

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Prof A. Amrutha Valli

LESSON-4

PHOTOSYNTHESIS AND PHOTOSYNTHETIC PIGMENTS

4.0 OBJECTIVE:

- **S**tudying microbial photosynthesis and photosynthetic pigments is to understand how these organisms capture light energy and convert it into chemical energy, and to explore the potential industrial and environmental applications of these processes and the pigments themselves.

STRUCTURE:

4.1. INTRODUCTION:

Photosynthetic organisms use light energy to fuel their biosynthetic processes. Oxygen is generated in oxygenic photosynthesis where water is used as the electron donor. In anoxygenic photosynthesis, organic or sulfur compounds are used as electron donors. Plants, algae and cyanobacteria carry out oxygenic photosynthesis, whereas the photosynthetic bacteria obtain energy from anoxygenic photosynthesis. Aerobic anoxygenic phototrophic bacteria use light energy in a similar way as the purple bacteria, and are a group of photosynthetic bacteria that grow under aerobic conditions.

Phototrophic organisms have a photosynthetic apparatus consisting of a reaction centre intimately associated with antenna molecules (or a light-harvesting complex). The antenna molecules and the reaction centre absorb light energy. The energy is concentrated at the reaction centre that is activated and initiates light-driven electron transport. Halophilic archaea convert light energy through a photophosphorylation process.

4.2. PHOTOSYNTHETIC MICROORGANISMS:

Microorganisms utilizing light energy include eukaryotic algae, and cyanobacteria, photosynthetic bacteria and aerobic anoxygenic phototrophic bacteria among the prokaryotes. The halophilic archaea synthesize ATP through photophosphorylation, but they are not considered to be photosynthetic organisms since they lack photosynthetic pigments.

Algae and cyanobacteria have similar photosynthetic processes, using chlorophyll, as plants. However, cyanobacteria are members of the proteobacteria according to their cell structure and ribosoma lRNA sequences (Table 4.1) Photosynthetic bacteria are different from other photosynthetic organisms. They have different photosynthetic pigments and do not use water as their electron donor. Some of them can grow chemoorganotrophically in the dark.

Table 4.1: Cyanobacteria

	Morphology	Cell division[a]	Heterocysts	N_2 fixation
Unicellular group	single cells	single	–	+[b]
Pleurocapsa group	single cells	multiple	–	+[b]
Oscillatoria group	filaments	single	–	–
Heterocystous group	filaments	single	+	+

[a] Cell divides into two daughter cells (single) or into more than two daughter cells (multiple).
[b] Several species do not fix N_2.

4.3. CYANOBACTERIA:

Cyanobacteria (also known as blue-green algae) grow photolithotrophically and fix CO2 through the Calvin cycle. They do not generally require growth factors except for some that require vitamin B12. They are classified into four groups according to their morphology. These are a unicellular group, the Pleurocapsa group, the Oscillatoria group and a heterocystous group (Table 1). N2 is fixed by some members of the unicellular cyanobacteria and the Oscillatoria group, and by all members of the heterocystous group. Under N2-fixing conditions, some of the cells within the filaments of heterocystous group cyanobacteria transform into heterocysts that lack photosystem II and this protects the nitrogenase from O2).

4.4. ANAEROBIC PHOTOSYNTHETIC BACTERIA:

Photosynthetic bacteria are grouped according to their photosynthetic pigments and the electron donors used. These are purple bacteria, green bacteria and heliobacteria. Purple and green bacteria are further divided into purple non-sulfur and purple sulfur bacteria, and green sulfur and filamentous anoxygenic phototrophic bacteria (Table 4.2).

The purple sulfur bacteria include members of the Chromatiaceae and Ectothiorhodospiraceae within the-proteobacteria. The former accumulates sulfur granules intracellularly and the latter extracellularly.

The purple non-sulfur bacteria are more diverse, belonging to "- and #-proteobacteria. They grow photosynthetically under anaerobic conditions, and many of them can grow chemo organotrophically under aerobic conditions. The purple non-sulfur bacteria grow under all electron-accepting conditions (aerobic respiratory, anaerobic respiratory and fermentative conditions) in addition to anaerobic photosynthetic conditions. The purple sulfur and nonsulfur bacteria have pheophytin–quinone-type reaction centres.

The photosynthetic green bacteria include two physiologically and phylogenetically distinct groups. These are the strictly anaerobic and obligately photolithotrophic green sulfur bacteria, and the filamentous anoxygenic photolithotrophic bacteria that are facultatively anaerobic. These have different reaction centres. The latter have the

Table 4.2: Photosynthetic Bacteria

	Purple bacteria		Green bacteria			
Character	Non-sulfur	Sulfur	Sulfur	FAPB	Heliobacteria	AAPB
BCHL	*a*, *b*	*a*, *b*	*a*, *c*, *d*, *e*	*a*, *c*, *d*	*g*	*a*
H_2S as e^- donor	±[a]	+	+	+	−	−
S accumulation	−	intracellular[b]	extracellular	−	−	−
H_2 as e^- donor	+	+	+	+	−	−
Organics as e^- donor	+	+	−	+	+	+
Carbon source	CO_2 organics	CO_2 organics	CO_2 organics	CO_2 organics	organics	organics
Aerobic respiration	+	−	−	+	−	+
CO_2 fixation	Calvin cycle	Calvin cycle	reductive TCA cycle	Calvin cycle[c]	−	−

FAPB, filamentous anoxygenic phototrophic bacteria; AAPB, aerobic anoxygenic phototrophic bacteria; BCHL, bacteriochlorophyll.
[a] Depending on the strain.
[b] Members of the family *Ectothiorhodospiraceae* accumulate sulfur granules extracellularly.
[c] Species of the genus *Chloroflexus* employ the 3-hydroxypropionate cycle.
Note: The halophilic archaea use light energy through photophosphorylation.

pheophytin–quinone type, while the former has the iron–sulfur type. The green sulfur bacteria cannot grow heterotrophically, while the filamentous anoxygenic phototrophic bacteria can grow heterotrophically under aerobic dark conditions. The latter, members of the Chloroflexaceae, belong to a deep-branching lineage of bacteria. These are also called photosynthetic flexibacteria. They stain Gramnegative but lack lipopolysaccharide.

The photoheterotrophic heliobacteria include three genera: Heliobacterium, Heliobacillus and Heliophilum. They do not grow aerobically in the dark, and fix N2. They do not have photosynthetic organelles and the photosynthetic pigments, including the unique bacteriochlorophyll g, are located in the cytoplasmic membrane. They have an iron–sulfur-type reaction centre. Heliobacterial cells have several unusual features. They are extremely fragile and lyse when approaching the stationary phase. They stain Gram-negative but lack lipopolysaccharide like the filamentous anoxygenic phototrophic bacteria, and do not fix CO2. Phylogenetically they belong to the low G/C Gram-positive bacteria.

4.5. AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA:

Photosynthetic bacteria utilize light energy under anaerobic conditions and their photosynthetic pigments are not synthesized under aerobic conditions. However, many bacteria are known to synthesize bacteriochlorophyll (BCHL) a and carotenoids under aerobic conditions.

They can harvest light energy while they respire oxygen. These are referred to as quasi-photosynthetic bacteria or aerobic anoxygenic phototrophic bacteria. They comprise at least 11% of the total microbial community in the upper open ocean. They inhabit a variety of locations, including the extreme environments of acidic mine drainage waters, hot springs and deep ocean hydrothermal vent plumes.

Aerobic anoxygenic phototrophic bacteria foundi n seawater include species of Erythrobacter, Roseibium, Roseivivax, Roseobacter, Roseovarius and Rubrimonas. Freshwater is the habitat of other aerobic anoxygenic phototrophic bacteria including species of Sandaracinobacter, Erythromonas, Erythromicrobium, Roseococcus, Porphyrobacter and Acidiphilium. These have the pheophytin–quinone-type reaction centre. These genera include not only aerobic phototrophs but also species unable to synthesize BCHL a. It is not clear if the ability to synthesize the photosynthetic pigment was lost or transferred through lateral gene transfer during their evolution. Aerobic anoxygenic photosynthesis is also known in species of Bradyrhizobium, syntrophically growing on the stems of tropical legume plants. Although these are obligate aerobes with high carotenoid and low BCHL contents, they are closely related to purple photosynthetic bacteria in several aspects.

Some of the aerobic anoxygenic phototrophic bacteria divide in an unusual manner. Budding in addition to binary division occurs in Porphyrobacter neustonensis and Erythromonas ursincola.

Ternary fission and branching are exhibited by Erythromicrobium ramosum and Erythromicrobium hydrolyticum.

4.6. PHOTOSYNTHETIC PIGMENTS:

Photosynthetic pigments include chlorophylls, carotenoids and phycobiliproteins in plants and cyanobacteria: phycobiliproteins are not found in photosynthetic bacteria (Table 4.3). Bacteria use bacteriochlorophylls in place of chlorophyll, and bacteriopheophytin to replace pheophytin.

Table 4.3: Photosynthetic Pigments

Pigment	Cyanobacteria	Purple bacteria	Green bacteria	Heliobacteria	Aerobic anoxygenic phototrophic bacteria
Reaction centre pigment	CHL *a*	BCHL *a*, *b*	BCHL *a*	BCHL *g*	BCHL *a*[a]
Antenna pigment	phycobiliprotein CHL *a*	BCHL *a* or *b*	BCHL *c*, *d* or *e*	BCHL *g*	BCHL *a*
Main carotenoid	dicyclic	aliphatic	aryl	aliphatic	dicyclic, aliphatic[b]

[a] BCHL *a* with Zn^{2+} or Mg^{2+}.
[b] Various carotenoids depending on the strain.

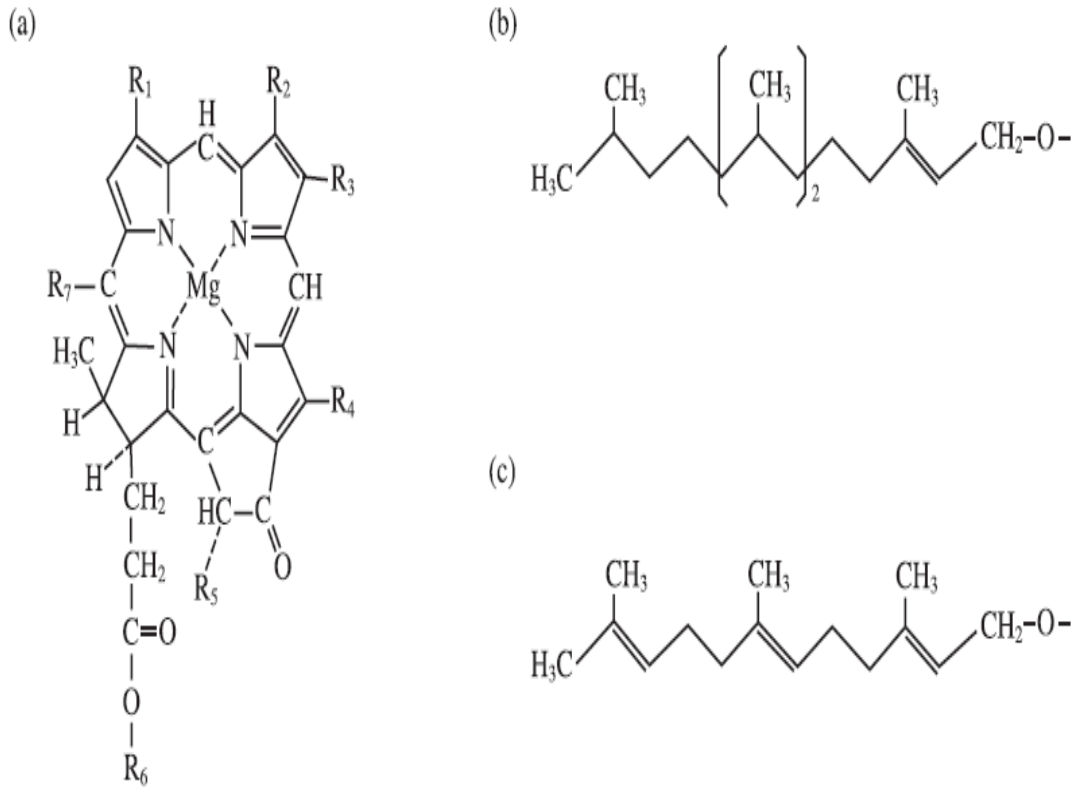


Fig 4.1: The Structure of Chlorophyll (see Table 11.4 for the side chains R1–R7). (a) chlorophyll; (b) phytyl group; (c) farnesyl group.

Chlorophylls:

Chlorophylls have a general structure of four pyrrole derivatives with covalently bound Mg2þ (Figure 4.1). They form a complex with proteins embedded in the membranes of the photosynthetic apparatus.

There are several structurally different chlorophylls that have different side chains from the pyrrole rings (Table 4.4). These include chlorophyll a, and bacteriochlorophyll (BCHL) a, b, c, d, e, and g. Bacteriochlorophyll a with Zn2þ in place of Mg2þ occurs in aerobic anoxygenic phototrophic bacteria. As listed in Table, cyanobacteria possess chlorophyll a as do plants. Green bacteria have reaction centres with BCHL a and antenna molecules with BCHL c, d or e. In contrast, purple bacterial reaction centres and antenna molecules contain BCHL a or b. Aerobic anoxygenic phototrophic bacteria contain BCHL a with Zn2þ or Mg2þin both the reaction centre and antenna molecules. Cyanobacteria with CHL a absorb relatively short wavelength light while light of wavelength over 700nm is absorbed by photosynthetic bacteria containing BCHL a or b. The purple bacteria absorb near infrared light of wavelength around 800nm (see Figure 4.4).

Carotenoids:

In addition to chlorophylls, photosynthetic organisms have carotenoids. They absorb light over wavelengths of 400–600nm and transfer the energy to chlorophylls, and also protect biological materials from photooxidation caused by reactive oxygen derivatives. The common carotenoid in cyanobacteria is #-carotene. Over 30 different carotenoids are known in purple bacteria and in aerobic anoxygenic phototrophic bacteria, and spirilloxanthin (without a bezene ring in its structure) is the most common among them. The typical carotenoid of green bacteria is isorenieratene (Figure). The major carotenoid in heliobacteria is neurosporene that is similar in structure to spirilloxanthin.

Table 4.4: The Structure and Maximum Absorbance of Chlorophylls

Table 11.4. *The structure and maximum absorbance of chlorophylls*

		BCHL					
Side chain[a]	CHL *a*	*a*	*b*	*c*	*d*	*e*	*g*
R1	$-CH=CH_2$	$-CO-CH_3$	$-CO-CH_3$	$-CHOH-CH_3$	$-CHOH-CH_3$	$-CHOH-CH_3$	$-CH=CH_2$
R2	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-CHO$	$-CH_3$
R3	$-C_2H_5$	$-C_2H_5$	$=CH-CH_3$	$-C_2H_5$	$-C_2H_5$	$-C_2H_5$	$=CH-CH_3$
R4	$-CH_3$	$-CH_3$	$-CH_3$	$-C_2H_5$	$-C_2H_5$	$-C_2H_5$	$-CH_3$
R5	$-CO-OCH_3$	$-CO-OCH_3$	$-CO-OCH_3$	$-H$	$-H$	$-H$	$-CO-OCH_3$
R6	phytyl	phytyl	phytyl	farnesyl	farnesyl	farnesyl	geranyl-geranyl
R7	$-H$	$-H$	$-H$	$-CH_3$	$-H$	$-CH_3$	$-H$
Maximum absorbance (nm)	680–685	850–910	1020–1035	745–760	725–745	715–725	788, 670

CHL, chlorophyll; BCHL, bacteriochlorophyll.
[a]The positions of side chains are shown in Figure 11.1.

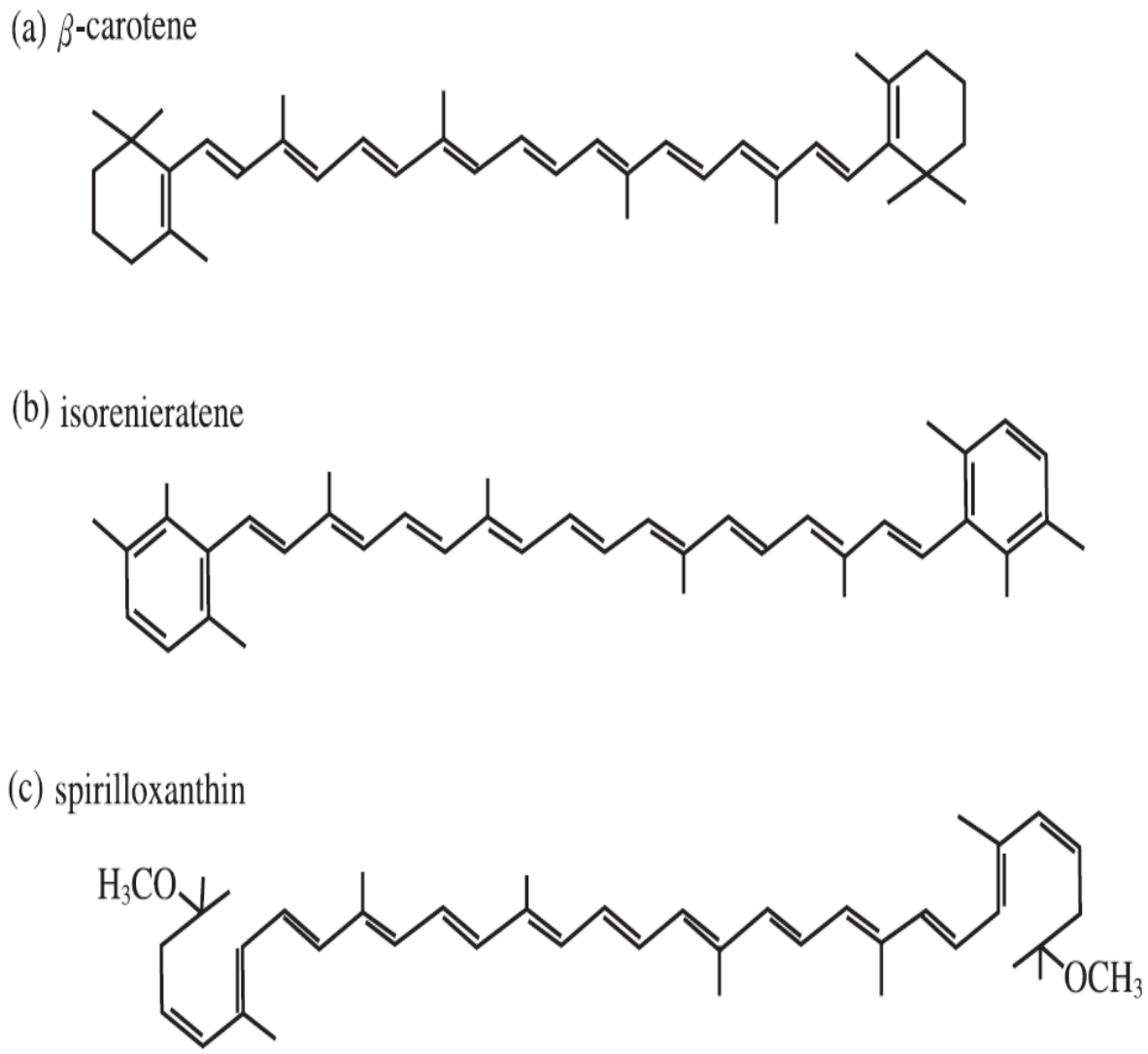


Fig 4.2: Common carotenoids in cyanobacteria and photosynthetic bacteria. Main carotenoids in (a) cyanobacteria, (b) green bacteria, and (c) purple bacteria.

Phycobiliproteins:

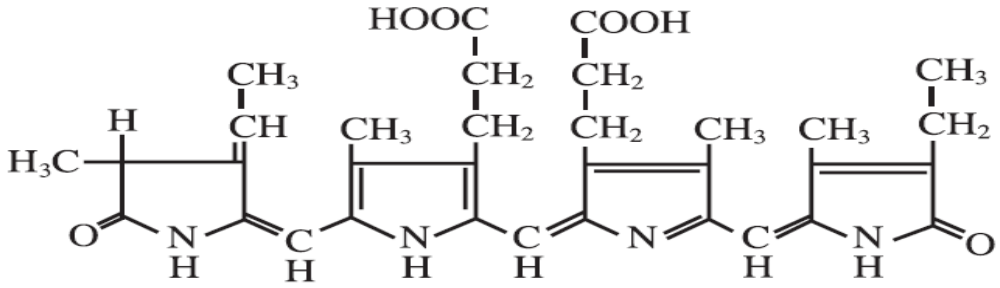
Phycobiliproteins are soluble proteins containing bilin, which has a structure of four pyrroles in a linear form and is found in photosynthetic eukaryotes and cyanobacteria (Figure 4.3). Cyanobacteria with a blue-green colour contain allophycocyanin and phycocyanin, while phycoerythrin is the phycobiliprotein in red-coloured cyanobacteria. This protein is not found in photosynthetic bacteria.

Pheophytin:

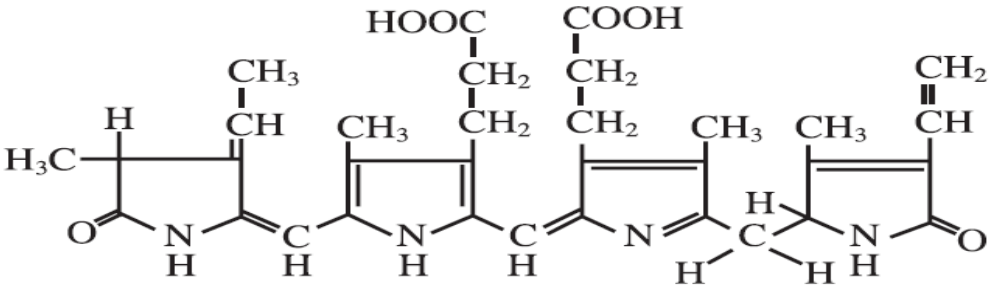
This pigment has the structure of chlorophyll but without Mg2þ or Zn2þ. Cyanobacteria and photosynthetic eukaryotes possess pheophytin, while bacteriopheophytin is found in photosynthetic bacteria with the pheophytin–quinone-type reaction centre serving

as an electron carrier. This pigment is not found in green sulfur bacteria and heliobacteria that have the iron–sulfur-type reaction centre.

Before its function was established, pheophytin was regarded as a chlorophyll degradation product.



phycocyanobilin



phycoerythrobilin

Fig 4.3: The Structure of Phycocyanobilins and Phycobiliproteins

4.7. ABSORPTION SPECTRA OF PHOTOSYNTHETIC CELLS:

Each photosynthetic organism has specific photosynthetic pigments in different ratios, and absorbs light of a specific wavelength depending on their pigments (Figure 4.4). Cyanobacteria with CHL a, carotenoids and phycobiliproteins absorb light at wavelengths shorter than 700 nm. On the other hand, photosynthetic bacteria absorb light at wavelengths shorter than 600 nm with carotenoids and the BCHLs absorb light at wavelengths above 700 nm. Green bacterial cells absorb light at wavelengths between 700 and 800 nm, and also below 600 nm, using BCHL a, b and e and carotenoids in their antenna molecules. The heliobacteria contain BCHL g that has maximum light absorption at 788 nm, and shows a similar absorption spectrum to green bacteria. BCHL a and b absorb long wavelength light over 800nm in purple bacteria. Aerobic anoxygenic phototrophic bacteria absorb light at 450nm with carotenoids and at 750nm with BCHL a.

Cyanobacteria absorb light at wavelengths shorter than 700 nm with CHL a. Light of longer wavelengths is absorbed by photosynthetic bacteria that possess BCHLs.

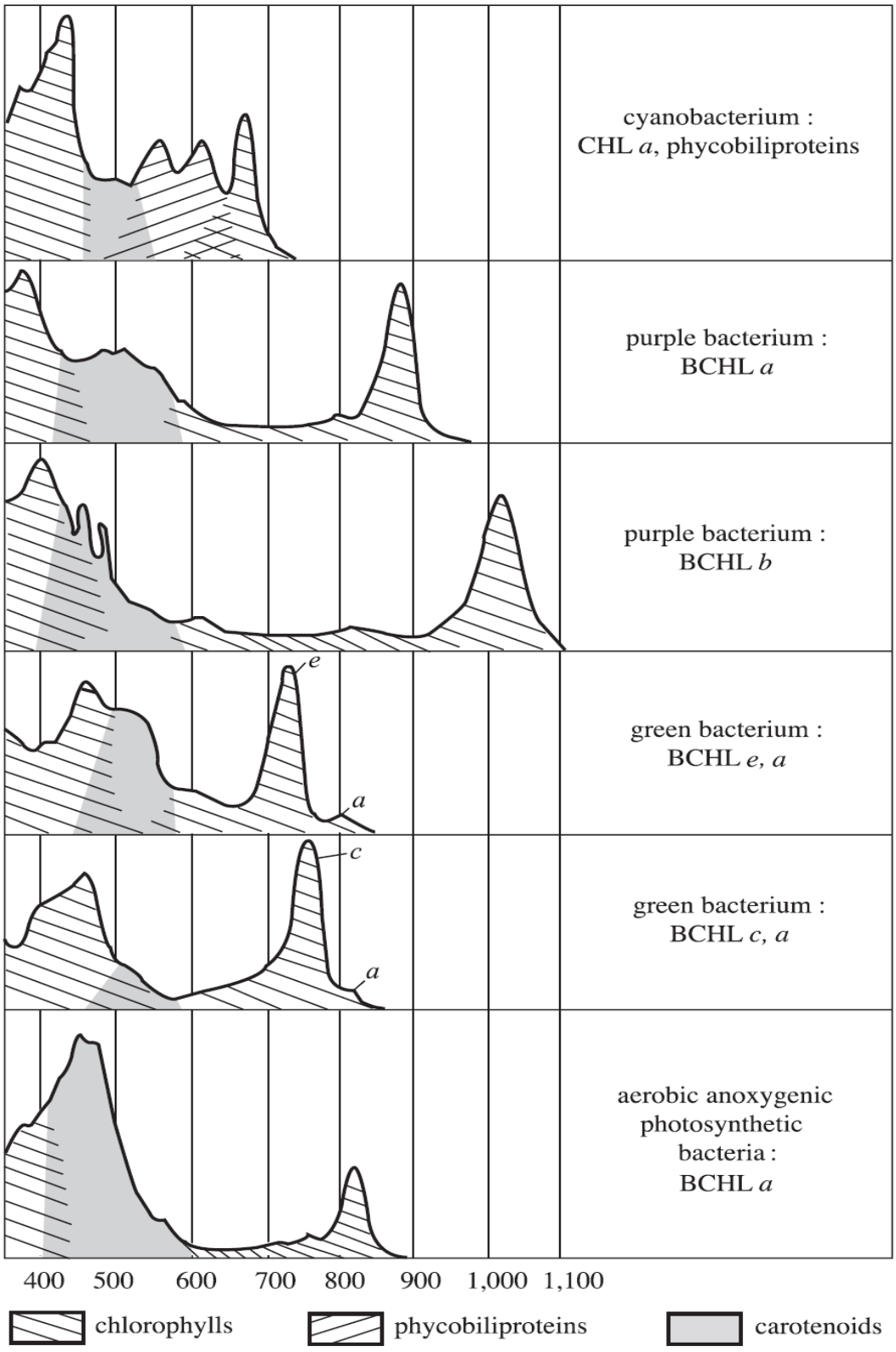


Fig 4.4: Absorption Spectra of Photosynthetic Organisms. The Photosynthetic Pigments Possessed Determine the Absorption Spectra of Photosynthetic Cells

4.8. SUMMARY:

Microbial photosynthesis converts light energy into chemical energy using specialized pigments, occurring in two stages: light-dependent reactions (capturing light via pigments like chlorophylls/ bacteriochlorophylls and carotenoids, producing ATP/NADPH) and light-independent reactions (using that energy to fix CO2 into sugars). Different microbes use unique pigments (e.g., phycobilins in cyanobacteria, rhodopsins in archaea) to absorb various light wavelengths, allowing them to thrive in diverse environments, with both oxygenic (producing O2) and anoxygenic (not producing O2) forms existing.

4.9. SELF ASSESSMENT

1) Photosynthetic microorganisms
2) Anaerobic photosynthetic bacteria
3) Aerobic anoxygenic phototrophic bacteria
4) Photosynthetic pigments
5) Absorption spectra of photosynthetic cells

4.10. REFERENCES:

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Prof K. Mallikarjuna

LESSON-5

PHOTOCHEMISTRY OF PHOTOSYSTEMS OXYGENIC AND ANOXYGENIC PHOTOSYNTHESIS

5.0 OBJECTIVE:

- Microbial photochemistry involves converting light energy into chemical energy using pigments like chlorophylls and bacteriochlorophylls. The key distinction between oxygenic and anoxygenic photosynthesis lies in the electron donor used and whether oxygen is produced as a byproduct.

STRUCTURE:

5.1. INTRODUCTION:

Photosynthetic organisms utilize light energy to reduce NADPþ and to synthesize ATP through the proton motive force. This energy transduction is facilitated by the photosynthetic pigments and electron carriers arranged in the photosynthetic apparatus. Separate photosynthetic structures are not found in the heliobacteria and aerobic anoxygenic phototrophic bacteria. Their reaction centres and antenna molecules are located at the cytoplasmic membrane. The reaction centre is the key component for the primary events in the photochemical conversion of light into biological energy. Coupling to secondary electron donors and acceptors allows the electrons and accompanying protons to be transferred to other components of the photosynthetic apparatus synthesizing ATP or reducing NADP +. Photosynthetic reaction centres can be classed in two categories based on the nature of the electron acceptors. Those of the purple bacteria, filamentous anoxygenic phototrophic bacteria, and photosystem II of cyanobacteria belong to the pheophytin–quinone type, while

the iron–sulfur type is found in green sulfur bacteria, heliobacteria and photosystemI of cyanobacteria. Pheophytin (or bacteriopheophytin) and iron–sulfur centres participate in the electron transfer reactions of each type of reaction centre.

5.2. THYLAKOIDS OF CYANOBACTERIA:

Cyanobacteria have thylakoids with the photosynthetic pigments arranged similarly to the chloroplasts of eukaryotic cells. The thylakoid has a bilayer membrane structure consisting of galactosyl diglyceride containing one or two galactose molecules in place of the phosphate of phospholipids (Figure). Thylakoids convert light energy into biological energy in the cytoplasm of the cyanobacteria. Chlorophyll a and proteins form the reaction centre on the thylakoid membrane. A small fraction of chlorophyll a is found in the reaction centre, and the majority forms antenna molecules known as the phycobilisome with phycobiliproteins and carotenoids. In addition to chlorophyll a, reaction centres have various electron carriers that convert the light energy into a proton motive force. Photosystem I contain [Fe-S] proteins and quinones, and photosystem II contains pheophytin and quinones. The phycobilisomes occupy the cytoplasmic side of the reaction centre (Figure 5.1).

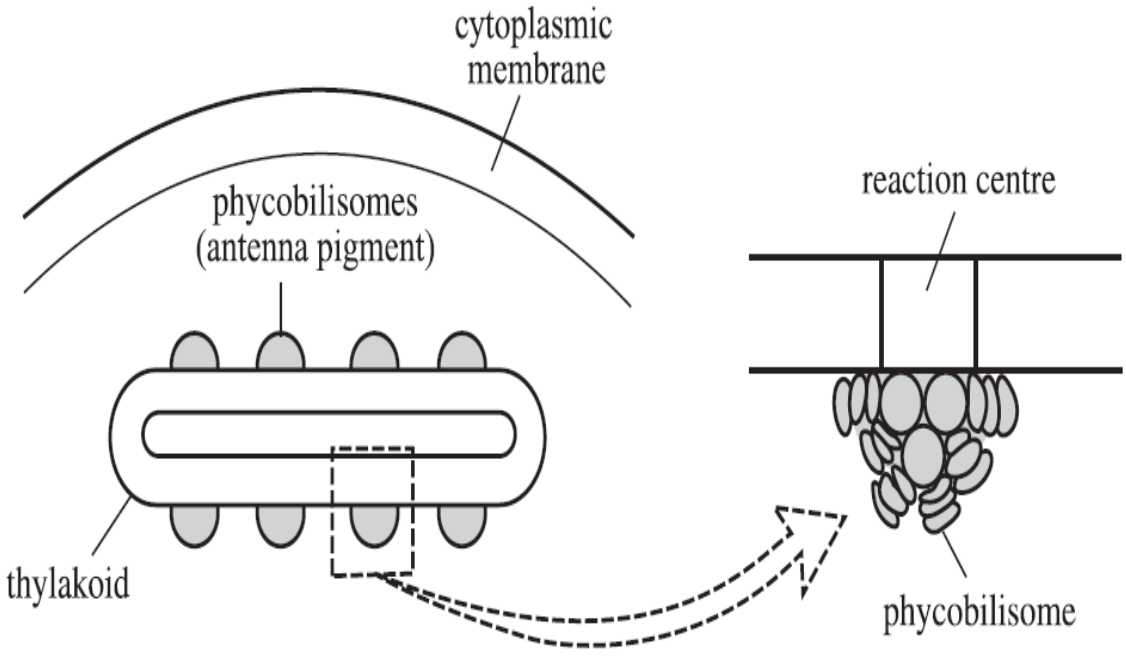

Fig 5.1: The Thylakoid, the Photosynthetic Organelle in Cyanobacteria

The thylakoid with a galactosyl diglyceride bilayer membrane structure is the intracellular photosynthetic organelle in cyanobacteria. The reaction centres are spread on the membrane, and phycobilisomes are attached to the reaction centres which are associated with the electron transport chains to use light energy for proton motive force generation and for the reduction of NAD(P)þ.

5.3. GREEN BACTERIA:

Green bacteria have a photosynthetic apparatus called the chlorosome on their cytoplasmic membrane. Chlorosomes contain antenna molecules, and their baseplates are bound to the reaction centre that is a part of the cytoplasmic membrane (Figure 5.2). The chlorosome has the structure of a galactosyl diglyceride monolayer membrane filled with rod-shaped antenna molecules. Bacteriochlorophyll (BCHL) c, d or e constitute the antenna molecules together with carotenoids. The baseplate contains BCHL a that transfers photons to the reaction centre. The reaction centre contains BCHL a and the electron transport chains. In addition to BCHLs, [Fe-S] proteins are found in the reaction centres of green sulfur bacteria, and bacteriopheophytin (BPHE) in those of filamentous anoxygenic phototrophic bacteria.

The obligate anaerobe Chlorobium tepidum thrives in anaerobic aquatic environments where sulfide is available with very dim light.

To capture light efficiently this bacterium contains about 200!250 chlorosomes per cell with more than 200%103 BCHL c molecules per chlorosome. Thus, this bacterium contains up to 50%106 BCHL c molecules per cell.

5.4. PURPLE BACTERIA:

The purple bacteria have a less developed photosynthetic structure than cyanobacteria and the green bacteria. An intracellular membrane structure contains antenna molecules and reaction centres (Figure 5.3). This is a phospholipid bilayer membrane continuous with the cytoplasmic membrane. The shape of the intracellular membrane structure varies depending on the strain.

5.5. HELIOBACTERIA AND AEROBIC ANOXYGENIC:

Phototrophic bacteria These organisms are the least developed in terms of the photosynthetic apparatus and lack differentiated structures such as chlorosomes or intracytoplasmic membranes. The antenna molecules and reaction centres reside within the cytoplasmic membrane.

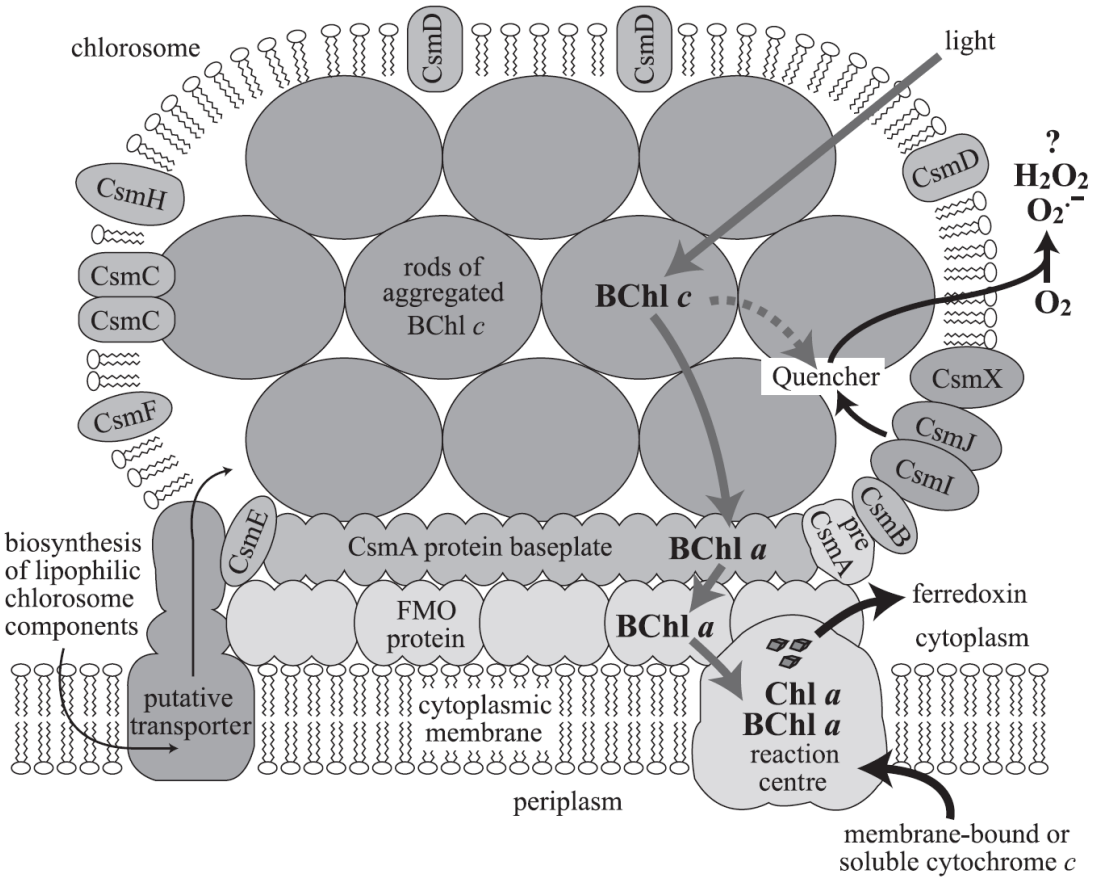

Fig 5.2: Chlorosome bound to the cytoplasmic membrane in green bacteria.

(Modified from Arch. Microbiol. 182:265–276, 2004) The monolayered chlorosome contains antenna molecules, and its baseplate is bound to the reaction centre that is a part of the cytoplasmic membrane.

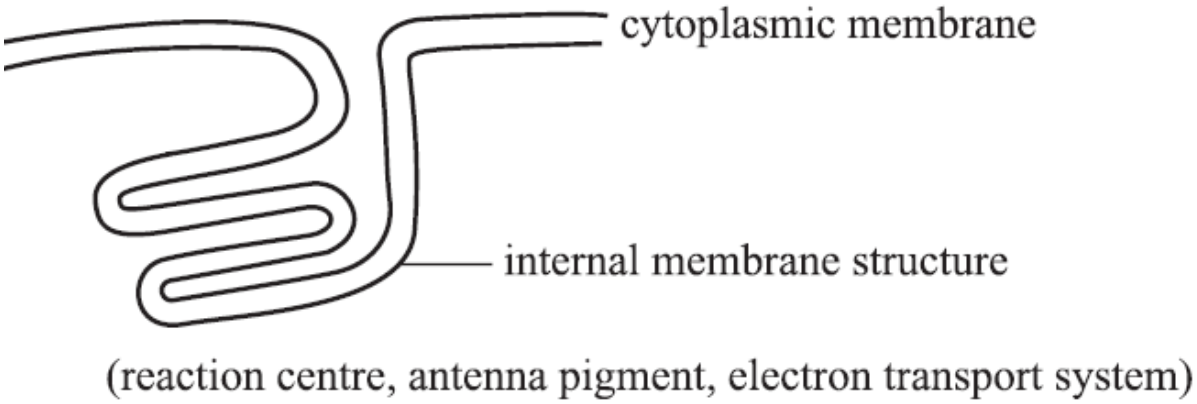

Fig 5.3: The Photosynthetic Apparatus in Purple Bacteria

The intracellular membrane structure, continuous with the cytoplasmic membrane, contains antenna molecules and the reaction centres.

5.6. LIGHT REACTIONS:

Photosynthesis is a process utilizing light energy for biosynthesis. It can be divided into light reactions that convert light energy into biological energy and dark reactions that utilize the biological energy in biosynthesis.

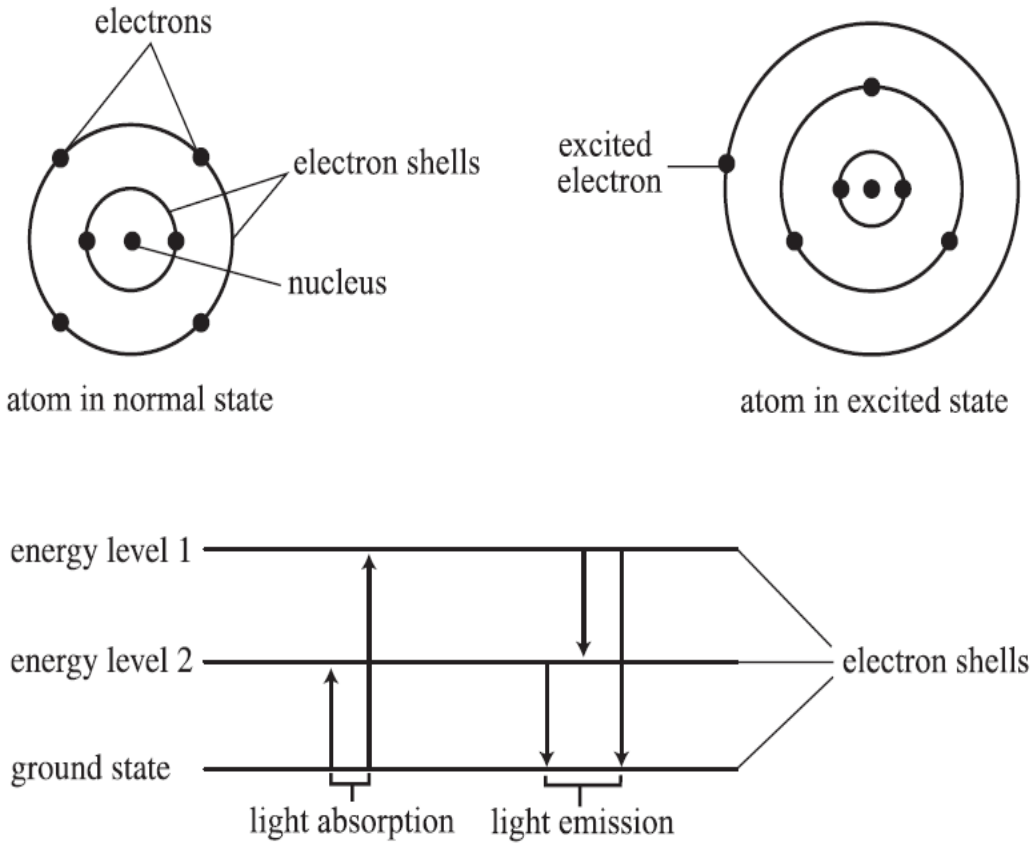

Fig 5.4: Photon Exciting an Electron of a Light-Absorbing Pigment Molecule

The excitation takes place when the energy of the photon is similar to the energy needed to excite the electron. The excited electron returns to the original unexcited state coupled to one of three reactions. These reactions are

1) Transfer of energy to the adjacent molecule exciting its electron (resonance transfer),
2) Oxidation of the excited pigment transferring an electron to a second compound or
3) Emission of fluorescent light.

Properties of Light:

Light is a form of electromagnetic radiation which travels in rhythmic waves transported in discrete particle units called photons. The number of photons per unit time is the intensity of the light. The energy carried by the photons is related to the frequency of the wave.

The higher the frequency, the higher the energy carried. When a photon is radiated to a surface, it may be reflected, transmitted, or absorbed. Different pigments absorb photons of different wavelengths depending on the nature of the absorbing pigment. When a photon is absorbed by a pigment, the energy of light is converted to kinetic energy in the form of an excited electron. When a molecule (or atom) absorbs a photon, an electron is boosted to a higher energy level by transferring an electron from the normal shell to the outer shell (Figure).

Excitation of Antenna Molecules:

The excited electron is very unstable and returns to the original unexcited state coupled to one of three reactions. These reactions are (1) transfer of energy to the adjacent molecule exciting its electron (resonance transfer), (2) reduction of a second compound (oxidation, photo-induced charge separation) or (3) emission of fluorescent light.

The antenna molecules are excited on absorbing photons. The energy gained by the pigments through such excitation is referred to as the exciton. The exciton is transferred from antenna molecules to the reaction centre through resonance transfer, and this reaction takes about 0.1 picosecond. This energy is referred to as resonance energy. The electron transport reaction at the reaction centre is initiated by the oxidation with the consumption of resonance energy.

Resonance transfer takes place from molecules of a higher exciton to those with a lower exciton. For this reason, photosynthetic pigments are arranged in such a way in the phycobilisome of the cyanobacterial thylakoid and in the bacterial photosynthetic apparatus to facilitate such resonance transfer.

Electron Transport:

When the resonance energy excites the reaction centre chlorophyll, its redox potential becomes very low, enough to transfer electrons to a lower potential electron carrier. The oxidized chlorophylls are reduced again, either oxidizing externally supplied electron donor(s) in a process known as non-cyclic electron transport or by taking the original electrons through the cyclic electron transport system.11.4.3.1 Photosystem I and II in cyanobacteria As in photosynthetic eukaryotes, cyanobacteria have photosystem II to supply reducing power, oxidizing water through non-cyclic electron transport, as well as photosystem I to generate a proton motive force through cyclic electron transport. Chlorophyll a serve as the photosynthetic pigment in the reaction centres of both photosystems.

The reaction centre complex of photosystem I have maximum absorption at a wavelength of 700 nm while that of photosystem II is at 680 nm. These are referred to as RCI or P700, and RCII or P680, respectively. The differences in the maximum absorption of chlorophyll a are due to the proteins forming the complex.

When excited by photons, the redox potential of RCI (P700) decreases to !1.0 V from þ0.5 V. The excited P700 reduces the primary acceptor (A0, CHL a). Electrons are transferred from A0 to ferredoxin through phylloquinone and several [Fe-S] centres. The electrons from the reduced ferredoxin are transferred back to P700 through the cytochrome bf complex and plastocyanin in a process known as cyclic electron transport (Figure 5.5).

The free energy changes in cyclic electron transport are conserved as a proton motive force transporting protons into the thylakoid. Alternatively, electrons are transferred from the reduced ferredoxin to NADPþ. Photosystem II replaces the electrons used to reduce NADPþ in photosystem I. When RCII absorbs light, the redox potential decreases from þ1.0V to !0.8 V. Electrons move from the excited RCII to pheophytin before being transferred to photosystem I via various electron carriers (Figure).

The P680 (RCII) of photosystem II has a redox potential of þ1.0V which is higher than that ofO2/H2O. Oxidized P680 is reduced, oxidizing water to molecular oxygen. This reaction is catalyzed by a manganoprotein. Since O2 is evolved, this is referred to as oxygenic photosynthesis.

5.7. GREEN SULFUR BACTERIA:

Green sulfur bacteria utilize light energy through anoxygenic cyclic electron transport, similar to that of cyanobacterial photosystem I, with a P840 iron–sulfur-type reaction centre. Bacteriochlorophyll a (BCHL a) is the photosynthetic pigment of P840 (Figure 5.6). Antenna molecules on the chlorosome transfer the exciton to the reaction centre on the cytoplasmic membrane. BCHL a mediates exciton transfer through the baseplate. The normal state P840 has a redox potential of þ0.3 V, and this decreases to lower than !1.0 V, low enough to reduce the primary acceptor (A0, BCHL a) when excited by photons.

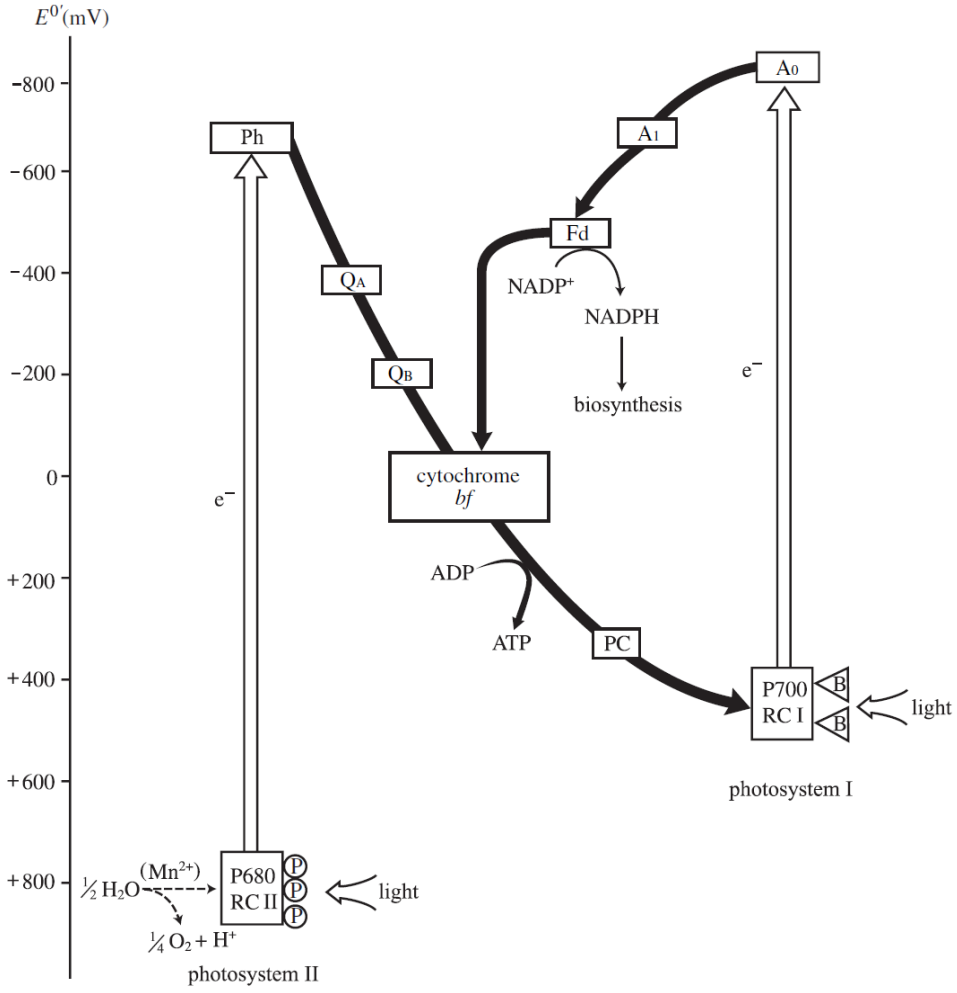

Fig 5.5: Photosynthetic Electron Transport Chains of Cyanobacteria

Cyanobacteria have two different reaction centres, RCI or P700, and RCII or P680. These consist of chlorophyll a and a protein complex. When RCI absorbs photons, chlorophyll a is excited and transfers electrons to the primary acceptor (A0, CHL a). Electrons are transferred from A0 to ferredoxin through phylloquinone (A1) and several [Fe-S] centres. The electrons from the reduced ferredoxin are transferred back to P700 through the cytochrome bf complex and plastocyanin (PC) in a process known as cyclic electron transport. The free energy changes in cyclic electron transport are conserved as a proton motive force transporting protons into the thylakoid. NADPþ is reduced, taking electrons from the ferredoxin (Fd). To replace the electrons channelled to NADPH, RCII oxidizes water reducing pheophytin (Ph) and transferring the electrons to the cyclic electron transport chain. Electron transport at RCII is referred to as non-cyclic electron transport. Ph, pheophytin; QA and QB, plastoquinone A and B, respectively; PC, plastocyanin; A0, primary electron acceptor (CHL a); A1, phylloquinone; Fd, ferredoxin.

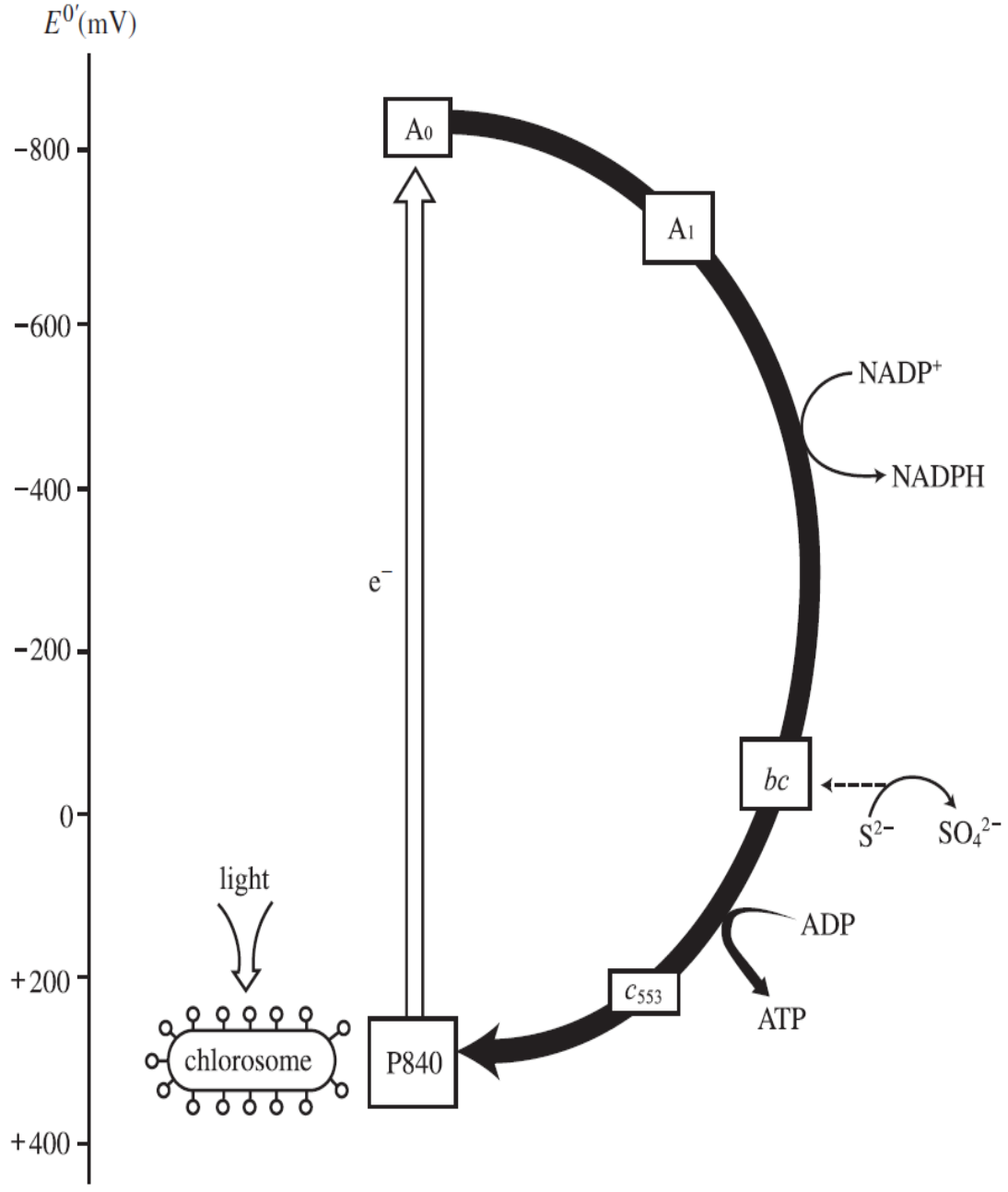

Fig 5.6: Photosynthetic Electron Transport in Green Bacteria

Green bacteria have only cyclic electron transport, and reduced sulfur or organic compounds serve as the electron donor. A0, primary electron acceptor (BCHLa); A1, a quinone compound; bc, cytochrome bc complex; c553, cytochrome c553.

Electrons either flow back to P840 generating a proton motive force or are transferred to NAD(P)þ. Electrons that are consumed to reduce NAD(P)þ are replaced by oxidizing electron donors such as sulfide. These organisms cannot grow chemoorganotrophically under aerobic conditions. On the other hand, the other members of the green bacteria, the filamentous anoxygenic phototrophic bacteria, can grow chemoorganotrophically under aerobic conditions. The latter have a different photosynthetic electron transport system from that of the green sulfur bacteria which is similar to that of the purple bacteria (see Figure), with a pheophytin–quinone-type reaction centre. Heliobacteria have P788 as the reaction centre, which is different from the green sulfur bacteria, although the photosynthetic mechanisms are similar.

5.8. PURPLE BACTERIA:

Purple bacteria have a reaction centre with maximum absorption at 870 nm, which is excited by photons with a decrease in redox potential. The excited P870 reduces bacteriopheophytin to begin the cyclic electron transport for the generation of a proton motive force. NAD(P)þ is reduced through reverse electron transport oxidizing quinol. Sulfur and organic compounds are used as electron donors reducing cytochrome c2 (Figure). Purple sulfur bacteria cannot grow under aerobic conditions, and purple non-sulfur bacteria grow chemoorganotrophically under aerobic conditions.

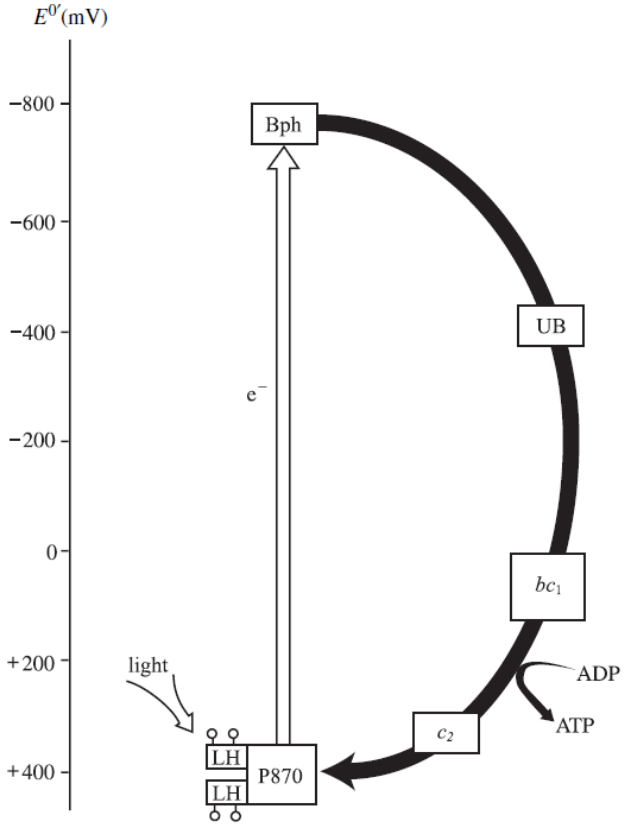

Fig. 5.7

Light reactions in purple bacteria. Purple bacteria utilize light energy to generate a proton motive force which is used to synthesize ATP or reduce NAD(P)þ through reverse electron transport.

Bph, bacteriopheophytin; UB, ubiquinone; bc1, cytochrome bc1 complex; c2, cytochrome c2. 11.4.3.4 Aerobic anoxygenic photosynthetic bacteria These bacteria synthesize bacteriochlorophyll and carotenoids only under aerobic conditions. They do not use light energy under anaerobic conditions. They have a pheophytin–quinone-type reaction centre. The cytoplasmic membrane accommodates the reaction centres and antenna molecules. They have a similar cyclic electron transport system as the purple bacteria, as depicted in Figure, but NAD(P)H is supplied from the metabolism of organic compounds.

Summary Table of Differences

Feature	Oxygenic Photosynthesis	Anoxygenic Photosynthesis
Organisms	Cyanobacteria, algae, plants	Purple bacteria, green sulfur bacteria, etc.
Electron Donor	Water (H_2O)	H_2S, $S_2O_3^{2-}$, H_2, organic compounds
Oxygen Produced	Yes	No
Photosystems	Photosystem I and Photosystem II	Only one photosystem (Type I or Type
Main Pigments	Chlorophylls *a*, *b*, carotenoids	Bacteriochlorophylls, carotenoids
Electron Flow	Non-cyclic (Z-scheme) and cyclic	Primarily cyclic

5.9. SUMMARY:

Microbial photochemistry involves two main types of photosynthesis: **oxygenic** and **anoxygenic**, which differ primarily in their electron donor, photosystem composition, and byproduct.

Oxygenic Photosynthesis **Organisms:** Performed by cyanobacteria, algae, and plants. **Electron Donor:** Uses water (H2O) as the electron donor. **Byproduct:** Produces molecular oxygen (O2) as a byproduct from the splitting (photolysis) of water. **Photosystems:** Involves both Photosystem II (PSII, P680 reaction center) and Photosystem I (PSI, P700 reaction center) operating in series (the Z-scheme).**Mechanism:** Light energy drives a non-yclic electron flow from water, through an electron transport chain, to NADP , producing ATP and NADPH. A proton gradient is generated across the membrane to power ATP synthase.

Pigments: Primarily uses chlorophyll b, along with accessory pigments like carotenoids and phycobilins.

Anoxygenic Photosynthesis **Organisms:** Performed by various anaerobic bacteria, including purple sulfur bacteria, green sulfur bacteria, and heliobacteria. **Electron Donor:** Uses electron donors other than water, such as hydrogen sulfide (H2S), elemental sulfur, or organic molecules. **Byproduct:** Does not produce oxygen. The byproduct depends on the electron donor (e.g., elemental sulfur is produced when H2Sis used). **Photosystems:** Involves only a single photosystem, which can be a type I reaction center (like in green sulfur bacteria) or a type II reaction center (like in purple bacteria), but not both. **Mechanism:** Electron flow is primarily cyclic, generating a proton gradient for ATP synthesis. Reducing power (NADH/NADPH) for carbon fixation is often produced via "reverse electron flow", which requires additional energy input. **Pigments:** Uses bacteriochlorophylls, which absorb light at longer (infrared) wavelengths than chlorophylls, allowing them to thrive in different environmental niches.

5.10. SELF-ASSESSMENT:

1) Thylakoids of Cyanobacteria
2) Green bacteria (Oxygenic Photosynthesis)
3) Purple Bacteria, Heliobacteria and Aerobic Anoxygenic
4) Light Reactions
5) Green Sulfur Bacteria
6) Purple Bacteria

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Prof K. Mallikarjuna

LESSON-6

PHOTOSYNTHETIC CARBON REDUCTION METABOLISM

6.0 OBJECTIVE:

- To understand Photosynthetic carbon reduction metabolism in photolithotrophs.

STRUCTURE:

6.1. INTRODUCTION:

According to their carbon sources, phototrophs are classified into photoorganotrophs and photolithotrophs. With a few exceptions, cyanobacteria are photolithotrophs, and the green bacteria and purple bacteria can grow photolithotrophically fixing CO2 as well as photoorganotrophically (Table 6.1). Heliobacteria and aerobic anoxygenic photosynthetic bacteria do not fix CO2.

6.2. CO2 FIXATION:

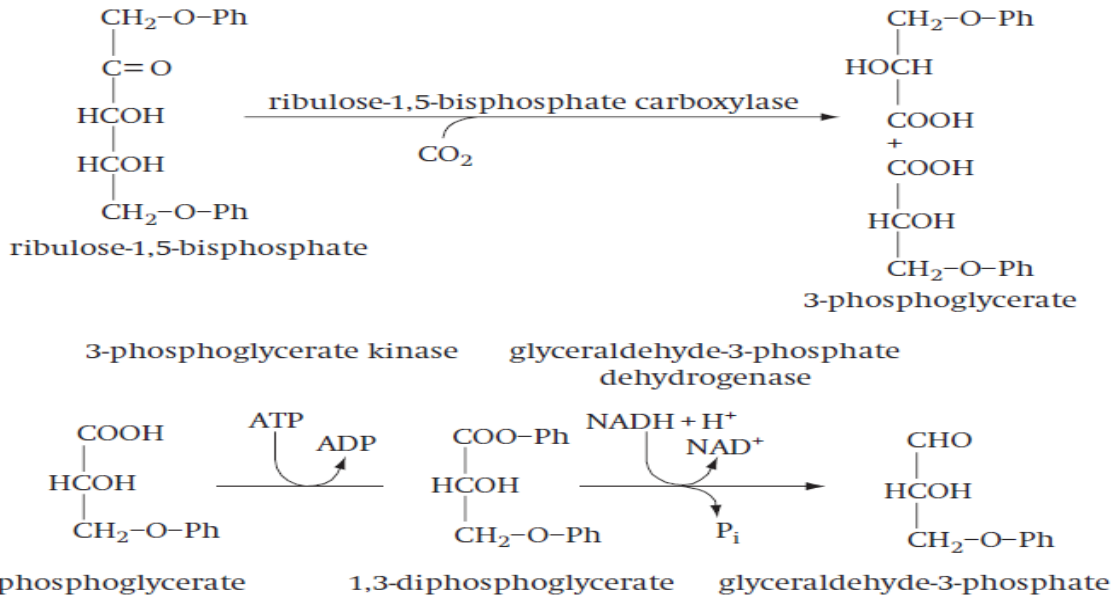
The Calvin cycle is the most common CO2 fixing mechanism in phototrophs. The key enzymes of the Calvin cycle, phosphoribulokinase and ribulose-1,5-bisphosphate carboxylase, are not found in green sulfur bacteria and in one of the filamentous anoxygenic phototrophic bacteria, Chloroflexus aurantiacus. Green sulfur bacteria fix CO2 into acetyl-CoA through the reductive TCA cycle, and a less common 3-hydroxypropionate pathway is employed by Chloroflexus aurantiacus. CO2 is fixed through the Calvin cycle in cyanobacteria, purple bacteria and filamentous anoxygenic phototrophic bacteria (Table 6.1).

6.3. CARBON METABOLISM IN PHOTOORGANOTROPHS:

Most photosynthetic bacteria use simple organic compounds as their carbon sources and electron donors with ATP and NAD(P)H generated from the light reactions.

Calvin Cycle:

As shown in Figure 6.1, CO2 is condensed to ribulose-1,5-bisphosphate to produce two molecules of 3-phosphoglycerate, which is reduced to glyceraldehyde-3-phosphate.



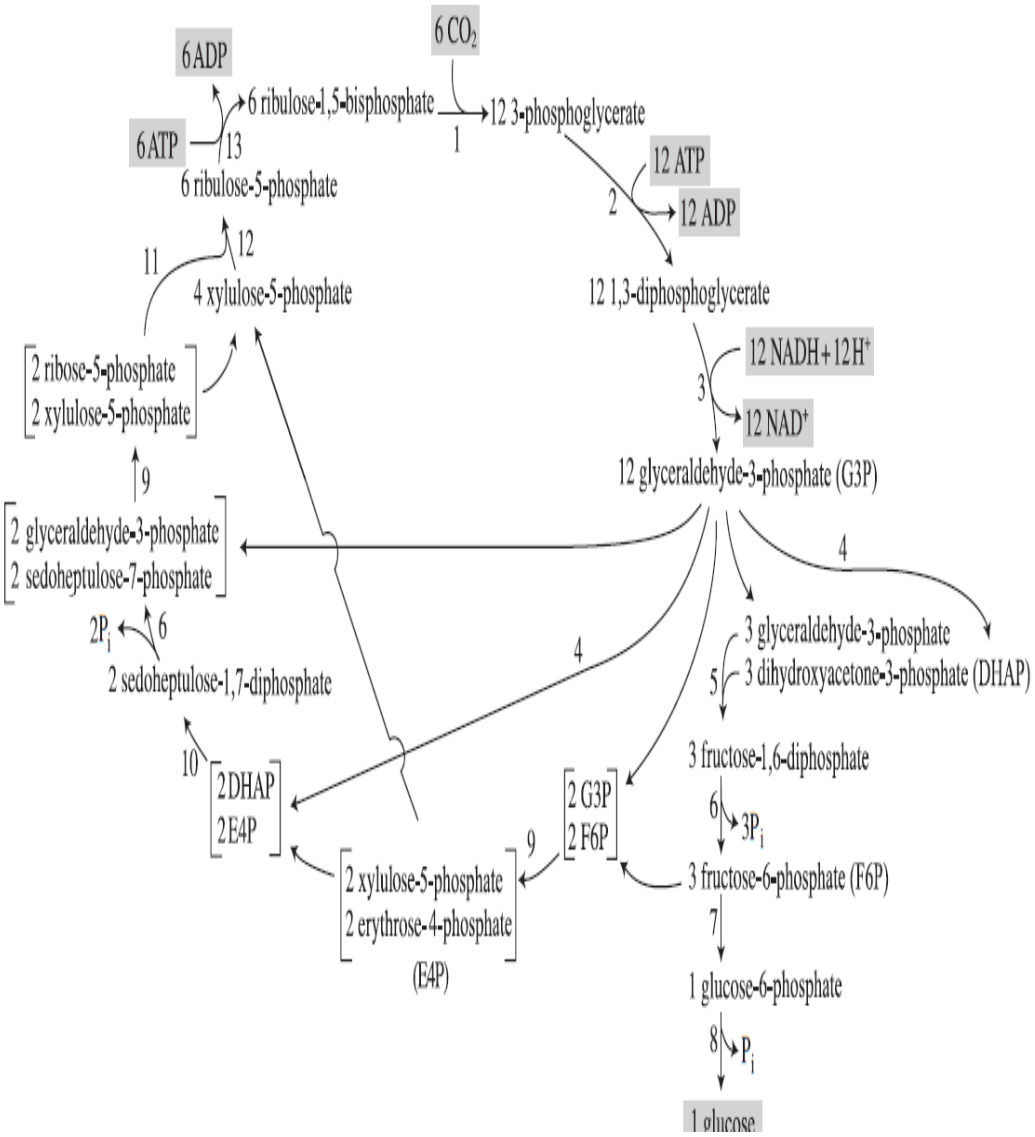
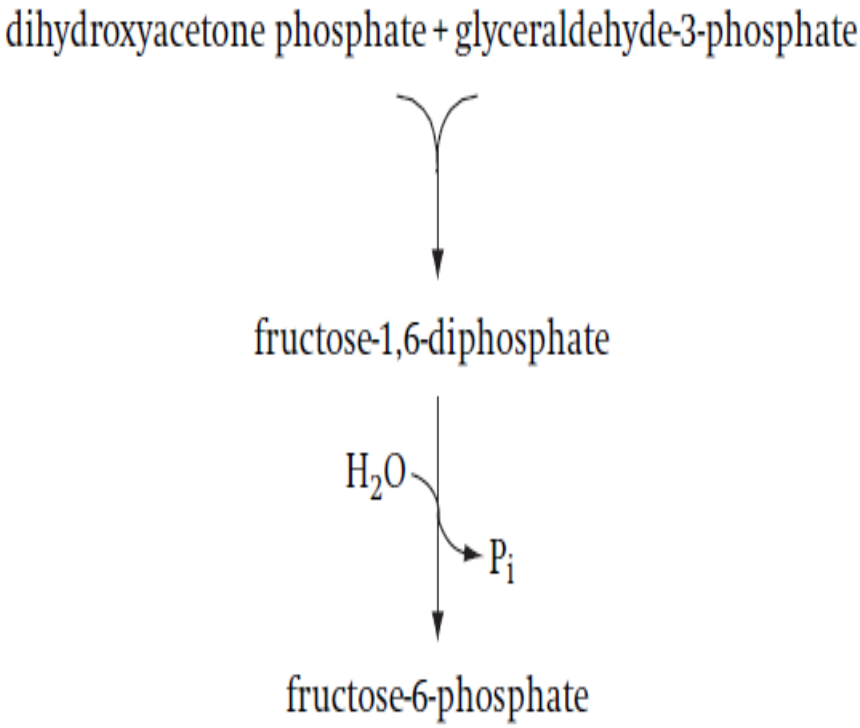


Fig 6.1: CO2 Fixation Through the Calvin Cycle

1, ribulose-1, 5-bisphosphate carboxylase; 2, 3-phosphoglycerate kinase; 3, glyceraldehyde-3-phosphate dehydrogenase; 4, triose phosphate isomerase; 5, fructose-1, 6-diphosphate aldolase; 6, fructose-1,6-diphosphatase; 7, glucose-6-phosphate isomerase; 8, glucose-6-phosphatase; 9, transketolase; 10, sedoheptulose-1,7-diphosphate aldolase (the same as number 5); 11, ribose-5-phosphate isomerase; 12, ribose-5-phosphate-3-epimerase; 13, phosphoribulokinas

A molecule of glyceraldehyde-3-phosphate is isomerized to dihydroxyacetone phosphate before being condensed to fructose-1,6-diphosphate with the second glyceraldehyde-3-phosphate molecule through the reverse reactions of the EMP pathway. Fructose-1,6-diphosphate is dephosphorylated to fructose-6-phosphate by the action of fructose-1,6-diphosphatase:



Through carbon rearrangement reactions similar to those of the HMP pathway, two molecules of fructose-6-phosphate (2C6) and six molecules of glyceraldehyde-3-phosphate (6C3) are converted tosix molecules of ribulose-5-phosphate (6C5) before being phosphorylated to ribulose-1,5-bisphosphate to begin the next round of reactions. The net result of these complex reactions is the synthesis of fructose-6-phosphate from 6CO2 consuming 18ATP and 12NAD(P)H:

$$6CO_2 + 18ATP + 12NAD(P)H + 12H^+ \longrightarrow \text{fructose-6-phosphate} + 18ADP + 18P_i + 12NAD(P)^+$$

ATP is consumed in the reactions catalyzed by 3-phosphoglycerate kinase and phosphoribulokinase, and glyceraldehyde-3-phosphate dehydrogenase oxidizes NAD(P)H.

Key Enzymes of the Calvin Cycle:

Ribulose-1,5-bisphosphate carboxylase and phosphoribulokinase are key enzymes of the Calvin cycle, and are resent only in the organisms fixing CO2 through this highly energy-demanding pathway with a few exceptions (see below). Their activities are controlled at the transcriptional level and also after they are expressed. The enzymes are encoded by cbb genes organized in cbb operons differing in size and composition depending on the organism. In a facultative chemolithotroph, Ralstonia eutropha, the transcription of the operons, which may form regulons, is strictly controlled, being induced during chemolithotrophic growth but repressed to varying extents during heterotrophic growth. CbbR is a transcriptional regulator and the key activator protein of cbb operons. The cbbR gene is located adjacent to its cognate operon. The activating function of CbbR is modulated by metabolites which signal the nutritional state of the cell to the cbb system. Phosphoenolpyruvate is a negative effector of CbbR, whereas NADPH is a coactivator of the protein. In the photolithotrophs Rhodobacter capsulatus and Rhodobacter sphaeroides, a global twoc omponent signal transduction system, RegBA, serves this function.

Different cbb control systems have evolved in the diverse chemolithotrophs characterized by different metabolic capabilities. Phosphoribulokinase activity is regulated by similar physiological signals. NADH activates enzyme activity, while AMP and phosphoenolpyruvate (PEP) are inhibitory. The increase in NADH concentration means the cells are ready to grow, activating the Calvin cycle. On the other hand, the biosynthetic pathway cannot be operated under a poor energy state with an increased AMP concentration. When a facultative chemolithotroph is provided with organic carbon, enzyme activity is inhibited by PEP. Similarly, 6-phosphogluconate inhibits ribulose-1,5-bisphosphate arboxylase activity.

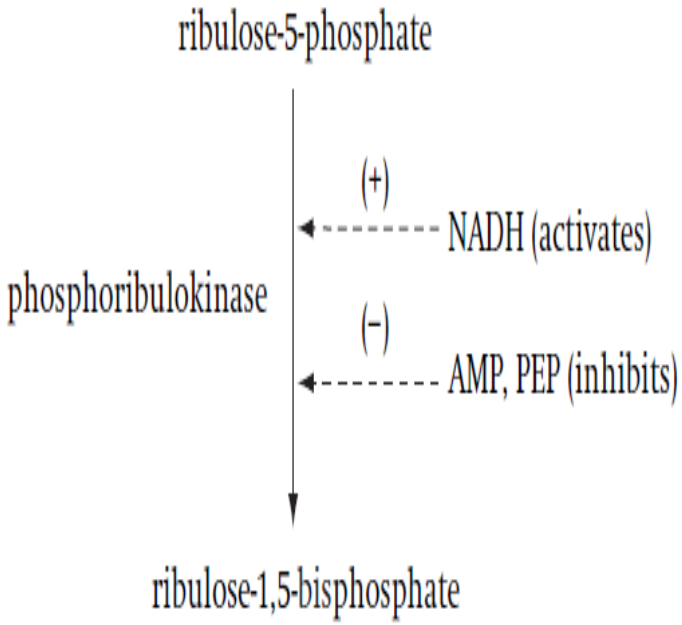


Fig 6.1: Ribulose-1,5-bisphosphate carboxylase is the most abundant single protein on Earth and is synthesized by all organisms fixing CO2 through the Calvin cycle including plants. This enzyme is typically categorized into two forms. Type I, the most common form, consists of eight large and eight small subunits in a hexadecameric (L8S8) structure. This type is

widely distributed in CO2-fixing organisms, including all higher plants, algae, cyanobacteria, and many chemolithotrophic bacteria. The type II enzyme, on the other hand, consists of only large subunits (Lx), the number of which may be 2, 4 or 8 in different organisms. Type II ribulose-1,5-bisphosphate carboxylase is found in anaerobic purple photosynthetic bacteria and in some chemolithotrophs. Both types are found in some bacteria, especially in sulfur bacteria such as *Halothiobacillus neapolitanus* (formerly Thiobacillus neapolitanus), *Thiomonas intermedia* (formerly Thiobacillus intermedius) and Thiobacillus denitrificans, in photolithotrophs such as Rhodobacter sphaeroides and Rhodobacter capsulatus, and in the obligately chemolithotrophic hydrogen bacterium Hydrogenovibrio marinus.

These have genes for both type I and type II enzymes. In addition to type I and type II enzymes, two novel type III and type IV enzymes have been revealed by the complete genome sequences of some archaea and bacteria in which the Calvin cycle is not yet known. The type III enzyme is found in many thermophilic archaea including Thermococcus (formerly Pyrococcus) kodakaraensis, Methanococcus jannaschii and Archaeoglobus fulgidus. The gene for the enzyme from Thermococcus kodakaraensis has been cloned and expressed in Escherichia coli. The recombinant enzyme shows carboxylase activity with a decameric structure, but the function has not been elucidated. The existence of the type III enzyme in archaea is not limited to thermophilic organisms. The complete genomes of the mesophilic heterotrophic methanogens Methanosarcina acetivorans, Methanosarcina mazei, and Methanosarcina barkeri were also found to contain genes encoding putative ribulose-1,5-bisphosphate carboxylase proteins, but these were not found in *Methanobacterium thermoautotrophicum* and *Methanococcus maripaludis.*

These methanogenic archaea fix CO2 through the acetyl-CoA pathway. It is not known what the function of the carboxylase enzyme is in these organisms. Microbial genome sequences have revealed open reading frames with a similar sequence to that of ribulose-1,5-bisphosphate carboxylase in Bacillus subtilis and green sulfur photosynthetic bacteria. This protein is referred to as type IV and is involved in oxidative stress response or sulfur metabolism. Green sulfur photosynthetic bacteria fix CO2 through the reductive TCA cycle.

Ribulose-1,5-bisphosphate carboxylase is encapsulated in protein to form microcompartments named carboxysomes in many, but not all, chemolithotrophs. The rate of CO2 fixation is much higher than expected from the CO2 concentration in the cytoplasm and the low affinity of the enzyme for CO2. The carboxysomes are believed to have a function in concentrating CO2 to achieve the higher rate.

6.4. PURPLE BACTERIA, HELIOBACTERIA AND AEROBIC ANOXYGENIC PHOTOSYNTHETIC BACTERIA:

As photoorganotrophs, purple non-sulfur bacteria preferentially use organic compounds as their carbon source. Sugars are metabolized through the EMP or ED pathway depending on the organism. CO2 from glycolysis is fixed under photosynthetic conditions (Figure 6.1). Under dark conditions, purple non-sulfur bacteria can grow with or without molecular oxygen.

Acetate is converted to acetyl-CoA before being metabolized through the TCA cycle and glyoxylate cycle, or by pyruvate: ferredoxin oxidoreductase to pyruvate. CO2 is fixed to consume the excess reducing equivalents generated from the metabolism of organic compounds under photosynthetic conditions.

For this reason, purple bacteria require CO2 for photoorganotrophic growth on compounds more reduced than acetate. Butyrate metabolism is a good example of this (Figure 6.2).

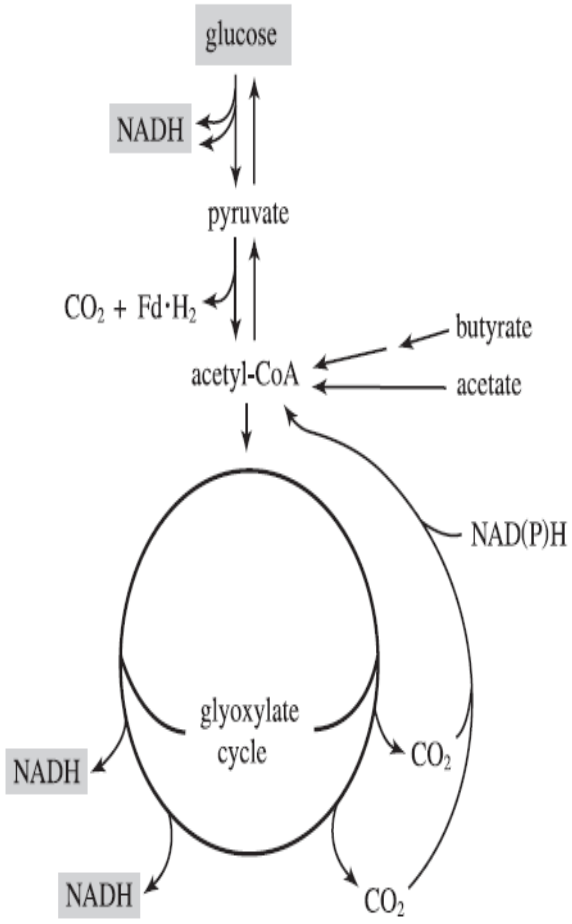


Fig 6.2: Photoorganotrophic Metabolism in Purple Bacteria

Green Sulfur Bacteria:

Green sulfur bacteria obtain energy required for their growth from the light reactions, and can use simple organic carbon sources such as acetate, but not as the electron donor. Acetate is assimilated only when CO2 and sulfur compounds are available as electron donors. On the other hand, filamentous anoxygenic phototrophic bacteria can use organic electron donors in phototrophic metabolism and can grow chemoorganotrophically like purple non-sulfur bacteria.

Cyanobacteria:

The majority of cyanobacteria grow photolithotrophically, and few grow photoorganotrophically bligately photolithotrophic cyanobacteria cannot use glucose, though they metabolize glycogen as storage materials with glycolytic enzymes. It is likely that they do not have sugar transport systems. The photoorganotrophic cyanobacteria use glucose but cannot use other organic compounds because

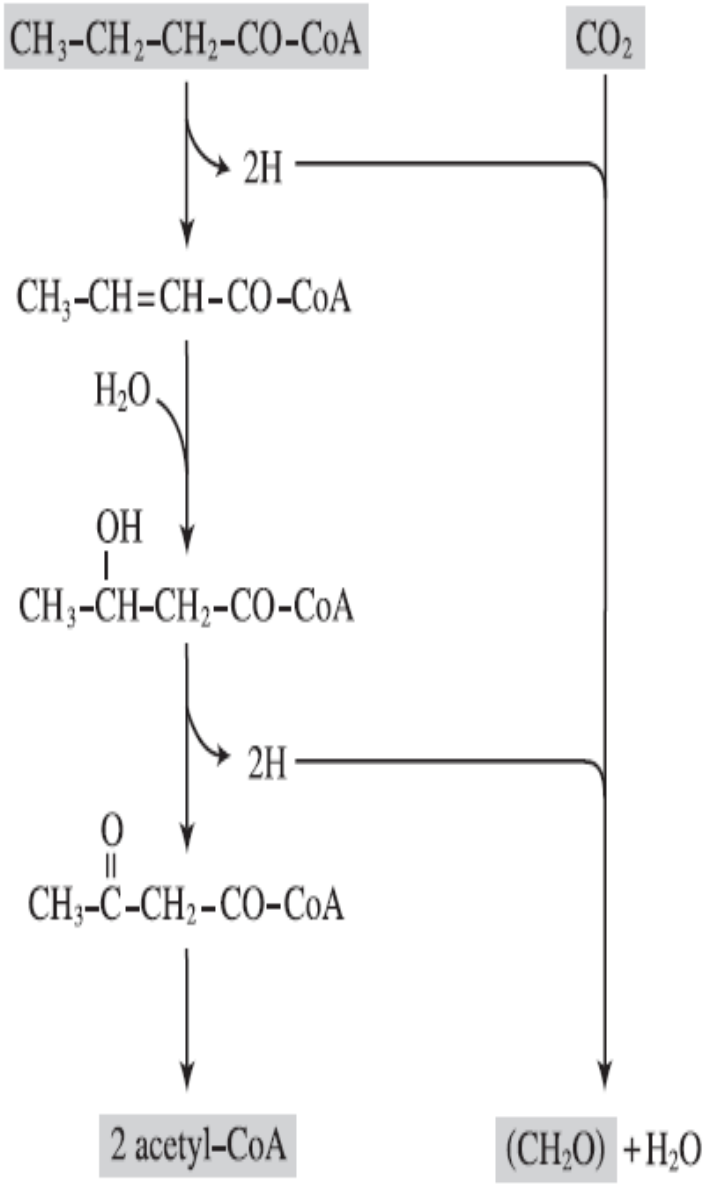


Fig 6.3: Butyrate Metabolism by Purple Bacteria

Heliobacteria and aerobic anoxygenic photosynthetic bacteria supplement their energy requirements through light reactions while growing as chemoorganotrophs. They are devoid of a functional TCA cycle. They metabolize glucose through the oxidative HMP cycle (Figure 6.4) as in *Thiobacillus novellus*. The cyanobacteria cannot grow under dark conditions.

6.5. PHOTOPHOSPHORYLATION IN HALOPHILIC ARCHAEA:

Halophilic archaea, including species of Halobacterium, can grow at a NaCl concentration of over 2.5M. They swim away from the light when enough electron donors and O2 are available. With a limited O2 supply, they move towards the light. They generate a proton motive force transporting Hþ and Cl^- across the membrane using light energy in a process known as photophosphorylation. A group of proteins known as rhodopsins facilitate motility and photophosphorylation. Sensory rhodopsin II is synthesized for movement away

from the light when the dissolved O2 (DO) concentration is high, and light attracts halophilic archaea under a limited O2 supply. Sensory rhodopsin I is synthesized with bacteriorhodopsin and halorhodopsin under low DO conditions. With light energy, bacteriorhodopsin exports Hþ and halorhodopsin imports Ci to generate a proton motive force. These rhodopsins are purple proteins boundwith retinal (Figure 6.4). Rhodopsins have a similar structure, with retinal forming a Schiff's base with a lysine residue of the peptide. The Schiff's base releases Hþ when it receives light. Bacteriorhodopsin exports H+, and the sensory rhodopsins pass the information to transducer proteins to control phototaxis (Figure 6.3).

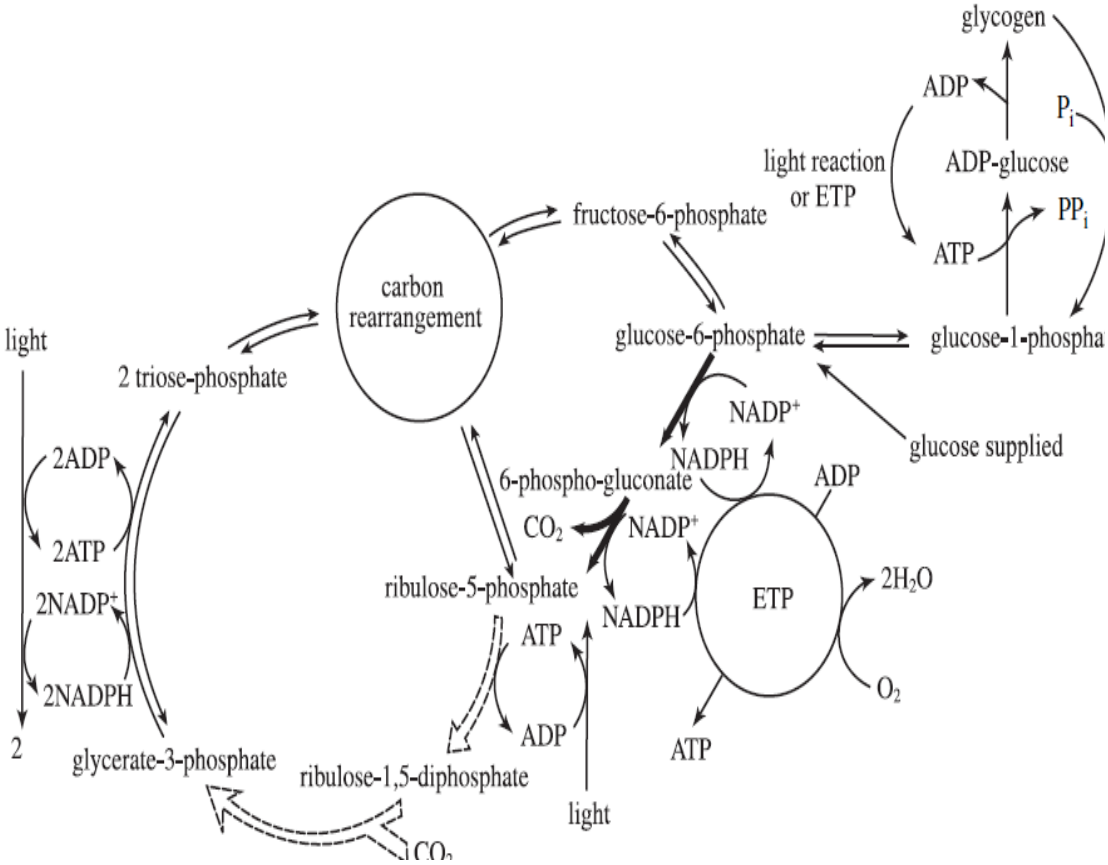


Fig 6.3: Carbon Metabolism in Cyanobacteria

Photoorganotrophic cyanobacteria metabolize glucose through the oxidative HMP cycle (thick solid lines and carbon rearrangement reactions) and fix CO2 through the Calvin cycle (double lines). Some enzymes participate in both metabolic pathways.

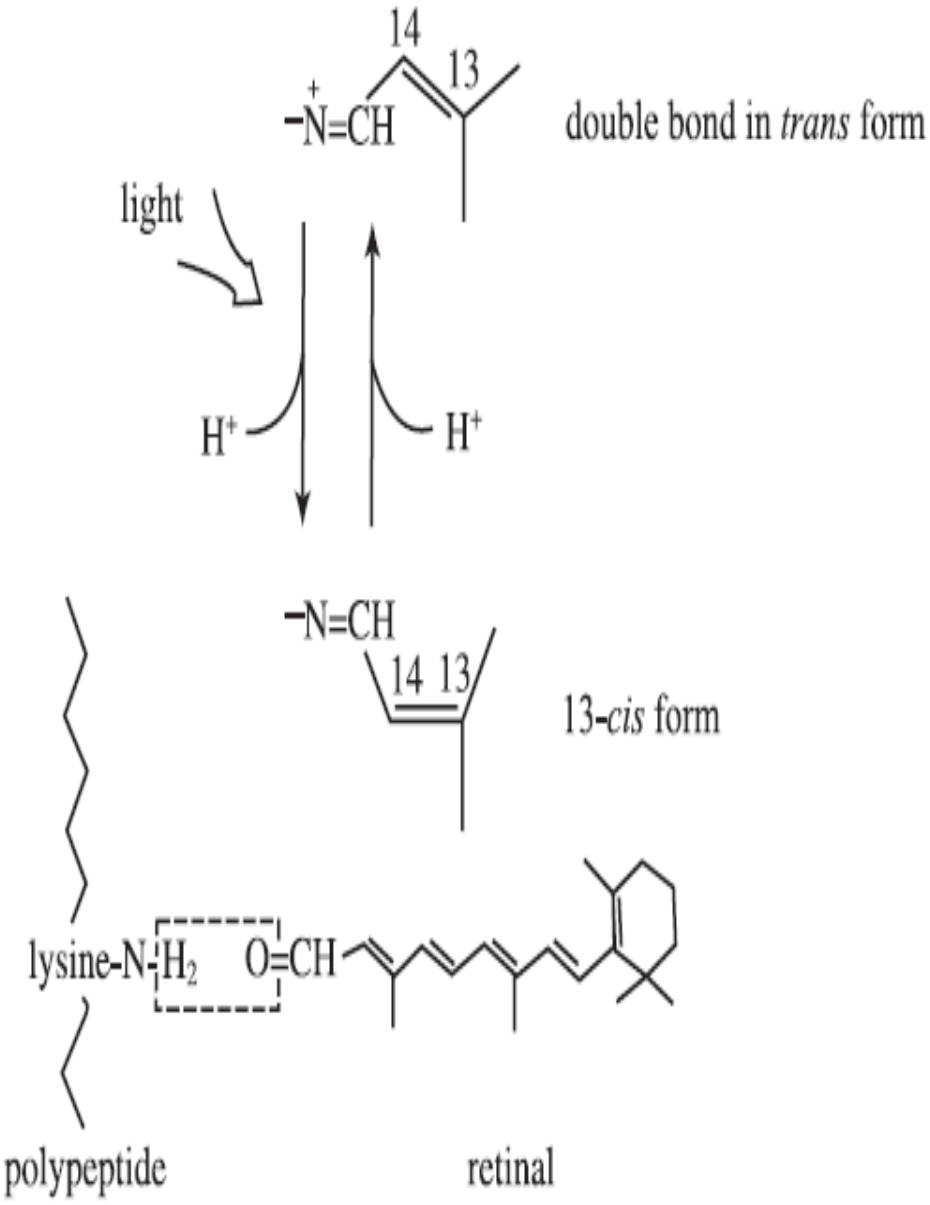


Fig 6.4: Structure of retinal bound to rhodopsins in species of Halobacterium. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 9.20. Springer, New York)

Rhodopsin has the structure of a Schiff's base between the chromophore retinal and a lysine residue of the apoprotein. The Schiff's base exports Hþ with light energy. Bacteriorhodopsin and halorhodopsin convert light energy into a proton motive force. Sensory rhodopsin II is synthesized in order for them to move away from the light at high dissolved O2 concentrations (.BR, bacteriorhodopsin; HR, halorhodopsin; SRI, sensory rhodopsin I; HtrI, transducer I; SRII, sensory rhodopsin II; HtrII, transducer II).

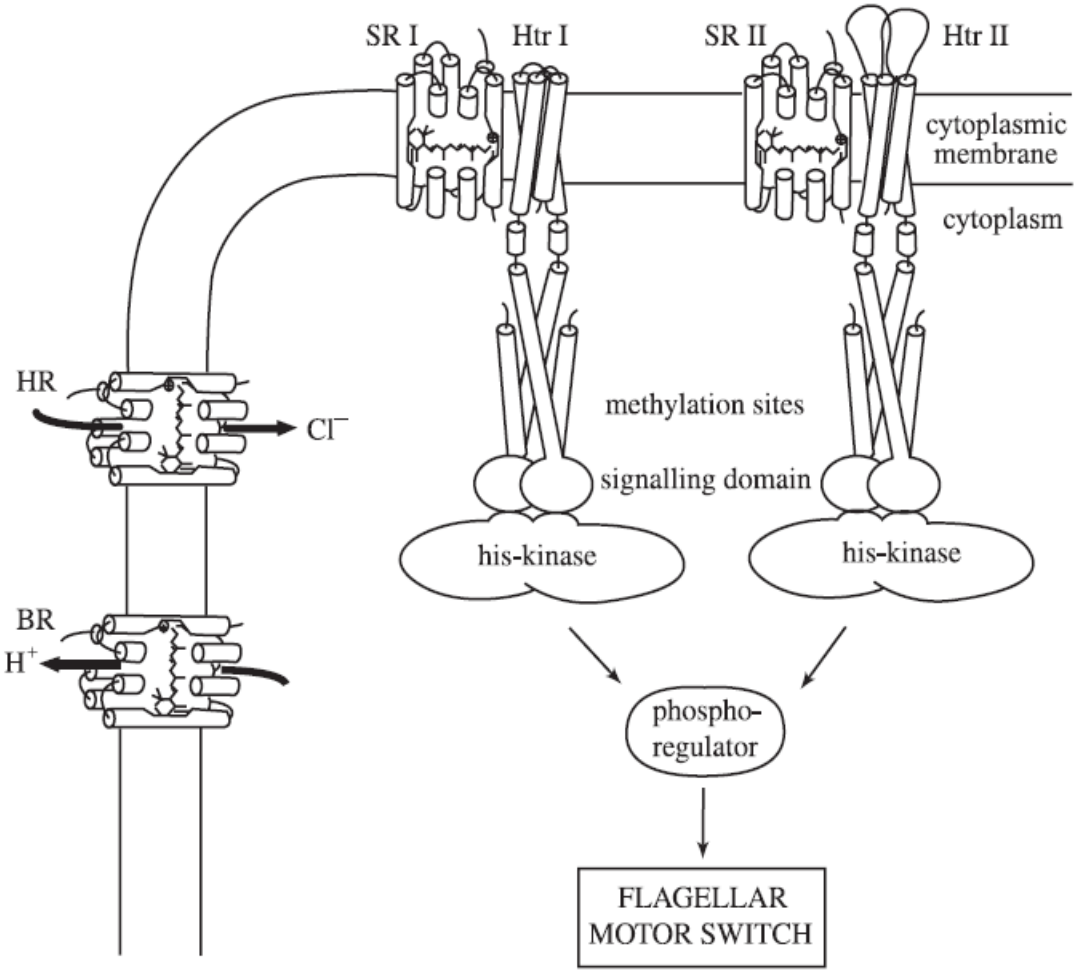


Fig 6.5: Rhodopsins and their functions in halophilic archaea. (Mol. Microbiol. 28:1051–1058,1998)

Halophilic archaea have sensory rhodopsin I and II to control phototaxis, and bacteriorhodopsin and halorhodopsin to generate a proton motive force utilizing light energy. Bacteriorhodopsin exports H+, and halorhodopsin imports Cl^-, utilizing light energy. Sensory rhodopsins with a similar structure pass the information to the transducer proteins to control phototaxis. At low dissolved O2 concentrations, the organisms move towards the light through the actions of sensory rhodopsin I and transducer I to use it.

6.6. SUMMARY:

Microbial photosynthetic carbon reduction is the process by which microorganisms use light energy to convert inorganic carbon (specifically carbon dioxide, CO2) into organic compounds, such as carbohydrates. This process occurs in two main stages: light-dependent reactions and light-independent (dark) reactions. Light-Dependent Reactions. In this stage, photosynthetic pigments (chlorophylls in cyanobacteria; bacteriochlorophylls in other bacteria) absorb light energy. This energy is used to drive electron transport chains embedded

in specialized membranes, which generates: **ATP**: An energy-storage molecule. **NADPH (or NADH)**: A reducing agent (source of electrons). Microbes exhibit two types of photosynthesis based on their electron source: **Oxygenic Photosynthesis**: Practiced by cyanobacteria and algae, it uses water (H2O) as the electron donor, releasing oxygen (O2) as a product. **Anoxygenic Photosynthesis**: Used by purple and green sulfur bacteria, it uses electron donors other than water, such as hydrogen sulfide (H2S) or organic molecules, and thus does not produce oxygen.

Light-Independent Reactions (Carbon Fixation) the ATP and NADPH/NADH produced in the light reactions provide the necessary energy and reducing power to "fix"CO2into organic molecules. This occurs through several distinct metabolic pathways depending on the microorganism: **Calvin-Benson Cycle**: The most common pathway in cyanobacteria and many other photosynthetic microbes, where the enzyme RuBisCO catalyzes the initial capture of CO2 and incorporates it into a five-carbon sugar, ribulose 1,5-bisphosphate (RuBP).**Reductive TCA Cycle (rTCA)**: Used by green sulfur bacteria, this pathway runs the normal, oxidative TCA cycle in reverse to assimilate CO2.**3-Hydroxypropionate Cycle/Bi-cycle**: Found in filamentous anoxygenic phototrophs like *Chloroflexus aurantiacus*. **Reductive Acetyl-CoA Pathway (Wood-Ljungdahl)**: Used by some anaerobic bacteria (acetogens and methanogens) to convert CO2into acetyl-CoA. Ultimately, these pathways produce three-carbon sugars (triose phosphates) and other intermediates, which are used as building blocks to synthesize essential cellular components like glucose, lipids, and amino acids. The excess fixed carbon is often stored as glycogen or other storage compounds

6.7. SELF-ASSESSMENT:

1) Carbon Metabolism in photoorganotrophs Calvin-Benson Cycle
2) Reductive TCA Cycle (rTCA)
3) Reductive Acetyl-CoA Pathway (Wood-Ljungdahl)
4) Photophosphorylation in Halophilic Archaea

6.8. REFERENCES:

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3) Nelson and Cox. 2000; Lehninger Principles of Biochemistry
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology
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Prof K. Mallikarjuna

LESSON-7

CARBON METABOLISM IN CHEMOLITHOTROPHS

7.0 OBJECTIVE:

- To understand carbon assimilation pathway in chemolithotrophic bacteria.

STRUCTURE:

7.1. INTRODUCTION:

CO2 fixation pathways in chemolithotrophs The Calvin cycle is the most common CO2 fixation pathway in aerobic chemolithotrophs and in photolithotrophs, and some fix CO2 through the reductive TCA cycle. The anaerobic chemolithotrophs, including methanogens, homoacetogens and sulfidogens, employ the acetyl-CoA pathway to fix CO2. A fourth CO2 fixation pathway, the 3-hydroxypropionate cycle, is known in some chemo- and photolithotrophs. The Calvin cycle is the only known CO2-fixing metabolism in eukaryotes.

7.2. REDUCTIVE TCA CYCLE:

The thermophilic H2 bacterium Hydrogenobacter thermophilus is an obligate chemolithotroph. This bacterium does not have enzymes of the Calvin cycle, and fixes CO2 through the reductive TCA cycle (Figure 7.1). This cyclic pathway is the CO2 fixation process in some archaea including species of Thermoproteus and Sulfolobus, and in the photosynthetic green sulfur bacteria. This CO2-fixing metabolism shares TCA cycle enzymes that catalyze the reverse reactions. ATP: citrate lyase, fumarate reductase and 2-ketoglutarate: ferredoxin oxidoreductase replace the TCA cycle enzymes that do not catalyze the reverse reactions, i.e. citrate synthase, succinate dehydrogenase and 2- 2-ketoglutarate dehydrogenase. The reductive TCA cycle can be summarized as:

$$2CO_2 + 3NADH + 3H^+ + Fd{\cdot}H_2 + CoASH + 2ATP \longrightarrow$$

$$CH_3CO\text{-}CoA + 3NAD^+ + 3H_2O + Fd + 2ADP + 2P_i$$

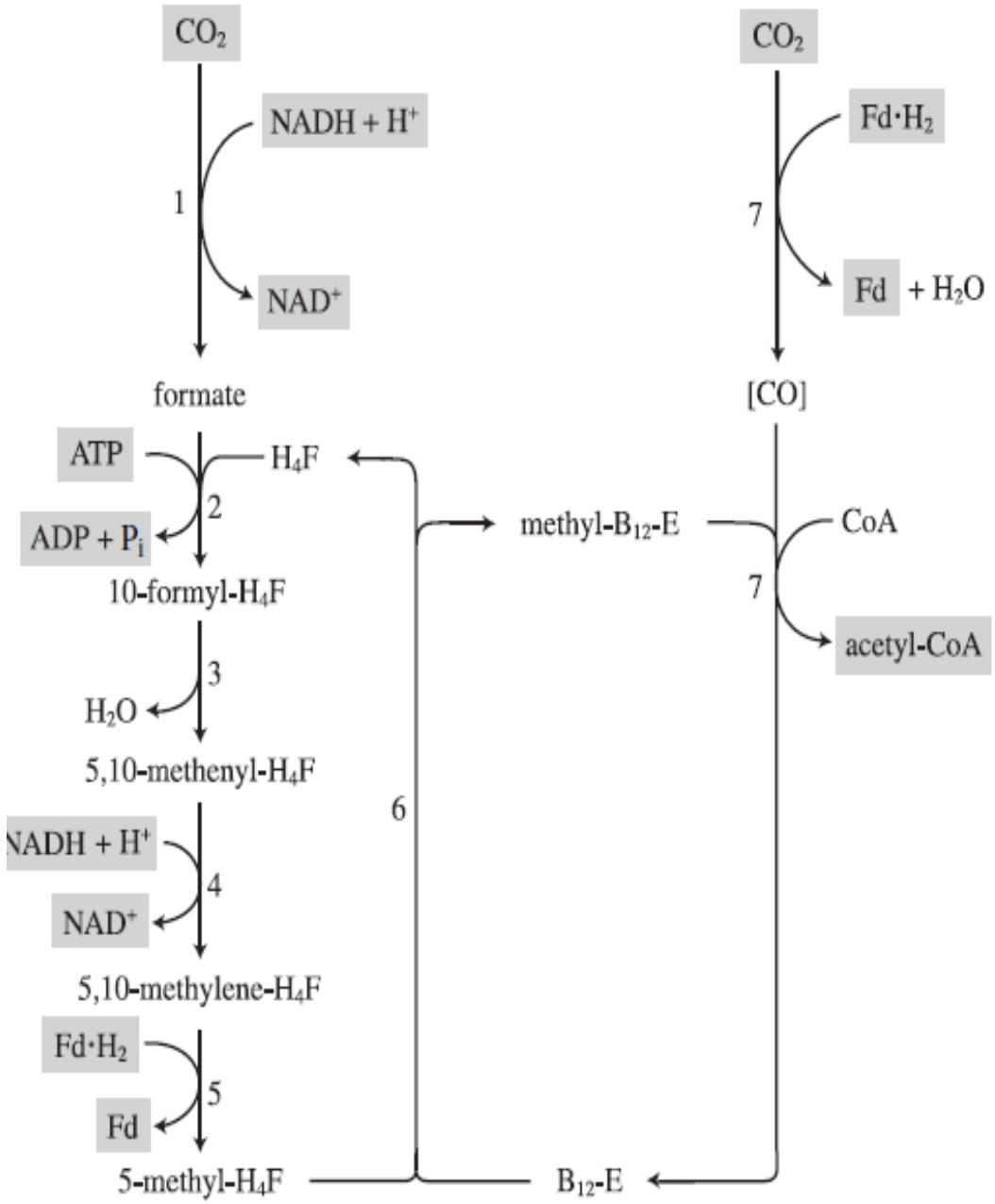

Fig. 7.1: The Acetyl-CoA Pathway – An Anaerobic CO2 Fixation Mechanism

The [CO]-bound carbon monoxide (CO) dehydrogenase (7) synthesizes acetyl-CoA with methyl-corrinoid. This pathway is also called the CO dehydrogenase (CODH) pathway, or the Wood–Ljungdahl pathway.

1, formate dehydrogenase;2, formyl-tetrahydrofolate (H4F) synthetase; 3, methenyl-H4F cyclohydrolase; 4, methylene-H4F dehydrogenase; 5, methylene-H4F reductase; 6, H4F:B12 methyltransferase; 7, carbon monoxide dehydrogenase. [CO], enzyme-bound carbon monoxide; methyl-B12-E, methylcorrinoid.

7.3. ANAEROBIC CO2 FIXATION THROUGH THE ACETYL-COA PATHWAY:

The acetyl-CoA pathway is employed for CO2 fixation by anaerobic chemolithotrophs including sulfidogens, methanogens and homoacetogens (Figure 7.2). Formate dehydrogenase reduces CO2 to formate that is bound to the C1-carrier tetrahydrofolate (H4F) to be reduced to methyl-H4F. This methyl-group is transferred to coenzyme B12 (corrinoid). A second CO2 molecule is reduced to the enzyme-bound form of [CO] by carbon monoxide dehydrogenase (CODH). [CO]-bound CODH synthesizes acetyl-CoA taking the methyl-group from methyl-corrinoid. CODH is a dual-function enzyme catalyzing CO oxidation/CO2 reduction and acetyl-CoA synthesis/cleavage. This enzyme can also be called acetyl-CoA synthase. This pathway has different names. ‘Acetyl-CoA pathway’ is commonly used since acetyl-CoA is the final product. ‘CODH pathway’ implies the pivotal role of the enzyme, while the ‘Wood–Ljungdahl pathway’ is another name to honour those who elucidated the pathway.

The pathway can be summarized as:

$$2CO_2 + 2NADH + 2H^+ + 2Fd{\cdot}H_2 + CoASH + ATP \longrightarrow$$

$$CH_3CO\text{-}CoA + 2NAD^+ + 3H_2O + 2Fd + ADP + P_i$$

In comparison with the reductive TCA cycle, this pathway consumes one ATP less, and 2NADH and 2Fd&H2 are oxidized while the reductive TCA cycle oxidizes 3NADH and 1Fd&H2. Additional energy is conserved in the form of a sodium motive force at the reaction catalyzed by methylene-tetrahydrofolate reductase. This comparison shows that the anaerobic process is more efficient than the reductive TCA cycle.

7.4. CO2 FIXATION THROUGH THE 3-HYDROXYPROPIONATE CYCLE:

CO2 is reduced to glyoxylate in a green gliding bacterium, *Chloroflexus aurantiacus*, through a prokaryote-specific 3-hydroxypropionate cycle (Figure 7.2). A similar, but not identical, pathway appears to operate in CO2 fixation by chemolithotrophic acidophilic archaea such as *Acidianus brierleyi*, *Acidianus ambivalens*, *Metallosphaera sedula*, and *Sulfolobus metallicus.*

Acetyl-CoA is carboxylated to malonyl-CoA by ATP-dependent acetyl-CoA carboxylase before being reduced to 3-hydroxypropionate. A bifunctional enzyme, malonyl-CoA reductase, catalyzes this two-step reductive reaction. 3-hydroxypropionate is reduced to propionyl-CoA. A single enzyme, propionyl-CoA synthase, catalyzes the three reactions from 3-hydroxypropionate to propionyl-CoA via 3-hydroxypropionyl-CoA and acrylyl-CoA consuming NADPH and ATP (AMPþPPi). Propionyl-CoA is carboxylated to methylmalonyl-CoA followed by isomerization of methylmalonyl-CoA to succinyl-CoA.

Succinyl-CoA is used for malate activation by CoA transfer, forming succinate and malyl-CoA; succinate in turn is oxidized to malate by the TCA cycle enzymes. Malyl-CoA is cleaved by malyl-CoA lyase with regeneration of the starting acetyl-CoA molecule and production of the first net CO2 fixation product, glyoxylate.

In the second cycle, pyruvate is synthesized from glyoxylate fixing another molecule of CO2. Acetyl-CoA is condensed with CO2 and converted to propionyl-CoA as in the first cycle. Glyoxylate and propionyl-CoA are condensed to erythro-3-methylmalyl-CoA before being cleaved to acetyl-CoA and pyruvate via erythro-3- methylmalyl-CoA, mesaconyl-CoA, and citramalate. Pyruvate is the product of the second cycle through the reduction of glyoxylate and CO2 with the regeneration of acetyl-CoA, the primary CO2 acceptor molecule.

There are three unique processes in this CO2 fixation pathway involving multifunctional enzymes that are not present in other chemolithotrophs. A bifunctional enzyme, malonyl-CoA reductase, catalyzes the two-step reduction of malonyl-CoA to 3-hydroxypropionate (reaction 2 in Figure 7.3). 3-hydroxypropionate is further metabolized to propionyl-CoA catalyzed by a trifunctional enzyme, propionyl-CoA synthase (reaction 3 in Figure). Another bifunctional enzyme, malyl-CoA lyase/erythro-3-methylmalyl-CoA lyase, cleaves malyl-CoA to acetyl-CoA and glyoxylate (reaction 9 in Figure 7.3), and condenses glyoxylate and propionyl-CoA to erythro-3-methylmalyl-CoA (reaction 10 in Figure 7.3).

The 3-hydroxypropionate cycle can be summarized as:

$$3CO_2 + 6NADPH + 6H^+ + X + 5ATP \longrightarrow$$

$$pyruvate + 6NADP^+ + 3H_2O + XH_2 + 3ADP + 2AMP + 2PP_i + 3P_i$$

X is an unknown electron carrier reduced by succinate dehydrogenase. Some prokaryotes grow by using reduced inorganic compounds as their energy source and CO2 as the carbon source. These are called chemolithotrophs. The electron donors used by chemolithotrophs include nitrogen and sulfur compounds, Fe(II), H2, and CO. The Calvin cycle is the most common CO2 fixation mechanism, and the reductive TCA cycle, acetyl-CoA pathway and 3-hydroxypropionate cycle are found in some chemolithotrophic prokaryotes. Some can use organic compounds as their carbon source while metabolizing an inorganic electron donor. This kind of bacterial metabolism is referred to as mixotrophy.

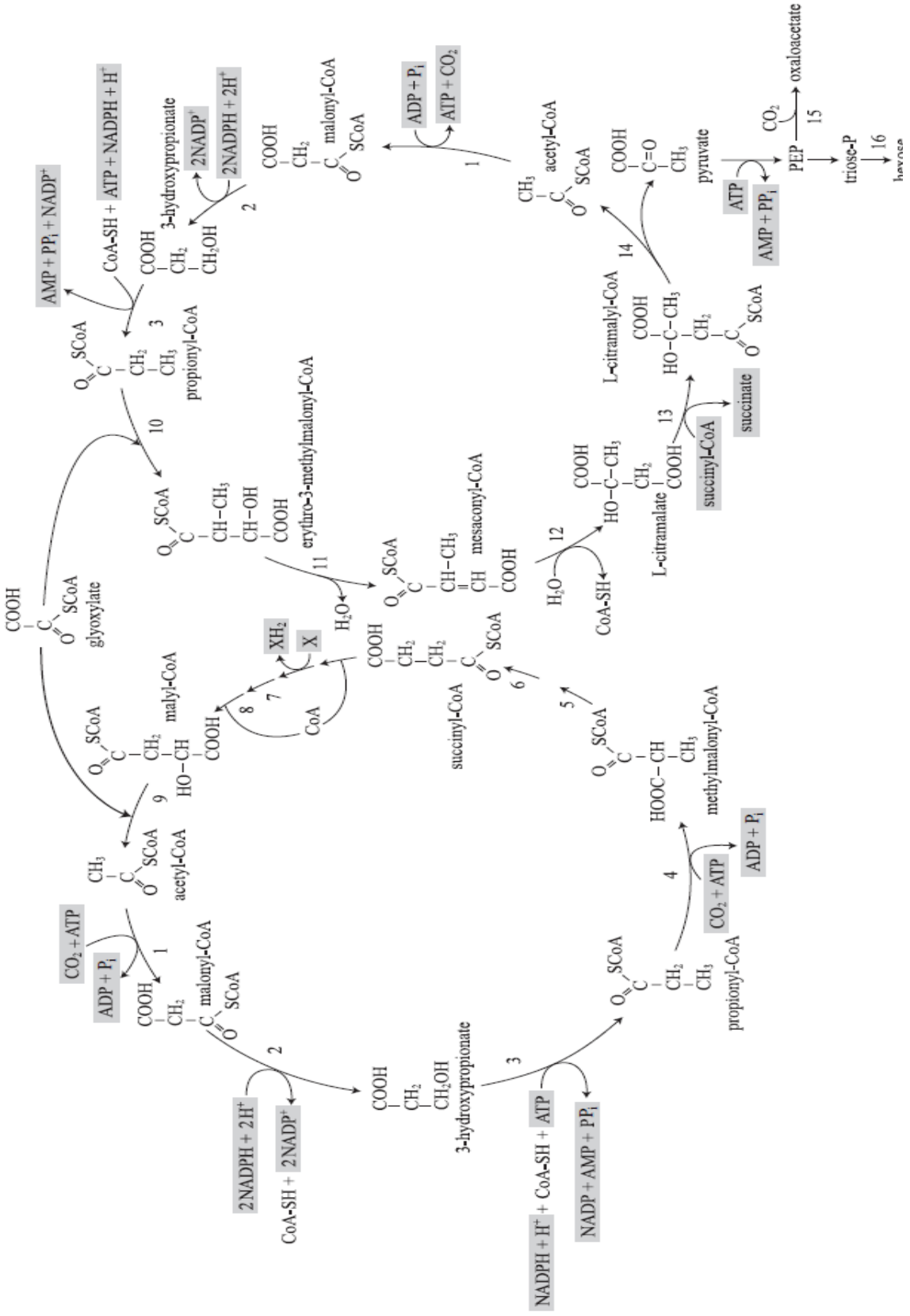

Fig 7.3: CO2 Fixation in Photosynthetic Chloroflexus Aurantiacus through the 3-Hydroxypropionate Cycle

(J. Bacteriol. 184:5999–6006, 2002) (Left) The 3-hydroxypropionate cycle for CO2 fixation with glyoxylate as the first net CO2 fixation product. This pathway involves part of the TCA cycle. An intermediate, 3-hydroxypropionate, was isolated and the cyclic pathway was named after this intermediate. (Right)

The glyoxylate assimilation cycle. This metabolism is known only in *Chloroflexus aurantiacus* and chemolithotrophic acidophilic archaea such as *Acidianus brierleyi*, *Acidianus ambivalens*, *Metallosphaera sedula*, and *Sulfolobus metallicus*.1, acetyl-CoA carboxylase; 2, malonyl-CoA reductase (bifunctional); 3, propionyl-CoA synthase; 4, propionyl-CoA carboxylase; 5, methylmalonyl-CoA epimerase; 6, methylmalonyl-CoA mutase; 7, citrate cycle enzymes (succinate dehydrogenase, fumarate hydratase); 8, succinyl-CoA:L-malate CoA transferase; 9, L-malyl-CoA lyase; 10, erythro-3-methylmalyl-CoA lyase; 11,3-methylmalyl-CoA dehydratase; 12, mesaconyl-CoA hydratase; 13, succinyl-CoA:L-citramalate CoA transferase; 14, citramalyl-CoA lyase; 15, phosphoenolpyruvate carboxylase; 16,gluconeogenesis enzymes.

7.5. REVERSE ELECTRON TRANSPORT:

As with chemoorganotrophs, metabolism of chemolithotrophs requires ATP and NAD(P)H for carbon metabolism and biosynthetic processes. Some of the electron donors used by chemolithotrophs have a redox potential higher than that of NAD(P)þ/NAD(P)H (Table 8.1). Electrons from these electron donors are transferred to coenzyme Q or to cytochromes. Some of the electrons are used to generate a proton motive force reducing O2 while the remaining electrons reduce NAD(P)þ to NAD(P)H through a reverse of the electron transport chain. The latter is an uphill reaction and coupled with the consumption of the proton motive force **(Figure 7.4**).

This is referred to as reverse electron transport. In most cases,electron donors with a redox potential lower than NAD(P)+/NAD(P)H are oxidized and this is coupled with the reduction of coenzyme Q or cytochromes for the efficient utilization of the electron donors at low concentration. The energy consumed in reverse electron transport from cytochrome c to NAD(P)+ is about five times the energy generated from the forward electron transport process.

Table 7.1: Redox Potential of Inorganic Electron Donars used by Chemiliothotrophs

Electron donating reaction	Redox potential ($E^{0'}$, V)
$CO + H_2O \longrightarrow CO_2 + 2H^+ + 2e^-$	–0.54
$SO_3^{2-} + H_2O \longrightarrow SO_4^{2-} + 2H^+ + 2e^-$	–0.45
$H_2 \longrightarrow 2H^+ + 2e^-$	–0.41
$NAD(P)H + H^+ \longrightarrow NAD(P)^+ + 2H^+ + 2e^-$	–0.32
$H_2S \longrightarrow S^0 + 2H^+ + 2e^-$	–0.25
$S^0 + 3H_2O \longrightarrow SO_3^{2-} + 6H^+ + 4e^-$	+0.05
$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^-$	+0.42
$NH_4^+ + 2H_2O \longrightarrow NO_2^- + 8H^+ + 6e^-$	+0.44
$Fe^{2+} \longrightarrow Fe^{3+} + e^-$	+0.78
$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$	+0.86

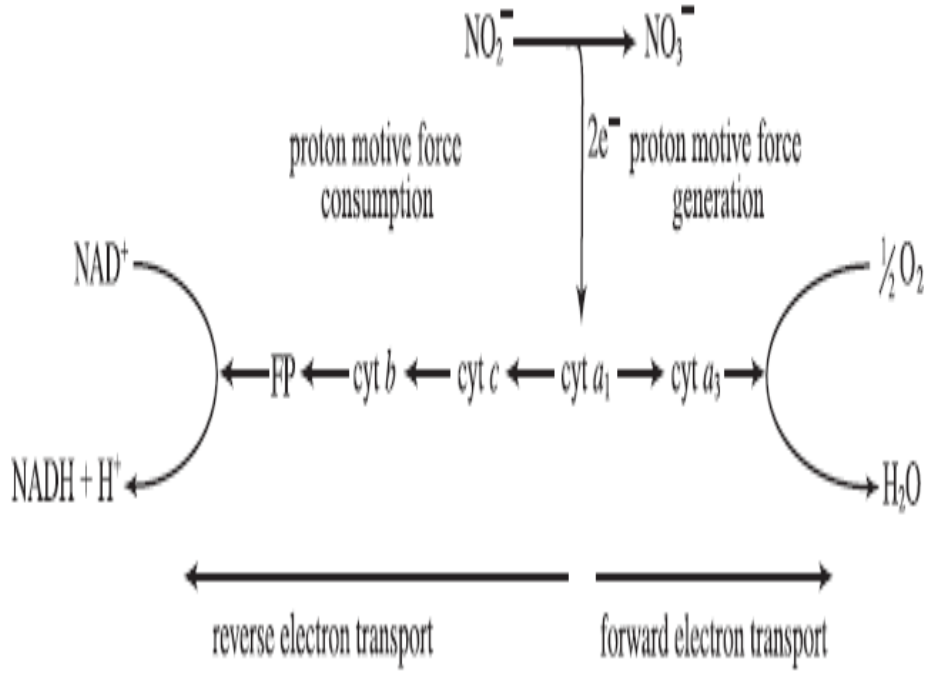

Fig 7.4: Reverse Electron Transport to Reduce NAD(P)þ in a Nitrite Oxidizer, Nitrococcus mobilis

Nitrococcus mobilis oxidizes nitrite as the electron donor reducing cytochrome a1. Some of the electrons are consumed to reduce O2 and generate a proton motive force (forward electron transport). The remaining electrons are transferred to NAD(P)þ to supply reducing power for biosynthesis. The latter process requires energy in the form of a proton motive force, and is referred to as reverse electron transport.

Energy Expenditure in CO2 Fixation:

Assuming that the electrons consumed in CO2 fixation processes are in the same energy state, efficiency can be compared in terms of ATP required for the synthesis of acetyl-CoA after normalization of the different products. Fructose-1,6-phosphate is produced in the Calvin cycle consuming 18ATP. When fructose-1,6-phosphate is metabolized through the EMP pathway to 2 pyruvate and then to 2 acetyl-CoA, 4ATP are generated. This gives a net 7ATP/acetyl-CoA from the Calvin cycle. The same parameters are 2ATP and 1ATP for the reductive TCA cycle and acetyl-CoA pathways, respectively.

Pyruvate is produced consuming 5ATP to 3ADP and 2AMP through the 3-hydroxypropionate cycle. Assuming that PPi is hydrolyzed to 2Pi without conserving energy, the ratio of ATP consumed/acetyl-CoA (pyruvate) is 7. From this comparison, it can be seen that energy efficiency varies from 1 to 7 ATP/acetyl-CoA in different CO2 fixation mechanisms.

Chemolithotrophs: Unable to Use Organics

Chemolithotrophs are divided into obligate and facultative chemolithotrophs. Obligate chemolithotrophs include a majority of the nitrifiers, some species of thiobacilli, and Hydrogenobacter thermophilus and Hydrogenovibrio marinus among the H2-oxidizing bacteria. Ever since the discovery of chemolithotrophic bacteria over a century ago, a satisfactory explanation has not been made as to why the obligate chemolithotrophs cannot use organic electron donors and what the advantage is to be obligately chemolithotrophic. They have to supply 12-carbon compounds needed for biosynthesis (see Table 7.1) from CO2 fixation products. Some of the obligate chemolithotrophs store polyglucose and other carbohydrates as carbon and energy reserves, so must be able to derive energy from their dissimilation. Several hypotheses have been proposed to explain why obligate chemolithotrophs cannot use organic compounds as energy and carbon sources.

These are:

1) Obligate chemolithotrophs cannot use organic carbon because the organisms are unable to transport them into cells. a potential fructose permease has been revealed in Nitrosomonas europaea through complete sequence determination of its genome, and this organism, as an obligate chemolithotroph, metabolizes fructose mixotrophically. However, amino acids and other monomers can diffuse into cells and are incorporated into biosynthesis in many chemolithotrophs.

2) Chemolithotrophs are unable to synthesize ATP from NADH oxidation. With a few exceptions, obligate chemolithotrophs couple the oxidation of their electron donors to the reduction of quinone or cytochromes, and supply reducing equivalents for biosynthesis through reverse electron transport. The oxidation of organic compounds reduces NADþ. Chemolithotrophs might lack enzymes for forward electron transport from NADH which makes them unable to develop a proton motive force via the electron transport chain from NADH. This hypothesis cannot explain the ATP levels sustained in many chemolithotrophs the metabolism of organic storage materials.

3) All the obligate chemolithotrophs do not have a functional TCA cycle to assimilate organic substrates. They do not synthesize 2-ketoglutarate dehydrogenase, and supply oxaloacetate, succinate, 2-ketoglutarate, etc. through the incomplete TCA fork). Pyridine nucleotides are not reduced in the incomplete TCA fork. 2-ketoglutarate dehydrogenase genes have been identified in many but not all of the chemolithotrophs examined. As the genes for the enzyme are repressed in facultative chemolithotrophs under the conditions for using an inorganic electron donor, the genes in obligate chemolithotrophs are regulated in such a way that they are repressed permanently.
4) Obligate chemolithotrophs might not be able to use organic electron donors due to metabolic control. The facultative chemolithotroph Ralstonia eutropha (Alcaligenes eutrophus), metabolizes fructose through the ED pathway. When H2 is supplied to the fructose culture, the bacterium stops growing due to the inability to use fructose as carbon source, because the enzymes for fructose metabolism are inhibited by H2. When a mixture of H2/CO2/O2 is supplied, growth is resumed. These hypotheses do not provide completely plausible reasons for the inability of obligate chemolithotrophs to grow on organic electron donors. The mechanism might be much more complex than expected.

7.6. SUMMARY:

Reductive TCA Cycle (rTCA): Used by green sulfur bacteria, this pathway runs the normal, oxidative TCA cycle in reverse to assimilate CO2.**3-Hydroxypropionate Cycle/Bi-cycle**: Found in filamentous anoxygenic phototrophs like *Chloroflexus aurantiacus*. **Reductive Acetyl-CoA Pathway (Wood-Ljungdahl)**: Used by some anaerobic bacteria (acetogens and methanogens) to convert CO2into acetyl-CoA. Ultimately, these pathways produce three-carbon sugars (triose phosphates) and other intermediates, which are used as building blocks to synthesize essential cellular components like glucose, lipids, and amino acids. The excess fixed carbon is often stored as glycogen or other storage compounds

7.7. SELF ASSESSMENT:

1) Reductive TCA Cycle
2) Anaerobic CO2 Fixation through the acetyl-CoA Pathway
3) CO2 Fixation through the 3-hydroxypropionate Cycle
4) Reverse Electron Transport

7.8. REFERENCES

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Prof K. Mallikarjuna

LESSON-8

CHEMOLITHOTROPHY

8.0 OBJECTIVE:

- To under the prokaryotic mode of energy transfer using chemical energy to transfer the electron to carbon metabolism.

STRUCTURE:

8.1. INTRODUCTION:

Some prokaryotes grow by using reduced inorganic compounds as their energy source and CO2 as the carbon source. These are called chemolithotrophs. The electron donors used by chemolithotrophs include nitrogen and sulfur compounds, Fe(II), H2, and CO. The Calvin cycle is the most common CO2 fixation mechanism, and the reductive TCA cycle, acetyl-CoA pathway and 3-hydroxypropionate cycle are found in some chemolithotrophic prokaryotes. Some can use organic compounds as their carbon source while metabolizing an inorganic electron donor. This kind of bacterial metabolism is referred to as mixotrophy.

8.2. NITRIFICATION:

A group of bacteria oxidize ammonia to nitrite that is further oxidized to nitrate by another group of bacteria in an energy generating process known as nitrification. They are all

Gram-negative, mostly obligately chemolithotrophic, and have an extensive membrane structure within the cytoplasm except for Nitrosospira tenuis. A separate group of bacteria oxidize nitrite to nitrate. These organisms are referred to as nitrifying bacteria and are widely distributed in soil and water. The nitrogen cycle cannot be completed without them.

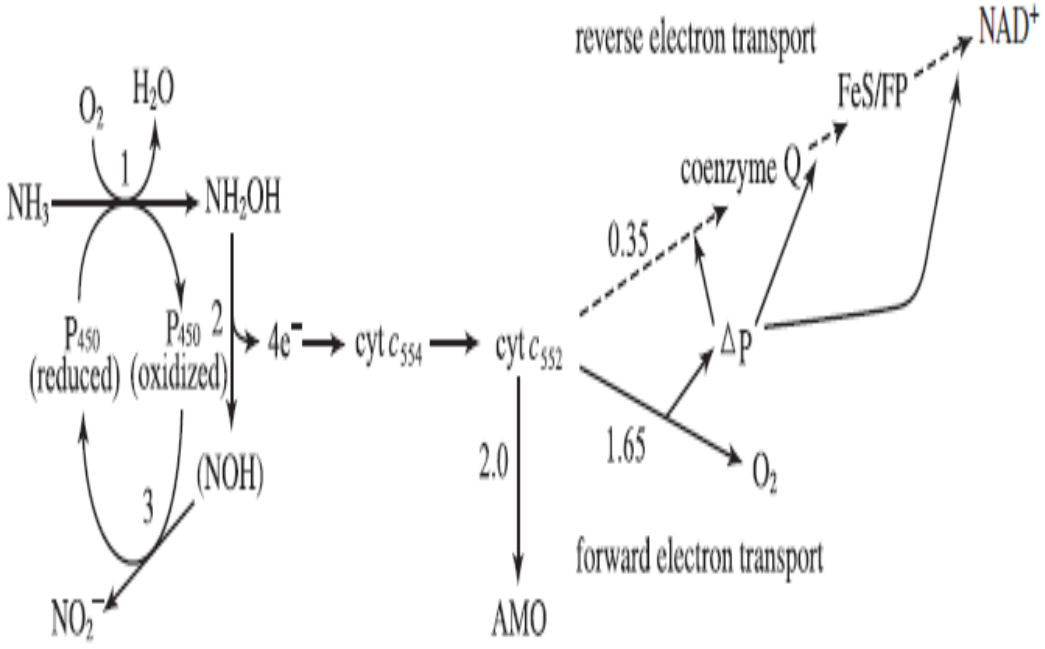


Fig 8.1: Ammonia Oxidation to Nitrite by Denitrifiers.

(Modified from Arch. Microbiol.178:250–255, 2002)

Since the redox potential of NH2OH/NH3 (þ0.899 V) is higher than that of ½O2/H2O, ammonia monooxygenase (1) oxidizes ammonia, consuming 2e- from the reduced form of cytochrome c552 mediated by P450. Hydroxylamine oxidation is coupled to the reduction of cytochrome c554. Out of the four electrons released in the oxidation of NH2OH by H2O, two electrons are consumed by AMO where they are used for the oxidation of ammonia. The other 1.65 electrons are routed to cytochrome oxidase to generate a proton motive force and the remaining 0.35 electrons pass to NADþ through reverse electron transport to supply reducing power for biosynthesis.Ammonia and nitrite oxidizers fix CO2 through the Calvin cycle.

8.2.1. Ammonia Oxidation:

NH3 is oxidized in a two-step reaction via hydroxylamine (NH2OH) and nitroxyl (NOH) in reactions catalyzed by ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (H2O) (Figure 8.2).

$$2NH_3 + 3O_2 \longrightarrow 2NO_2^- + 2H^+ + 2H_2O \quad (\Delta G^{0'} = -272\ kJ/mol\ NH_3)$$

NH3 is oxidized to hydroxylamine by AMO consuming two electrons available from the oxidation of hydroxylamine, probably through a membrane-bound cytochrome c552. Since the redox potential of NH2OH/NH3 (þ0.899 V) is higher than that of½O2/H2O, ammonia monooxygenase oxidizes ammonia consuming 2e" from the reduced form of cytochrome c552 mediated by P450, as shown in Figure 8.2. Hydroxylamine oxidation to nitrite is coupled to the reduction of cytochrome c554.

$$2NH_3 + O_2 \xrightarrow{AMO} 2NH_2OH \qquad (\Delta G^{0'} = +16\ kJ/mol\ NH_3)$$

$$\text{hydroxylamine} + H_2O \xrightarrow{HAO} NO_2^- + 5H^+ + 4e^-$$

HAO from Nitrosomonas europaea is a homotrimer with each subunit containing eight c-type hemes, giving a total of 24 hemes. Seven of the hemes in each subunit are covalently attached to the protein by two thioester linkages. The eighth heme, designated P-460, is an unusual prosthetic group, and has an additional covalent bond through a tyrosine residue. The P-460 heme is located at the active site. The function of the c-hemes is believed to be the transfer of electrons from the active site of P-460 to cytochrome c554.

The four electrons released from the oxidation of NH2OH by HAO in Nitrosomonas europaea are channelled through cytochrome c554 to a membrane-bound cytochrome c552. Two of the electrons are routed back to AMO, where they are used for the oxidation of ammonia, while 1.65 electrons are used to generate a proton motive force through cytochrome oxidase and 0.35 are used to reduce NAD(P)þ through reverse electron transport. Out of 3/2 electrons, 2/2e" are consumed in ammonia oxidation by the monooxygenase, and the remaining 2e" are used to generate ip through ETP and NAD(P)H through the reverse electron transport.

Although ammonia oxidizers are known as obligate chemolithotrophs, they can utilize a limited number of organic compounds including amino acids and organic acids. The complete genome sequence of Nitrosomonas europaea has revealed a potential fructose permease, and this bacterium metabolizes fructose and pyruvate mixotrophically.

8.2.2. Nitrite Oxidation:

Nitrite produced from the oxidation of ammonia is used by a separate group of bacteria as their energy source (Table 8.2):

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \quad (\Delta G^{0'} = -74.8\ kJ/mol\ NO_2^-)$$

Nitrite oxidoreductase (NOR) oxidizes nitrite to nitrate, reducing cytochrome a1. It is a membrane-associated iron-sulfur molybdoprotein, and is part of an electron transfer chain which channels electrons from nitrite to molecular oxygen. NOR in Nitrobacter hamburgensis is a heterodimer consisting of ! and " subunits. The NOR of Nitrobacter winogradskyi is composed of three subunits as well as heme a1, heme c, non-heme iron and molybdenum. This enzyme transfers electrons to the cytochrome c oxidase through a membrane-bound cytochrome c (Figure 8.3). Since the free energy change is small, only one H+ is transported coupled to these reactions. The electron transfer is not well understood. Hydride ion (H") is transferred from NO2 to cytochrome c consuming the inside negative membrane potential,

Table 8.1: Repsentative Types of Nitrifying Bacteria

Organism	Characteristics
$NH_3 \longrightarrow NO_2^-$	
Nitrosomonas europaea	soil, fresh water, seawater, sewage works
Nitrosospira (Nitrosovibrio) tenuis	soil
Nitrosococcus nitrosus	soil
Nitrosococcus oceanus	seawater
Nitrosospira briensis	soil
Nitrosolobus multiformis	soil
$NO_2^- \longrightarrow NO_3^-$	
Nitrococcus mobilis	seawater
Nitrobacter winogradskyi	soil, fresh water, seawater, facultative chemolithotroph
Nitrospina gracilis	seawater
Nitrospira marina	seawater

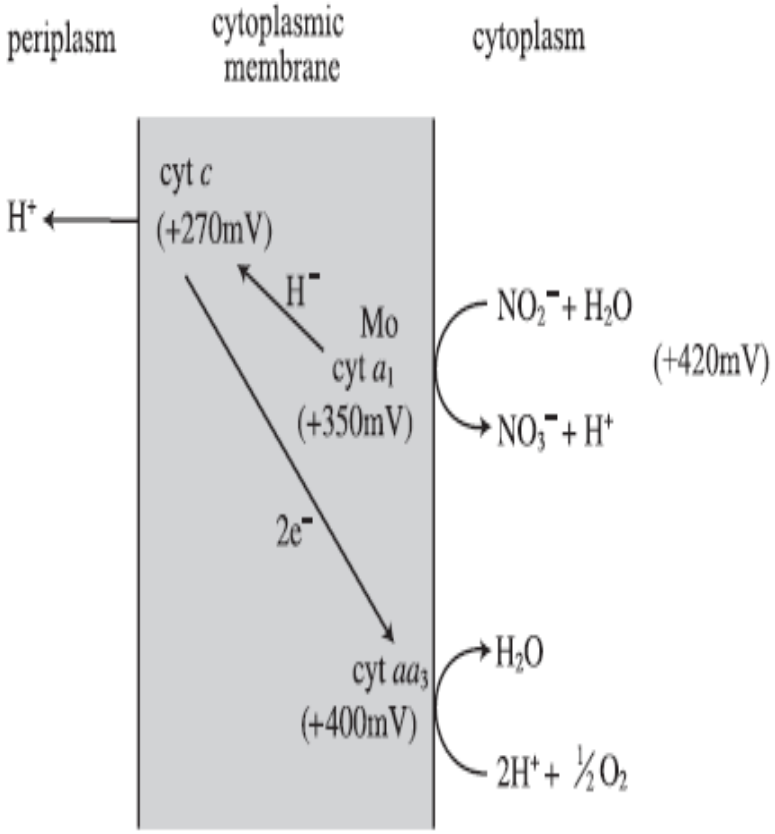


Fig 8.2: The electron transport system in nitrite oxidation by *Nitrobacter winogradskyi*. (Dawes, E. A. 1986, Microbial Energetics, Figure 9.4. Blackie & Son, Glasgow)

The oxidation of nitrite transports 1Hþ across the membrane because electron transfer from NOR with a redox potential of þ420mV to cytochrome c (þ270 V) is an uphill reaction. Nitrite oxidizers are obligate chemolithotrophs with the exception of Nitrobacter winogradskyi, which is a facultative chemolithotroph.

8.2.3 Anaerobic Nitrification:

Some bacteria of the phylum Planctomycetes oxidize ammonia under naerobic conditions using nitrite as the electron acceptor. These are known as ANAMMOX bacteria. Nitrosomonas europaea oxidizes ammonia under anaerobic conditions using nitrogen dioxide (NO2) as the electron acceptor.

8.3. SULFUR BACTERIA AND THE OXIDATION OF SULFUR COMPOUNDS:

Certain prokaryotes can use inorganic sulfur compounds including sulfide (HS"), elemental sulfur (S0), thiosulfate (HS2O3"), and sulfite (HSO3") as their energy source. These are known as sulfur bacteria.

8.3.1 Sulfur Bacteria

To distinguish them from photolithotrophic sulfur bacteria, chemolithotrophic sulfur bacteria are referred to as colourless sulfur bacteria. These are phylogenetically diverse and include bacteria and archaea. They are grouped either according to the location of sulfur deposition after sulfide is oxidized (Table 8.3) or by their ability to use polythionate. Many can thrive at the aerobic–anaerobic interface where sulfide produced by sulfate-reducing bacteria diffuses from anaerobic regions. These organisms have to compete with molecular oxygen for sulfide, which is rapidly oxidized with molecular oxygen. Some other sulfur bacteria are acidophilic, oxidizing pyrite (FeS2), and include species of Thiobacillus. The genus Thiobacillus is designated as small Gram-negative rod-shaped bacteria

Table 8.3: Sulfur Bacteria

1. Accumulating sulfur intracellularly	
Gliding, filamentous cells	*Beggiatoa, Thiothrix, Thioploca*
Gliding, very large unicells	*Achromatium*
Immotile or motile with flagella, cocci or rod-shaped	
immotile, rod	*Thiobacterium*
motile with flagella, rod	*Macromonas*
motile with flagella, cocci	*Thiovulum*
motile with flagella, vibrioid	*Thiospira*

2. Accumulating sulfur extracellularly				
	Thiobacillus	*Thiomicrospira*	*Thioalkalimicrobium*	*Thioalkalivibrio*
Morphology	rod	vibrioid	rod to spirillum	rod to spirillum
Flagellum	+	+	+	+
DNA G + C (%)	34–70	48	61.0–65.6	48.0–51.2
Growth pH	1–8.5	5.0–8.5	7.5–10.6	7.5–10.6
Chemolithotrophy	facultative	obligate	obligate	obligate

3. Thermophilic			
	Thermothrix	*Sulfolobus*	*Acidianus*
Classification	Gram-negative	archaeon	archaeon
Morphology	rod	cocci	cocci
DNA G + C content (%)	?	36–38	~31
Growth temperature (°C)	40–80	50–85	45–95
Chemolithotrophy	facultative	facultative	facultative

Source: Applied and Environmental Microbiology, **67**, 2873–2882, 2001.

deriving energy from the oxidation of one or more reduced sulfur compounds including sulfides, thiosulfate, polythionate and thiocyanate. They fix CO2 through the Calvin cycle. However, they are

diverse phylogenetically in terms of 16S ribosomal RNA gene sequences, DNA GþC content and DNA homology in addition to physiological differences. Many species have been reclassified to Paracoccus, Acidiphilium, Thiomonas, Thermithiobacillus, Acidithiobacillus and Halothiobacillus but the classification of several species is still uncertain. For convenience, Thiobacillus is used here. In addition to species of Beggiatoa, Thiobacillus and Thiomicrospira within the sulfur bacteria, many other prokaryotes can oxidize sulfur compounds mixotrophically or chemolithotrophically.

These include bacteria such as species of Aquaspirillum, Aquifex, Bacillus, Paracoccus, Pseudomonas, Starkeya and Xanthobacter, and archaea such as species of Sulfolobus and Acidianus. Species of the Gram-negative bacterial genus Thermothrix, and the archaea Sulfolobus and Acidianus are thermophiles.

Acidianus brierleyi uses elemental sulfur not only as an electron donor under aerobic conditions, but also as an electron acceptor to reduce hydrogen under anaerobic conditions.

Species of Thiobacillus and Thiomicrospira can be isolated from diverse ecosystems including soil, freshwater and seawater. Sulfolobus acidocaldarius is an archaeon isolated from an acidic hot spring. In addition to reduced sulfur compounds, Thiobacillus ferrooxidans and Sulfolobus acidocaldarius oxidize Fe(II) to Fe(III) as their electron donor. Thiobacillus ferrooxidans and *Thiobacillus thiooxidans* grow optimally at around pH 2.0 and oxidize metal sulfides solubilizing the metals. Low quality ores are treated with these bacteria to recover metals such as Cu, U, and others. This process is referred to as bacterial leaching. This property can also be applied to remove sulfur (in pyrite, FeS2) from coal. *Thiobacillus denitrificans* is an anaerobe which oxidizes sulfur compounds using nitrate as the electron acceptor.

Filamentous sulfur bacteria of the genera *Thioploca and Beggiatoa* accumulate nitrate in intracellular vacuoles. Nitrate, acting as electron acceptor, is reduced to ammonia with sulfide or sulfur as electron donors in these bacteria. Many nitrate-accumulating sulfur bacteria inhabit the sediments of upwelling areas characterized by high sediment concentrations of soluble sulfide, and low levels of dissolved oxygen.

The ecological implication of nitrate ammonification is that nitrogen is conserved within the ecosystem. Thiomargarita namibiensis is another sulfur bacterium with vacuoles containing nitrate. This bacterium has not been isolated in pure culture, but is found in sediments in the coastal waters of Namibia measuring up to 0.75mm in size, which is about 100 times bigger than a normal sized bacterium.

In addition to the acidophilic and neutrophilic sulfur bacteria, alkalophilic sulfur bacteria thrive in alkaline soda lakes. These are species of *Thioalkalimicrobium* and *Thioalkalivibrio*. They accumulate elemental sulfur extracellularly before oxidizing it when sulfide is depleted. Members of the former genus are obligate aerobes, while some of the latter genus are facultative anaerobes using nitrate as electron acceptor (Section 9.1.4). An anaerobic Gram-negative bacterium is also known that can grow chemolithotrophically using HS as the electron donor and arsenate as the electron acceptor.

8.3.2. Biochemistry of Sulfur Compound Oxidation:

Sulfur compound oxidation mechanisms have not been clearly established, partly because sulfur chemistry is complicated and also because different organisms have different oxidative mechanisms

that use different enzymes and coenzymes. Figure 8.4 outlines the sulfur compound oxidation pathways in bacteria and archaea. These oxidize HS^-, S0, $HS2O3^-$ and HSO^-through a common pathway transferring electrons to the electron transport chain to generate a proton motive force.

Inorganic sulfur oxidation enzyme systems are best known in *Paracoccus tentotrophus,* a facultative chemolithotrophic Gram-negative

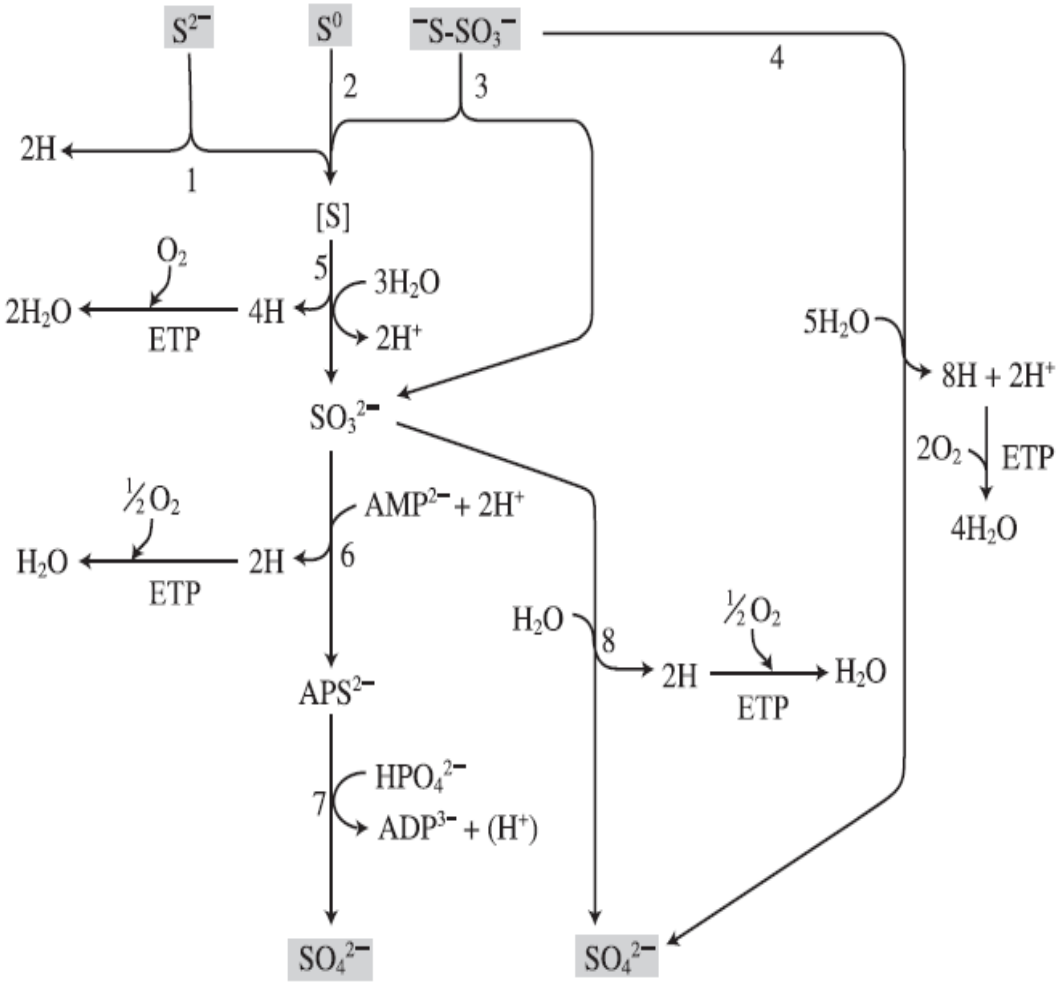


Fig 8.3: Oxidation of sulfur compounds by Thiobacillus ferrooxidans.

(Dawes, E. A. 1986, Microbial Energetics, Figure 9.5. Blackie & Son, Glasgow)

Sulfide and sulfur are converted to polysulfide before being oxidized further. Thiosulfate is metabolized to sulfite and polysulfide by rhodanese (3) or directly oxidized to sulfate by the thiosulfateoxidizing multienzyme complex (4).

Sulfur compound oxidation is coupled to the reduction of cytochrome b or c. 1, sulfide-oxidizing enzyme; 2, conversion to polysulfide; 3, rhodanese; 4, thiosulfate-oxidizing multienzyme complex; 5, sulfuroxidizing enzyme; 6, APS reductase; 7, ADP sulfurylase; 8, sulfite cytochrome c reductase.

The genes for sulfur oxidation (Sox) are encoded in the sox gene cluster consisting of 15 genes. The Sox system reconstituted from SoxB, SoxCD, SoxXA and SoxYZ oxidizes HS –, S0, HS2O3" and HSO3– reducing cytochrome c, but each of the proteins is catalytically inactive in vitro. Other gene products include HS"-oxidizing enzyme, c-type cytochromes, regulator proteins and others. These proteins are believed to catalyze the reactions shown in Figure 8.4. HS" and S0 are converted to polysulfide, [S], before being oxidized to sulfate. The reduced form of glutathione (GSH) is involved in the oxidation of sulfide to [S].

$$nS^{-} + GSH \longrightarrow GS_nSH + 2ne^{-}$$

Sulfur oxidase catalyzes [S] oxidation to sulfite. Sulfite is oxidized to sulfate either through the direct reaction catalyzed by sulfite cytochrome reductase (SCR) or through the reactions catalyzed by adenosine-50-phosphosulfate (APS) reductase and ADP sulfurylase. Direct oxidation appears to be far more widespread than the APS reductase pathway. More energy is conserved in the latter reactions than in the former. The nature of SCR is different among the sulfur oxidizers.

SCR of Thiobacillus thioparus is a cytoplasmic soluble enzyme containing molybdenum, and that of Paracoccus (Thiobacillus) pantotrophicus is a periplasmic enzyme containing Mo and c-type heme. It is not known if Mo is contained in the membrane SCR of Thiobacillus thiooxidans. The soluble and periplasmic SCRs oxidize sulfite coupled to the reduction of c-type cytochromes, while the membrane enzyme is coupled to the reduction of Fe(III). The electron transport chain is not known in detail, though genes of sulfur oxidizing enzymes have been characterized in many organisms. The mid-point redox potential of the sulfur compounds are:

$$S^0/H_2S = -0.25\,V$$

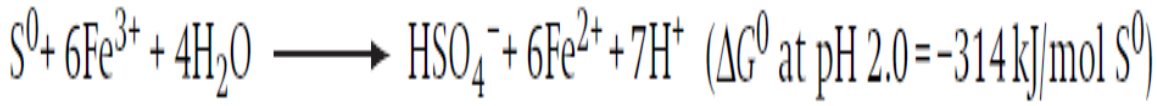
$$SO_3^{2-}/S = +0.5\,V$$

$$SO_4^{2-}/SO_3^{2-} = -0.454\,V$$

The cell yield on sulfide is higher in the anaerobic denitrifier Thiobacillus denitrificans than in Thiobacillus thiooxidans. This shows that some sulfur compounds are oxidized by oxidases directly reducing molecular oxygen in the aerobic bacterium without energy conservation, while the oxidative reactions are coupled to denitrification with energy conservation.

A sulfur bacterium Paracoccus denitrificans (Thiosphaera pantotropha) can grow chemolithotrophically oxidizing carbon disulfide (CS2) or carbonyl sulfide (COS).

Thiobacillus ferrooxidans and Sulfolobus acidocaldarius can use Fe(III) as their electron acceptor and S0 as the electron donor.



8.3.3. Carbon Metabolism in Colourless Sulfur Bacteria:

All species of the genus Thiomicrospira and some species of *Thiobacillus* and *Sulfolobus* are obligate chemolithotrophs. Other species are either facultative chemolithotrophs or mixotrophs using organic compounds.

Obligately chemolithotrophic colourless sulfur bacteria fix CO2 through the Calvin cycle, while the reductive TCA cycle and the 3-hydroxypropionate cycle are employed in the archaea *Sulfolobus acidocaldarius* and *Acidianus* brierleyi, respectively.

8.4. IRON BACTERIA: FERROUS IRON OXIDATION:

Many microorganisms can oxidize Fe(II) to Fe(III). Some of these are known as iron bacteria and use the free energy generated from the oxidation. Many heterotrophic bacteria also oxidize Fe(II), but the function of such ferrous iron oxidation is not known and they do not conserve the free energy.

Fe(II) is used as the electron donor in some Gram-negative bacteria including Gallionella ferruginea, Thiobacillus ferrooxidans and species of Leptospirillum, and the archaea Sulfolobus acidocaldarius, Acidianus brierleyi and species of Ferroplasma. Thiobacillus ferrooxidans, Sulfolobus acidocaldarius and Acidianus brierleyi use sulfur compounds as their electron donors, but the others do not use them. Since Fe(II) is chemically oxidized easily at neutral pH, iron bacteria growing at neutral pH are microaerophilic and prefer a medium redox potential around 200–320mV at a slightly acidic pH of 6.0. Their growth can be observed in an agar gel with gradients of Fe(II) and O2 in opposite directions. Species.

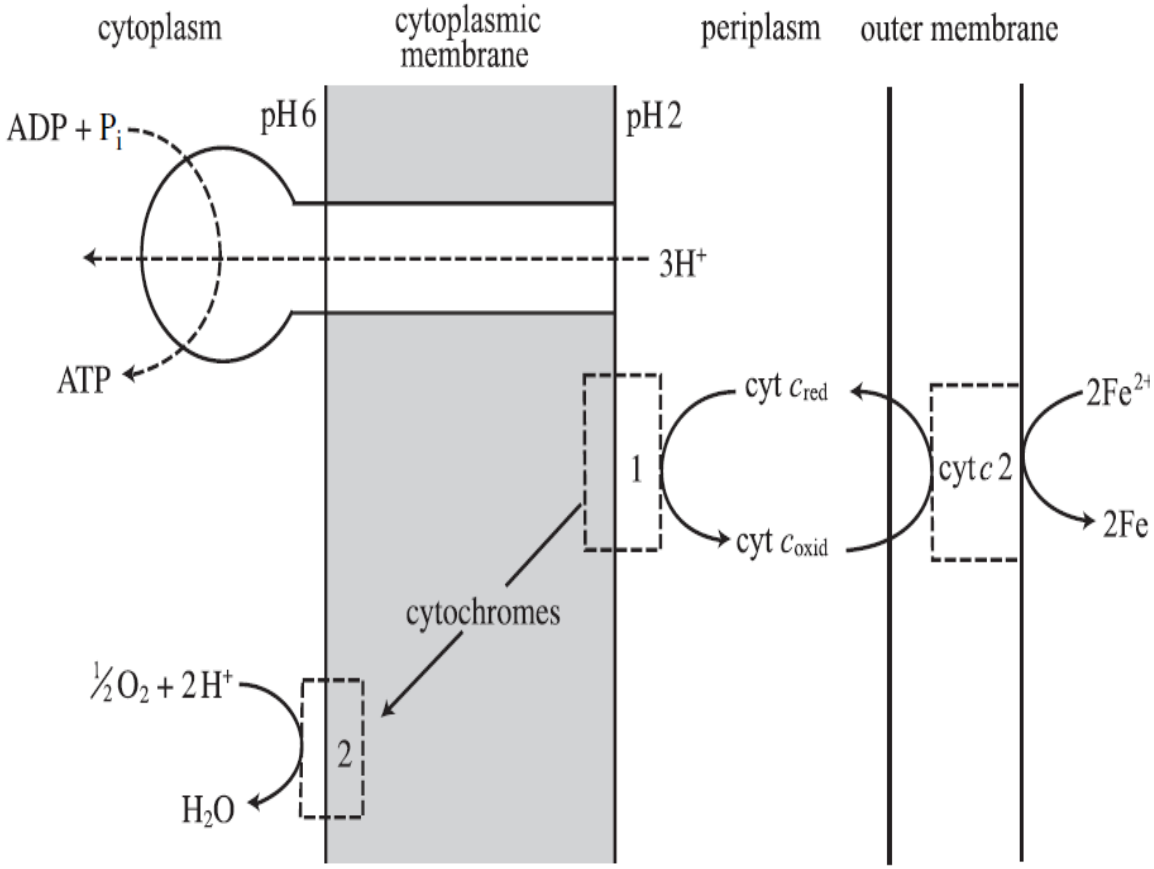


Fig 8.4: ATP synthesis coupled to the oxidation of Fe2+ in Thiobacillus ferrooxidans. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn, Figure 9.6. Springer, New York)1, rusticyanin; 2, cytochrome *Leptospirillum*, *Thiobacillus ferrooxidans* and *Sulfolobus acidocaldarius* grow optimally around pH 2.0 where the chemical oxidation of Fe(II) is slow. A water-insoluble mineral, pyrite, is the natural electron donor used by acidophilic iron bacteria including *Thiobacillus ferrooxidans*. They oxidize the electron donor at the cell surface, reducing an outer membrane c-type cytochrome which transfers electrons to the terminal oxidase located at the inner face of the cytoplasmic membrane (Figure 8.5).

Electron transfer involves rusticyanin, a copper containing blue protein. Rusticyanin is not involved in the electron transport chain that oxidizes sulfur compounds in this bacterium. The free energy change in Fe(II) oxidation is small since the redox potential of Fe(III)/Fe(II) is þ0.78 V which is very similar to the þ0.86 V of O2/H2O. The acidophilic Fe(II) oxidizers maintain their internal pH around neutrality with a Hþ gradient of 103"106 (Section 5.7.3). They maintain a low or inside positive membrane potential to compensate for the large potential generated by the H+ gradient. Electron transfer from the periplasmic region to cytochrome oxidase contributes to the membrane potential and proton consumption by the oxidase contributes to the proton gradient part of the proton motive force.

The hyperthermophilic archaeon *Sulfolobus acidocaldarius* grows chemolithotrophically using sulfide and Fe(II) as its electron donors, like *Thiobacillus ferrooxidans*. The former is a facultative chemolithotroph while the latter is an obligate chemolithotroph. Species of *Leptospirillum, Gallionella ferruginea* and *Thiobacillus ferrooxidans* fix CO2 through the Calvin cycle, while the reductive TCA cycle (Section 8.8.2) and 3-hydroxypropionate cycle (Section 8.8.4) are employed by Sulfolobus acidocaldarius and Acidianus brierleyi, respectively.

An archeaon, Ferroglobus placidus, oxidizes Fe(II) under anaerobic conditions using nitrate as the electron acceptor. This is unusual since the redox potential of the electron donor (E00, Fe(III)/Fe(II)¼þ0.78 V) Pi An archeaon, Ferroglobus placidus, oxidizes Fe(II) under anaerobic conditions using nitrate as the electron acceptor. This is unusual since the redox potential of the electron donor

$$(\dot{E}^{0'}, \text{Fe(III)/Fe(II)} = +0.78\ \text{V}$$

is higher than that of the electron acceptor (E^0, NO_3^-/NO_2^- þ0.42 V). Several dissimilatory perchlorate-reducing bacteria including Dechlorosoma suillum use Fe(II) as their electron donor . In addition to Fe(II), As(III) and U(IV) can be used as electron donors by various prokaryotes. These are discussed later.

8.5. HYDROGEN OXIDATION:

8.5.1 Hydrogen-Oxidizing Bacteria:

Various bacteria grow chemolithotrophcally on a H2þCO2 mixture.With a few exceptions (e.g. Hydrogenobacter thermophilus and Hydrogenovibrio marinus), these are facultative chemolithotrophs (Table 8.4). They are phylogenetically diverse, and are grouped not on their chemolithotrophy but on their heterotrophic characteristics.

8.5.2. Hydrogenase:

The bacteria listed in Table 8.4 use hydrogen as their electron donor to grow chemolithotrophically with the aid of hydrogenase. Most of them have a cytochrome-dependent particulate hydrogenase on their cytoplasmic membrane and Ralstonia eutropha (*Alcaligenes eutrophus*) and Nocardia autotrophica possess a NADP-dependent soluble hydrogenase in addition to the particulate enzyme (Figure 8.6). Only the soluble enzyme is found in the third group which includes Alcaligenes denitrificans, *Alcaligenes ruhlandii* and *Rhodococcus opacus*. The soluble hydrogenase reduces NADP, and the particulate enzyme transfers electrons from H2 to coenzyme Q of the electron transport chain.

The soluble hydrogenase gene in *Ralstonia eutropha* is plasmid encoded. Since the affinity of the soluble enzyme for the substrate is low, this enzyme cannot oxidize the substrate at low concentrations. The particulate hydrogenase has a high affinity for the substrate, enabling the bacterium to use hydrogen at low concentrations.

Organisms with only the particulate hydrogenase employ reverse electron transport to reduce NAD(P)þ. In most cases, the hydrogenase from hydrogen-oxidizing bacteria cannot produce hydrogen. These enzymes are referred to as uptake hydrogenases to differentiate

them from those of anaerobic bacteria. The anaerobic bacterial enzymes are called evolution (or production) hydrogenases. The function of the evolution hydrogenase is to dispose of electrons generated from fermentative metabolism by reducing protons.

$$2H^+ + 2e^- \text{ (reduced ferredoxin)} \xrightleftharpoons{\text{evolution hydrogenase}} H_2$$

$$H_2 + \text{electron carrier (oxidized)} \xrightarrow{\text{uptake hydrogenase}} \text{electron carrier (reduced)}$$

Organism	Hydrogenase: Soluble (NAD^+-dependent)	Hydrogenase: Particulate (cytochrome-dependent)
Facultative chemolithotroph		
Gram-negative		
Alcaligenes denitrificans	+	−
Ralstonia eutropha (*Alcaligenes eutrophus*)	+	+
Alcaligenes latus	−	+
Alcaligenes ruhlandii	+	−
Aquaspirillum autotrophicum	−	+
Azospirillum lipoferum	−	+
Derxia gummosa	−	+
Flavobacterium autothermophilum	?	+
Microcyclus aquaticus	−	+
Paracoccus denitrificans	−	+
Pseudomonas facilis	−	+
Pseudomonas hydrogenovara	−	+
Ralstonia eutropha	+	+
Renobacter vacuolatum	−	+
Rhizobium japonicum	−	+
Xanthobacter flavus	−	+
Gram-positive		
Arthrobacter sp.	−	+
Bacillus schlegelii	−	+
Mycobacterium gordonae	?	+
Nocardia autotrophica	+	−
Rhodococcus opacus	+	−
Obligate chemolithotroph		
Hydrogenobacter thermophilus	−	+
Hydrogenovibrio marinus	−	+[a]

[a] The particulate hydrogenase in this bacterium is NAD^+-dependent and catalyzes the reverse reaction.

The particulate hydrogenase of the thermophilic chemolithotrophic Hydrogenovibrio marinus is NADP-dependent and catalyzes the reverse reaction.

8.5.3. Anaerobic H2-oxidizers:

It has been stated that some anaerobic respiratory prokaryotes can grow on H2þCO2 with an appropriate electron acceptor. They include some sulfidogens, methanogens and

homoacetogens (Section 9.5). In addition to these, H2-oxidizing anaerobic chemolithotrophs have been isolated from hydrothermal vents.*Desulfurobacterium crinifex, Thermovibrio ammonificans and Thermovibrio ruber use H2 as the electron donor reducing nitrate* to ammonia, or S0 to HS". These are strict anaerobes. Species of Caminibacter and

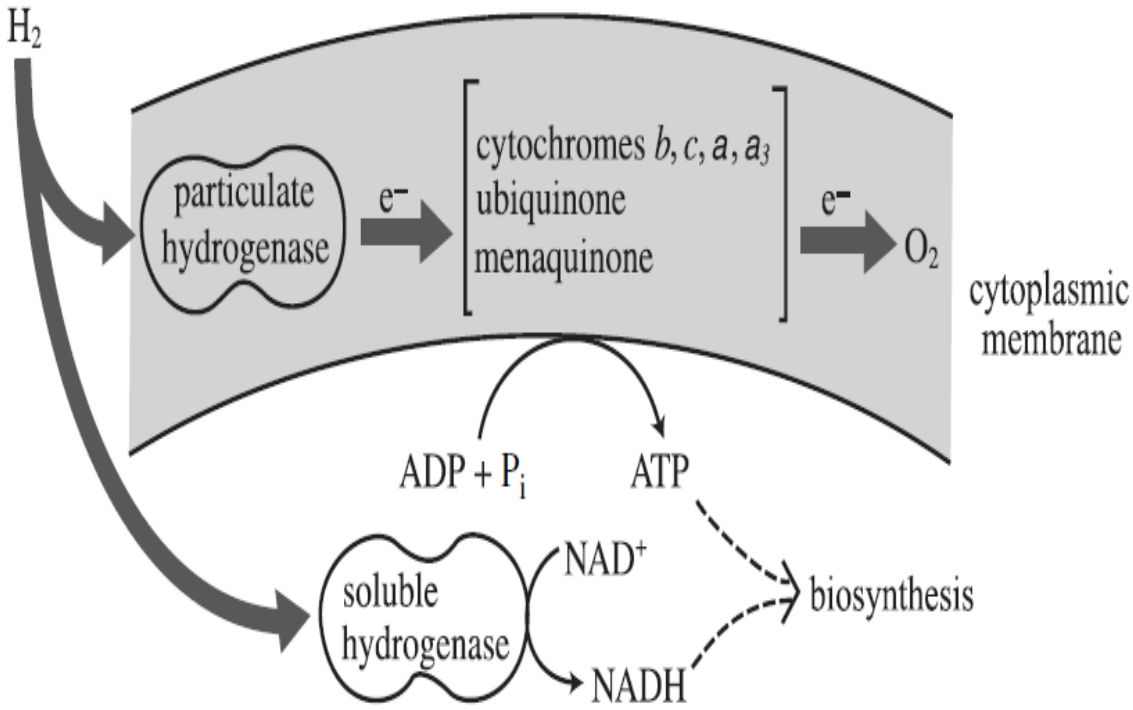


Fig 8.5: Hydrogen utilization by Ralstonia eutropha. (Alcaligenes eutrophus) (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 9.1. Springer, New York)

This bacterium has a NADP dependent soluble hydrogenase in addition to the particulate enzyme. The soluble enzyme reduces pyridine nucleotide for biosynthesis, and the particulate hydrogenase channels electrons from hydrogen directly to the electron transport chain to generate a proton motive force.

Hydrogenomonas thermophila are microaerophilic H2-oxidizers using O2, nitrate or elemental sulfur as electron acceptors. Another strict anaerobe, *Balnearium lithotrophicum*, oxidizes H2 reducing nitrate to ammonia, but cannot reduce sulfur. A *Dechloromonas* sp. isolated from a sewage works grows chemolithotrophically on H2 and perchlorate. Ferroglobus placidus, a strictly anaerobic archaeon, grows chemoautotrophically oxidizing hydrogen coupled to nitrate reduction.

8.5.4. CO2 Fixation in H2-Oxidizers:

The reductive TCA cycle is used to fix CO2 in obligately chemolithotrophic Hydrogenobacter thermophilus, while all the other hydrogen bacteria tested to date fix CO2 through the Calvin cycle.

Hydrogenomonas thermophila are microaerophilic H2-oxidizers using O2, nitrate or elemental sulfur as electron acceptors. Another strict anaerobe, Balnearium lithotrophicum, oxidizes H2 reducing nitrate to ammonia, but cannot reduce sulfur. A Dechloromonas sp. isolated from a sewage works grows chemolithotrophically on H2 and perchlorate. Ferroglobus placidus, a strictly anaerobic archaeon, grows chemoautotrophically oxidizing hydrogen coupled to nitrate reduction.

8.5.4 CO2 Fixation in H2-Oxidizers:

The reductive TCA cycle is used to fix CO2 in obligately chemolithotrophic Hydrogenobacter thermophilus, while all the other hydrogen bacteria tested to date fix CO2 through the Calvin cycle.

Table	*Typical carboxydobacteria*
Acinetobacter sp. Strain IC-I	
Alcaligenes (Carbophilus) carboxidus	
Comamonas (Zavarzinia) compransoris	
Pseudomonas (Oligotropha) carboxidovorans	
Pseudomonas carboxidoflava (Hydrogenovibrio pseudoflava)	
Pseudomonas carboxidohydrogena	
Pseudomonas gazotropha	

dehydrogenases of methanogens and homoacetogens. The latter are dual function enzymes catalyzing CO oxidation and acetyl-CoA synthesis in both directions (Sections 9.4.3 and 9.5.2). They are soluble enzymes and use low redox potential electron carriers such as F420 and ferredoxin in methanogens and homoacetogens, respectively. The membrane-bound aerobic enzyme in the carboxydobacteria catalyzes CO oxidation only, reducing coenzyme Q:

$$CO + H_2O \xrightarrow{\text{CO dehydrogenase}} CO_2 + 2H^+ + 2e^-$$

As stated above, carboxydobacteria can use CO efficiently at low concentrations employing CO dehydrogenase to reduce coenzyme Q with a redox potential of around zero V,much higher than "0.54 V, the redox potential of CO2/CO. Carboxydobacteria reduce NAD(P)þ through a reverse electrontransport chainnot directly coupled to CO oxidation. The Calvin cycle is the CO2-fixing mechanism in carboxydobacteria. Many methanogens and homoacetogens can use CO as their electron donor.

8.6. SUMMARY:

Anoxygenic Photosynthesis Organisms: Performed by various anaerobic bacteria, including purple sulfur bacteria, green sulfur bacteria, and heliobacteria. Electron Donor: Uses electron donors other than water, such as hydrogen sulfide (H2S), elemental sulfur, or organic molecules. Byproduct: Does not produce oxygen. The byproduct depends on the electron donor (e.g., elemental sulfur is produced when H2Sis used). Photosystems: Involves only a single photosystem, which can be a type I reaction center (like in green sulfur bacteria) or a type II reaction center (like in purple bacteria), but not both. Mechanism: Electron flow is primarily cyclic, generating a proton gradient for ATP synthesis. Reducing power (NADH/NADPH) for carbon fixation is often produced via "reverse electron flow", which requires additional energy input. Pigments: Uses bacteriochlorophylls, which absorb light at longer (infrared) wavelengths than chlorophylls, allowing them to thrive in different environmental niches.

8.7. SELF ASSESSMENT:

1) Nitrification
2) Ammonia Oxidation
3) Nitrite Oxidation
4) Anaerobic Nitrification
5) Sulfur Bacteria and the Oxidation of Sulfur Compounds
6) Sulfur Bacteria
7) Biochemistry of Sulfur Compound Oxidation
8) Carbon Metabolism in Colourless Sulfur Bacteria
9) Iron Bacteria: Ferrous Iron Oxidation
10) Hydrogen Oxidation

8.8. REFERENCES:

1) Reddy and Reddy (2005). Microbial Physiology.
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th Edition.
3) Nelson and Cox.2000; Lehninger Principles of Biochemistry.
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology.
5) Caldwell, D.R.1995. Microbial Physiology and Metabolism.
6) David White.1995. The Physiology and Biochemistry of Prokaryotes.
7) Gottschalk, G. Bacterial Metabolism.

Prof K. Mallikarjuna

LESSON-9

RESPIRATION -1

9.0 OBJECTIVE:

- ……..
  …

STRUCTURE:

9.1. INTRODUCTION:

Escherichia coli can grow on a simple medium containing glucose and mineral salts and this bacterium can synthesize all cell constituents using materials provided in this medium. Glucose is metabolized through the Embden – eyerhof–Parnas (EMP) pathway and hexose monophosphate (HMP) pathway and the metabolic product, pyruvate, is decarboxylated oxidatively to acetyl-CoA to be oxidized through the tricarboxylic acid (TCA) cycle. Twelve intermediates of these pathways are used as carbon skeletons for biosynthesis (Table 9.1). Heterotrophs that utilize organic compounds other than carbohydrates convert their substrates into one or more of these intermediates. For this reason, glucose metabolism through glycolysis and the TCA cycle is called central metabolism.

Eukaryotes metabolize glucose through the EMP pathway to generate ATP, pyruvate and NADH, and the HMP pathway is needed to supply the metabolic intermediates not available from the EMP pathway such as pentose-5-phosphate and erythrose-4-phosphate, and NADPH. Most prokaryotes employ similar mechanisms, but some prokaryotes metabolize glucose through unique pathways known only in prokaryotes, e.g. the Entner–Doudoroff (ED) pathway and phosphoketolase (PK) pathway.

Some prokaryotes have genes for the ED pathway in addition to the EMP pathway:

Genes for these pathways are expressed at the same time in several prokaryotes including a thermophilic bacterium (Thermotoga maritima), a thermophilic archaeon (Thermoproteus tenax) and a halophilic archaeon (Halococcus saccharolyticus). Escherichia

coli metabolizes glucose via the EMP pathway, but gluconate is oxidized through the ED pathway. Modified EMP and ED pathways are quite common in archaea. Carbohydrates are phosphorylated before they are metabolized in most cases. It is believed that phosphorylated intermediates are less likely to diffuse away through the cytoplasmic membrane. Some bacteria and archaea also phosphorylate intermediates of glucose metabolism in modified glycolytic pathways.

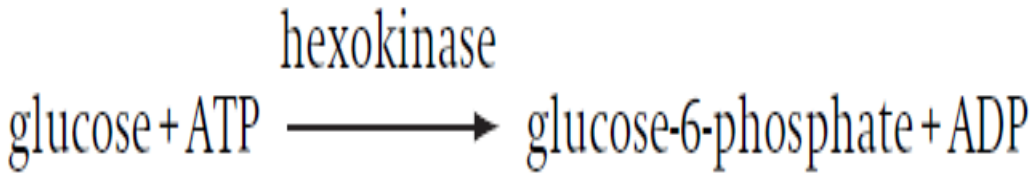
Table 9.1. Metabolic Intermediates used as Carbon Skeletons for Biosynthesis

Table | *Metabolic intermediates used as carbon skeletons for biosynthesis*

Carbon skeleton	From	Precursor for
Glucose-6-phosphate	EMP	polysaccharides
Fructose-6-phosphate	EMP	murein
Ribose-5-phosphate	HMP	nucleic acids
Erythrose-4-phosphate	HMP	amino acids
Triose-phosphate	EMP	lipids
3-phosphoglycerate	EMP	amino acids
Phosphoenolpyruvate	EMP	amino acids
Pyruvate	EMP	amino acids
Acetyl-CoA	Pyruvate	fatty acids
2-ketoglutarate	TCA	amino acids
Succinyl-CoA	TCA	amino acids
Oxaloacetate	TCA	amino acids

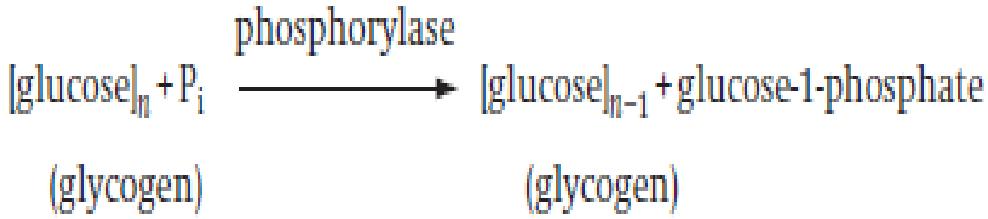
9.1 EMP PATHWAY:

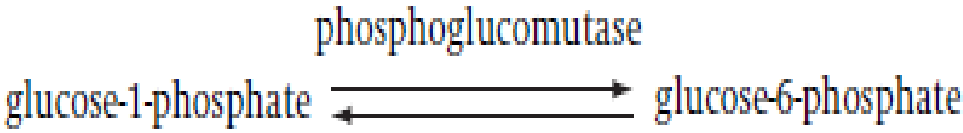
Many anaerobic and enteric bacteria transport glucose via group translocation (phosphotransferase system, PTS, Section 3.5) in the form of glucose-6-phosphate. Glucose transported through active transport is phosphorylated by hexokinase:



Hexokinase can phosphorylate other hexoses such as mannose, and requires Mg2þ for activity. The enzyme cannot catalyze the reverse reaction.

Glucose-6-phosphate can also be obtained from glycogen: phosphorylase





Glucose-6-phosphate is a precursor for the biosynthesis of polysaccharides as well as a substrate of the EMP pathway (Figure 9.1), which is the commonest glycolytic pathway in all kinds of organisms.

9.1.1 Phosphofructokinase (PFK): key enzyme of the EMP pathway

Glucose-6-phosphate is isomerized to fructose-6-phosphate before being phosphorylated to fructose-1,6-diphosphate by the action of phosphofructokinase (PFK). These two reactions require Mg2+.

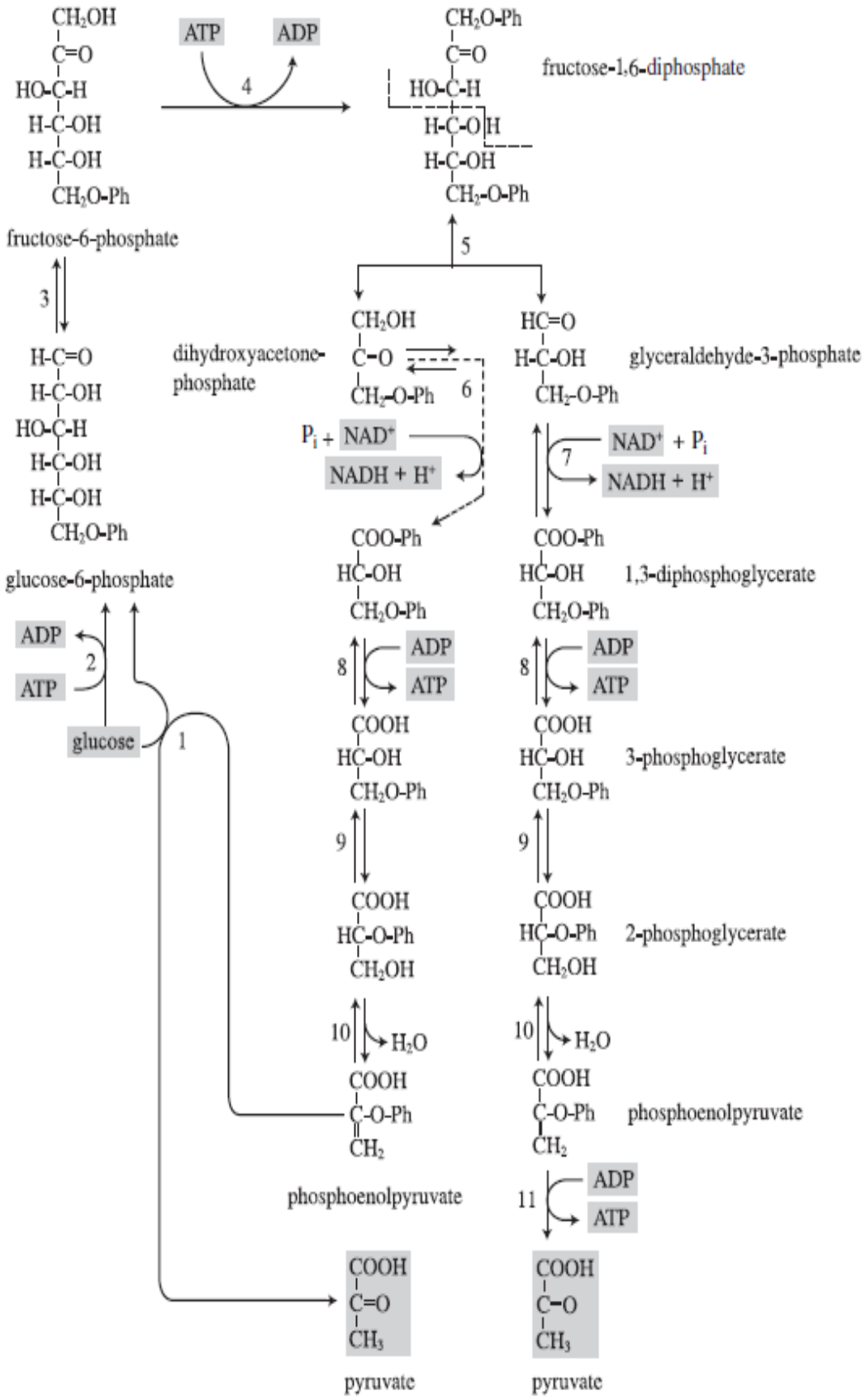


Fig 9.1 Glucose Oxidation via the Embden–Meyerhof–Parnas Pathway

Glucose is phosphorylated to glucose-6-phosphate by PEP: glucose phosphotransferase (1) during group translocation (phosphotransferase system, PTS) or by hexokinase (2) after uptake via active transport.

3, glucose-6-phosphate isomerase; 4, phosphofructokinase; 5, fructose diphosphate aldolase; 6, triose-phosphate isomerase; 7, glyceraldehyde-3-phosphate dehydrogenase; 8, 3-phosphoglycerate kinase; 9, phosphoglyceratemutase; 10, enolase; 11, pyruvate kinase

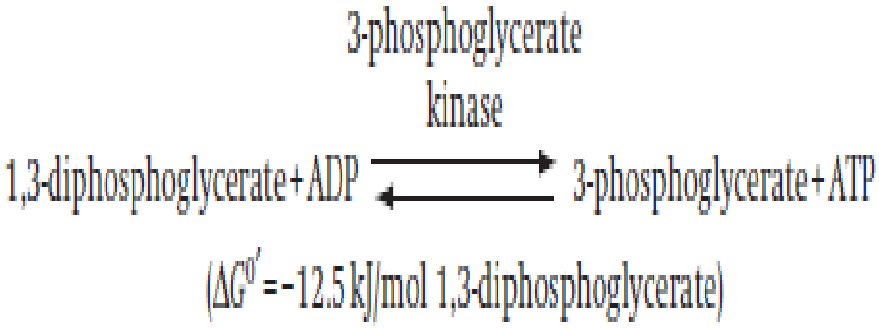
Glucose-6-phosphate isomerase catalyzes the reverse reaction, but phosphofructokinase does not. The irreversibility of an enzyme is due to thermodynamic reasons, and many enzymes that do not catalyze the reversible reaction are regulated. PFK is the key enzyme of the EMP pathway. If this enzyme is present in a given prokaryote, it can be assumed that this organism catabolizes glucose through the EMP pathway. Fructose-6-phosphate is the precursor of amino sugars and their polymers such as murein.

Fructose-1,6-diphosphate aldolase cleaves fructose-1,6-diphosphate to two molecules of triose-phosphate. This aldolase catalyzes the reverse reaction, and participates in gluconeogenesis, producing hexose-phosphate when non-carbohydrate substrates are used as carbon sources.

9.1.2 ATP Synthesis and Production of Pyruvate:

Triose-phosphate isomerase equilibrates dihydroxyacetone phosphate and glyceraldehyde-3-phosphate produced from fructose-1,6-diphosphate. Under standard conditions the equilibrium shifts to the formation of dihydroxyacetone phosphate (!G00¼#7.7 kJ/mol glyceraldehyde-3-phosphate), but the reverse reaction is favoured because glyceraldehyde-3-phosphate is continuously consumed in subsequent reactions. Phospholipids are synthesized from glyceraldehyde-3- phosphate (Section 6.6.2).

Glyceraldehyde-3-phosphate is oxidized to 1,3-diphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase. This endergonic reaction (!G00¼þ6.3kJ/mol glyceraldehyde-3-phosphate) is efficiently pulled by the following exergonic reaction catalyzed by 3-phosphoglycerate kinase (!G00¼#12.5kJ/mol 1,3-phosphoglycerate). This enzyme requires Mg2þ, as domost kinases, and ATP generation in this reaction is an example of substrate-level-phosphorylation. 3-phosphoglycerate is the starting material for the synthesis of amino acids, serine, glycine and cysteine



3-phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase which requires 2,3-diphosphoglycerate as a coenzyme. 2-phosphoglycerate is dehydrated to phosphoenolpyruvate (PEP) by an enolase in the presence of divalent cations such as Mg2þ and Mn2þ. PEP is used to generate ATP with the reaction of the last enzyme in the EMP pathway in the presence of Mg2þ and Kþ. PEP supplies energy in group translocation, and is used to synthesize aromatic amino acids (Section 6.9.4). Glyceraldehyde-3-phosphate is an intermediate in the HMP and ED pathways and the reactions from this triose-phosphate are shared with both these pathways.

Four ATPs are synthesized and two high energy phosphate bonds are consumed in this pathway, resulting in a net gain of two ATPs per glucose oxidized. The NADH reduced in the glycolytic pathway is reoxidized in aerobic (Section 5.8) and anaerobic respiration (Chapter 9), and in fermentation (Chapter 8), reducing various electron acceptors depending on the organism and on their availability.

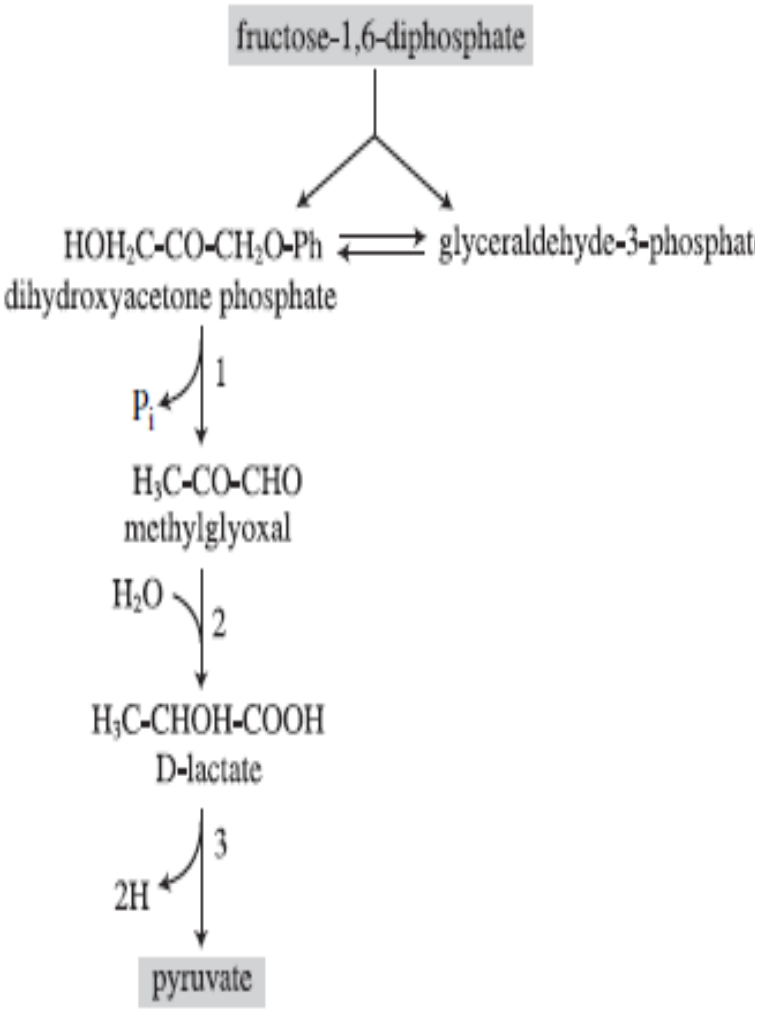


Fig 9.2: The Methylglyoxal By pass, a modified EMP pathway under phosphate limited conditions. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 5.15. Springer, New York)

Under phosphate-limited conditions, bacteria such as Escherichia coli metabolize dihydroxyacetone phosphate to pyruvate to supply precursors for biosynthesis with a reduced ATP yield.1, methylglyoxal synthase; 2, glyoxalase; 3, lactate oxidase

9.2. PEP SYNTHESIS PATHWAY:

Microbes growing on non-carbohydrate compounds produce PEP through pyruvate, oxaloacetate or malate. Phosphoenolpyruvate (PEP) synthetase is widespread in microbes:

$$\text{pyruvate} + \text{ATP} \xrightarrow{\text{PEP synthetase}} \text{PEP} + \text{AMP} + \text{P}_i$$
$$(\Delta G^{0'} = -8.4\ \text{kJ/mol pyruvate})$$

Pyruvate-phosphate dikinase is another enzyme that synthesizes PEP from pyruvate. This enzyme is found in Acetobacter xylinum growing on ethanol and in Propionibacterium shermanii growing on lactate

$$\text{pyruvate} + \text{ATP} + \text{P}_i \underset{\text{pyruvate phosphate dikinase}}{\rightleftharpoons} \text{PEP} + \text{AMP} + \text{PP}_i$$

Pyruvate-phosphate dikinase is another enzyme that synthesizes PEP from pyruvate. This enzyme is found in Acetobacter xylinum growing on ethanol and in Propionibacterium shermanii growing on lactate:

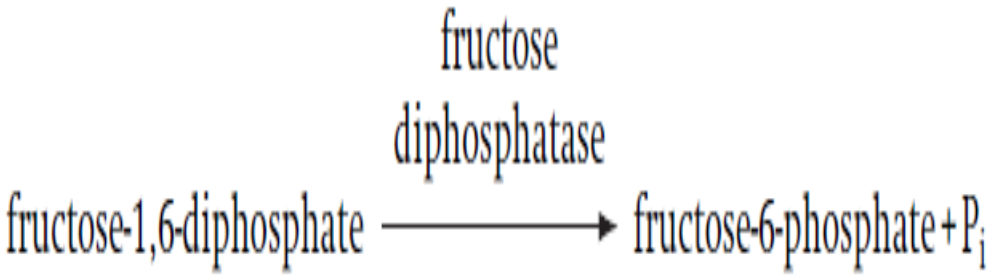
$$\text{oxaloacetate} + \text{ATP} \underset{\text{PEP carboxykinase}}{\rightleftharpoons} \text{PEP} + \text{CO}_2 + \text{ADP}$$

Malate can be converted either through oxaloacetate or directly by the action of malate enzyme:

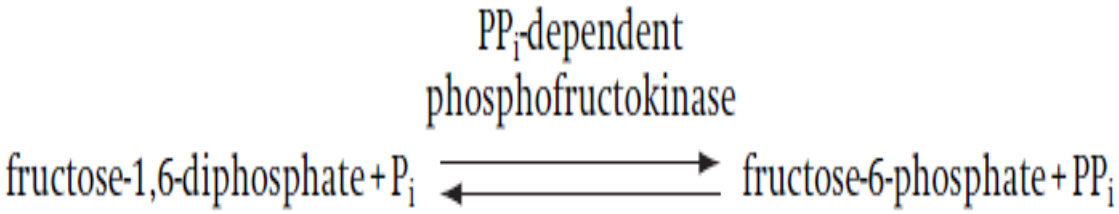
$$\text{malate} + \text{NAD}^+ + \text{H}^+ \underset{\text{malate enzyme}}{\rightleftharpoons} \text{pyruvate} + \text{NADH} + \text{CO}_2$$

9.2.2. Fructose Diphosphatase:

PEP can be converted to fructose-1,6-diphosphate by the EMP pathway enzymes since they catalyze the reverse reactions. However, phosphofructokinase does not catalyze the reverse reaction and fructose-1,6-diphosphate is dephosphorylated to fructose-6-phosphate and Pi by fructose diphosphatase:



Pyrophosphate (PPi)-dependent phosphofructokinase is found in a hyperthermophilic archaeon Thermoproteus tenax and this enzyme catalyzes the reverse reaction. Similar reactions are known in plants and bacteria. This enzyme may be a component of gluconeogenesis:



9.3. HEXOSE MONOPHOSPHATE (HMP) PATHWAY:

When Escherichia coli grows on glucose as the sole carbon and energy source, about 72% of the substrate is metabolized through the EMP pathway, and the HMP pathway consumes the remaining 28%. This is because the EMP cannot meet all the requirements for biosynthesis. The HMP pathway provides the biosynthetic metabolism with pentose-5-phosphate, erythrose-4-phosphate and NADPH. This pathway is also called the pentose phosphate pathway. NADPH is used to supply reducing power in biosynthetic processes. NADPþ is reduced only by isocitrate dehydrogenase (Section 5.2) when glucose is metabolized through the EMP pathway and TCA cycle. NADH is seldom used in biosynthetic reactions. Most of the NADPH needed for biosynthesis arises from the HMP pathway.

9.3.1. HMP Pathway in Three Steps:

For convenience, the HMP pathway can be discussed in three steps (Figure 9.4). During the initial step of the HMP pathway,

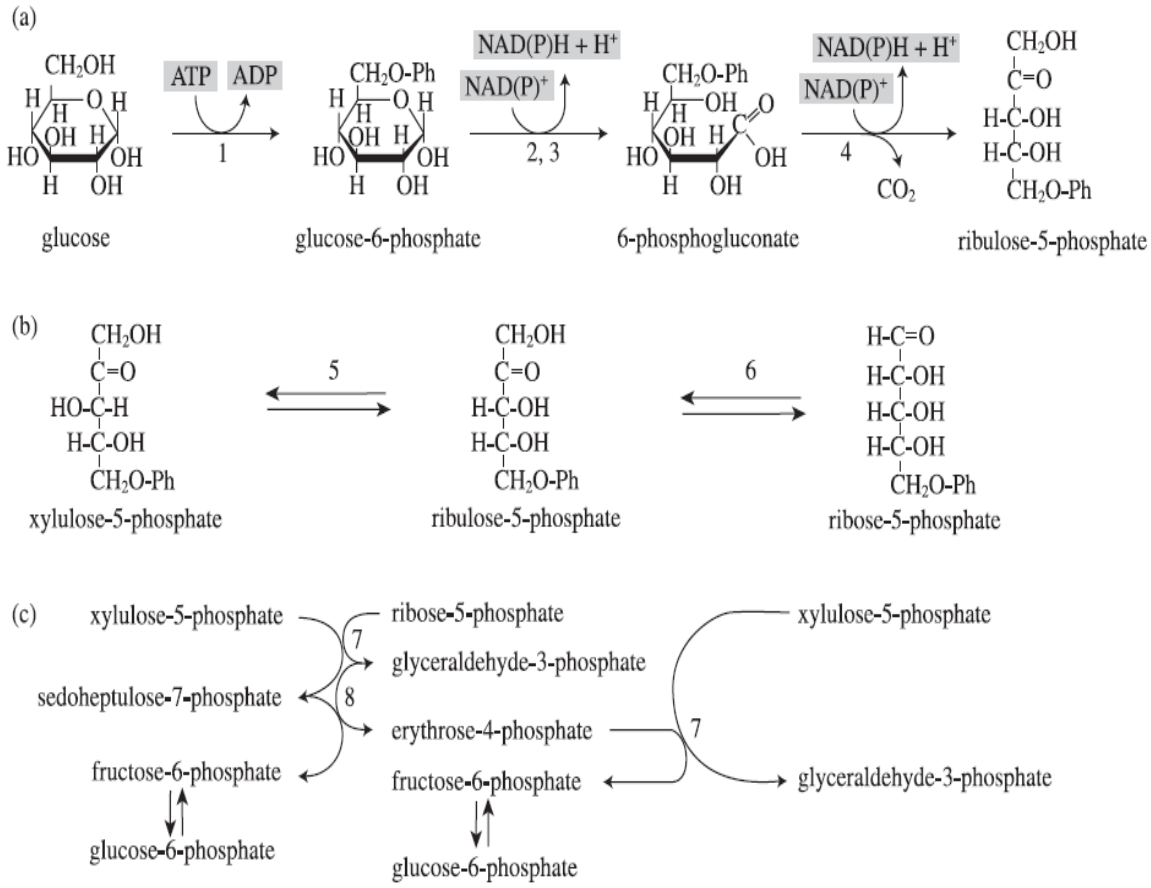
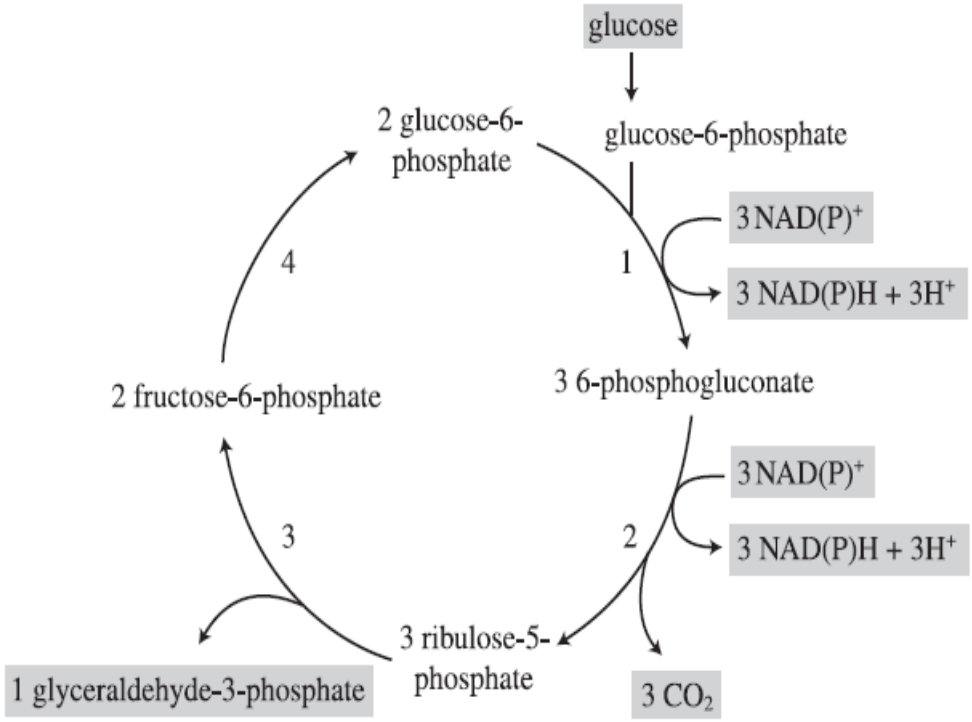


Fig 9.3: Hexose monophosphate (pentose phosphate) pathway.Glucose is oxidized to ribulose-5-phosphate coupled to the reduction of NADPH (a). Isomerase and epimerase convert ribulose-5-phosphate to ribose-5-phosphate and xylulose- 5-phosphate (b). Transaldolase and transketolase rearrange pentose-5-phosphates into glucose- 6-phosphate and glyceraldehyde-3-phosphate involving erythrose-4-phosphate (c). Nucleotide synthesis starts with ribose- 5-phosphate, and aromatic amino acids are produced from erythrose- 4-phosphate and phosphoenolpyruvate. NADPH supplies reducing power during the biosynthetic processes. 1, hexokinase; 2, glucose-6-phosphate dehydrogenase;3, lactonase; 4, 6-phosphogluconate dehydrogenase; 5, ribulose-5-phosphate-3-epimerase; 6, ribose-5-phosphate isomerase;7, transketolase; 8, transaldolase glucose-6-phosphate is oxidized to ribulose-5-phosphate and CO2,reducing NADPþ. Glucose-6-phosphate dehydrogenase, lactonase and 6-phosphogluconate dehydrogenase catalyze these reactions. In the following reactions, ribulose-5-phosphate is converted to ribose- 5-phosphate and xylulose-5-phosphate by the action of isomerase and epimerase. Finally, the pentose-5-phosphates are transformed to glucose-6-phosphate and glyceraldehyde-3-phosphate through carbon rearrangement by transaldolase and transketolase. A transaldolase transfers a 3-carbon fragment, and a 2-carbon fragment transfer is catalyzed by a transketolase. HMP can be summarized as: Glucose-6-phosphate + 2NADP+ glyceraldehyde-3-phosphate + 3CO2 + 2NADPH + 2H+

Ribose-5-phosphate is the precursor for nucleotide synthesis, and aromatic amino acids are produced from erythrose-4-phosphate. NADPH supplies reducing power during biosynthesis. Some eukaryotic microorganisms metabolize more glucose through the HMP pathway when they use nitrate as their nitrogen source. They use NADPH in assimilatory nitrate reduction).

9.3.2 Additional functions of the HMP pathway in addition to supplying precursors and reducing power for biosynthesis from glucose, the HMP and related pathways have some other functions. HMP is the major glycolytic metabolism in microbes that (1) utilize pentoses, and (2) do not possess other glycolytic activities. The HMP cycle is also employed for the complete oxidation of sugars in bacteria lacking a functional TCA cycle.



9.3.2.1 Utilization of Pentoses:

Pentoses are phosphorylated and metabolized to fructose- 6-phosphate and glyceraldehyde-3-phosphate through steps 2 and 3 of the HMP pathway.

9.3.2.2 Oxidative HMP Cycle:

Thiobacillus novellus and Brucella abortus oxidize glucose completely although they lack a functional EMP or ED pathway. Glucose is oxidized through the oxidative HMP cycle (Figure 9.5). Glyceraldehyde- 3-phosphate is oxidized to pyruvate as in the EMP pathway. The HMP cycle is found in species of Gluconobacter which do not have a functional TCA cycle. These bacteria possess the incomplete TCA fork to meet the supply of biosynthetic precursors

9.4 Entner–Doudoroff (ED) Pathway:

9.9.1 Glycolytic pathways in some Gram-negative bacteria in addition to the EMP pathway, unique glycolytic pathways are also known in prokaryotes. The ED pathway was first identified in Pseudomonas saccharophila by Entner and Doudoroff, and this turned out to be the main glycolytic pathway in prokaryotes that do not possess enzymes of the EMP

pathway. In addition to species of Pseudomonas, the ED pathway functions as the main glycolytic pathway in other Gram-negative bacteria such as Zymomonas and Azotobacter species, and gluconate is metabolized through this pathway in some other Gram-negative bacteria including Escherichia coli, and insome coryneform bacteria such as species of Arthrobacter and Cellulomonas (Table 9.2).

9.4.2 Key Enzymes of the ED Pathway:

In the first two reactions of the ED pathway, glucose-6-phosphate is converted to 6-phosphogluconate via phosphogluconolactone, as in the HMP pathway. 6-phosphogluconate is dehydrated to 2-keto-3- deoxy-6-phosphogluconate (KDPG) by 6-phosphogluconate dehydratase.

KDPG aldolase splits its substrate into pyruvate and glyceraldehyde- 3-phosphate (Figure 94). The latter is oxidized to pyruvate as in the EMP pathway. The key enzymes of this pathway are 6-phosphogluconate dehydratase and KDPG aldolase.

Two ATP are generated and one high energy phosphate bond is consumed with a net gain of one ATP per glucose oxidized in this pathway.

9.4.3. Modified ED Pathways:

Unusually, some prokaryotes oxidize glucose and the intermediates are phosphorylated before being metabolized in a similar manner as in the ED pathway.

9.4.3.1. Extracellular oxidation of glucose by Gram-Negative Bacteria

Sugars are metabolized after they are phosphorylated, the latter process probably preventing their loss through membrane diffusion. Negatively charged phosphorylated sugars and their intermediates

Table *Major glycolytic pathways in prokaryotes*

Organism	EMP	ED
Arthrobacter species	+	+/−[a]
Azotobacter chroococcum	+	−
Ralstonia eutropha (*Alcaligenes eutrophus*)	−	+
Bacillus subtilis	+	−
Cellulomonas flavigena	+	+/−[a]
Escherichia coli and enteric bacteria	+	+/−[a]
Pseudomonas saccharophila	−	+
Rhizobium japonicum	−	+
Thiobacillus ferrooxidans	−	+
Xanthomonas phaseoli	−	+
Thermotoga maritima	+	+[b]
Thermoproteus tenax	+[c]	+[b,d]
Halococcus saccharolyticus	+[c,e]	+[b,d,f]
Halobacterium saccharovorum	−	+[d]
Clostridium aceticum	−	+[d]
Sulfolobus acidocaldarius	−	+[d]

+, present; −, absent.
[a] When gluconate is used as energy and carbon source.
[b] Enzymes for EMP and ED pathways are expressed simultaneously.
[c] Modified EMP pathway.
[d] Modified ED pathway.
[e] Fructose.
[f] Glucose.

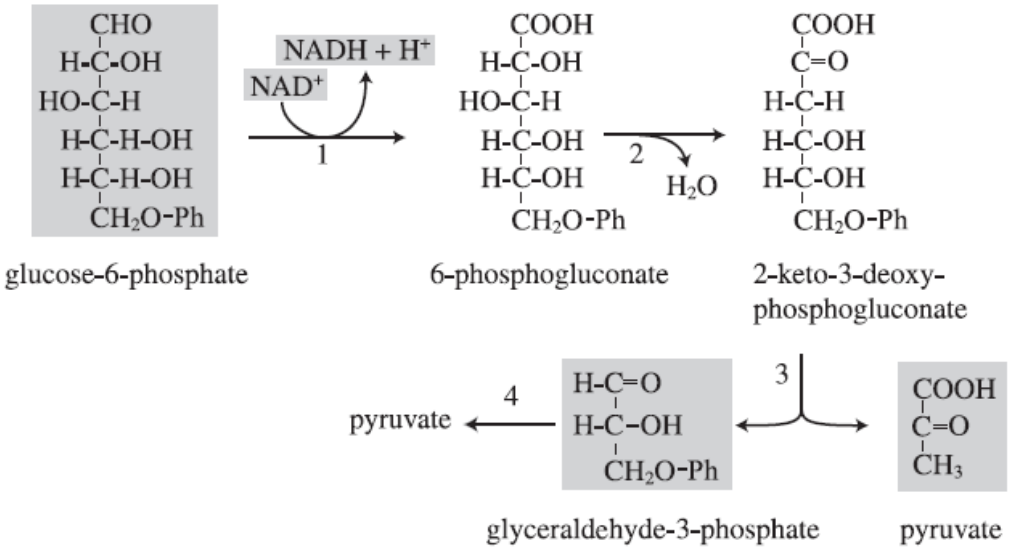


Fig 9.6: Entner–Doudoroff (ED) Pathway

This metabolism is known only in prokaryotes, mainly Gram-negative bacteria, that do not possess the EMP pathway. 1, glucose-6-phosphatedehydrogenase; 2,6-phosphogluconate dehydratase; 3, 2-keto-3-deoxy-6-phosphogluconate aldolase; 4, as in the EMP pathway are less permeable to the membrane. However, some strains of Pseudomonas oxidize glucose extracellularly when the glucose concentration is high. These bacteria possess glucose dehydrogenase and gluconate dehydrogenase on the periplasmic face of the cytoplasmic membrane. When glucose is depleted, gluconate and 2-ketogluconate are transported through specific transporters and hen the glucose concentration is high, some strains of Pseudomonas oxidize glucose at the periplasmic region. Glucose dehydrogenase and gluconate dehydrogenase are phosphorylated, consuming ATP (Figure 9.7). 2-keto-6-phosphogluconate is reduced to 6-phosphogluconate by a NADPH-dependent reductase. Glucose dehydrogenase and gluconate dehydrogenase in these bacteria are quinoproteins containing pyrroloquinoline quinone (PQQ, methoxatin) as a prosthetic group).

The reduced form of PQQ transfers electrons to cytochrome c of the electron transport chain. Gluconate and 2-ketogluconate are uncommon in nature, and few microbes use these compounds. The ability to oxidize glucose and to use its products might therefore be advantageous for those organisms capable of doing this. A group translocation (phosphotransferase system) negative mutant of Escherichia coli synthesizes PQQ-containing glucose dehydrogenase and metabolizes glucose in the same way as the Pseudomonas species described above.

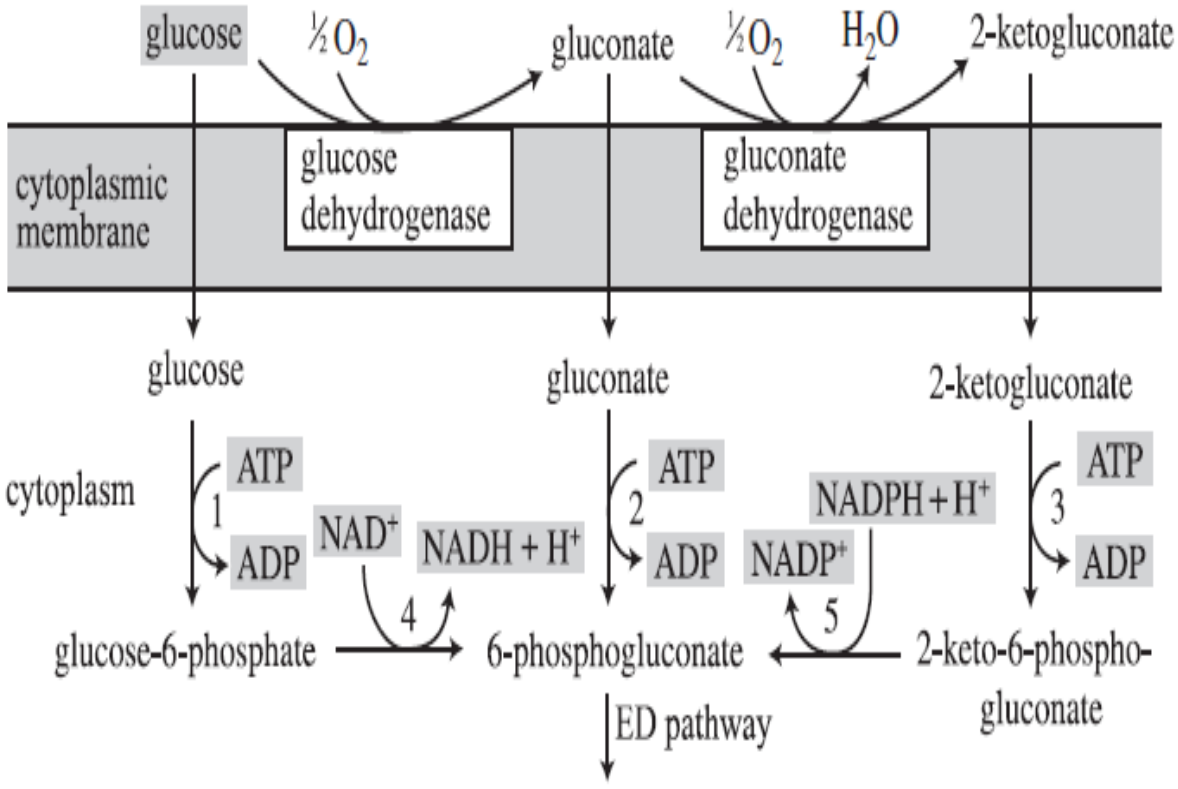


Fig 9.7: Cytoplasmic Membrane Quinoproteins Containing Pyrroloquinoline Quinone (PQQ).

The reduced PQQ transfers electrons to cytochrome c, and gluconate and 2-ketogluconate are transported through specific transporters to be metabolized in a similar way as in the ED pathway when glucose is depleted.1, hexokinase; 2, gluconate kinase; 3, 2-ketogluconate kinase; 4, glucose-6-phosphate dehydrogenase; 5, 2-keto-6- phosphogluconate reductase.

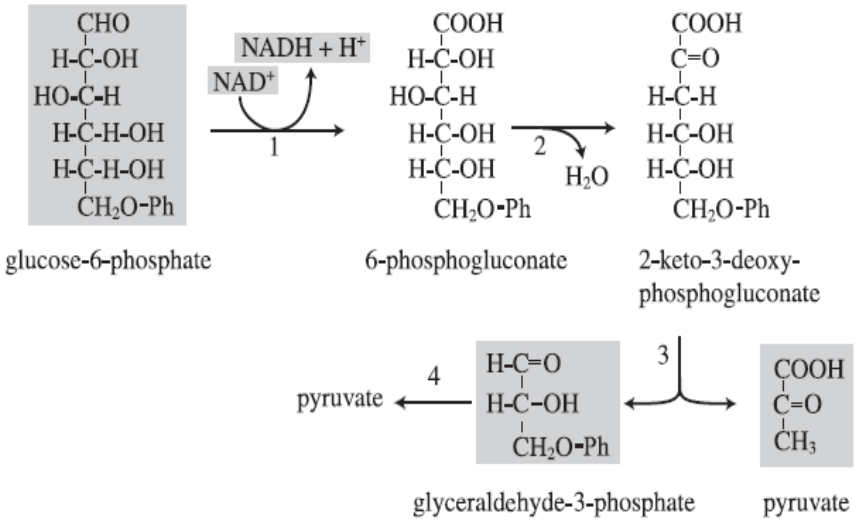
Oxidative HMP Cycle:

Organisms lacking functional EMP or ED pathways, or a functional TCA cycle, oxidize glucose through the oxidative HMP cycle. Glyceraldehyde-3-phosphate is oxidized to pyruvate as in the EMP pathway.

1, glucose-6-phosphate dehydrogenase; 2, 6- phosphogluconate dehydrogenase; 3, carbon rearrangement as in the HMP pathway; 4, glucose -6- phosphate isomerase.

Table . *Metabolic intermediates used as carbon skeletons for biosynthesis*

Carbon skeleton	From	Precursor for
Glucose-6-phosphate	EMP	polysaccharides
Fructose-6-phosphate	EMP	murein
Ribose-5-phosphate	HMP	nucleic acids
Erythrose-4-phosphate	HMP	amino acids
Triose-phosphate	EMP	lipids
3-phosphoglycerate	EMP	amino acids
Phosphoenolpyruvate	EMP	amino acids
Pyruvate	EMP	amino acids
Acetyl-CoA	Pyruvate	fatty acids
2-ketoglutarate	TCA	amino acids
Succinyl-CoA	TCA	amino acids
Oxaloacetate	TCA	amino acids



The Entner–Doudoroff (ED) pathway. This metabolism is known only in prokaryotes, mainly Gram-negative bacteria, that do not possess the EMP pathway.1, glucose-6-phosphate dehydrogenase; 2, 6-phosphogluconate dehydratase;3, 2-keto-3-deoxy-6- phosphogluconate aldolase; 4, as in the EMP pathway are less permeable to the membrane. However, some strains of Pseudomonas oxidize glucose extracellularly when the glucose concentration is high. These bacteria possess glucose dehydrogenase and gluconate dehydrogenase on the periplasmic face of the cytoplasmic membrane. When glucose is depleted, gluconate and 2-ketogluconate are transported through specific transporters and

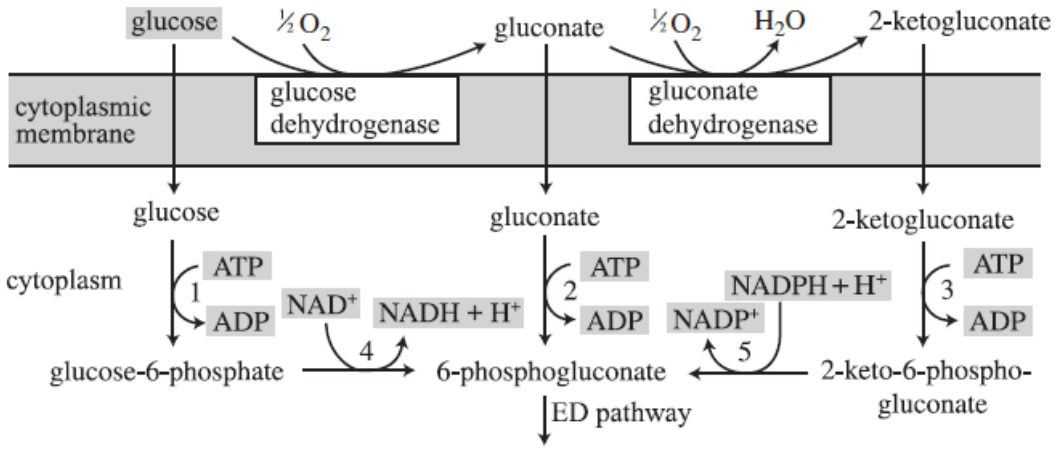


Fig 9.7: Glucose utilization O2 O2 H2O by some species of Pseudomonas in a high glucose environment. (Dawes, E. A. 1986, Microbial Energetics, Figure 3.5. Blackie & Son, Glasgow)

When the glucose concentration is high, some strains of Pseudomonas oxidize glucose at the periplasmic region. Glucose dehydrogenase and gluconate dehydrogenase are cytoplasmic membrane quinoproteins containing pyrroloquinoline quinone (PQQ). The reduced PQQ transfers electrons to cytochrome c, and gluconate and 2-ketogluconate are transported through specific transporters to be metabolized in a similar way as in the ED pathway when glucose is depleted.1, hexokinase; 2, gluconate kinase; 3, 2-ketogluconate kinase; 4, glucose-6-phosphate dehydrogenase; 5, 2-keto-6-phosphogluconate reductase phosphorylated, consuming ATP (Figure 9.7). 2-keto-6-phosphogluconate is reduced to 6-phosphogluconate by a NADPH-dependent reductase.Glucose dehydrogenase and gluconate dehydrogenase in these bacteria are quinoproteins containing pyrroloquinoline quinone (PQQ, methoxatin) as a prosthetic group.

The reduced form of PQQ transfers electrons to cytochrome c of the electron transport chain. Gluconate and 2-ketogluconate are uncommon in nature, and few microbes use these compounds. The ability to oxidize glucose and to use its products might therefore be advantageous for those organisms capable of doing this.

A group translocation (phosphotransferase system) negative mutant of Escherichia coli synthesizes PQQ-containing glucose dehydrogenase and metabolizes glucose in the same way as the Pseudomonas species described above.

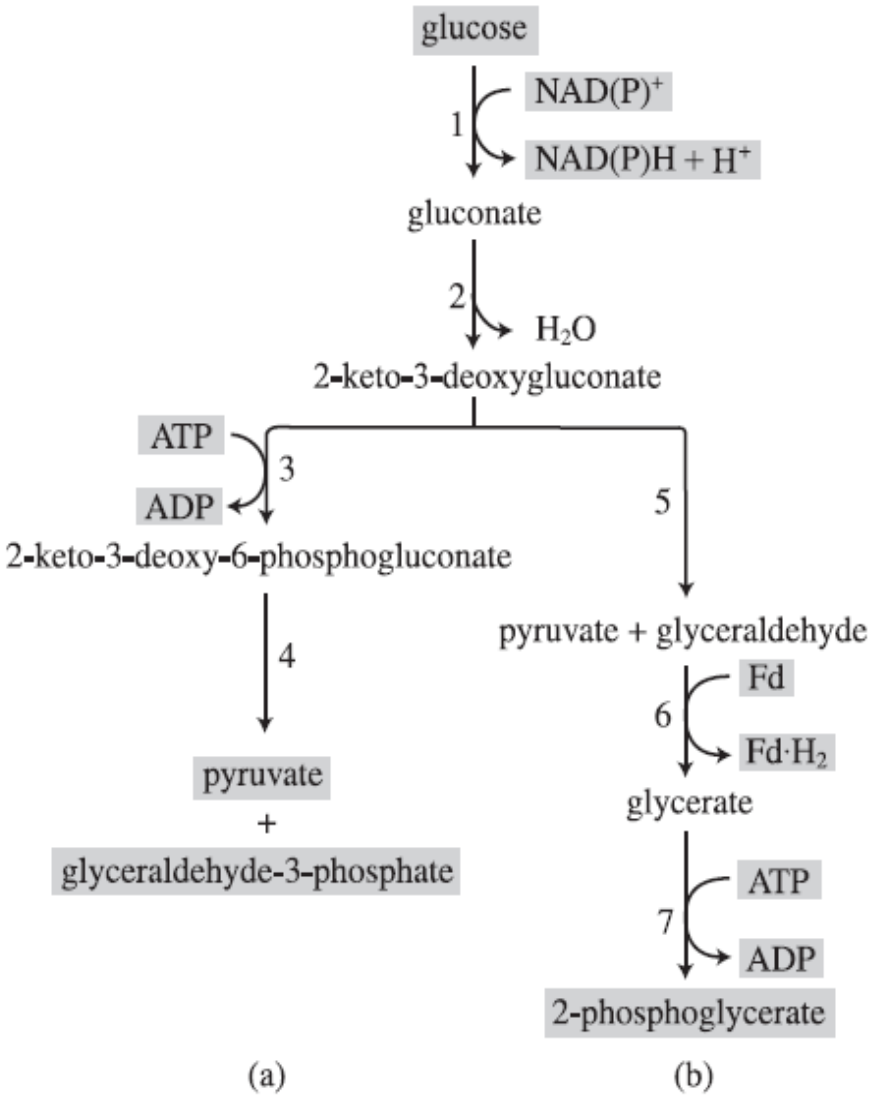
9.4.3.2. Modified ED Pathways in Archaea:

Hyperthermophilic archaea belonging to the genera Sulfolobus, Thermoplasma, and Thermoproteus metabolize glucose to pyruvate and glyceraldehyde without phosphorylation in a similar way as the ED pathway, and a halophilic archaeon, Halobacterium saccharovorum, oxidizes glucose to 2-keto-3-deoxygluconate, which is phosphorylated before metabolism through the ED pathway (Figure 9.8).

The archaeal glucose dehydrogenase is a NAD(P)þ-dependent enzyme. Some eubacteria, including Clostridium aceticum and Rhodopseudomonas sphaeroides, metabolize glucose in a similar mechanism to this halophilic archaeon.

9.5. PHOSPHOKETOLASE PATHWAYS:

Lactate is the sole glucose fermentation product in homofermentative lactic acid bacteria (LAB), while the heterofermentative LAB produce acetate and ethanol in addition to lactate from glucose. The former ferment glucose through the EMP pathway and the phosphoketolase (PK) pathway is employed in the latter and



in bifidus bacteria. A heterofermentative bacterium, Leuconostoc mesenteroides, produces lactate and ethanol from glucose through the PK pathway, involving one PK active on xylulose-5-phosphate (Figure 9.9). Lactate and acetate are produced from glucose by Bifidobacterium bifidum with two PKs active on fructose-6-phosphate and xylulose-5-phosphate (Figure 9.10). Facultatively homofermentative LAB ferment pentoses and low concentrations of glucose through the PK pathway to produce lactate, acetate and ethanol

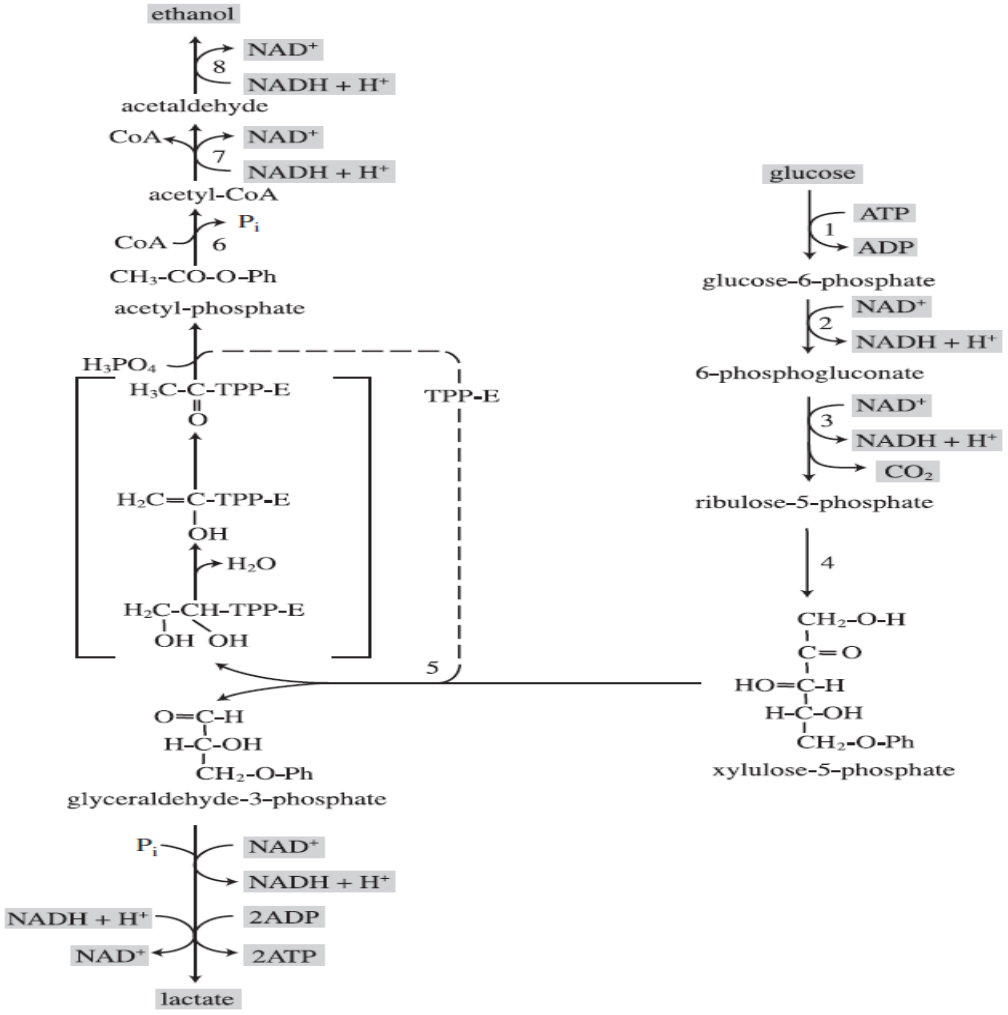
9.5.1 Glucose Fermentation by Leuconostoc Mesenteroides:

Heterofermentative LAB, including, Leuconostoc mesenteroides, ferment glucose to lactate, ethanol and carbon dioxide

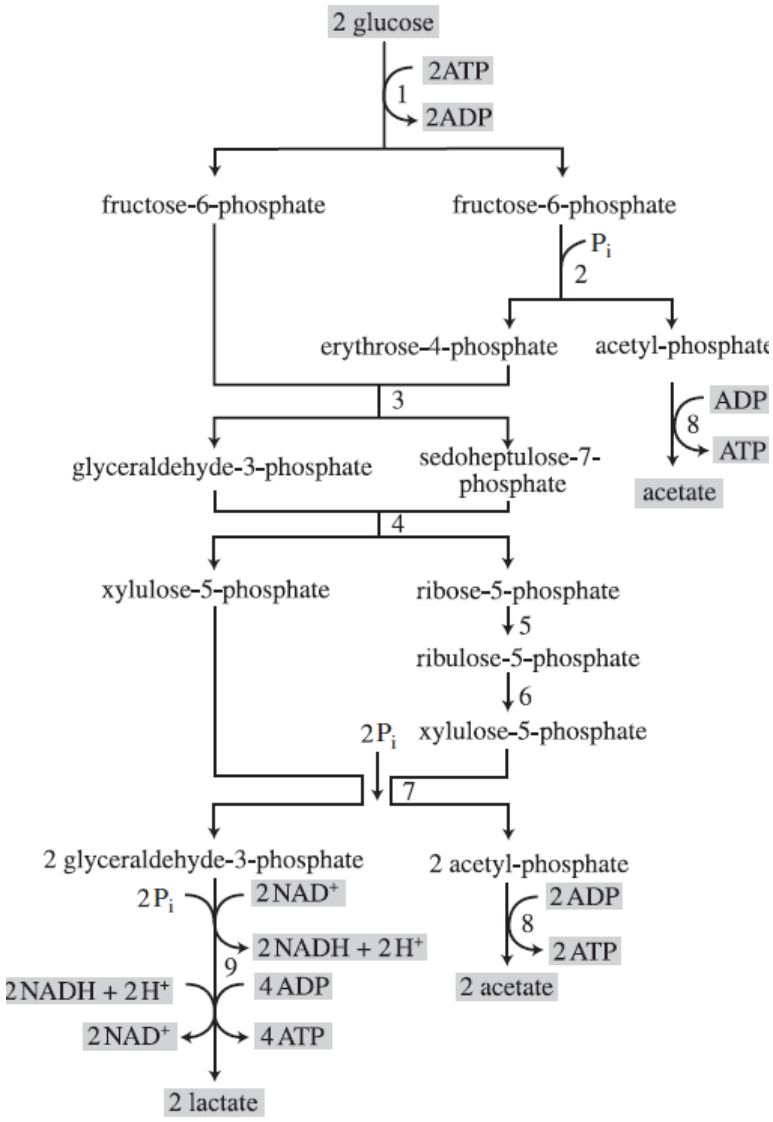
$$C_6H_{12}O_6 \longrightarrow CH_3CHOHCOOH + CH_3CH_2OH + CO_2$$

These bacteria oxidize glucose-6-phosphate to ribulose-5-phosphate as in the HMP pathway before being converted to xylulose-5-phosphate by an epimerase. Phosphoketolase splits the pentose-5-phosphate to glyceraldehyde-3- hosphate and acetyl-phosphate. Acetyl-phosphate is reduced to ethanol to regenerate NAD+ and pyruvate is produced from the triose-phosphate as in the latter part of the EMP pathway.

Since LAB have a restricted electron transport chain they use pyruvate and acetyl-phosphate as electron acceptors in the reactions catalyzed by lactate dehydrogenase, acetaldehyde dehydrogenase and alcohol dehydrogenase to regenerate NADþ from NADH (Section 8.4). For this reason Leuconostoc mesenteroides synthesizes one more ATP from pentoses than hexoses. In the hexose fermentation,



acetyl-phosphate is reduced to ethanol to oxidize NADH (reactions 7 and 8 in Figure 9.9), which is reduced by glucose-6-phosphate dehydrogenase (reaction 2 in Figure 9.9) and 6-phosphogluconate dehydrogenase (reaction 3 in Figure 9.9). Acetyl-phosphate is used to synthesize ATP in the reaction catalyzed by acetate kinase in pentose fermentation. Thiamine pyrophosphate (TPP) is a prosthetic group in phosphoketolase. TPP binds glycoaldehyde, and the complex is dehydrated before being phosphorylated to acetyl-phosphate. PK in Leuconostoc mesenteroides is active on xylulose-5-phosphate, but not fructose-6- phosphate. Hexose fermentation results in the net gain of 1 ATP.



9.6. SUMMARY:

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9.7. SELF ASSESSMENT:

1) PEP Synthesis Pathway
2) Hexose Monophosphate (HMP) Pathway
3) Entner–Doudoroff (ED) Pathway
4) Phosphoketolase Pathways

9.8. REFERENCES:

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Prof. V. Umamaheswara Rao

LESSON-10

TRICARBOXYLIC ACID (TCA) CYCLE, ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

10.0 OBJECTIVE:

- This chapter explained the mechanisms of pyruvate oxidation, electron transport and oxidative phosphorylation

STRUCTURE:

10.1. INTRODUCTION:

Pyruvate produced from glycolysis and other metabolic pathways is metabolized in various ways depending on the organism and growth conditions. Pyruvate is either used as a precursor for biosynthesis, or is oxidized completely to CO2 under aerobic conditions. This chapter is devoted to the mechanisms of pyruvate oxidation, electron transport and oxidative phosphorylation. 10.1 Oxidative decarboxylation of pyruvate Pyruvate is oxidized by the pyruvate dehydrogenase complex to acetyl-CoA and CO2 reducing NADþ under aerobic conditions. The pyruvate dehydrogenase complex consists of 24 molecules of pyruvate dehydrogenase containing thiamine pyrophosphate (TPP), 24 molecules of dihydrolipoate acetyltransferase containing dihydrolipoate and 12 molecules of dihydrolipoate dehydrogenase containing flavin adenine dinucleotide (FAD). In addition, NADþ and coenzyme A participate in the reaction (Figure 10.1). This reaction is irreversible, and takes place in the mitochondrion in eukaryotic cells.

The reaction can be summarized as:

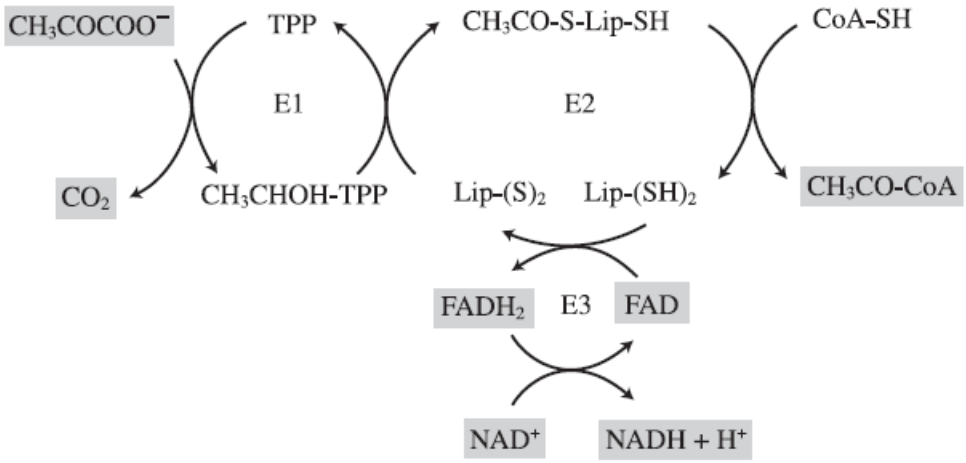


Fig 10.1: Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex. Pyruvate dehydrogenase (E1) decarboxylates pyruvate and its prosthetic group thiamine pyrophosphate (TPP) binds the resulting hydroxyethyl group, which binds to the lipoate (Lip) of dihydrolipoate acetyltransferase (E2), reducing its disulfide. E2 transfers an acetyl group to coenzyme A to form acetyl-CoA. Dihydrolipoate dehydrogenase (E3) transfers electrons from reduced lipoate to NADþ.

10.2. TRICARBOXYLIC ACID (TCA) CYCLE

This cyclic metabolic pathway was discovered by Krebs and his colleagues in animal tissue. It is referred to as the tricarboxylic acid (TCA) cycle, Krebs cycle or citric acid cycle. Acetyl-CoA produced by the pyruvate dehydrogenase complex is completely oxidized to CO2, reducing NADþ, NADPþ and FAD. These reduced electron carriers are oxidized through the processes of electron transport and oxidative phosphorylation to form the proton motive force and to synthesize ATP.

The TCA cycle provides not only reducing equivalents for ATP synthesis but also precursors for biosynthesis. The TCA cycle or related metabolic processes are indispensable, providing biosynthetic precursors for all forms of cells except mycoplasmas which take required materials from their host animal cells.

10.2.1. Citrate SYNTHESIs and the TCA Cycle:

Citrate synthase synthesizes citrate from acetyl-CoA and oxaloacetate (Figure 10.2). This is an exergonic reaction (DG00¼#32.2 kJ/mol acetyl-CoA) and irreversible. The reverse reaction is catalyzed by a separate enzyme, ATP: citrate lyase, in the reductive TCA cycle.

Citrate is converted to isocitrate catalyzed by aconitase. Isocitrate is oxidized to 2-ketoglutarate by isocitrate dehydrogenase. In most bacteria this enzyme is NADPþ dependent, but two separate enzymes are found in eukaryotes using NADP+ or NAD+.

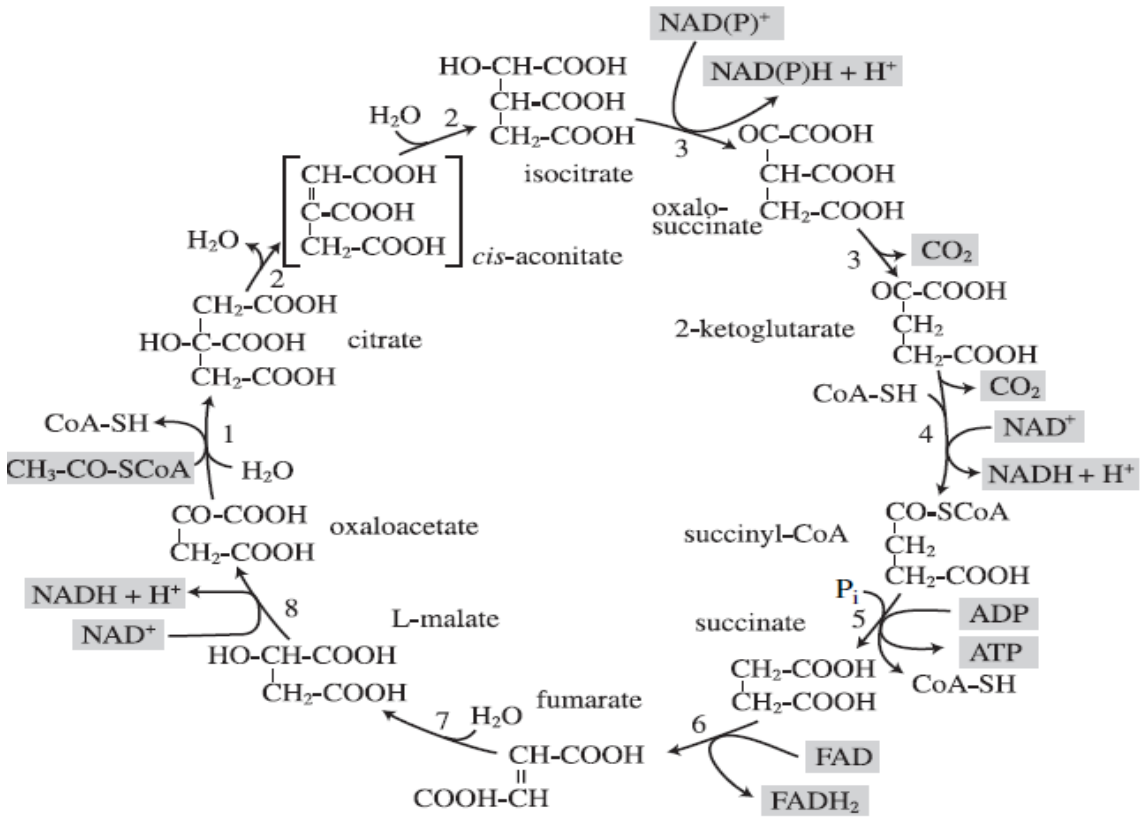


Fig 10.2: Acetyl-CoA oxidation through the tricarboxylic acid cycle. 1, citrate synthase; 2, aconitase; 3, isocitrate dehydrogenase;4, 2-ketoglutarate dehydrogenase complex; 5, succinate thiokinase (succinyl-CoA synthetase); 6, succinate dehydrogenase; 7, fumarase (fumarate hydratase); 8, malate dehydrogenase.

The 2-ketoglutarate dehydrogenase complex oxidizes its substrate to succinyl-CoA. As with the pyruvate dehydrogenase complex, this enzyme complex consists of many peptides and cofactors, and catalyzes oxidative decarboxylation producing acyl-CoA. This is another irreversible reaction in the TCA cycle. The reverse reaction is catalyzed by 2-ketoglutarate synthase (2-ketoglutarate: ferredoxin oxidoreductase) in the reductive TCA cycle to fix CO2 (Section 10.4.2). Some anaerobic fermentative bacteria do not have this enzyme. They supply the precursors for biosynthesis through the incomplete TCA fork.

Glutamate and related amino acids are synthesized from 2-ketoglutarate (Section 6.4.3). Succinyl-CoA is the precursor for porphyrin synthesis which provides the chemical nucleus of cytochromes and chlorophyll (Section 6.7). Both carbons of acetyl-CoA are liberated as CO2 in the two reduction reactions. Like all acyl-CoA derivatives, succinyl-CoA has a high energy linkage. This energy is conserved as ATP through the succinate thiokinase (succinyl-CoA synthetase) reaction producing succinate. This is an example of substrate-level phosphorylation. Guanosine triphosphate (GTP) is synthesized in the mitochondrion by these reactions in eukaryotic cells.

Succinate is oxidized to fumarate by succinate dehydrogenase. Since the redox potential of fumarate/succinate (#0.03V) is considerably higher than NADþ/NADH (#0.32 V), NAD(P)þ cannot be reduced in this reaction. The prosthetic group of succinate

dehydrogenase, FAD, is reduced. Electrons of the reduced succinate dehydrogenase are transferred to coenzyme Q of the electron transport chain.

Fumarate is hydrated to malate by fumarase before being reduced to oxaloacetate bymalate dehydrogenase reducing NAD+.

Oxaloacetate is then ready to accept acetyl-CoA for the next round of the cycle. Oxaloacetate is used to synthesize amino acids, and decarboxylated to phosphoenolpyruvate (PEP) in gluconeogenesis (Section 4.2.1). The TCA cycle can be summarized as:

$$CH_3\text{-}CO\text{-}CoA + 3NAD(P)^+ + FAD + ADP + P_i + 3H_2O \longrightarrow 2CO_2 + 3NAD(P)H + FADH_2 + ATP + 3H^+ + CoA\text{-}SH$$

The reduced electron carriers channel electrons to the electron transport chain to synthesize ATP through the proton motive force.

10.2.2 Regulation of the TCA Cycle:

The TCA cycle is an amphibolic pathway serving anabolic needs by producing ATP as well as catabolic needs by providing precursors for biosynthesis. Consequently, this metabolism is regulated by the energy status of the cell and the availability of biosynthetic precursors.

In addition, oxygen regulates the TCA cycle since the reduced electron carriers are recycled, consuming oxygen as the electron acceptor. Oxygen controls the expression of genes for TCA cycle enzymes. Facultative anaerobes do not synthesize 2-ketoglutarate dehydrogenase under anaerobic conditions without alternative electron acceptors such as nitrate. The activity is lower with nitrate than with oxygen as the electron acceptor. In Gram-negative bacteria, including Escherichia coli, the regulatory proteins FNR and Arc regulate the transcription of many genes for aerobic and anaerobic metabolism. A FNR protein with a similar function is also known in Gram-positive Bacillus subtilis.

Citrate synthase is regulated to control the TCA cycle. Generally, this enzyme is repressed with the accumulation of NADH and ATP or 2-ketoglutarate. This accumulation means that the cell has enough energy and precursors for biosynthesis. Gram-negative bacteria have two different citrate synthase enzymes, one repressed by NADH and the other unaffected. Gram-positive bacteria have only one enzyme and this is not repressed by NADH. Instead, ATP inhibits this enzyme. AMP activates the citrate synthase inhibited by NADH in some bacteria.

10.3. REPLENISHMENT OF TCA CYCLE INTERMEDIATES:

Some intermediates of the TCA cycle serve as precursors for biosynthesis. For efficient operation of this cyclic metabolism, the intermediates used for biosynthesis should be replenished otherwise the concentration of oxaloacetate would be too low to start the TCA cycle. Oxaloacetate is replenished through a process called the anaplerotic sequence (Figure 10.3).

10.3.1. Anaplerotic Sequence:

Bacteria growing on carbohydrates synthesize oxaloacetate from pyruvate or phosphoenolpyruvate (PEP) as shown in Figure 10.3. Many organisms, from bacteria to mammals, carboxylate pyruvate to oxaloacetate and this is catalyzed by pyruvate carboxylase consuming ATP:

$$\text{pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$$

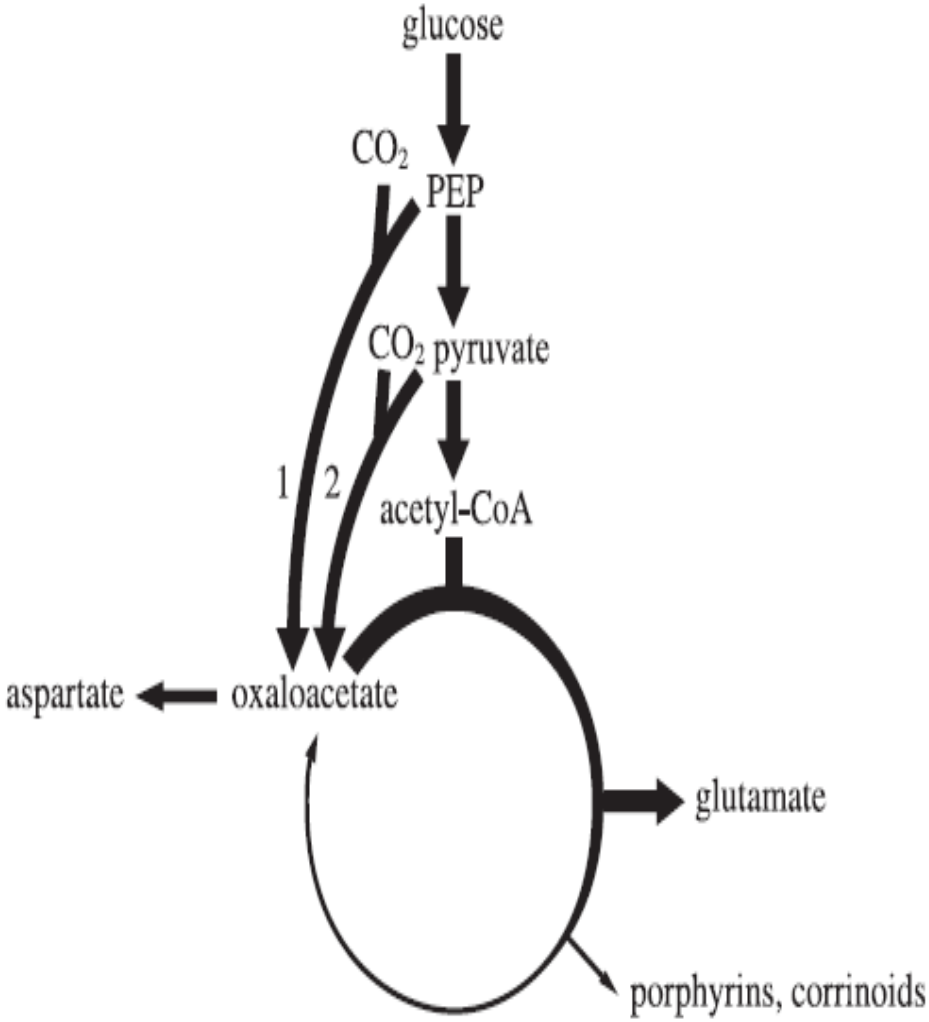


Fig 10.3: Anaplerotic sequence in bacteria growing on carbohydrates. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.410. Springer, New York) 1, PEP carboxylase; 2, pyruvate carboxylase.

This enzyme needs biotin. Acetyl-CoA activates this enzyme in many bacteria, as in animals, but some bacteria such as Pseudomonas aeruginosa have a pyruvate carboxylase that is not activated by acetyl-CoA. A PEP carboxylase mutant of Escherichia coli is unable to grow in a glucose–mineral salts medium, but can grow when supplemented with TCA cycle

intermediates. This bacterium has PEP carboxylase as the anaplerotic sequence, not pyruvate carboxylase. This property is shared by many other bacteria including Bacillus anthracis, Thiobacillus novellus, Acetobacter xylinum and Azotobacter vinelandii

$$PEP + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$$

10.4. ELECTRON TRANSPORT (OXIDATIVE) PHOSPHORYLATION:

Electron carriers such as NAD(P)þ, FAD and PQQ are reduced during glycolysis and the TCA cycle. Electrons from these carriers enter the electron transport chain at different levels. Electron carriers are oxidized, reducing molecular oxygen to water through ETP to conserve free energy as the proton motive force (Dp) (Figure 10.21a).

104.1. Chemiosmotic Theory:

It took many years to elucidate how the free energy generated from ETP is conserved as ATP. Compounds with high energy bonds are not involved in ETP as in substrate-level phosphorylation. ATP is synthesized only with an intact membrane or membrane vesicles, and ATP synthesis is inhibited in the presence of uncouplers or ionophores.

From these observations, a chemiosmotic mechanism was proposed. According to this, export of charged particles is coupled to oxidation–reduction reactions to form an electrochemical gradient which is used for ATP synthesis. Hþ are the charged particles exported, and the electrochemical gradient is the proton motive force, consisting of the Hþ gradient (DpH) across the membrane and the membrane potential (Dy). The phospholipid membrane is impermeable to H+ and OH# and is suitable to maintain the proton gradient. Most of the electron carriers involved in ETP are arranged in the membrane, and the membrane-bound ATP synthase synthesizes ATP from ADP and Pi consuming the proton gradient.

10.4.2. Electron Carriers and the Electron Transport Chain:

Electron carriers involved in electron transport from NADH to molecular oxygen are localized in the mitochondrial inner membrane in eukaryotic cells and in the cytoplasmic membrane in prokaryotic cells. The mitochondrial electron transport chain is shown in Figure 10.15, and bacterial electron transport systems are shown in Figure 10.19. Bacterial systems are diverse depending on the species and strain as well as on the availability of electron acceptors.

10.4.2.1. Mitochondrial Electron Transport Chain:

Eukaryotic electron transport is discussed here as a model to compare with the process in prokaryotes. The mitochondrial electron transport chain consists of complex I, II,

III and IV. The overall reaction can be summarized as dehydrogenases (complex I and II) and an oxidase (complex IV) connected by quinone (including complex III).

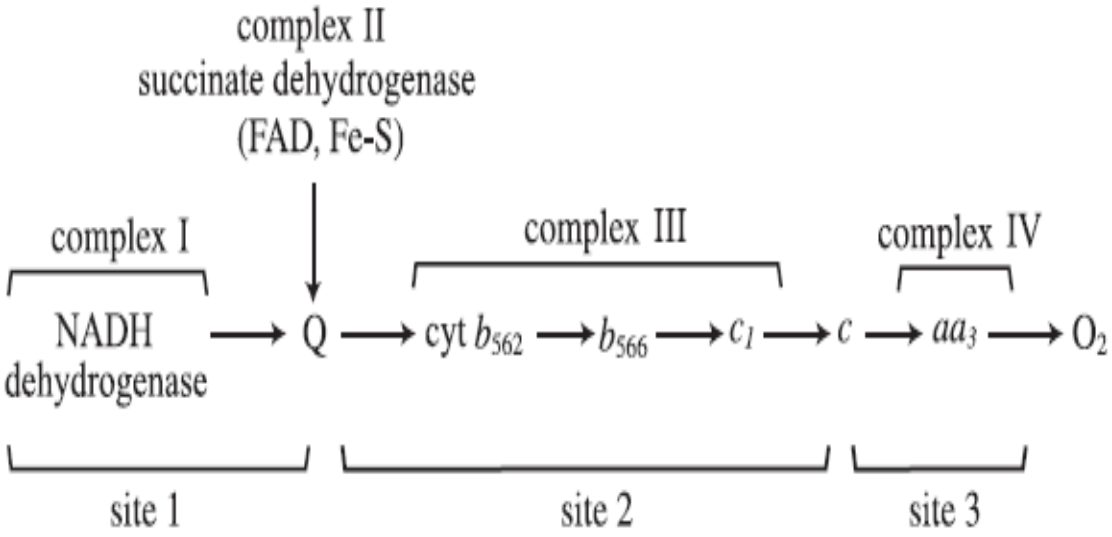


Fig 10.4: The eukaryotic electron transport chain localized in the mitochondrial inner membrane. (Dawes, E. A. 1986, Microbial Energetics, Figure 7.1. Blackie & Son, Glasgow) The electron transport chain forms four complexes in the mitochondrial inner membrane. These are referred to as complex I, II, III and IV.

Complex I has the lowest redox potential and consists of NADH dehydrogenase with FMN as the prosthetic group and [Fe-S] proteins. This complex is called NADH-ubiquinone reductase. Succinate dehydrogenase of the TCA cycle contains FAD as a prosthetic group, and forms complex II (succinate-ubiquinone reductase) with [Fe-S] proteins. Complex I and II transfer a pair of electrons to coenzyme Q from NADH and succinate, respectively. Complex III is ubiquinol-cytochrome c reductase, transferring electrons from reduced coenzyme Q (ubiquinol) to cytochrome c. This complex contains cytochrome b562, cytochrome b566, cytochrome c1 and [Fe-S] protein. Complex IV is referred to as cytochrome c oxidase. Among the electron transport processes, three steps generate enough free energy to synthesize ATP from ADP and Pi. These are shown in the figure as sites 1, 2 and 3.

NADH dehydrogenase oxidizes NADH, reduced in various catabolic pathways, to NADþ. This enzyme contains FMN as a prosthetic group and forms with [Fe-S] proteins a complex known as complex I or NADH-ubiquinone reductase. FMN is reduced with the oxidation ofnNADH and the [Fe-S] proteins mediate electron and proton transfer from FMNH2 to coenzyme Q. This reaction generates enough free energy to synthesize ATP, and the electron transfer from NADH to ubiquinone (coenzyme Q) is referred to as site 1 of ETP. In a mitochondrion and in most bacteria, protons are translocated by this complex, but sodium ions are exported by the complex I of certain bacteria including Vibrio alginolyticus (Section 10.7.4).

As a step in the TCA cycle, succinate dehydrogenase oxidizes succinate to fumarate, reducing its prosthetic group, FAD, before electrons are transferred to coenzyme Q. This enzyme forms complex II (or the succinate-ubiquinone reductase complex) of ETP with [Fe-S] proteins, cytochrome b558, and low molecular weight peptide. Other dehydrogenases containing FAD as a prosthetic group reduce coenzyme Q in a similar way. These include glycerol-3-phosphate dehydrogenase and acyl-CoA dehydrogenase.

Electrons from coenzyme Q are transferred to a series of reddish brown coloured proteins known as cytochromes. Cytochromes involved in mitochondrial electron transport are b562, b566, c1, c and aa3 as shown in Figure 10.110.

Two separate protein complexes mediate electron transfer from coenzyme Q to molecular oxygen through the cytochromes. These are ubiquinol-cytochrome c reductase (complex III) and cytochrome oxidase (complex IV).

Complex III transfers electrons from coenzyme Qto cytochrome c. This complex consists of [Fe-S] protein, and cytochromes b562, b566

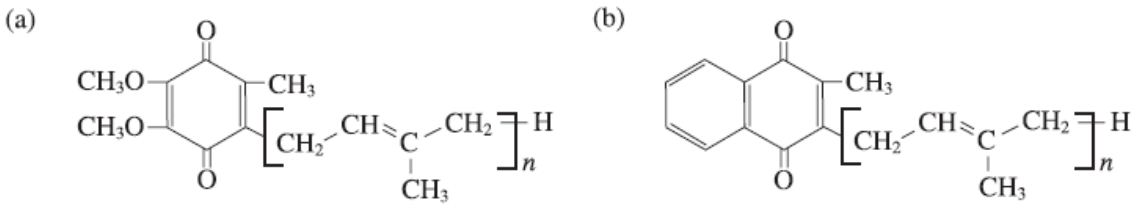


Fig 10.5: Structure of (a) ubiquinone and (b) menaquinone. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 2.9. Springer, New York) n¼4, 6, 8 or 10.

and c1. At this step, energy is conserved exporting protons (site 2). The cytochrome oxidase complex mediates electron transfer from reduced cytochrome c to molecular oxygen. Energy is also conserved at this step (site 3). This terminal oxidase complex contains cytochrome a and cytochrome a3.

10.5. ELECTRON CARRIERS:

Electron transport involves various electron carriers including flavoproteins, quinones, [Fe-S] proteins and cytochromes. Flavoproteins are proteins containing riboflavin (vitamin B2) derivatives as their prosthetic group. They are FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide). The redox potential of the flavoproteins varies not due to the flavin structure but due to the differences in the protein component.

Two structurally different quinones are involved in the electron transport process, ubiquinone and menaquinone, which serve as coenzyme Q. Quinones are lipid electron carriers, highly hydrophobic and mobile in the semi-solid lipid phase of the membrane. As shown in Figure 10.16, quinones have a side chain of 6, 8 or 10 isoprenoid units. These are named Q6, Q8 and Q10 according to the number of isoprenoid units. Ubiquinone is found in mitochondria, and bacteria have menaquinone (Figure 10.16). Both forms of quinones are

found in Gram-negative facultative anaerobes. The structure of coenzyme Q can be used as one characteristic for bacterial classification. Quinones can carry protons as well as electrons.

[Fe-S] proteins contain [Fe-S] cluster(s), usually [2Fe-2S] or [4Fe-4S]. The non-heme irons are attached to sulfide residues of the cysteines of the protein and acid-labile sulfur (Figure 10.17). The acid-labile sulfur is released as H2S at an acidic pH. [Fe-S] proteins participating in electron transport can carry protons as well as electrons. There are many different [Fe-S] proteins mediating not only the electron transport process in the membrane, but also various oxidation–reduction reactions in the cytoplasm. The redox potential of different [Fe-S] proteins spans from as low as #410mV (clostridial ferredoxin, Section 8.5) to þ350mV.

Many enzymes catalyzing oxidation–reduction reactions are [Fe-S] proteins including hydrogenase, formate dehydrogenase, pyruvate: ferredoxin oxidoreductase and nitrogenase. Cytochromes are hemoproteins. They are classified according to their prosthetic heme structures (Figure 10.18) and absorb light at 550–650 nm. Cytochrome b562 refers to a cytochrome b with the maximum wavelength absorption at 562 nm. Heme is covalently bound to the proteins in cytochrome c, and hemes are non-covalently associated with the protein in other cytochromes. Since cytochromes

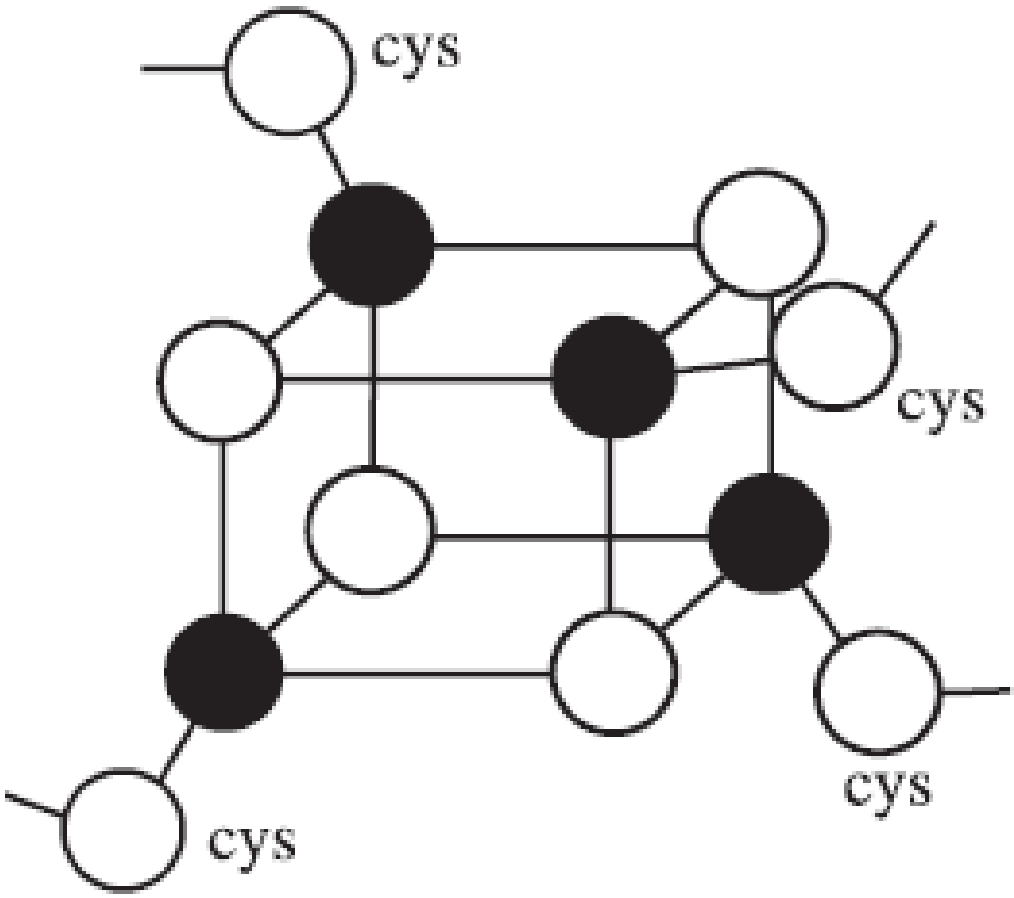


Fig 10.6: The structure of a [4Fe-4S] cluster. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 8.8. Springer, New York) *, sulfur atom; *, iron atom. carry only one electron, electron transfer from reduced coenzyme Q to cytochrome requires two steps

10.6. DIVERSITY OF ELECTRON TRANSPORT CHAINS IN PROKARYOTES:

As in the mitochondrial electron transport chain, the prokaryotic electron transport chain is organized with dehydrogenase and oxidase connected by quinone. However, electron transport chains in prokaryotes are much more diverse since they use diverse electron donors and electron acceptors including those not used by any eukaryotes. (inorganic electron donors, chemolithotrophs). Chapter 9 contains exclusive discussion of the electron transport chains to electron acceptors other than O2 (anaerobic respiration). In this section, prokaryotic electron transport chains analogous to the mitochondrial system will be described.

The electron carriers involved in prokaryotic electron transport are diverse. As described earlier, menaquinone is used as coenzyme Q in addition to ubiquinone in prokaryotes, and diverse cytochromes participate in prokaryotic electron transport mainly in relation to the terminal oxidase, which leads to branched electron transport chains depending on the availability of O2. Some bacteria such as Paracoccus denitrificans and Alcaligenes eutrophus have very similar electron transport systems to mitochondria.

They have cytochrome aa3 as the terminal oxidase while others use cytochrome d or o in its place. Cytochrome o has a b-type heme, and cytochrome d has a different heme structure (Figure 10.18). They are not only structurally different but also show different responses towards respiratory inhibitors, and form branched electron transport pathways (Figure 10.19). This diversity of electron transport systems is closely related to bacterial growth under a variety of conditions.

The terminal oxidases in bacteria have different affinities for O2. Under O2-limited conditions, cytochrome d replaces the normal terminal oxidase, cytochrome aa3, in Klebsiella pneumoniae and *Haemophilus parainfluenzae*. Similarly, *Paracoccus denitrificans* and *Alcaligenes eutrophus* use cytochrome o as their terminal oxidase. The high affinity cytochrome d and o enables the bacteria to use the electron acceptor (O2) efficiently at low concentrations. In nitrogen-fixing *Azotobacter vinelandii*, cytochrome d functions as the terminal oxidase under nitrogen fixing conditions with less energy conservation than the normal oxidase (Figure 10.19). Cytochrome d keeps the intracellular O2 concentration low to protect the O2-labile nitrogenase).

In branched bacterial electron transport systems, the number of sites for energy conservation is less than that on the mitochondrial system (Section 10.8.4). This might enable a survival strategy under certain conditions but at the expense of reduced energy conservation.

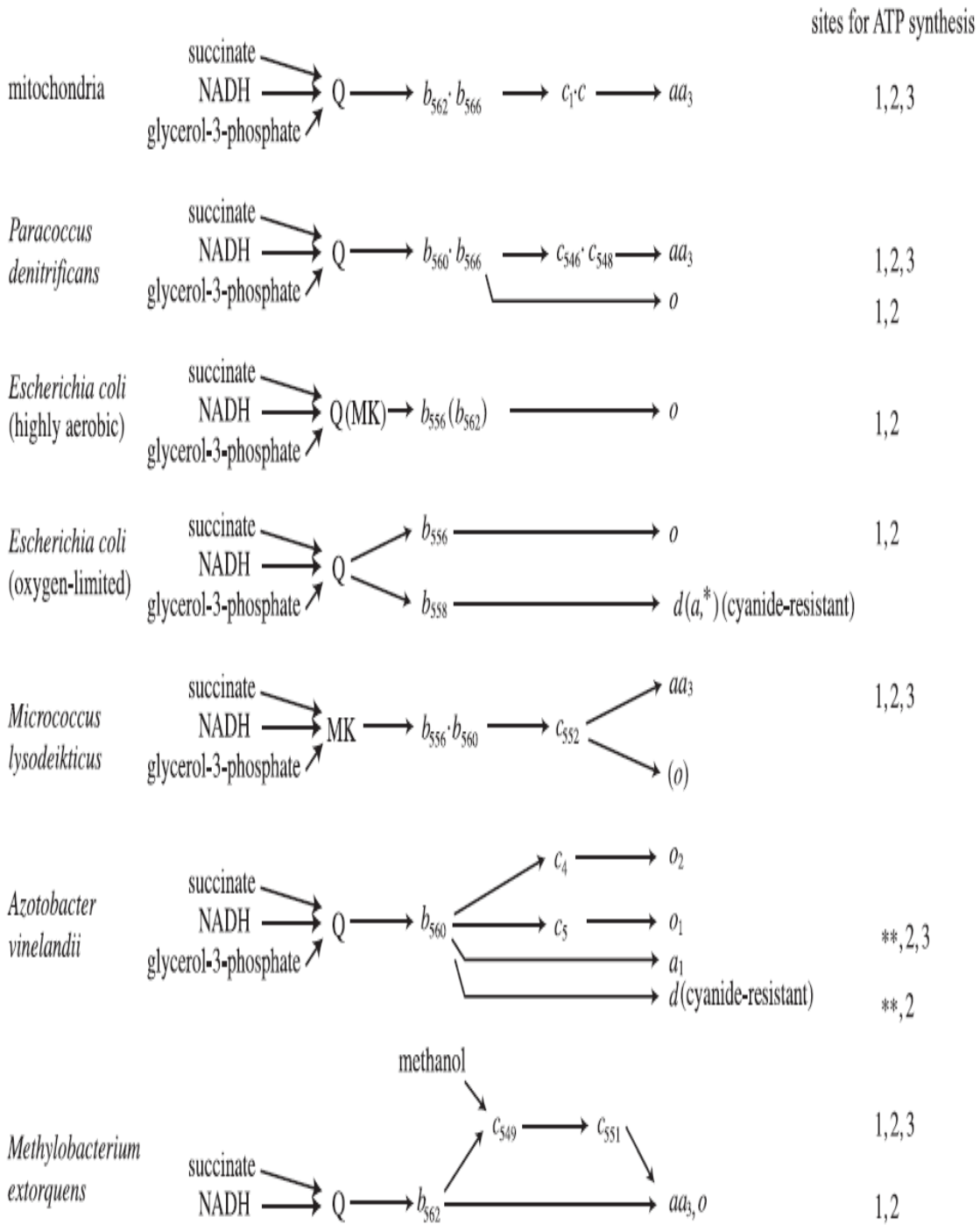


Fig 10.7: With a few exceptions, the dehydrogenases of chemolithotrophs reduce quinone or cytochrome and this is coupled to the oxidation of their inorganic electron donors. They cannot directly reduce NAD(P)+, which is needed for biosynthetic purposes. They transfer electrons from the reduced quinone or cytochrome to NAD(P)þ in an uphill reaction, consuming the proton motive force, known as reverse electron transport. Reverse electron transport is possible because complexes I and III can catalyze the reverse reactions.

Complex IV cannot catalyze the reverse reaction to use water as the source of electrons. Water is used as the electron source in oxygenic photosynthesis through a different reaction.

10.7. INHIBITORS OF ELECTRON TRANSPORT PHOSPHORYLATION (ETP):

Inhibitors of ETP are grouped into three kinds according to their mechanism of action: electron transport inhibitors, uncouplers and ATPase inhibitors. Electron transport inhibitors interfere with the enzymes and electron carriers involved in the electron transport system. They inhibit not only ATP synthesis but also oxygen consumption. Rotenone, amytal and piericidin A inhibit NADH dehydrogenase, and 2-n-heptyl-4- hydroquinoline-N-oxide (HQNO), antimycin A, cyanide (CN#) and azide have their own specific inhibition sites (Figure 10.20).

Uncouplers increase Hþ permeability through the membrane, thus dissipating the proton motive force. The proton motive force becomes too low to be used to synthesize ATP in the presence of uncouplers, but the O2 consumption rate increases in the presence of the uncouplers. The term uncoupler means that ATP synthesis is not coupled to O2 consumption. Further discussion occurs later (Section 10.8.5).

ATP synthase inhibitors block the membrane-bound ATPase preventing ATP synthesis even with a high proton motive force. N, N0- dicyclohexyl carbodiimide (DCCD) and oligomycin are well-known ATPase inhibitors. They bind the Fo part of the membrane-bound F1Fo-ATPase blocking the path for Hþ. Fomeans the oligomycin-binding component of the F1Fo-ATPase.

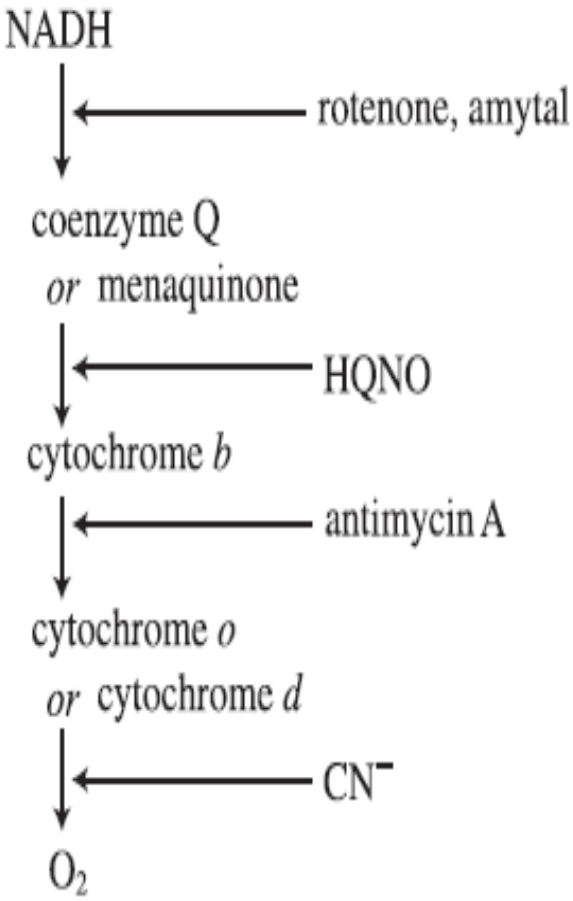
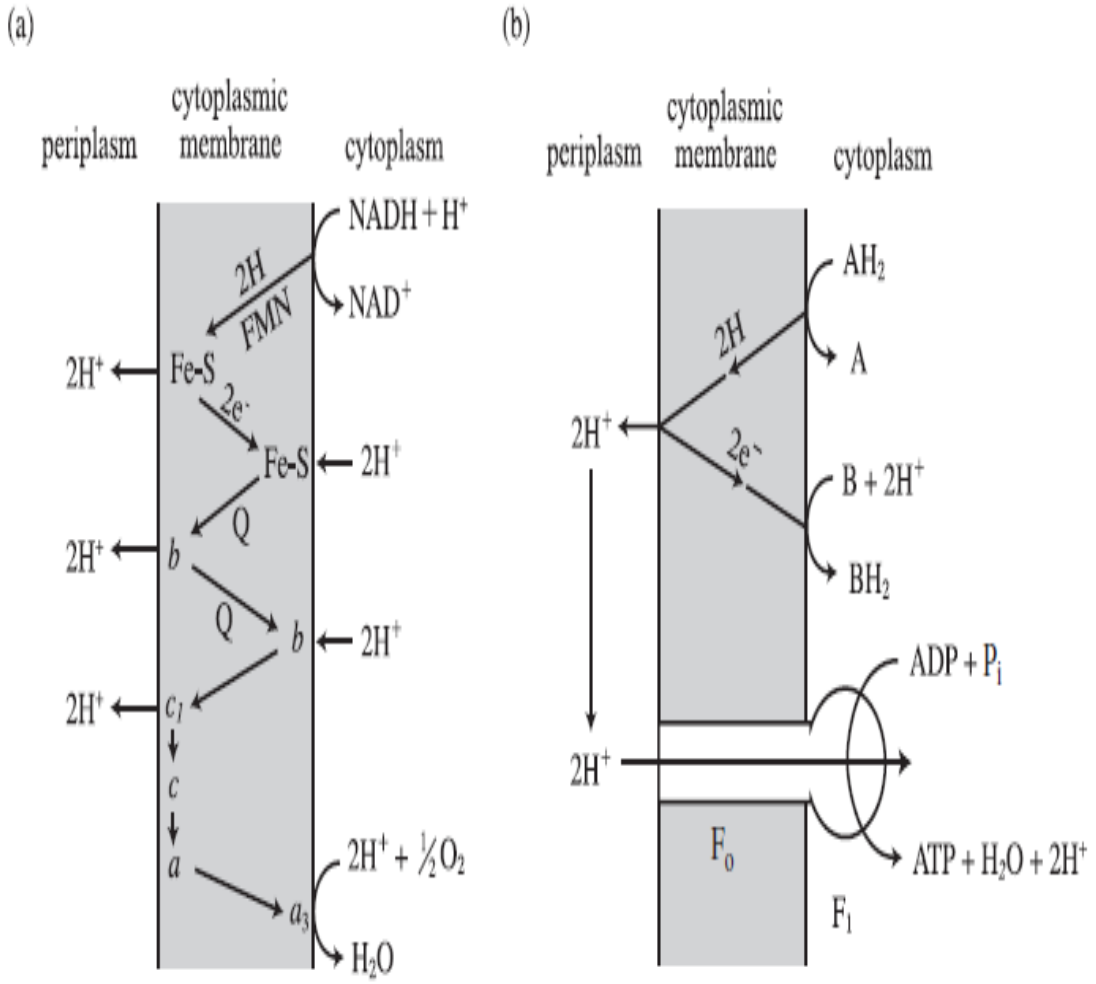


Fig. 10.7: Electron transport inhibitors and the sites of their action. Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 2.17. Springer, New York) HQNO, 2-n-heptyl-4-hydroquinoline- N-oxide.

10.8. TRANSHYDROGENASE:

Nicotinamide nucleotide transhydrogenase is known in many prokaryotes and catalyzes the following reaction:



Formation of proton motive force and ATP synthesis through ETP. (Dawes, E. A. 1986, Microbial Energetics, Figure 7.3. Blackie & Son, Glasgow) Since the electron carriers are arranged in the mitochondrial inner membrane and prokaryotic cytoplasmic membrane in such a way that electrons move from the inner face to the outer face of the membrane with H+, and in the reverse direction withoutHþ, H+ is exported during the electron transport process (a). The membrane-bound ATPase synthesizes ATP, consuming the protonmotive forcewith Hþ flow tothe low Hþ concentration side (b).

10.9. SUMMARY:

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10.10. SELF ASSESSMENT:

1) Tricarboxylic Acid (TCA) Cycle
2) Replenishment of TCA Cycle Intermediates
3) Electron Transport (Oxidative) Phosphorylation
4) Electron Carriers
5) Diversity of Electron Transport Chains in Prokaryotes
6) Inhibitors of Electron Transport Phosphorylation (ETP)
7) Transhydrogenase

10.11. REFERENCES:

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Prof. V. Umamaheswara Rao

LESSON-11

ANAEROBIC FERMENTATION

11.0 OBJECTIVE:

- The primary objectives for studying bacterial anaerobic fermentation generally fall into two broad categories: understanding the fundamental biological processes and leveraging these processes for industrial and environmental applications.

STRUCTURE:

11.1. ETHANOL FERMENTATION:

Saccharomyces cerevisiae ferments carbohydrates through the EMP pathway to ethanol, and the ED pathway is used by Zymomonas mobilis. Pyruvate is decarboxylated to acetaldehyde, which is used as the electron acceptor. Acetaldehyde is reduced to ethanol, which consumes the electrons generated during the glycolytic process where ATP is generated through SLP (Figure 11.1). Saccharomyces cerevisiae

Pyruvate decarboxylase has thiamine pyrophosphate as a prosthetic group as in pyruvate dehydrogenase. Pyruvate decarboxylase is known mainly in eukaryotes. In addition to Zymomonas mobilis, this enzyme is found in a facultative anaerobe, Erwinia amylovora, and in a strictly anaerobic acidophile, Sarcina ventriculi. Pyruvate decarboxylase is a key enzyme of ethanol fermentation.

It should be noted that ethanol is produced through different reactions in saccharolytic clostridia, heterofermentative lactic acid bacteria and enteric bacteria. These bacteria oxidize pyruvate to acetyl-CoA before reducing it to ethanol. They do not possess pyruvate decarboxylase. Ethanol production in clostridia is catalyzed by the following reactions:

$$CH_3\text{–}CO\text{–}COOH \xrightarrow{\text{pyruvate decarboxylase}} CH_3\text{–}CHO + CO_2$$

$$CH_3\text{–}CHO + NADH + H^+ \xrightarrow{\text{alcohol dehydrogenase}} CH_3\text{–}CH_2OH + NAD^+$$

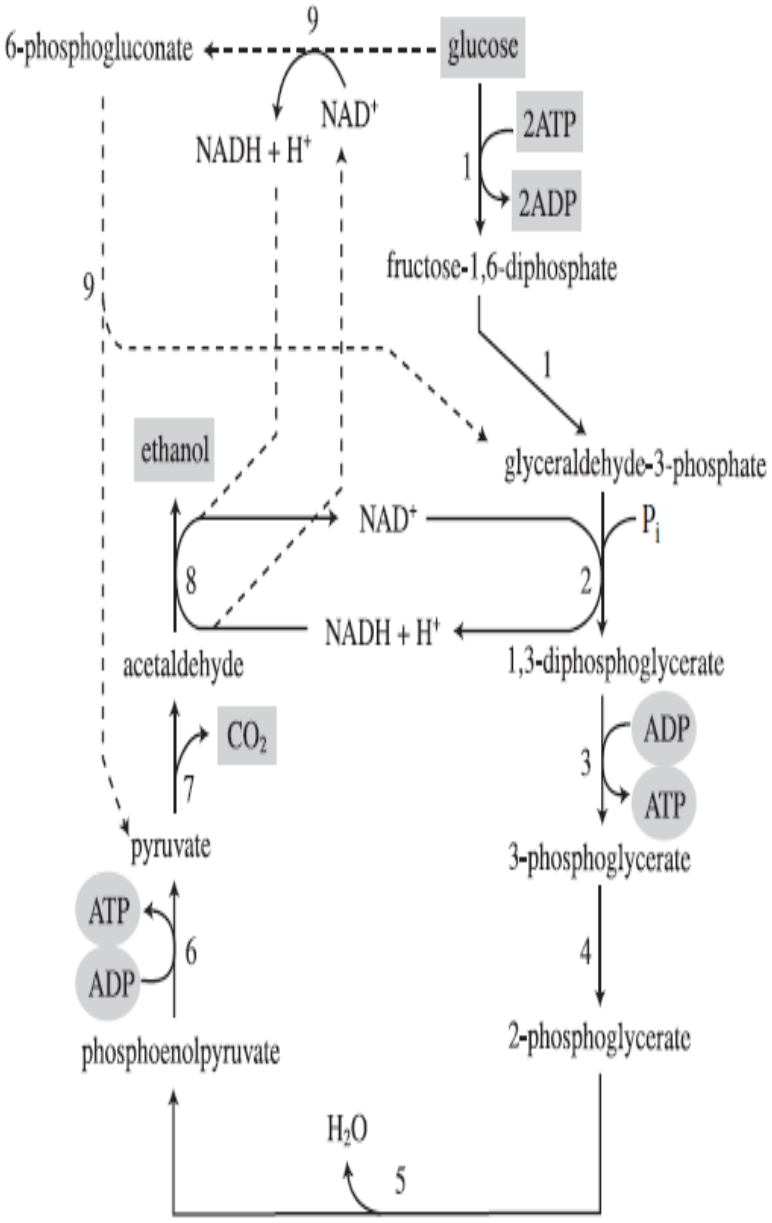
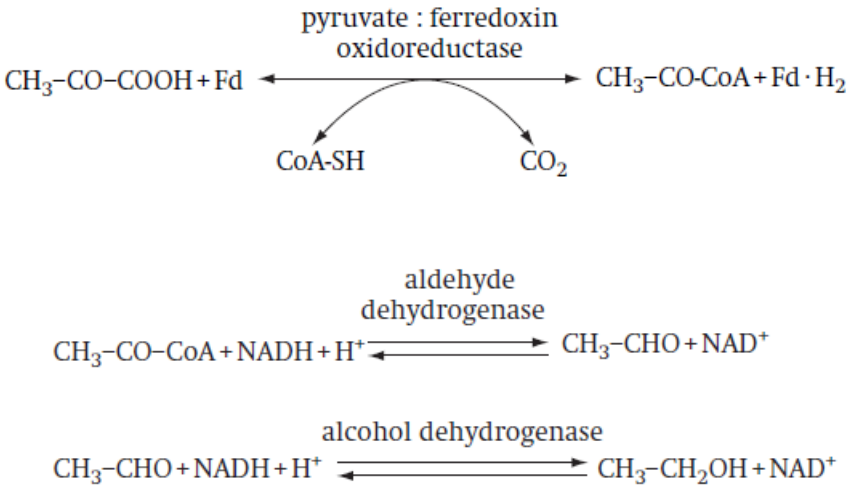


Fig 11.1: Ethanol fermentation by Saccharomyces cerevisiae and Zymomonas mobilis. 1-6, EMP pathway (in solid lines); 7, pyruvate decarboxylase; 8, alcohol dehydrogenase; 9, ED pathway (in dotted lines) Generates 2 ATP from 1 hexose molecule but a single ATP results from 1 hexose molecule in Zymomonas Mobilis.



Ethanol fermentation through pyruvate decarboxylase in a linear fermentative pathway does not produce any by-products except CO2 and water, while ethanol fermentation through acetyl-CoA is a branched fermentative pathway and produces various fermentation products such as lactate, acetate and H2. Thermophilic anaerobes ferment various carbohydrates including cellulose and pentoses through acetyl-CoA to ethanol. Among them are *Thermoanaerobium brockii*, *Thermoanaerobacter ethanolicus* and *Clostridium thermocellum*.

11.2. LACTATE FERMENTATION:

Lactate is a common fermentation product in many facultative and obligate anaerobes. Some bacteria produce lactate as a major fermentation product and these are referred to as lactic acid bacteria (LAB).

Most LAB have a limited ability to synthesize monomers for biosynthesis and vitamins which are needed as growth factors.

LAB are regarded as obligate anaerobes but they can use oxygen, synthesizing cytochromes when hemin is provided in the medium. Some LAB produce only lactate from sugars while others produce acetate and ethanol in addition to lactate (Table 11.2). The former are referred to as homofermentative and the latter heterofermentative LAB.

Homofermentative LAB ferment sugars through the EMP pathway and heterofermentative LAB ferment sugars through the phosphoketolase pathway.

11.2.1. Homolactate Fermentation:

Homofermentative LAB include most species of Lactobacillus, Sporolactobacillus, Pediococcus, Enterococcus and Lactococcus. They use hexoses through the EMP pathway to generate ATP. Lactate dehydrogenase reoxidizes the NADH reduced during the EMP pathway using pyruvate as the electron acceptor (Figure 11.2).

As fermentation proceeds, lactate is accumulated lowering the intracellular pH. Lactate dehydrogenase is active in acidic conditions producing lactate as the major product. Under alkaline conditions, homofermentative LAB produce large quantities of acetate and ethanol.

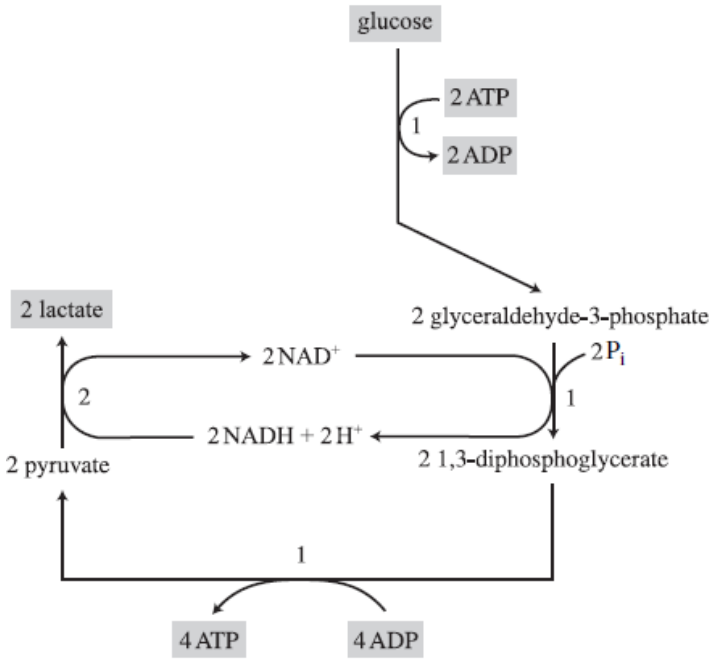


Fig 11.2: Homofermentative lactic acid fermentation. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 11.2. Springer, New York) 1, EMP pathway; 2, lactate dehydrogenase.

11.2.2. Heterolactate Fermentation:

Species of Leuconostoc and Bifidobacterium produce ethanol and acetate in addition to lactate. They employ a unique glycolytic pathway known as the phosphoketolase pathway. As shown in Figure 4.9, heterofermentative LAB like Leuconostoc mesenteroides oxidize glucose-6-phosphate to ribulose-5-phosphate. Epimerase converts ribulose-5-phosphate to xylulose-5-phosphate, before cleavage to glyceraldehyde-3-phosphate and acetyl-phosphate by the action of phosphoketolase. Glyceraldehyde-3-phosphate is metabolized to lactate as in the homolactate fermentation generating ATP.

Acetyl-phosphate is reduced to ethanol acting as the electron acceptor to oxidize the NADH reduced in the glucose-6-phosphate oxidation process. One ATP per hexose is available from this fermentation.

Pentoses are converted to xylulose-5-phosphate without reducing NADþ. In this case, acetyl- phosphate is not used as the electron acceptor but is used to synthesize ATP. Leuconostoc mesenteroides synthesizes 1 ATP from a molecule of hexose and 2 ATP from a molecule of pentose.

Bifidobacterium bifidum ferments 2 molecules of hexose to 2 molecules of lactate and 3 molecules of acetate employing two separate phosphoketolases, one active on fructose-6-phosphate and the other on xylulose-5-phosphate). This bacterium synthesizes 5 ATP from 2 molecules of glucose. Since hexose-6-phosphate is not metabolized through a reductive process, acetyl-phosphate is used to synthesize ATP as in pentosemetabolismby Leuconostoc mesenteroides.

11.3. BUTYRATE AND ACETONE–BUTANOL–ETHANOL FERMENTATIONS:

Spore-forming anaerobic Gram-positive bacteria without sulfate reducing ability are classified as the genus Clostridium. They are divided into saccharolytic and proteolytic clostridia according to their preferred electron donor. Saccharolytic clostridia ferment carbohydrates to butyrate and acetate, and proteinaceous compounds are fermented by proteolytic clostridia. The latter organisms are mostly pathogenic. Clostridial fermentation is a typical branched fermentative pathway. In addition to clostridia, species belonging to the genera *Butyri vibrio*, Eubacterium and Fusobacterium also produce butyrate (Table 11.3). All of these are obligate anaerobes.

11.3.1. Butyrate Fermentation:

Clostridium butyricum transports glucose by group translocation before metabolizing it to pyruvate. Pyruvate is oxidized to acetyl-CoA through a reaction known as a phosphoroclastic reaction catalyzed by pyruvate: ferredoxin oxidoreductase. This is different from the reaction catalyzed by pyruvate dehydrogenase (Figure 5.1). Hydrogenase can oxidize the reduced ferredoxin in this reaction to produce H2:

$$\text{ferredoxin} \cdot H_2 \xrightleftharpoons{\text{hydrogenase}} \text{ferredoxin} + H_2$$

11.3.1. Phosphoroclastic Reaction:

Pyruvate: ferredoxin oxidoreductase contains thiamine pyrophosphate as a prosthetic group, like pyruvate dehydrogenase, and catalyzes the following reactions:

$$CH_3\text{–}CO\text{–}COOH + \text{TPP-enzyme} \rightleftharpoons CH_3\text{–}CHOH\text{–}\text{TPP-enzyme} + CO_2$$

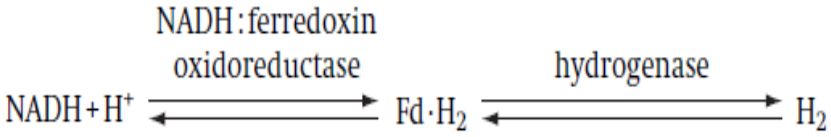
$$CH_3\text{–}CHOH\text{–}\text{TPP-enzyme} + \text{ferredoxin} + \text{CoA-SH} \rightleftharpoons \text{TPP-enzyme} + CH_3\text{–}\text{CO-CoA} + \text{ferredoxin} \cdot H_2$$

Table | *Anaerobes producing butyrate as their main fermentation product*

Butyribacterium methylotrophicum
Butyrivibrio fibrisolvens
Clostridium butyricum
C. kluyveri
C. pasteurianum
Eubacterium limosum
Fusobacterium nucleatum

11.3.2. Butyrate Formation:

Acetyl-CoA produced from the phosphoroclastic reaction is metabolized either to acetate through acetyl-phosphate or to butyrate through acetoacetyl-CoA (Figure 11.3). The NADH reduced during glycolysis is oxidized, reducing acetoacetyl-CoA to butyrate. When the hydrogen partial pressure is low, hydrogenase oxidizes reduced ferredoxin producing H2. Under this condition, the ferredoxin (oxidized)/ferredoxin(reduced) ratio is high, and NADH: ferredoxin oxidoreductase couples NADH oxidation to ferredoxin reduction. Since NADH is oxidized through these reactions, acetyl-CoA is not needed as the electron acceptor, and it is converted to acetyl-phosphate on which acetate kinase reacts to synthesize ATP.



In an undisturbed culture of Clostridium butyricum, 100mol glucose is fermented to 76mol butyrate and 42mol acetate. However, when H2 is continuously removed by shaking, the butyrate/acetate ratio becomes 1. This is due to the fact that the equilibrium shifts to the right in the above reactions. Since kinases synthesize ATP from acetyl-phosphate and butyryl-phosphate, 4 ATP are synthesized from glucose fermentation to acetate and 3 ATP from the butyrate fermentation.

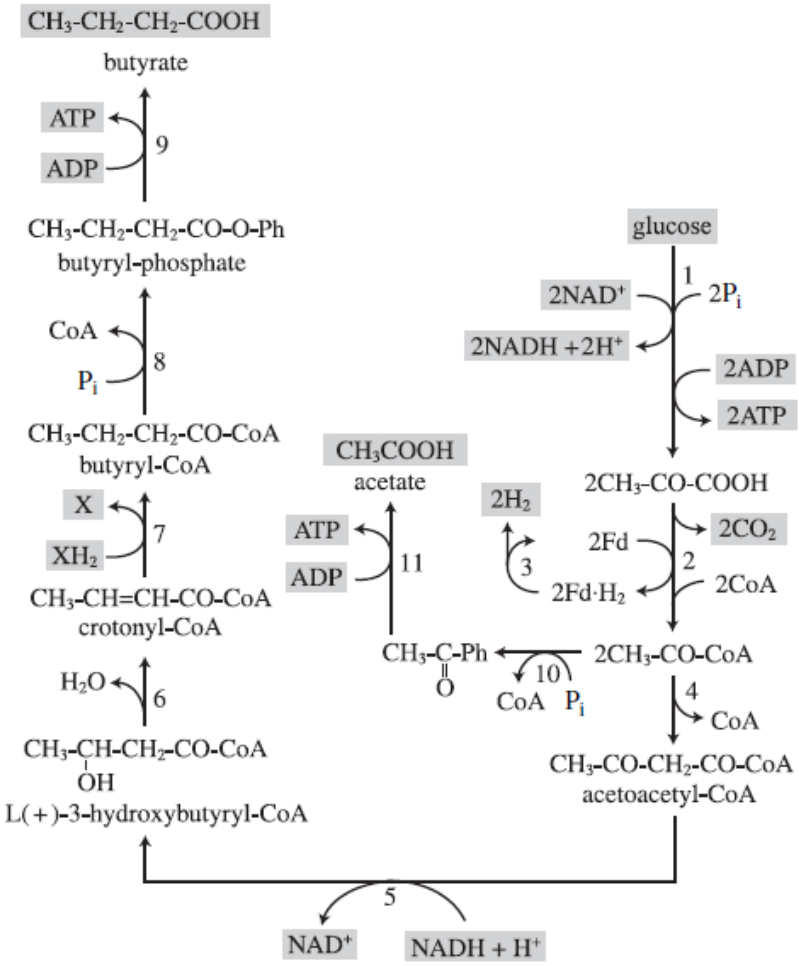


Fig 11.3: Butyrate-acetate fermentation of glucose. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 11.9. Springer, New York) 1, EMP pathway; 2, pyruvate: ferredoxin oxidoreductase; 3, hydrogenase; 4, acetyl-CoA-acetyltransferase (thiolase); 5, L(þ)-3- hydroxybutyryl-CoA dehydrogenase; 6, 3-hydroxyacyl- CoA hydrolase (crotonase); 7, butyryl-CoA dehydrogenase; 8, phosphotransbutyrylase; 9, butyrate kinase; 10, phosphotransacetylase; 11, acetate kinase.

11.4. Mixed Acid and Butanediol Fermentation:

11.4.1. Mixed Acid Fermentation:

Some Gram-negative facultative anaerobic bacteria ferment glucose, producing various products including lactate, acetate, succinate, formate, CO2 and H2. These include species of Escherichia, Salmonella, Shigella and Enterobacter (Figure 11.4). Glucose is metabolized through the EMP pathway. Phosphoenolpyruvate (PEP) carboxylase synthesizes oxaloacetate from PEP before being reduced to succinate. Pyruvate is either reduced to lactate

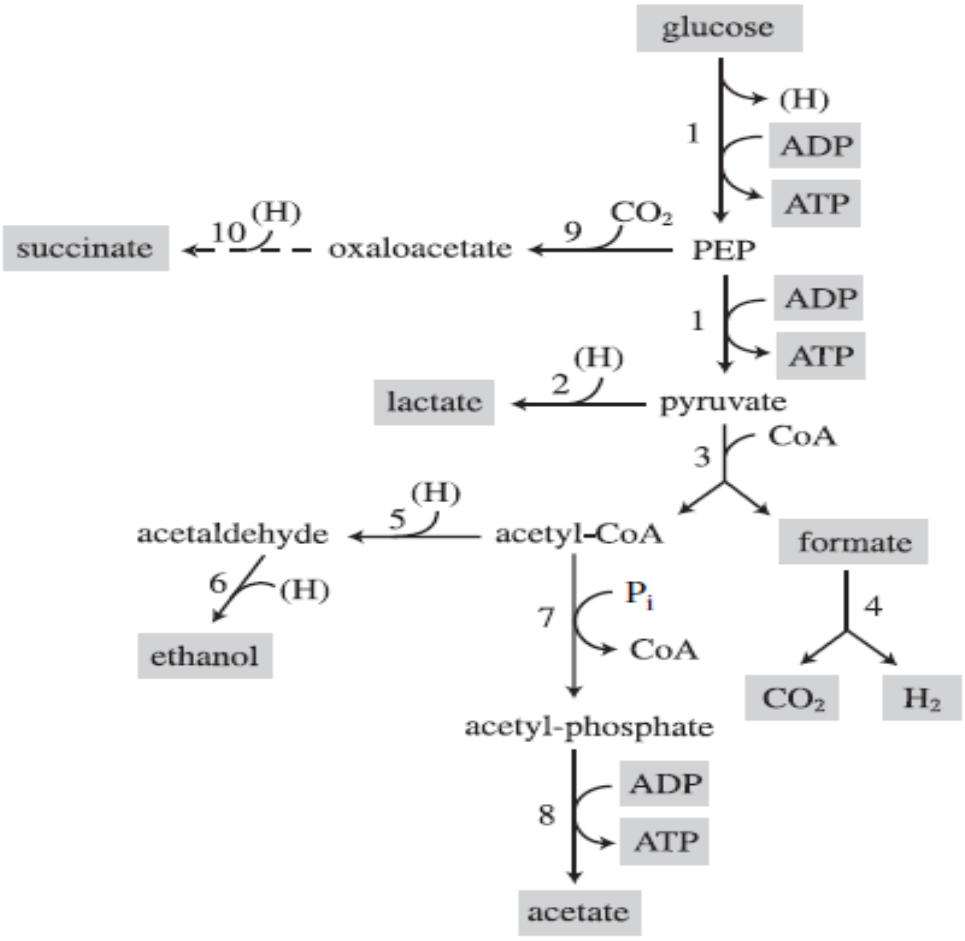


Fig 11.4: Mixed acid fermentation by some Gram negative facultative anaerobic bacteria. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 11.15. Springer, New York)

Facultative anaerobes belonging to the genera Escherichia, Salmonella, Shigella, Enterobacter and others ferment sugars to lactate, acetate, formate, succinate and ethanol in the absence of electron acceptors.

1, EMP pathway; 2, lactate dehydrogenase; 3, pyruvate: formate lyase; 4, formate: hydrogen lyase; 5, acetaldehyde dehydrogenase; 6, alcohol dehydrogenase; 7, phosphotransacetylase; 8, acetate kinase; 9, phosphoenolpyruvate (PEP) carboxylase; 10, enzymes of the TCA cycle by lactate dehydrogenase, or cleaved to acetyl-CoA and formate by pyruvate: formate lyase. According to the availability of electrons, acetyl-CoA is either reduced to ethanol or used to synthesize ATP.

Strictly anaerobic bacteria such as Anaerobiospirillum succiniciproducens and Actinobacillus succinogenes ferment carbohydrate mainly to succinate through a similar metabolism. In this case the succinate yield is as high as the amount of carbohydrate fermented. PEP carboxylase (reaction 9, Figure 11.4) fixes a large amount of CO2 in this fermentation.

11.4.2. Butanediol Fermentation:

Some Erwinia, Klebsiella and Serratia species produce 2,3-butanediol in addition to lactate and ethanol from pyruvate, the EMP pathway product. Pyruvate is the substrate for one of three enzymes in these bacteria. These are lactate dehydrogenase, pyruvate: formate lyase and 2-acetolactate synthase (Figure 11.5). The reactions catalyzed by these enzymes are similar to those of the mixed acid fermentation except for 2-acetolactate synthase. This enzyme condenses two molecules of pyruvate to 2-acetolactate that is further decarboxylated and reduced to 2,3-butanediol. A similar metabolism is found in Bacillus polymyxa during vegetative growth and in lactic acid bacteria fermenting citrate.

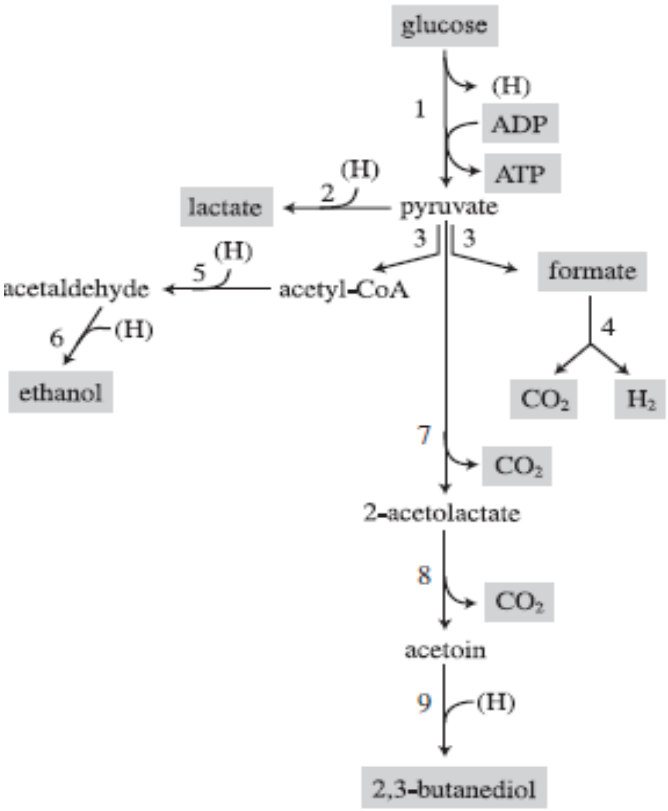
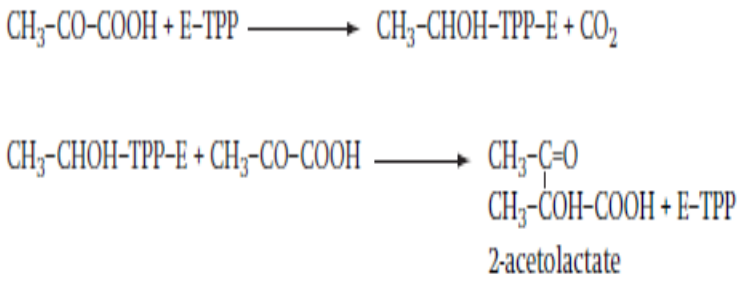


Fig 11.5: Butanediol fermentation by some Gram negative facultative anaerobic bacteria. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 11.15. Springer, New York) Facultative anaerobes belong to the genera Erwinia, Klebsiella and Serratia and produce 2,3-butanediol in addition to lactate and ethanol.

1, EMP pathway; 2, lactate dehydrogenase; 3, pyruvate: formate lyase; 4, formate: hydrogen lyase; 5, acetaldehyde dehydrogenase; 6, alcohol dehydrogenase; 7, 2-acetolactate synthase; 8, 2-acetolactate decarboxylase; 9, 2,3-butanediol dehydrogenase.

The first enzyme of this metabolism, 2-acetolactate synthase, is best characterized in Gram-negative facultative bacteria. This enzyme has thiamine pyrophosphate as a cofactor to catalyze the following reactions:



Under anaerobic conditions, 2,3-butanediol-producing facultative anaerobes produce acidic products, lowering the external and intracellular pH. 2-acetolactate synthase, which catalyzes the first reaction to produce 2,3-butanediol, has an optimum at pH 6.0.

When the intracellular pH drops, this enzyme becomes active to divert carbon flux from acid production to the neutral solvent. An enzyme catalyzing the same reaction catalyzes the first reaction of valine synthesis from pyruvate. The enzyme involved in valine synthesis has an optimum at pH 11.0, and also catalyzes the condensing reaction of 2-ketobutyrate and pyruvate to synthesize isoleucine. This enzyme is referred to as the pH 11.0 enzyme while the enzyme involved in 2,3-butanediol synthesis is referred to as the pH 6.0 enzyme.

Klebsiella pneumoniae, Klebsiella oxytoca and Enterobacter aerogenes ferment glycerol to various products including 2,3-butanediol (Figure 11.6). They oxidize a part of glycerol to pyruvate, and dispose

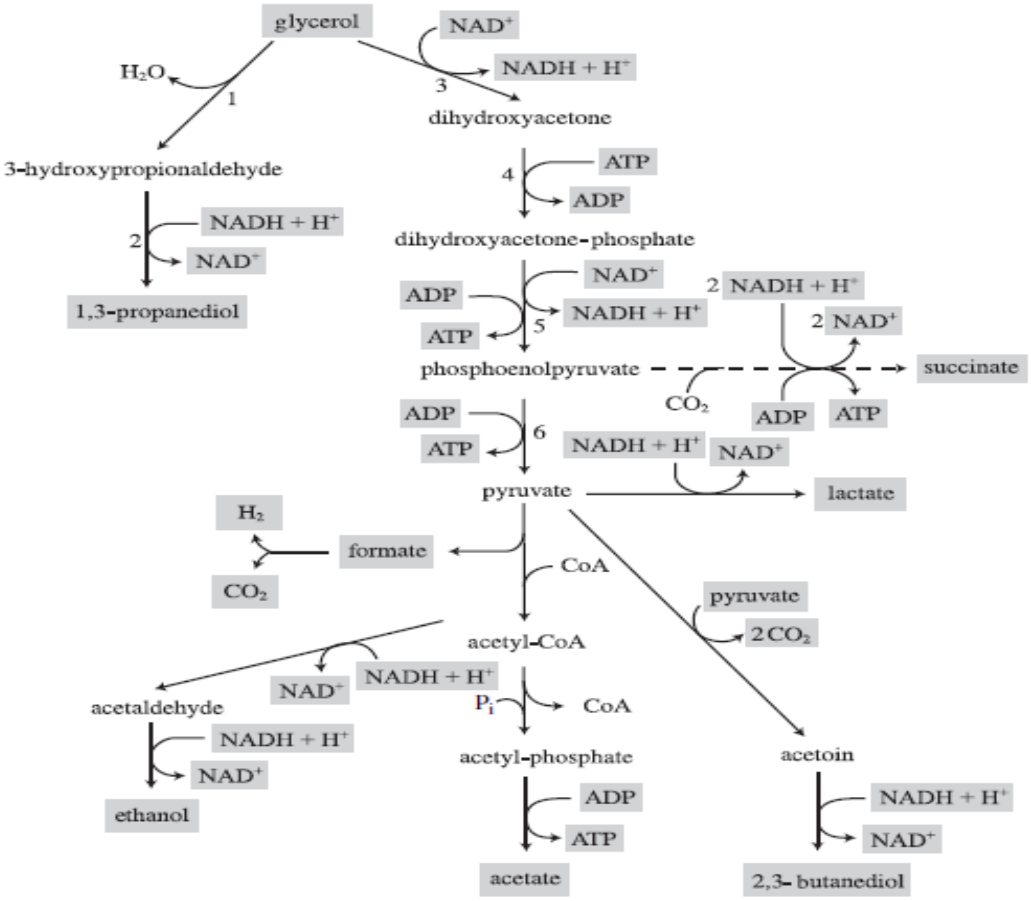


Fig 11.6: Glycerol fermentation by Klebsiella pneumoniae. (Appl. Microbiol. Biotechnol. 50:24–29, 1998) Glycerol is metabolized though a similar pathway as in the butanediol fermentation as well as being used as an electron acceptor to be reduced to 1,3-propanediol.1, glycerol dehydratase; 2, 1,3-propanediol dehydrogenase; 3, glycerol dehydrogenase;4, dihydroxyacetone kinase; 5, 6, enzymes in Figure 11.6.

of the resulting electrons to reduce the remaining glycerol to 1,3-propanediol. Pyruvate is metabolized as in the 2,3-butanediol fermentation. Glycerol is reduced to 1,3-propanediol by lactic acid bacteria while oxidizing carbohydrate (Section 11.4.6). These diols are important petrochemical intermediates.

11.5. PROPIONATE FERMENTATION:

Species of the genera Propionibacterium, Clostridium propionicum and Megasphaera elsdenii ferment carbohydrate or lactate to propionate, acetate, and CO2:3 glucose 4 propionate + 2 acetate + 2CO2 3 lactate 2 propionate + acetate + CO2 Lactate is the preferred substrate over carbohydrate in most propionate producers. They ferment glucose or lactate to propionate through either the acrylate pathway or the succinate–propionate pathway. Spore-forming Propionispora vibrioides ferments sugar alcohols such as mannitol, sorbitol and xylitol to propionate and acetate through an unknown pathway. Propionispora vibrioides ferments the aliphatic polyester poly(propylene adipate) to propionate.

11.5.1. Succinate-Propionate Pathway:

Species belonging to the genus Propionibacterium ferment lactate to propionate via succinate through this pathway (Figure 11.7). Lactate dehydrogenase with flavin oxidizes lactate to pyruvate. Two molecules of pyruvate are reduced to propionate as the electron acceptor while one pyruvate is oxidized to acetate through acetyl-CoA synthesizing 1 ATP. Some enzymes of this pathway are of interest. They are transcarboxylase requiring biotin, fumarate reductase

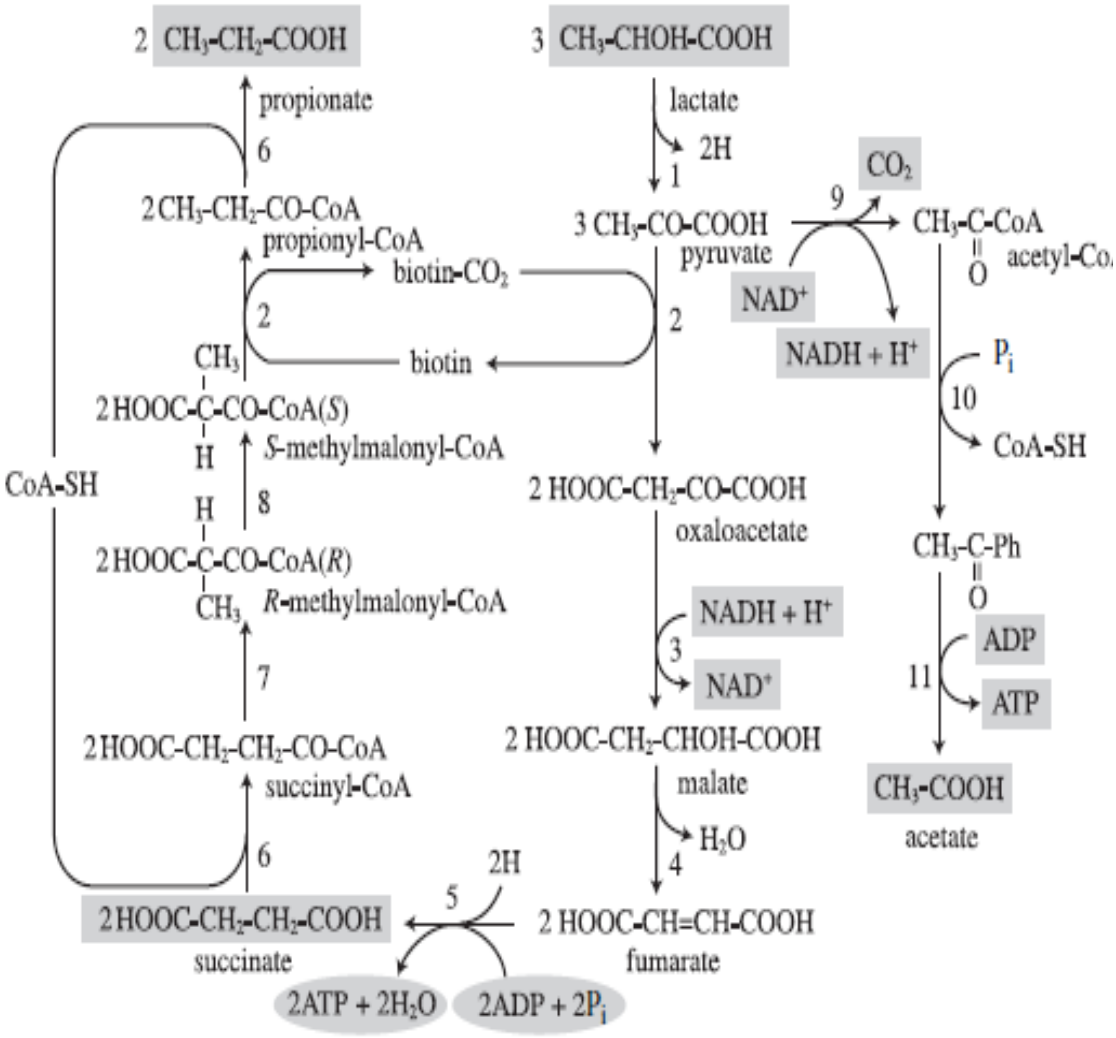


Fig 11.7: Succinate–propionate pathway in the genus Propionibacterium, which ferments lactate to propionate.

(Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 11.111. Springer, New York) 1, lactate dehydrogenase; 2, methylmalonyl-CoA: pyruvate transcarboxylase; 3, malate dehydrogenase; 4, fumarase; 5, fumarate reductase; 6, coenzyme A transferase; 7, methylmalonyl- CoA mutase; 8, methylmalonyl- CoA racemase; 9, pyruvate dehydrogenase; 10, phosphotransacetylase; 11, acetate kinase.

coupled to ATP synthesis and methylmalonyl-CoA mutase that uses coenzyme B12. In this fermentation 3 ATP are synthesized fermenting 3 lactate, one by acetate kinase and two by fumarate reductase.

Methylmalonyl-CoA: pyruvate transcarboxylase transfers a carboxyl group from methylmalonyl-CoA to pyruvate to form propionyl-CoA and oxaloacetate as in reaction 2 of Figure 11.16. Biotin is involved in this reaction as a cofactor.

Fumarate reductase couples the reduction of fumarate, using NADH as the electron donor, to the formation of a proton motive force as described previously (Section 5.11.6.1). When heme is provided, Bacteroides fragilis ferments carbohydrates to propionate and acetate through the succinate–propionate pathway with functional cytochromes. However, without heme, lactate and acetate are produced from the carbohydrate fermentation because fumarate reductase cannot function in the absence of functional cytochromes. As in lactic acid bacteria of the Lactococcus genus, this bacterium synthesizes the apoprotein of cytochromes.

Methylmalonyl-CoA mutase catalyzes the carbon-rearranging reaction to convert succinyl-CoA to methylmalonyl-CoA. This reaction requires coenzyme B12. Coenzyme B12 has the structure of 50-deoxyadenosylcobalamin (Figure 11.17). This coenzyme is required for various enzyme reactions involving carbon rearrangement. Vitamin B12 is produced commercially using Propionibacterium shermanii and related bacteria since they synthesize coenzyme B12 in large concentrations for methylmalonyl-CoA mutase.

Propionate producers through the succinate–propionate pathway excrete a small amount of succinate into the medium. In this case, methylmalonyl-CoA: pyruvate transcarboxylase cannot produce propionyl-CoA because the concentration of methylmalonyl-CoA becomes too low. To replace the excreted succinate, pyruvate or phosphoenolpyruvate are carboxylated to oxaloacetate which is reduced to succinate via malate:

$$\text{pyruvate} + \text{ATP} + P_i \xrightarrow{\text{pyruvate orthophosphate dikinase}} \text{PEP} + \text{AMP} + PP_i$$

$$\text{PEP} + CO_2 + \text{ADP} \xrightarrow{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{ATP}$$

$$\text{PEP} + CO_2 + P_i \xrightarrow{\text{PEP carboxytransphosphorylase}} \text{oxaloacetate} + PP_i$$

Since pyrophosphatase activity is low in species of Propionibacterium, the pyrophosphate produced in the above reactions is used to phosphorylate sugars, thus conserving the energy carried by this inorganic compound.

11.6. SUMMARY:

Bacterial anaerobic fermentation is a metabolic process where microorganisms break down carbohydrates into organic acids, gases (like CO2), or alcohols (like ethanol) in the absence of oxygen, generating energy (ATP) through glycolysis and substrate-level phosphorylation, essential for survival when oxygen isn't available, and widely used in industries for products like yogurt, biofuels, and probiotics, as well as occurring naturally in the gut. Key players include bacteria like *Lactobacillus* and *Clostridium*, producing useful compounds like lactic acid, acetic acid, or hydrogen.

11.7. SELF-ASSESSMENT:

1) Ethanol Fermentation
2) Lactate Fermentation
3) Butyrate Fermentation
4) Mixed Acid Fermentation
5) Butanediol Fermentation
6) Propionate Fermentation

11.8. REFERENCES:

1) Reddy and Reddy (2005). Microbial Physiology.
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th Edition
3) Nelson and Cox. 2000; Lehninger Principles of Biochemistry
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology.
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7) Gottschalk, G. Bacterial Metabolism

Prof. V. Umamaheswara Rao

LESSON-12

METHANOTROPH

12.0 OBJECTIVE:

- To understand how methane-eating microbes (methanotrophs) convert methane into biomass and valuable products, focusing on their role in mitigating the potent greenhouse gas methane, their potential for bioremediation.

STRUCTURE:

12.1. INTRODUCTION:

Methanotrophy and methylotrophy Methyltrophs are divided into methanotrophs and methylotrophs according to their ability to use methane. Methanotrophs use C1compounds but do not use multicarbon compounds. These are termed obligate methylotrophs. Methylotrophs do not use methane. Based on their carbon assimilation metabolism, methylotrophs are divided into heterotrophic methylotrophs and autotrophic methylotrophs. Autotrophic methylotrophs assimilate carbon dioxide through the Calvin cycle while heterotrophic methylotrophs assimilate formaldehyde through the ribulose monophosphate pathway or the serine–isocitrate lyase pathway . Methanotrophs are not known in eukaryotes, and methylotrophic yeasts assimilate formaldehyde through the xylulose monophosphate pathway.

12.2. CHARACTERISTICS OF METHANOTROPHS:

Methanotrophs use C1 compounds as their carbon and energy sources and are unable to use multicarbon compounds. For these reasons, they are referred to as obligate methylotrophs. In addition to their use of C1 compounds, they have some other characteristics. All of them are Gram-negative and have extensive intracellular Ortho cleavage (3-ketoadipate pathway of catechol and protocatechuate membrane structures as do

the nitrifiers. This membrane structure is used in the classification of methanotrophs). Spore and cyst forms of resting cells are known in all the obligate methylotrophs except species of Methylobacterium.

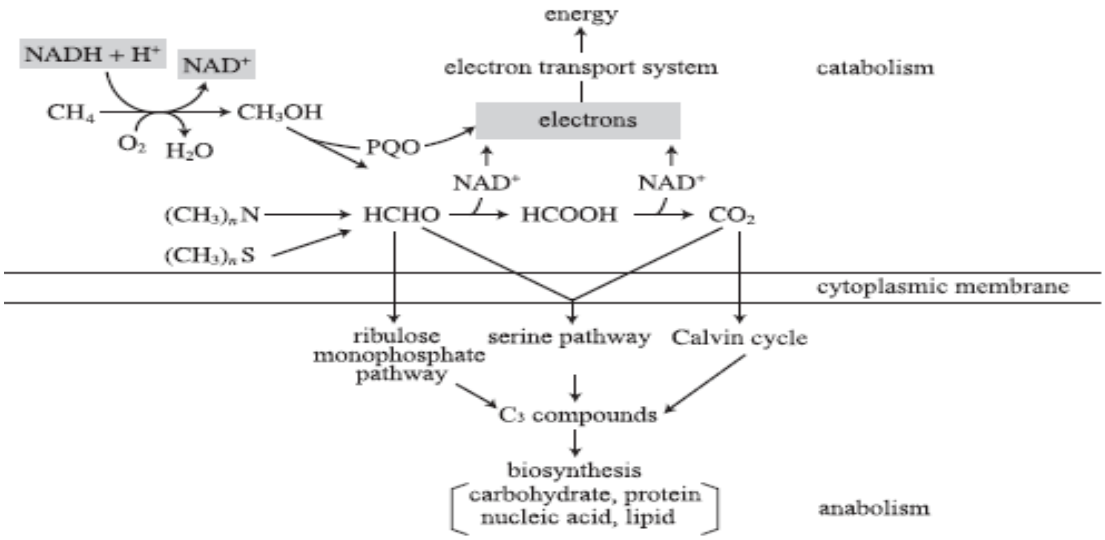


Fig. 12.1: Metabolism of One-Carbon Compounds

12.3. DISSIMILATION OF METHANE BY METHANOTROPHS:

Methane monooxygenase oxidizes methane to methanol using NADH as the cosubstrate (Figure 12.2). Electrons from methanol oxidation are channeled to the electron transport system for ATP synthesis. Formaldehyde or carbon dioxide are assimilated for biosynthesis of cell materials.

Table 12.1: Classification of Methanotrophs According to Their Characters

Physiological characteristics	Carbon assimilation pathway	Organisms	C1 compounds assimilated
Heterotrophic methylotrophy	serine–isocitrate lyase pathway	*Methylobacterium extorquens* AM1	methanol, MMA, formate
		Pseudomonas MA	MMA
		Methylbacterium organophilum	methane, methanol
	ribulose monophosphate pathway	*Arthrobacter* P1	MMA, DMA, TMA
		Bacillus PM6	MMA, DMA, TMA, TMO, tetramethylammonium
Autotrophic methylotrophy	Calvin cycle	Group 1. Phototrophic	
		Rhodopseudomonas spp.	methanol, CO, formate
		Group 2. Chemoautotrophic	
		Thiobacillus A2	methanol, MMA, formate
		Paracoccus denitrificans	methanol, MMA, formate, CO
		Pseudomonas carboxydovorans	CO
		Group 3.	
		Pseudomonas oxalaticus	formate
Obligate methyltrophy	serine–isocitrate lyase pathway	*Methylobacterium* spp.	methane
		Methylocystis spp.	methane
		Methylomonas methanooxidans	methane, methanol
		Methylosinus trichosporium	methane, methanol
	ribulose monophosphate pathway	*Methylomonas methanica*	methane, methanol
		Methylophilus methylotrophus	methanol, MMA, DMA
		Methanococcus capsulatus	TMA

MMA, monomethylamine; DMA, dimethylamine; TMA, trimethylamine; TMO, trimethyl-N-oxide; CO, carbon monoxide.

Table 12.2: Charecteristics of Methylotrophs

Methanotrophs	Morphology	Flagella	Resting cell	Intracellular membrane structure	Carbon assimilation pathway	G+C content (%)
Methylomonas	rod	polar	cyst-like body	I	RMP pathway	50–54
Methylobacter	rod	polar	thick-walled cyst	II	RMP pathway	50–54
Methylococcus	coccus	none	cyst-like body	I	RMP pathway	62
Methylosinus	rod, vibrioid	polar tuft	exospore	II	SIL pathway	62–66
Methylocystis	vibrioid	none	PHB-rich cyst	II	SIL pathway	?
Methylobacterium	rod	none	none	II	SIL pathway	58–66

I, multilayer membrane structure throughout the cell; II, double-layer membrane structure under the cell surface; RMP pathway, ribulose monophosphate pathway; SIL pathway, serine–isocitrate lyase pathway; PHB, polyhydroxybutyrate.

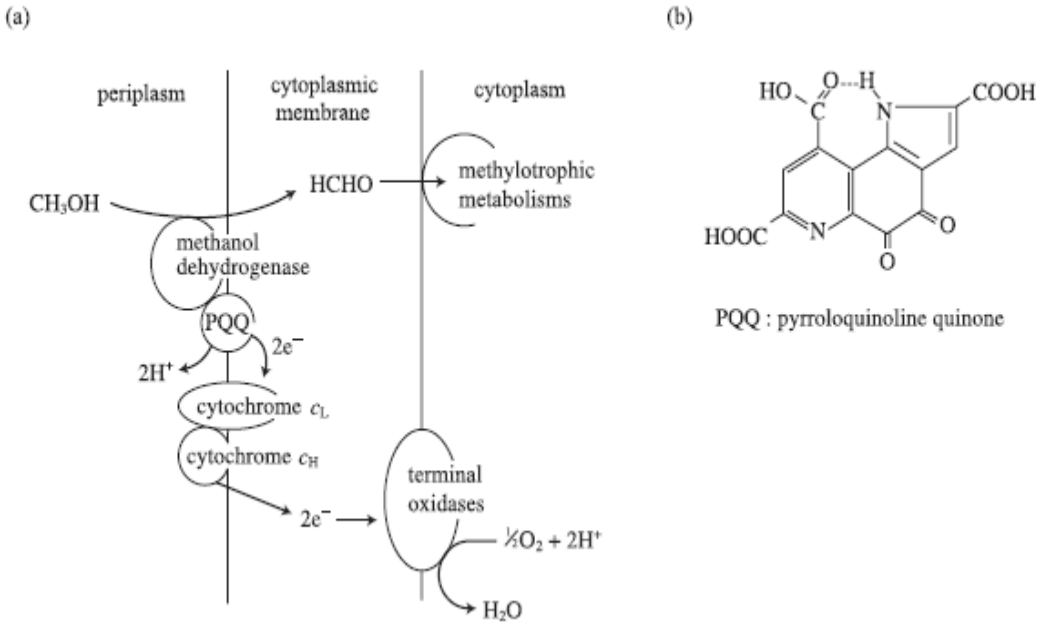
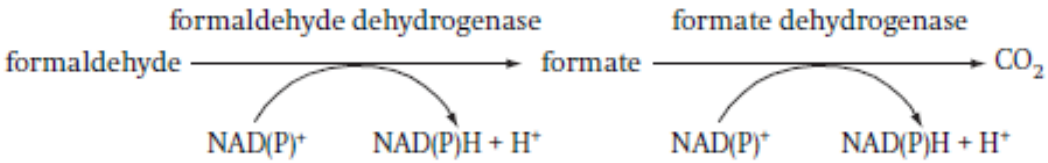


Fig 12.2: (a) Methanol dehydrogenase and the electron transport chain of the methylotroph, and (b) the structure of PQQ. Cytochrome cL: cytochrome c of low potential, cytochrome cH: cytochrome c of high potential.

Methanol dehydrogenase reduces pyrroloquinoline quinone (PQQ, Figure 12.3) coupled to the oxidation of methanol to formaldehyde. In addition to methanol dehydrogenase, PQQ with a redox potential of þ0.12 V serves as the coenzyme of glucose dehydrogenase in Pseudomonas putida and alcohol dehydrogenase in acetic acid bacteria Formaldehyde is oxidized to carbon dioxide either in free or in bound forms. Formaldehyde dehydrogenase and formate dehydrogenase oxidize formaldehyde in the free form to CO2 via formate in Gram-positive methylotrophs such as Amycolatopsis methanolica and in the autotrophic methylotroph Paracoccus denitrificans.



coenzyme. Methylobacterium extorquens has both sets of enzymes that catalyze oxidation of C1 compounds bound either to H4F or H4MTP. Since the enzyme activities are higher for C1 compounds bound toH4MTP, it is believed that formaldehyde is oxidized after being bound to H4MTP in this bacterium (Figure 12.2). H4F is used inmethylotrophsthat employ the serine–isocitrate lyase pathway to convert formaldehyde to cell materials (Figure 12.3) Methylophilus methylotrophus does not possess enzymes that oxidize formaldehyde in the free form or bound to C1 carriers. This bacterium oxidizes formaldehyde to CO2 through the ribulose monophosphate cycle (Figure 12.4).

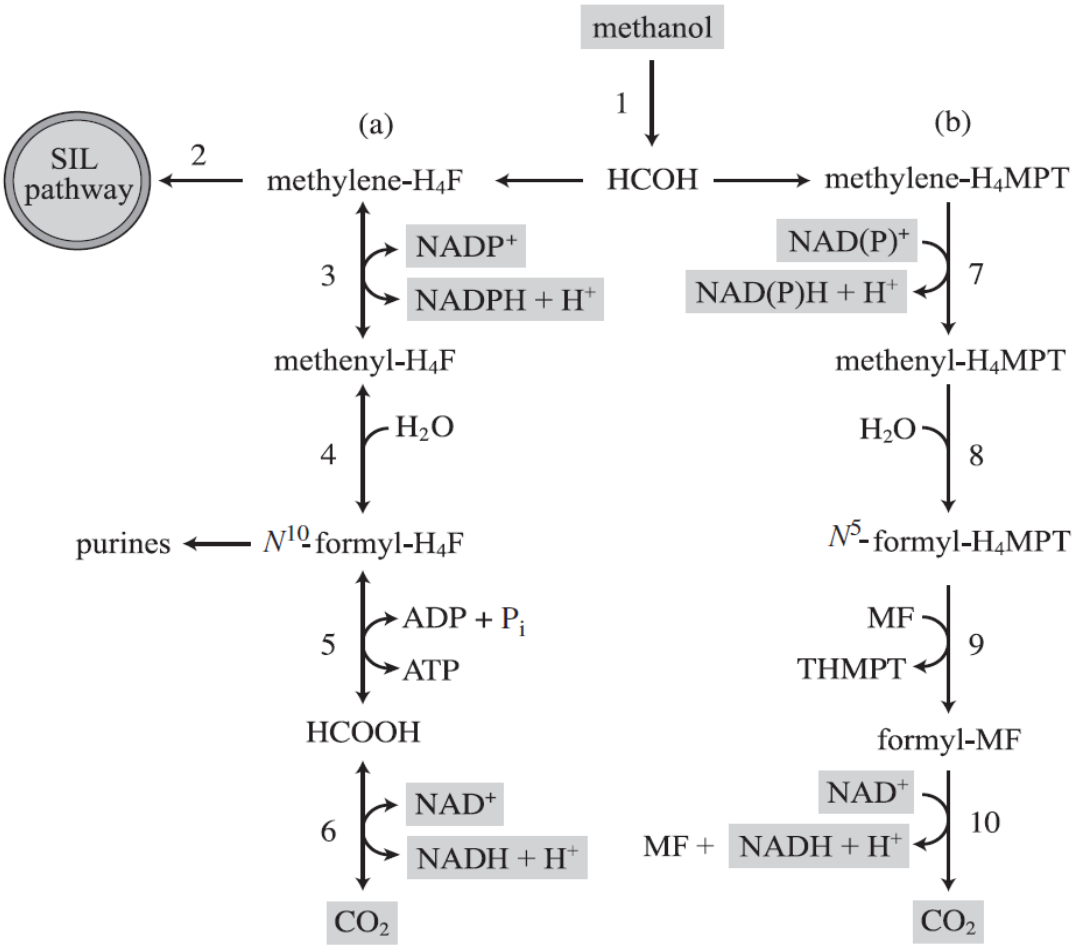


Fig 12.3: Methanol oxidation by Methylobacterium extorquens. (J. Bacteriol. 180:5351–5356, 1998)

This methylotrophic Gramnegative bacterium uses tetrahydrofolate (H4F) and tetrahydromethanopterin (H4MP) as C1 carriers, and has enzymes active on C1 compounds carried by H4F and H4MP. H4MP (b) is the main C1 carrier in this bacterium while other methylotrophs use H4F (a) as their main C1 carrier. 1, methanol dehydrogenase; 2, serine H4F hydroxymethyl transferase; 3, methylene H4F dehydrogenase;4, methenyl H4F cyclohydrolase; 5, formyl H4F synthetase; 6, formate dehydrogenase; 7, methylene H4MTP dehydrogenase; 8, methenyl H4MTP cyclohydrolase; 9, formyl methanofuran H4MTP formyltransferase; 10, formylmethanofuran (formyl-MF) dehydrogenase.

12.4. CARBON ASSIMILATION BY METHYLOTROPHS:

Obligate methylotrophs cannot use multicarbon compounds as their carbon source. Obligate methylotrophs and eterotrophic methylotrophs employ either the ribulose monophosphate (RMP) pathway to assimilate ormaldehyde or the serine–isocitrate lyase (SIL) pathway to assimilate formaldehyde and CO2. Autotrophic methylotrophs fix CO2 through the Calvin cycle. Methylotrophic yeasts growing on methanol employ yet another novel pathway, the xylulose monophosphate (XMP) pathway.

12.4.1. Ribulose Monophosphate (RMP) Pathway:

The RMP pathway that assimilates formaldehyde as triose phosphate is a collection of four different pathways all sharing the first two reactions. RMP accepts formaldehyde to form hexulose-6-phosphate that is isomerized to fructose-6-phosphate **(Figure 12.4).**

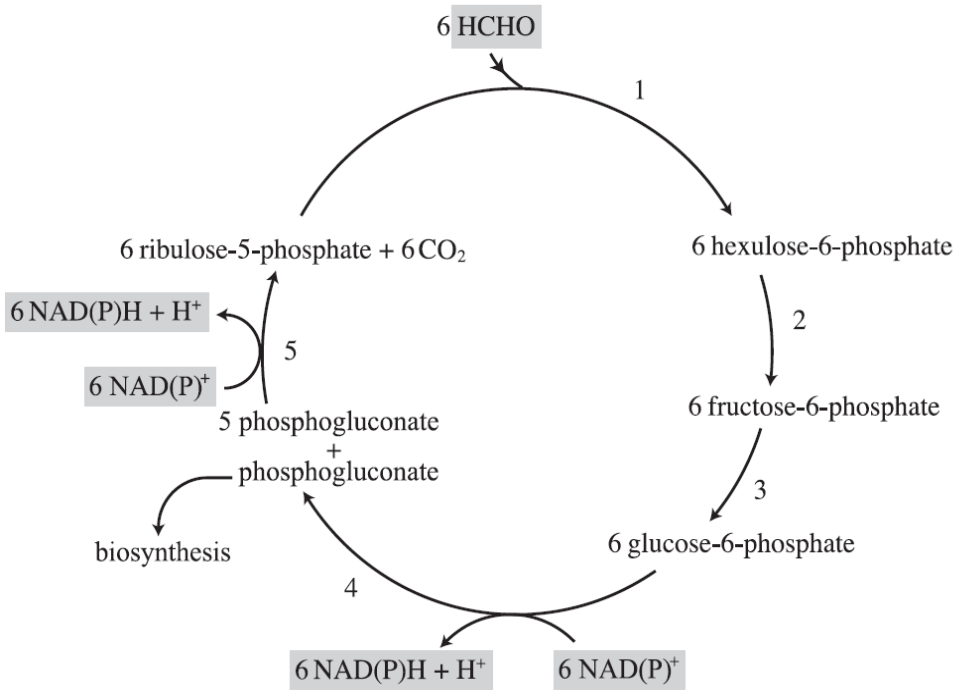


Fig. 12.4: Oxidation of formaldehyde through the ribulose monophosphate cycle in Methylophilus methylotrophus.1, hexulose phosphate synthase; 2, hexulose-6-phosphate isomerase;3, fructose-6-phosphate isomerase;4, glucose-6-phosphate dehydrogenase; 5, 6-phosphogluconate dehydrogenase.

Fructose-6-phosphate is cleaved through two alternative routes, one involving fructose-1,6-diphosphate aldolase (RMP-EMP variant, Figure 12.5a) and the other 2-keto-3-deoxy-6-phosphogluconate aldolase (RMP-ED variant, Figure 12.5b). The resulting triose phosphate and pyruvate are used in assimilatory metabolism. The remaining triose phosphate is used to regenerate ribulose-5-phosphate through carbon rearrangement with two molecules of fructose-6-phosphate. As shown in Figure 12.5, the carbon rearrangement takes place in two different ways depending on the organism: one involves transaldolase and transketolase (TA variant, Figure 12.5a) while the other involves fructose-1,6-diphosphate aldolase (FDA variant, Figure 12.5b).

Each variant of RMP pathway can be summarized as: EMP–TA variant: 3HCHOþATP! glyceraldehyde-3-phosphate EMP–FDA variant: 3HCHOþ2ATP! dihydroxyacetonephosphate ED–TA variant: 3HCHO ! pyruvateþ NAD(P)H ED–FDA variant: 3HCHOþATP!pyruvateþNAD(P)H

12.4.2. Serine–Isocitrate Lyase (SIL) Pathway:

Formaldehyde is the source of all the carbons of triose phosphate synthesized through the RMP pathway, but Methylosinus trichosporium uses formaldehyde and CO2 to synthesize 2-phosphoglycerate via acetyl-CoA. This metabolic pathway is referred to as the serine–isocitrate lyase (SIL) pathway. SIL can be considered in two parts: (1) acetyl-CoA synthesis from formaldehyde and CO2 through 2-phosphoglycerate and (2) acetyl-CoA conversion to serine via glyoxylate). Formaldehyde forms methylene-H4F with tetrahydrofolate (H4F) before condensing with glycine to serine.

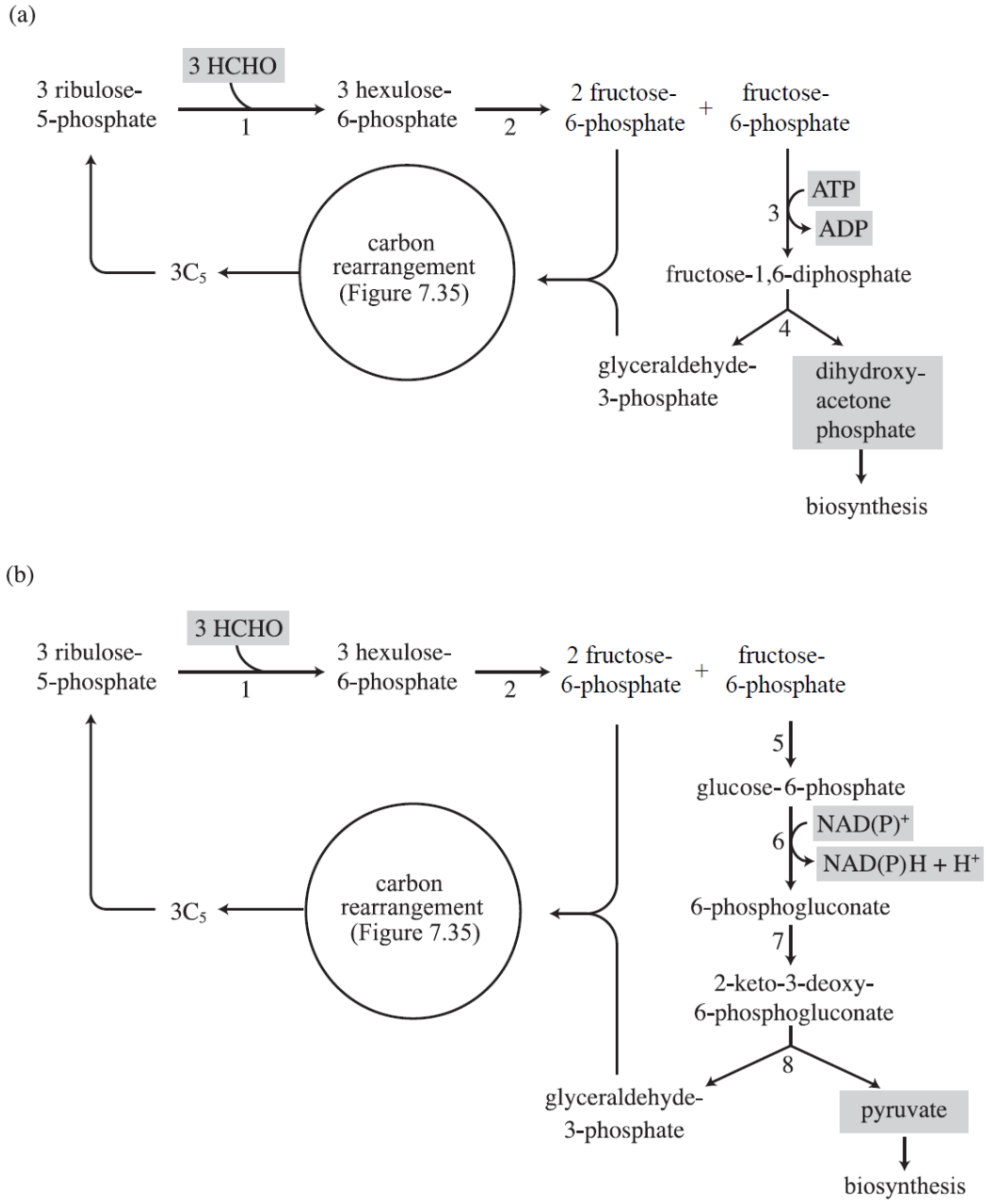


Fig 12.5: The ribulose monophosphate pathway. The carbon rearrangement reactions in the circles are shown in Figure 7.35. (a) RMP – Modified EMP pathway. (b) RMP – Modified ED pathway. 1, hexulose phosphate synthase; 2, hexulose phosphate isomerase; 3, phosphofructokinase; 4, fructose-1,6-diphosphatealdolase; 5, hexose phosphate isomerase; 6, glucose-6-phosphate dehydrogenase; 7, 6-phosphogluconate dehydratase; 8, 2-keto-3-deoxy-6-phosphogluconate aldolase.

12.5. SUMMARY:

Methanotrophy is the process by which microorganisms, known as **methanotrophs**, etabolize methaneCH4) as their sole source of carbon and energy. This crucial biological process serves as the only biological sink for methane and plays a vital role in mitigating the amount of this potent greenhouse gas released into the atmosphere.

12.6. SELF-ASSESSMENT

1) Carbon assimilation by Methylotrophs
2) Ribulose monophosphate (RMP) pathway
3) Serine–isocitrate lyase (SIL) pathway

12.7. REFERENCES:

1) Reddy and Reddy (2005). Microbial Physiology.
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th Edition
3) Nelson and Cox.2000; Lehninger Principles of Biochemistry
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology
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Prof. V. Umamaheswara Rao

LESSON-13

AMINO ACID BIOSYNTHESIS

13.0. OBJECTIVE:

- The study of microbial amino acid synthesis has several key objectives, ranging from fundamental biological understanding to practical industrial and medical applications.

STRUCTURE:

13.1. INTRODUCTION:

Amino acids are synthesized using carbon skeletons available from central metabolism. These are pyruvate, oxaloacetate, 2-ketoglutarate, 3-phosphoglycerate, phosphoenolpyruvate, erythrose-4-phosphate and ribose-5-phosphate (Table 6.5). Some amino acids are synthesized by different pathways depending on the organism. For convenience, those of Escherichia coli are discussed below.

13.2. THE PYRUVATE AND OXALOACETATE FAMILIES:

Pyruvate and oxaloacetate are converted to alanine and aspartate through reactions catalyzed by transaminase. In these reactions, glutamate is used as the -NH2 donor. Asparagine synthetase synthesizes asparagine from aspartate and ammonia consuming energy in the formof ATP in a similar reaction to that catalyzed by glutamine synthetase.

Table 13.1: Carbon Skeletons Used for Amino Acid Biosynthesis

Precursor	Amino acid
Pyruvate	alanine, valine, leucine
Oxaloacetate	aspartate, asparagine, methionine, lysine, isoleucine, threonine
2-ketoglutarate	glutamate, glutamine, arginine, proline
3-phosphglycerate	serine, glycine, cysteine
PEP and erythrose-4-phosphate	phenyalanine, tyrosine, tryptophan
Ribose-5-phosphate	histidine

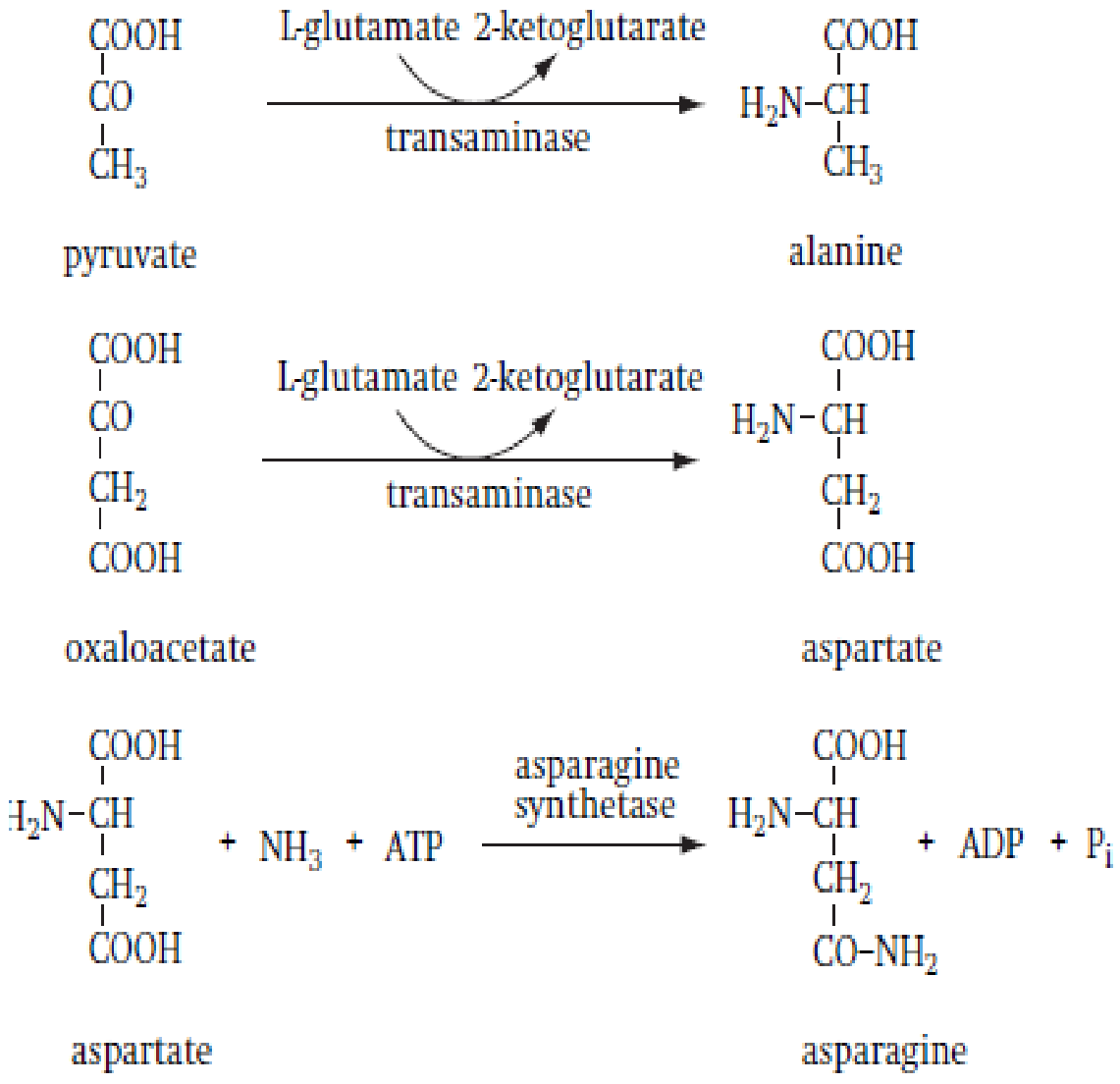


Fig 13.1: Threonine, methionine and lysine are produced from aspartate in addition to asparagine (Figure 13.1). An intermediate of lysine biosynthesis, diaminopimelate, is not found in protein, but is a precursor of murein, and so is ornithine, an intermediate in arginine biosynthesis Most prokaryotes employ the diaminopimelate pathway to synthesize lysine (Figure 13.1), but yeasts and fungi synthesize this amino acid from 2-ketoglutarate through the 2-aminoadipate pathway.

Threonine produced fromaspartate is deaminated to 2-ketobutyrate, which is used as the precursor for isoleucine biosynthesis. Two molecules of pyruvate are condensed to 2-acetolactate for the biosynthesis of valine and leucine. Since pyruvate and 2-ketobutyrate are similar in structure, a series of the same enzymes is used to synthesize isoleucine and valine.

13.3. THE PHOSPHOGLYCERATE FAMILY:

The EMP pathway intermediate 3-phosphoglycerate is converted to serine and then further to glycine and cysteine (Figure 13.3). The 2-ketoglutarate family Glutamate synthesized from 2-ketoglutarate through the reactions catalyzed by glutamate dehydrogenase or GOGAT is the precursor for the synthesis of proline, arginine and glutamine (Figure 13.4). N-acetylornithine deacetylase (reaction 9, Figure 13.5) has not been detected in coryneform bacteria, Pseudomonas aeruginosa and the yeast Saccharomyces cerevisiae. Instead, the reaction is catalyzed by N-acetylglutamate-acetylornithine acetyltransferase, coupling reactions 6 and 9 in Figure 13.3 in these organisms.

13.4. AROMATIC AMINO ACIDS:

The benzene ring of aromatic amino acids is formed from shikimate, which is produced from the condensation of erythrose-4-phosphate and phosphoenolpyruvate. Shikimate is further metabolized to

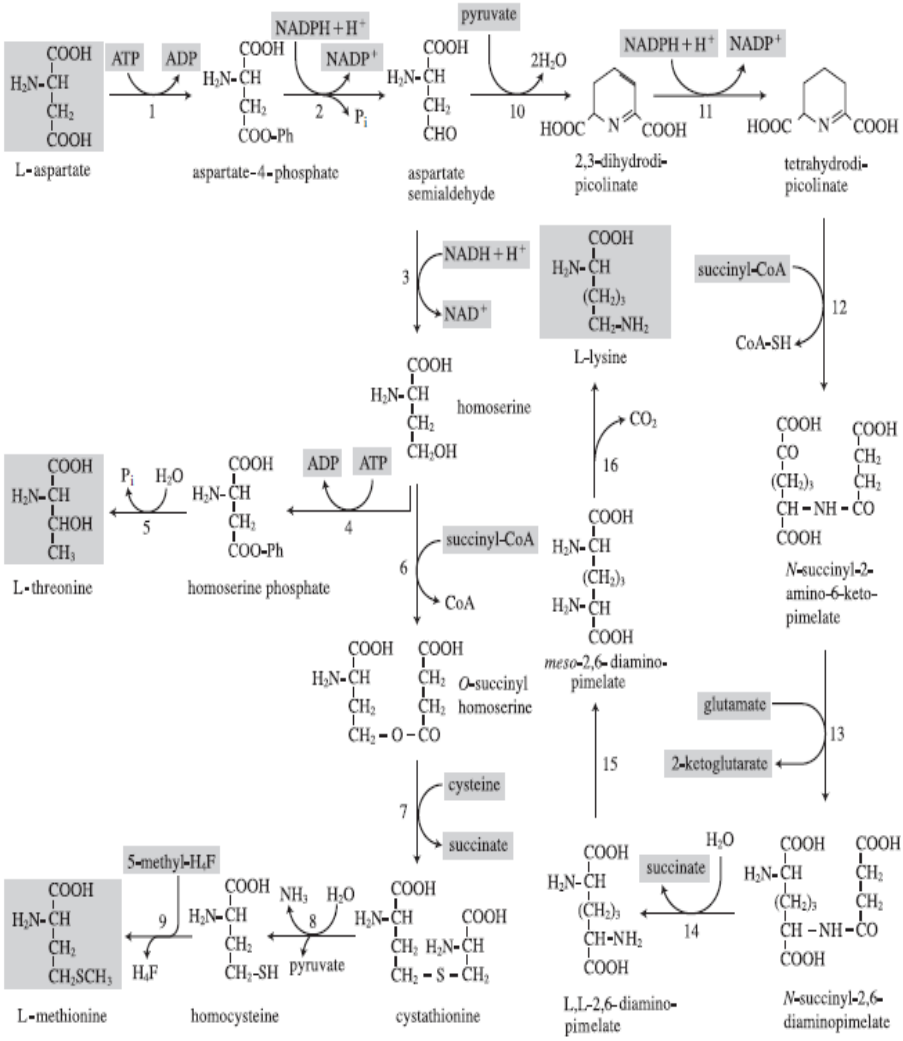


Fig 13.3: Biosynthesis of threonine, methionine and lysine from the common precursor, aspartate. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.6. Springer, New York) 1, aspartate kinase; 2, aspartate semialdehyde dehydrogenase; 3, homoserine dehydrogenase; 4, homoserine kinase; 5, threoninesynthase; 6, homoserine a cyltransferase; 7, cystathionine synthase; 8, cystathionine lyase; 9, homocysteine: 5-methyl-tetrahydrofolate methyltransferase; 10, dihydrodipicolinate synthase; 11, dihydrodipicolinate reductase; 12, tetrahydrodipicolinate succinylase; 13, glutamate: succinyl-diaminopimelate a minotransferase; 14, succinyl-diaminopimelate desuccinylase; 15, diaminopimelate epimerase; 16, diaminopimelate decarboxylase. H4F, tetrahydrofolate.

phenylpyruvate and p-hydroxyphenylpyruvate before being transaminated to phenylalanine and tyrosine, respectively. Transaminase catalyzes these reactions using glutamate as the -NH2 donor. Tryptophan is synthesized from indole-3-glycerol phosphate catalyzed by tryptophan synthase using serine as the -NH2 donor (Figure 13.6)

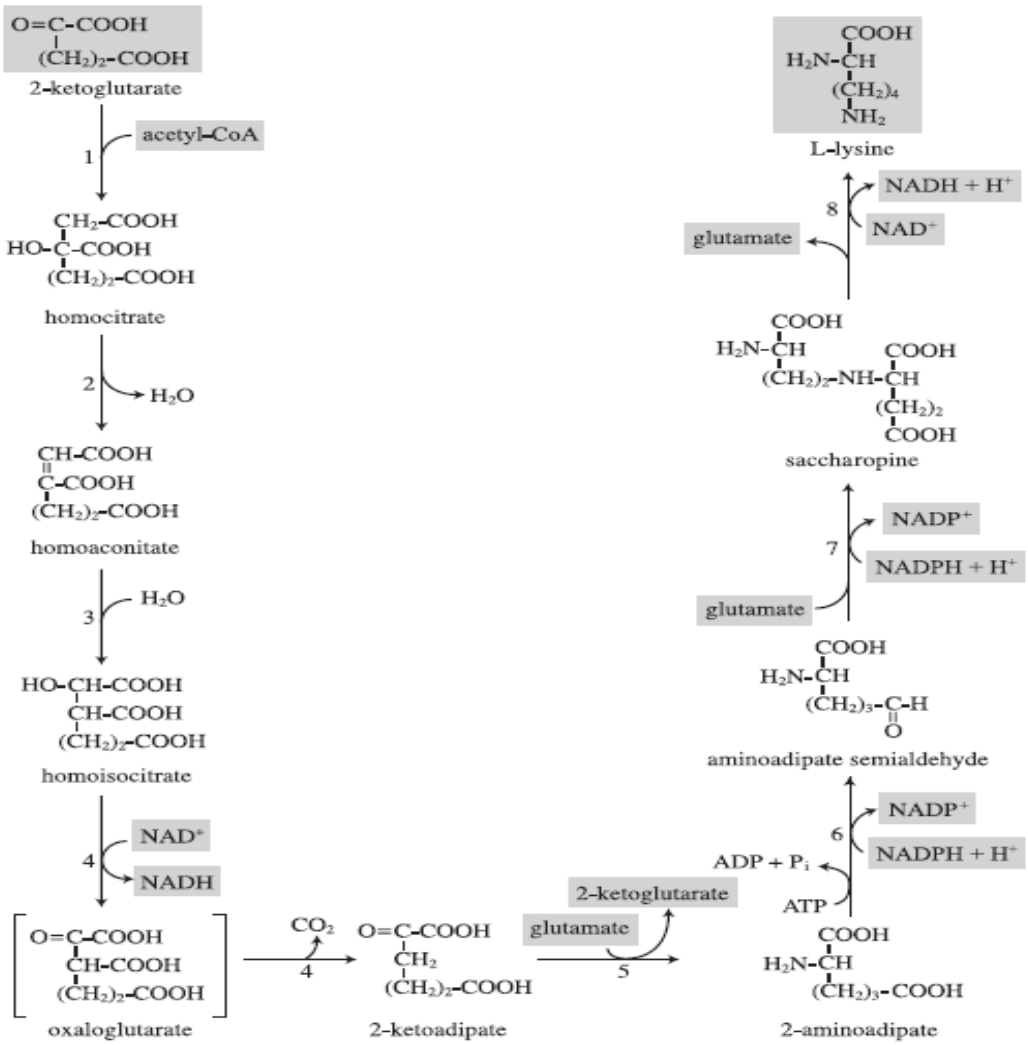


Fig 13.4: Lysine biosynthesis in yeasts and fungi through the 2-aminoadipate pathway. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.7. Springer, New York) 1, homocitrate synthase; 2, homocitrate dehydratase; 3, homoaconitate hydratase; 4, homoisocitrate dehydrogenase; 5, aminoadipate aminotransferase; 6, 2-aminoadipate semialdehyde dehydrogenase; 7, saccharopine dehydrogenase (glutamate forming); 8, saccharopine dehydrogenase (lysine-forming).

Indole-3-glycerol phosphate is formed through the condensation of anthranilate and 5-phospho-D-ribosyl-1-pyrophosphate (PRPP). PRPP is synthesized from ribose-5-phosphate taking pyrophosphate from ATP catalyzed by PRPP synthetase as below

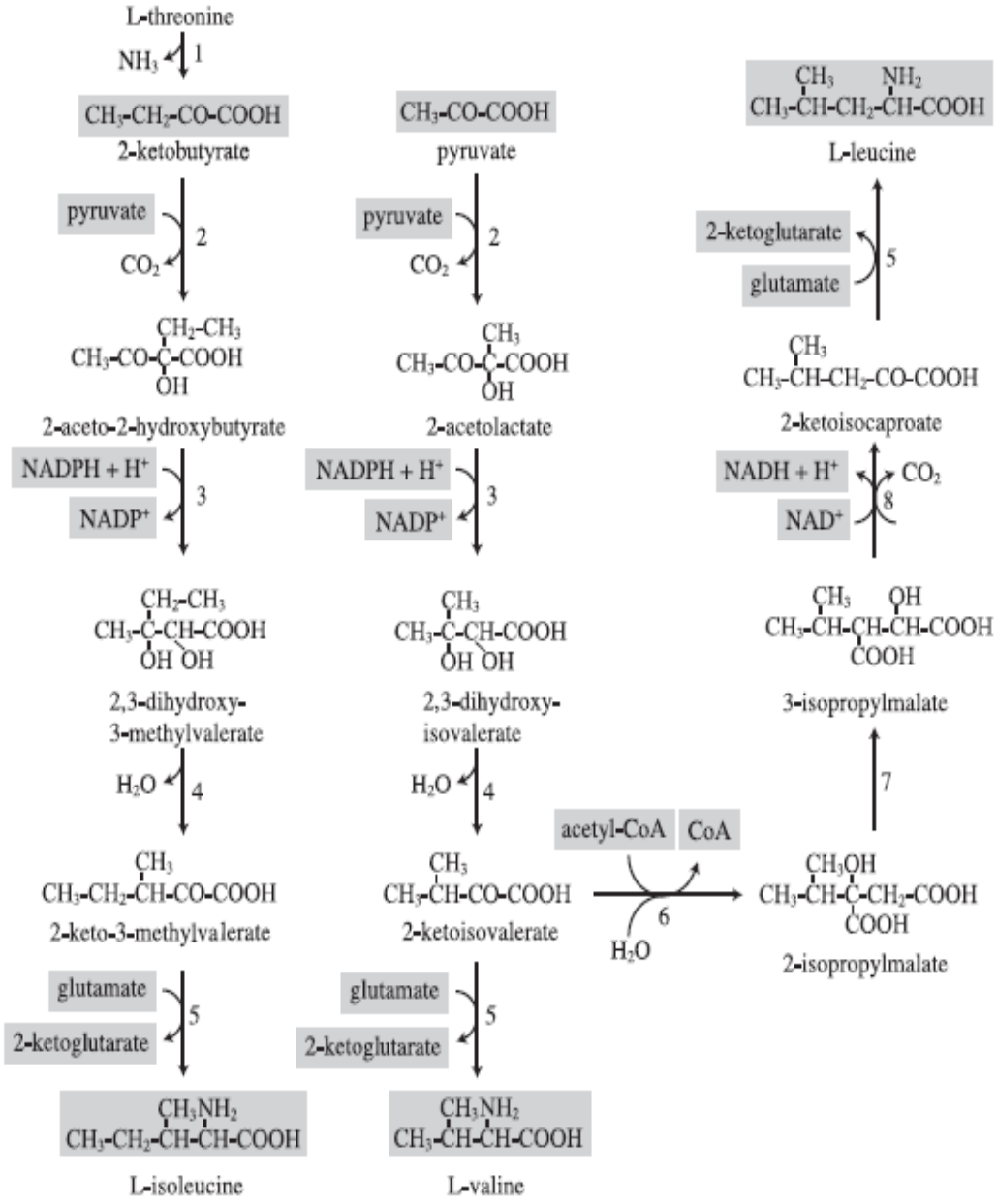
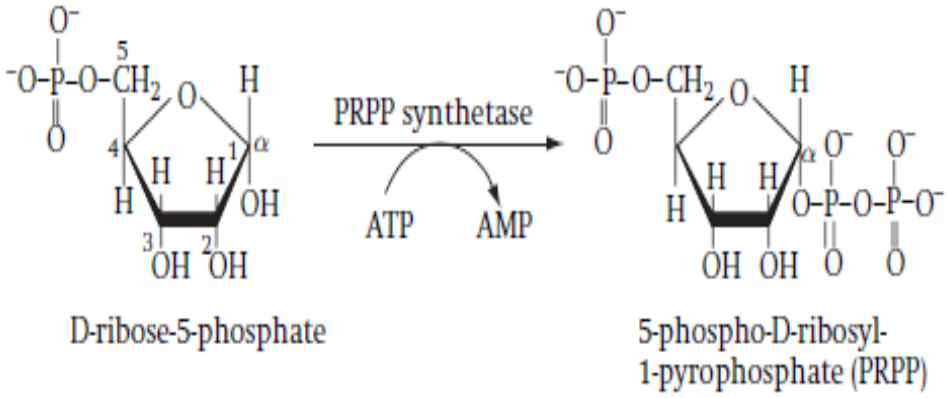


Fig 13.5: Biosynthesis of isoleucine, valine and leucine.

(Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.8. Springer, New York)

Isoleucine and valine are synthesized from 2-ketobutyrate and pyruvate, respectively. Since the precursors are similar in structure,the same enzymes catalyze the reactions.1, threonine dehydratase; 2, acetohydroxy acid synthase; 3, acetohydroxy acid isomeroreductase; 4, dihydroxy acid dehydratase; 5, transaminase; 6, alpha-isopropylmalate synthase; 7, isopropylmalate isomerase; 8, beta-isopropylmalate dehydrogenase.



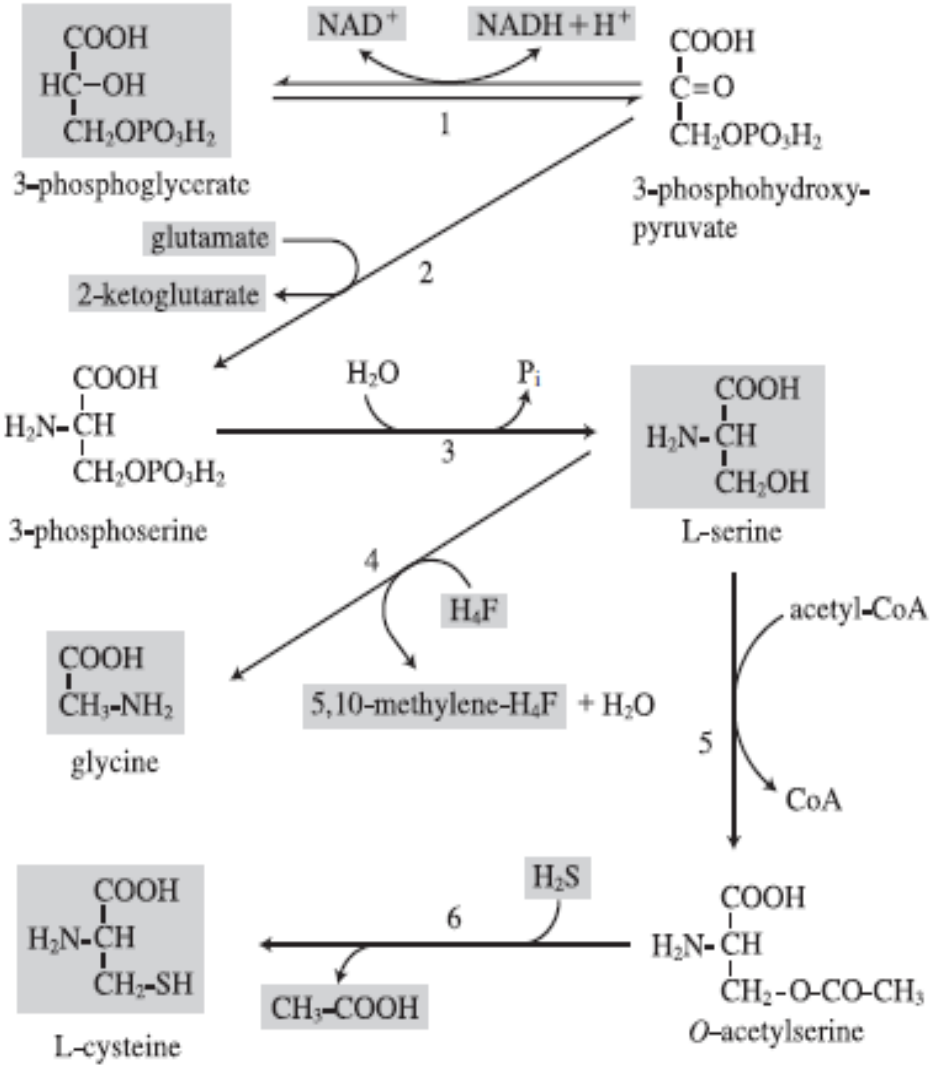


Fig 13.6: Serine, glycine and cysteine biosynthesis from 3-phosphoglycerate. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.9. Springer, New York)

1, phosphoglycerate dehydrogenase; 2, phosphoserine aminotransferase; 3, phosphoserine phosphatase; 4, serine hydroxymethyltransferase; 5, serine transacetylase; 6, O-acetylserinesulfhydrylase. H4F, tetrahydrofolate.

PRPP is the precursor for the synthesis of histidine and nucleotides. PRPP synthetase is regulated by a feedback inhibition mechanism by its biosynthetic products.

13.5. HISTIDINE BIOSYNTHESIS:

Histidine is produced from PRPP (Figure 6.19). 6.4.6 Regulation of amino acid biosynthesis Biosynthesis of amino acids is regulated according to their concentration. Enzyme activity is regulated by feedback inhibition while the.

13.6. ASSIMILATION OF INORGANIC NITROGEN:

Many cell constituents are nitrogenous compounds and include amino acids and nucleic acid bases. Nitrogen exists in various redox states ranging from !5 to þ3. Organic nitrogen is used preferentially over inorganic nitrogen by almost all microbes. When organic nitrogen, ammonia or nitrate is not available, some prokaryotes (within the bacteria and archaea) can reduce gaseous nitrogen to ammonia to meet their nitrogen requirements.

This process is known as nitrogen fixation and is not found in eukaryotes.

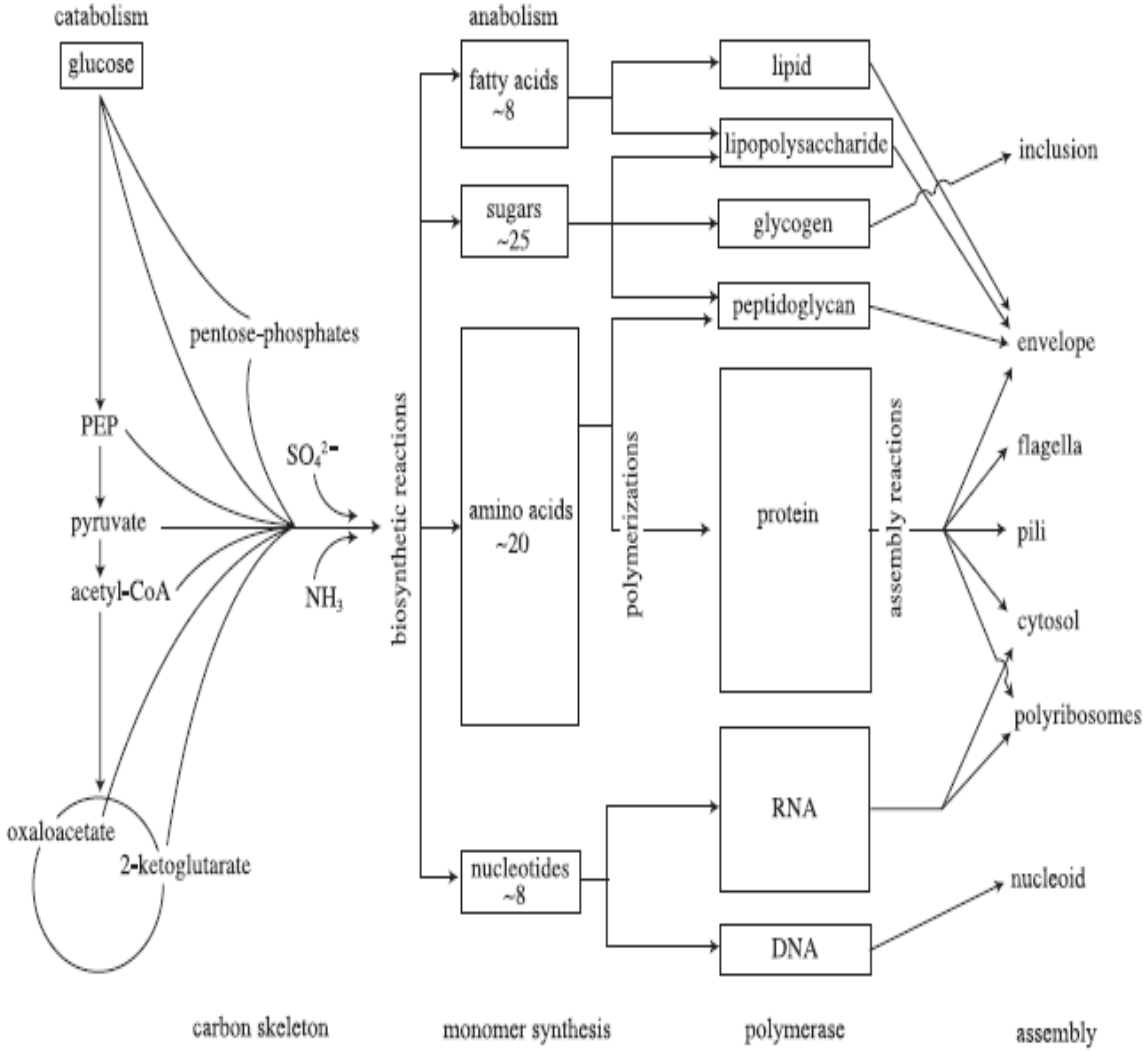


Fig 13.7: On the other hand, formaldehyde is bound to tetrahydrofolate (H4F) or to tetrahydromethanopterin (H4MTP) before being oxidized to CO2 inb many Gram-negative bacteria that convert formaldehyde to cell materials through the ribulose monophosphate or serine–isocitrate lyase pathway (Figure 13.7). H4MTP is regarded as a methanogen-specific coenzyme

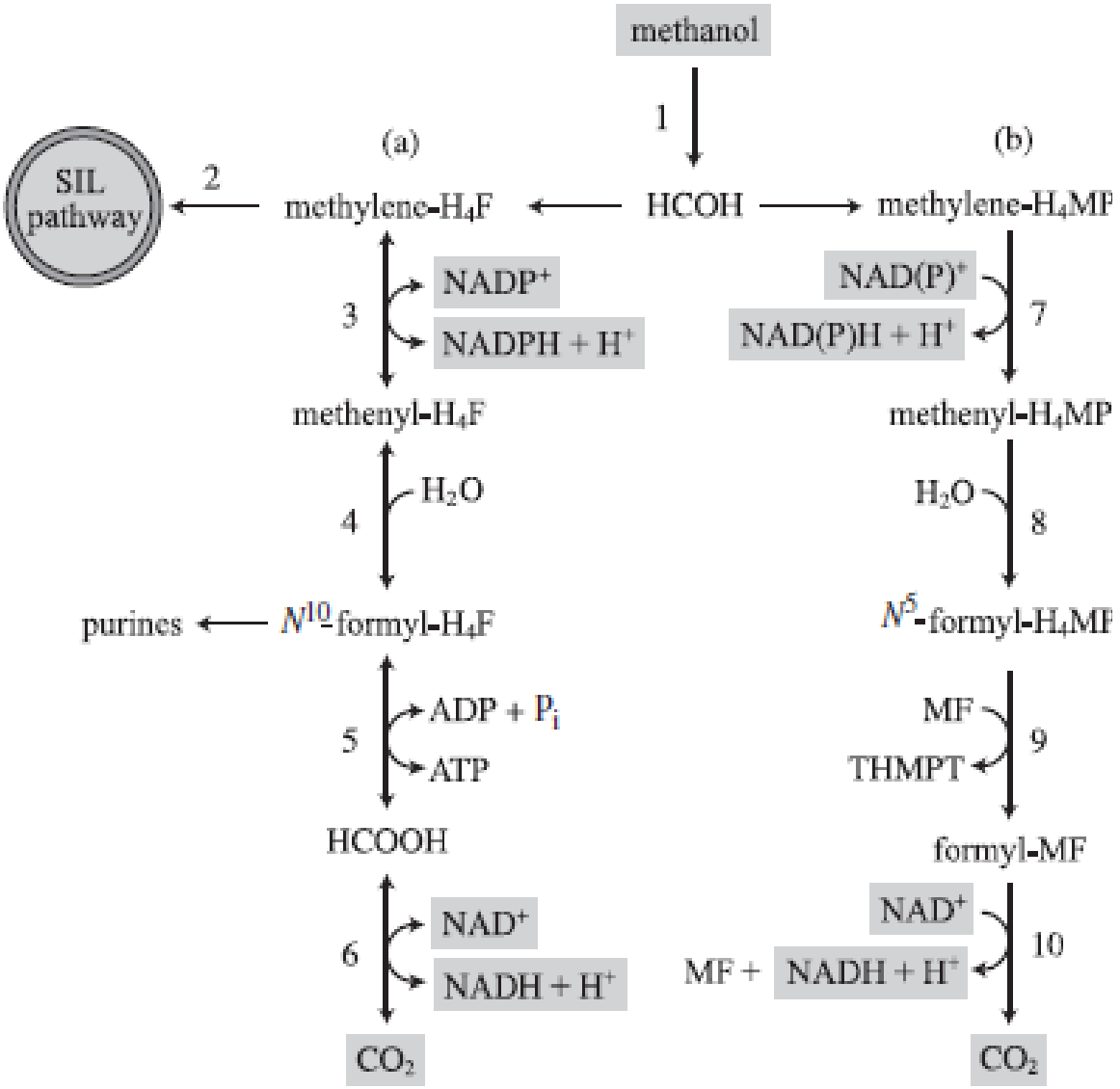


Fig 13.8: Methanol oxidation by Methylobacterium extorquens. (J. Bacteriol. 180:5351-5356, 1998). This methylotrophic Gramnegative bacterium uses tetrahydrofolate (H4F) and tetrahydromethanopterin (H4MP) as C1 carriers, and has enzymes active on C1 compounds carried by H4F and H4MP. H4MP (b) is the main C1 carrier in this bacterium while other methylotrophs use H4F (a) as their main C1 carrier. 1, methanol dehydrogenase; 2, serine H4F hydroxymethyltransferase; 3, methylene H4F dehydrogenase; 4, methenyl H4F cyclohydrolase; 5, formyl H4F synthetase; 6, formate dehydrogenase; 7, methylene H4MTP dehydrogenase; 8, methenyl H4MTP cyclohydrolase; 9, formyl methanofuran H4MTP formyltransferase; 10, formyl methanofuran (formyl-MF) dehydrogenase.

Methylobacterium extorquens has both sets of enzymes that catalyze oxidation of C1 compounds bound either to H4F (Figure 7.32a) Methylophilus methylotrophus does not possess enzymes that oxidize formaldehyde in the free form or bound to C1 carriers. This bacterium oxidizes formaldehyde to CO2 through the ribulose monophosphate cycle (fig 13.8).

13.7. SUMMARY:

Microbial amino acid synthesis involves complex metabolic pathways where microbes build amino acids from simpler precursors, primarily using glutamate and aspartate (derived from citric acid cycle intermediates like α-ketoglutarate and oxaloacetate) as central nitrogen donors, ultimately forming protein building blocks and other crucial molecules. These processes are vital for microbial growth and are heavily regulated, with organisms like *E. coli* and *C. glutamicum* serving as models for industrial production of valuable amino acids like lysine and glutamic acid (MSG) through fermentation, often using metabolic engineering.

13.8. SELF-ASSESSMENT:

1) Pyruvate and Oxaloacetate Families
2) The phosphoglycerate Family
3) Aromatic Amino Acids
4) Assimilation of Inorganic Nitrogen

13.9. REFERENCES:

1) Reddy and Reddy (2005). Microbial Physiology.
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th Edition
3) Nelson and Cox.2000; Lehninger Principles of Biochemistry
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology
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7) Gottschalk, G. Bacterial Metabolism

Dr. J. Madhavi

LESSON-14

LIPID BIOSYNTHESIS

14.0. OBJECTIVE:

- The primary objectives for studying microbial lipid synthesis revolve around producing sustainable alternatives to plant and animal oils for use in biofuels, food, cosmetics, and pharmaceuticals, and understanding the underlying biochemical and genetic mechanisms to optimize production.

STRUCTURE:

14.1. INTRODUCTION:

Phospholipids are essential cellular components as the major part of the membrane. Bacterial phospholipids are based on acylglyceride with an ester link between glycerol and fatty acids as in eukaryotic cells. The archaeal membrane contains phospholipids with an ether linkage between polyalcohol and polyisoprenoid alcohols. Fatty acids and polyisoprenoid alcohols are synthesized from acetyl-CoA.

14.2. FATTY ACID BIOSYNTHESIS:

Acetyl-CoA is converted to acyl-acyl carrier protein (acyl-ACP) through the action of seven enzymes. In eukaryotes these enzymes form a complex, but such a complex is not found in prokaryotes. Enzymes directly involved are 3-ketoacyl-ACP synthase, 3-ketoacyl- ACP reductase, 3-hydroxyacyl-ACP dehydratase and enoyl-ACP reductase. Isoenzymes are identified in all of them except 3-ketoacyl-ACP reductase (FabG). These isoenzymes have specific functions. Three isoenzymes of 3-ketoacyl-ACP synthase are I (FabB), II (FabF) and

III (FabH). FabA (3-hydroxydecanoyl-ACP dehydratase) and FabZ (3-hydroxyacyl-ACP dehydratase) have a similar function. Enoyl-ACP reductase also has three isozymes: I (FabI), II (FabK) and III (FabL). Fatty acid synthesis is initiated by 3-ketoacyl-ACP synthase III (FabH) catalyzing the formation of acetoacetyl-ACP from malonyl-ACP and acetyl-CoA. The other two 3-ketoacyl-ACP synthases do not react with malonyl-ACP, but catalyze the elongation reaction. Mutants (fabA! and fabB!) synthesize saturated fatty acids normally but the synthesis of unsaturated fatty acids is impaired. They are involved in unsaturated fatty acid synthesis.

14.3. SATURATED ACYL-ACP:

Acetyl-CoA is carboxylated to malonyl-CoA in a reaction catalyzed by acetyl-CoA carboxylase, and malonyl transacylase transfers the malonyl group to ACP. Acetyl-CoA carboxylase is a complex enzyme consisting of one molecule each of biotin carboxylase and biotin carboxyl carrier protein (BCCP), and two molecules of carboxyltransferase.

$$\text{BCCP} + \text{HCO}_3^- + \text{ATP} \xrightarrow{\text{biotin carboxylase}} \text{BCCP–COO}^- + \text{ADP} + \text{P}_i$$

$$\text{acetyl-CoA} + \text{BCCP–COO}^- \xrightarrow{\text{carboxyltransferase}} \text{malonyl-CoA} + \text{BCCP}$$

$$\text{malonyl-CoA} + \text{ACP} \xrightarrow{\text{malonyl transacylase}} \text{malonyl-ACP} + \text{CoA-SH}$$

Malonyl-ACP and acetyl-CoA condense to acetoacetyl-ACP, replacing the carboxyl group of malonyl-ACP with an acetyl group catalyzed by 3-ketoacyl-ACP synthase III (FabH). Acetoacetyl-ACP is reduced to 3-hydroxybutyryl-ACP by the action of 3-ketoacyl-ACP reductase (FabG) that uses NADPH as a coenzyme. Crotonyl-ACP is formed from 3-hydroxybutyryl-ACP through a dehydration reaction catalyzed by 3-hydroxyacyl-ACP dehydratase (FabZ). Enoyl-ACP reductase (FabI) reduces crotonyl-ACP to butyryl-ACP using NAD(P)H as a coenzyme. Butyryl-ACP condenses with malonyl-ACP to start the next cycle catalyzed probably by 3-ketoacyl-ACP reductase II (FabF) (Figure 14.1). The initiating 3-ketoacyl-ACP synthase III (FabH) does not catalyze the reverse reaction, and its regulation determines the rate of fatty acid synthesis.

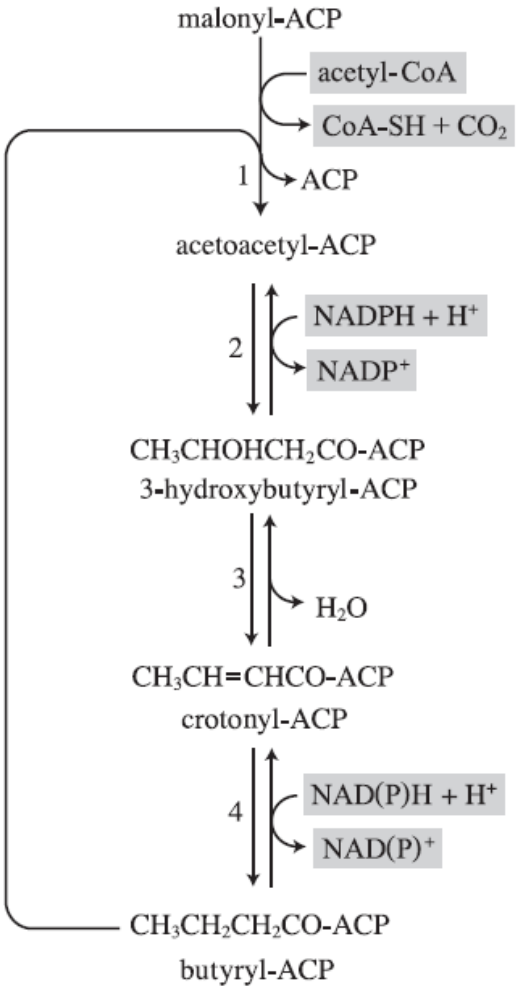


Fig 14.1: Synthesis of acyl-ACP. (Ann. Rev. Biochem. 74:791–831,2005)1, 3-ketoacyl-ACP synthase FabH for acety-CoA and malonyl-ACP, and FabF for further elongation); 2, 3-ketoacyl-ACP reductase (FabG); 3, 3-hydroxyacyl-ACP dehydratase (FabZ); 4, enoyl-ACP reductase (FabI).

14.4. BRANCHED ACYL-ACP:

Branched fatty acids are synthesized in two different ways. Branched building blocks such as isobutyryl-ACP or methylmalonyl- ACP result in branched fatty acids from similar reactions as in the straight-chain fatty acid biosynthetic pathway. The other pathway which synthesizes branched fatty acids involves methylation of unsaturated fatty acids as in the formation of cyclopropane fatty acids.

14.5. UNSATURATED ACYL-ACP:

Many unsaturated fatty acid residues are found in biological membranes. They are synthesized by two different mechanisms. These are the aerobic route found both in eukaryotes and prokaryotes and the anaerobic route which occurs in some bacteria. In the

anaerobic route the double bond is formed during fatty acid biosynthesis (Figure 14.2). In saturated fatty acid synthesis, 3-hydroxyacyl-ACP dehydratase (FabA) dehydrates 3-hydroxyacyl-ACP to trans-2,3-enoyl-ACP that can be reduced by enoyl-ACP reductase (FabI). For unsaturated fatty acid synthesis, trans-2,3-enoyl-ACP is isomerized to cis-3,4-enoyl-ACP by the bifunctional 3-hydroxyacyl-ACP dehydratase (FabA). Enoyl-ACP reductase (FabI) cannot reduce cis-3,4-enoyl-ACP. This cis-3,4-enoyl- ACP becomes a substrate for the elongation catalyzed by a separate 3-ketoacyl-ACP synthase (FabF). FabF is expressed constitutively and unstable under physiological temperature. More unsaturated fatty acids are produced at a reduced growth temperature when this enzyme becomes stable.

Saturated fatty acids are oxidized in an aerobic route to produce unsaturated fatty acids. Acyl-ACP oxidase catalyzes this reaction, consuming O2, to oxidize NADPH to water:

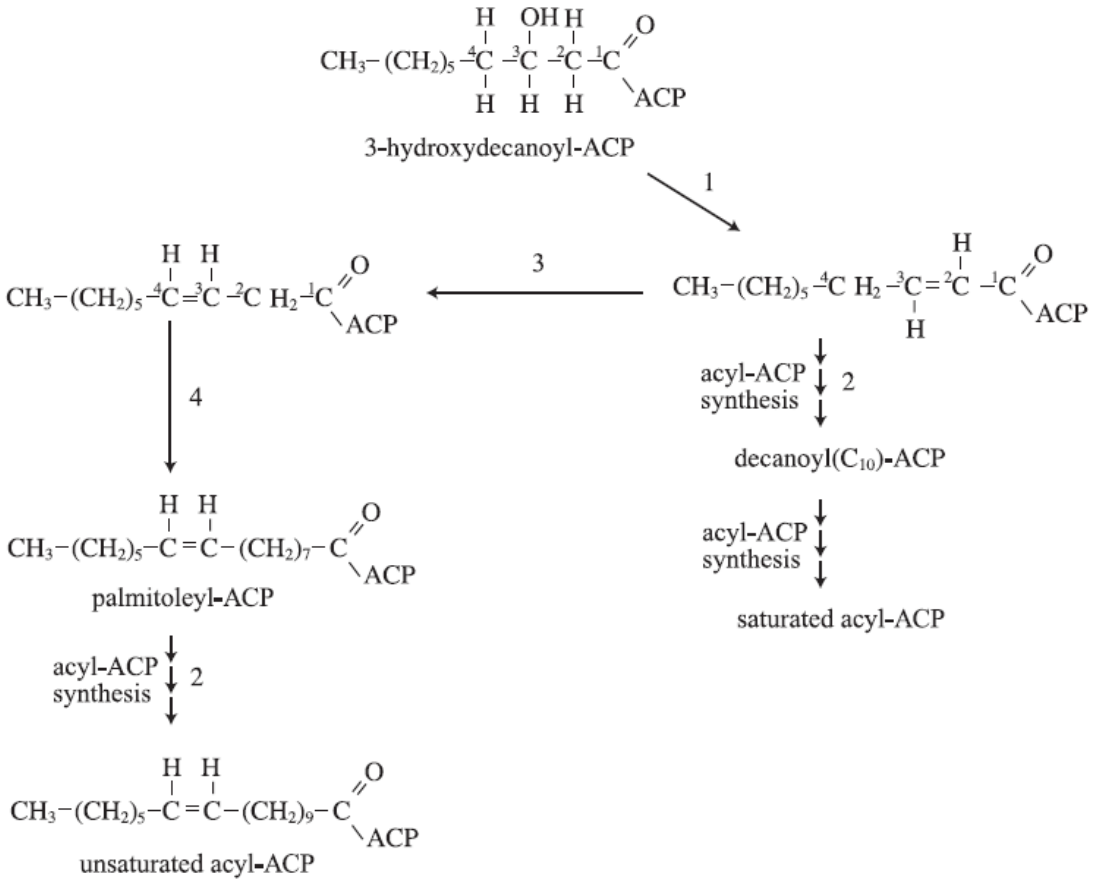
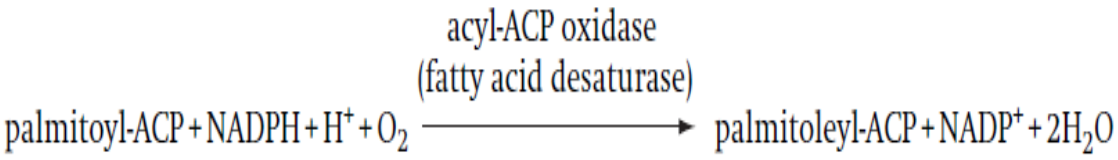


Fig 14.2: Unsaturated acyl-ACP synthesis by the anaerobic route. (Ann. Rev. Biochem. 74:791–831, 2005) The dual function 3-hydroxydecanoyl-ACP dehydratase (FabA) removes a water molecule from 3-hydroxydecanoyl-ACP, producing trans-2,3-enoyl-ACP (1). For the synthesis of saturated fatty acid residues, the double bond is reduced by 3-ketoacyl-ACP reductase (FabG, 2). On the other hand, the dual function 3-hydroxydecanoyl-ACP dehydratase (FabA) isomerizes trans-2,3-enoyl-ACP to cis-3,4-enoyl-ACP (3) that cannot be reduced by 3-ketoacyl-ACP reductase (FabG), but is condensed with malonyl-ACP (4) catalyzed by hydroxyacyl-ACP synthase I (FabB). FabA and FabB are the key enzymes of the synthesis of unsaturated fatty acids, and are expressed constitutively. The enzymes are unstable under physiological temperatures, and become stable, producing more unsaturated fatty acid at lower temperatures.



The fatty acid desaturase is a membrane-bound enzyme in bacteria while eukaryotes have soluble enzymes. The expression of this enzyme is activated when the membrane fluidity becomes low at a Unsaturated acyl-ACP synthesis by the anaerobic route.

The dual function 3-hydroxydecanoyl-ACP dehydratase (FabA) removes a water molecule from 3-hydroxydecanoyl-ACP, producing trans-2,3-enoyl-ACP (1). For the synthesis of saturated fatty acid residues, the double bond is reduced by 3-ketoacyl-ACP reductase (FabG, 2). On the other hand, the dual function 3-hydroxydecanoyl-ACP dehydratase (FabA) isomerizes trans-2,3-enoyl-ACP to cis-3,4-enoyl-ACP (3) that cannot be reduced by 3-ketoacyl-ACP reductase (FabG), but is condensed with malonyl-ACP (4) catalyzed by hydroxyacyl-ACP synthase I (FabB). FabA and FabB are the key enzymes of the synthesis of unsaturated fatty acids, and are expressed constitutively. The enzymes are unstable under physiological temperatures, and become stable, producing more unsaturated fattyacid at lower temperatures.

14.6. LIPID BIOSYNTHESIS:

Some fatty acid desaturases reduce fatty acid residues bound to ACP, and others in the form of phospholipids. The yeast Saccharomyces cerevisiae can grow fermentatively on glucose, but cannot grow under strictly anaerobic conditions unless supplemented with unsaturated fatty acids and ergosterol as growth factors. These lipids cannot be synthesized without molecular oxygen in yeast.

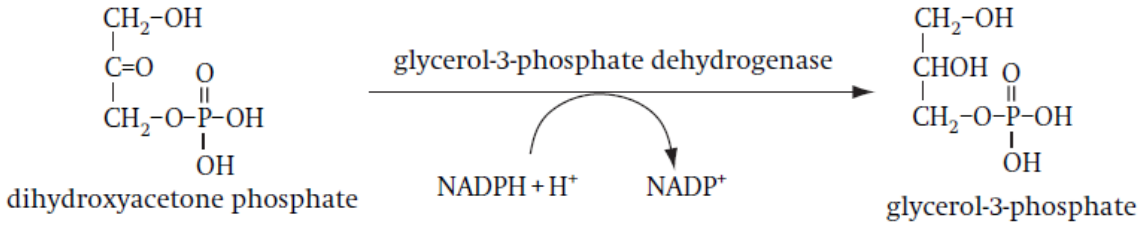
14.6.1. Regulation of Fatty Acid Biosynthesis:

The fatty acid composition of amembrane varies depending on growth conditions and culture age in a given bacterium. The rate of fatty acid synthesis is determined by the activity of the initiating enzyme, 3-ketoacyl-ACP synthase III (FabH). The length of the fatty acid depends on regulation of the 3-ketoacyl-ACP synthase II (FabF) activity. Bacteria growing at a sub-optimum temperature synthesize more unsaturated fatty acids to maintain membrane fluidity. Unsaturated fatty acid synthesis through the anaerobic route is increased through the increase in stability of the enzymes, 3-hydroxydecanoyl-ACP dehydratase (FabA) and 3-ketoacyl-ACP synthase I (FabB). In the aerobic route, more unsaturated fatty acids are produced through the increased expression of the fatty acid desaturase gene. In Bacillus subtilis,a gene for fatty acid desaturase is expressed under cold-shock conditions.

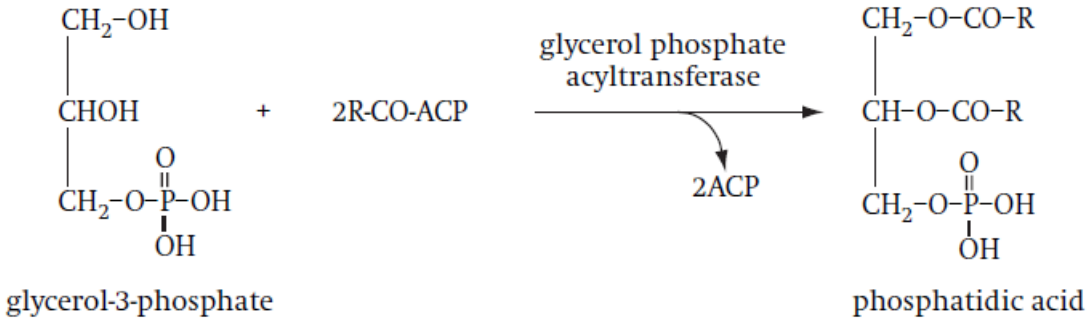
Escherichia coli synthesizes cyclopropane fatty acid (CFA) in the stationary phase. CFA-negative mutants are less resistant to freezing, which suggests that CFA is involved in survival of the bacterium. The CFA synthase gene is recognized by the stationary phase sigma factor of RNA polymerase ("s)

14.6.2. Phospholipid Biosynthesis:

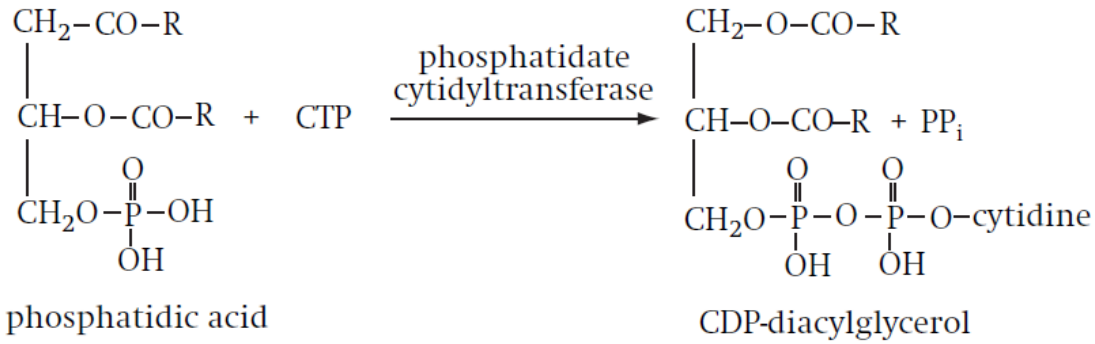
The EMP pathway intermediate, dihydroxyacetone phosphate, is reduced to glycerol-3-phosphate oxidizing NADPH:



Glycerol-3-phosphate acyltransferase then synthesizes phosphatidic acid consuming two acyl-ACPs. Phosphatidic acid serves as a precursor for the synthesis of phospholipids and triglycerides.



The bacterial cytoplasmic membrane contains phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol, and others. These phospholipids are phosphatidic acid derivatives containing alcohol esters linked to the phosphate residue. Phosphatidic acid is activated to CDP diacylglycerol, consuming cytidine 50-triphosphate (CTP) to receive alcohols.



Cytidine 50-monophosphate (CMP) is replaced with alcohols such as serine and inositol in a reaction catalyzed by alcohol-specific phosphatidyl transferase. Phosphatidylserine is decarboxylated to phosphatidylethanolamine before being synthesized to phosphatidylcholine through methylation using S-adenosylmethionine (SAM) as the source of the methyl group. Similar reactions are employed to produce phosphatidylinositol, phosphatidylglycerol and cardiolipin (Figure 14.3).

As in phosphatidylcholine synthesis, C1 units such as methyl groups are transferred in various reactions involving the C1 carrier's tetrahydrofolate (H4F) and SAM. H4F participates in the reactions which add or remove all forms of C1 units except carbonate, including methyl (-CH3), methylene (-CH2-), methenyl (-CH=), formyl (-CHO) and formimino (-CH=NH) as shown in Figure 6.25. SAM functions as a -CH3 donor. Methanogens have their own C1 carriers such as coenzyme M, tetrahydromethanopterin (H4MTP) and methanofuran (MF). Some of these are found in other archaea and in some eubacteria.

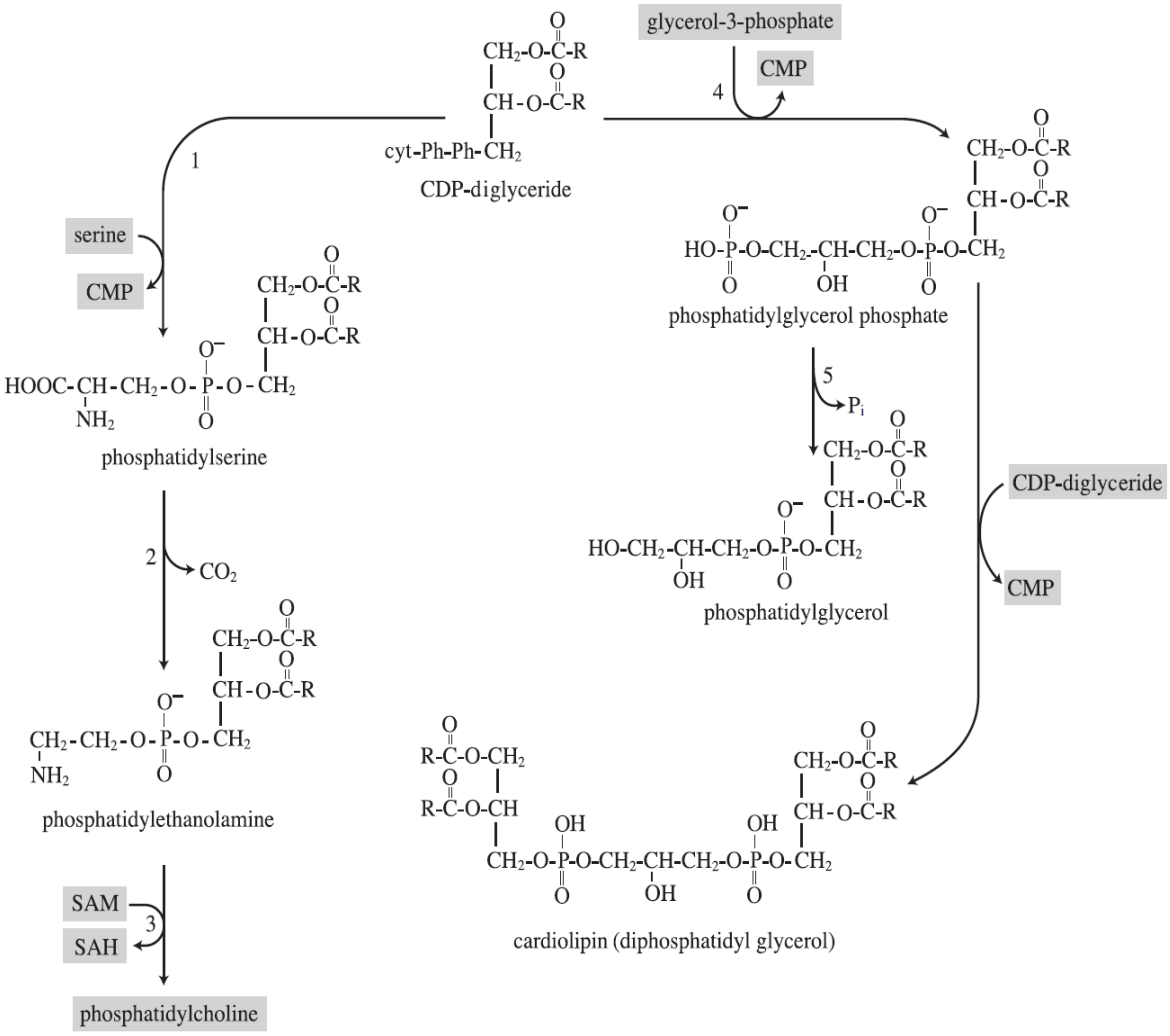


Fig 14.3: Phospholipid biosynthesis. Phosphatidic acid is activated to CDP-diglyceride in a reaction catalyzed by phosphatidate cytidyltransferase before an alcohol replaces CMP.

1, CDP-diacylglyceride: serine O-phosphatidyl transferase; 2, phosphatidylserine decarboxylase; 3, phosphatidylethanolamine methyltransferase; 4, CDP-diacylglyceride: glycerol-3-phosphate 3-phosphatidyltransferase; 5, phosphatidylglycerol phosphatase SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; CMP, cytidine 50-monophosphate.

14.7. SUMMARY:

Microbial lipid synthesis involves oleaginous microorganisms (yeasts, fungi, algae, bacteria) converting carbon sources (like sugars from biomass) into intracellular lipids (fats), primarily triacylglycerols, for energy storage or structural functions, often triggered by nutrient limitation (like nitrogen depletion) after initial cell growth, and serves as a sustainable source for biofuels (biodiesel) and food ingredients due to fast production and ability to use waste streams, utilizing pathways like fatty acid synthesis from acetyl-CoA and NADPH.

14.8. SELF-ASSESSMENT

1) Fatty acid biosynthesis
2) Saturated acyl-ACP
3) Branched acyl-ACP
4) Unsaturated acyl-ACP
5) Lipid Biosynthesis
6) Regulation of fatty acid biosynthesis
7) Phospholipid biosynthesis

14.9. REFERENCES:

1) Reddy and Reddy (2005). Microbial Physiology.
2) Freeman, W.H. (2001). Biochemistry, by Stryer, 5th Edition
3) Nelson and Cox.2000; Lehninger Principles of Biochemistry
4) Moat, A.G and J.W. Foster (1999). Microbial Physiology
5) Caldwell, D.R.1995. Microbial Physiology and Metabolism
6) David White.1995. The Physiology and Biochemistry of Prokaryotes
7) Gottschalk, G. Bacterial Metabolism

Dr. J. Madhavi

LESSON-15

BACTERIAL CELL WALL SYNTHESIS

15.0. OBJECTIVE:

- To understand fundamental bacterial physiology and to inform the development of novel antimicrobial therapies.

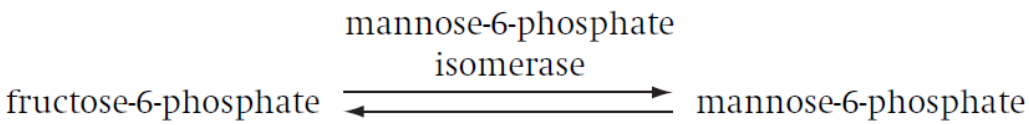
STRUCTURE:

15.1. INTRODUCTION:

Microbial cells contain various saccharides located in the cell wall, lipopolysaccharide of the outer membrane in Gram-negative bacteria, capsular material and glycogen. These polymers are synthesized from activated monomers derived from glucose-6-phosphate. (Fig.15.1). The latter can be produced not only from sugars but also from noncarbohydrate substrates through gluconeogenesis. (Fig15.2)

15.2. HEXOSE PHOSPHATE AND UDP-SUGAR:

Fructose-6-phosphate is isomerized to mannose-6-phosphate:



Polysaccharides containing galactose are synthesized from UDP galactose which is converted fromglucose-6-phosphate in three steps.

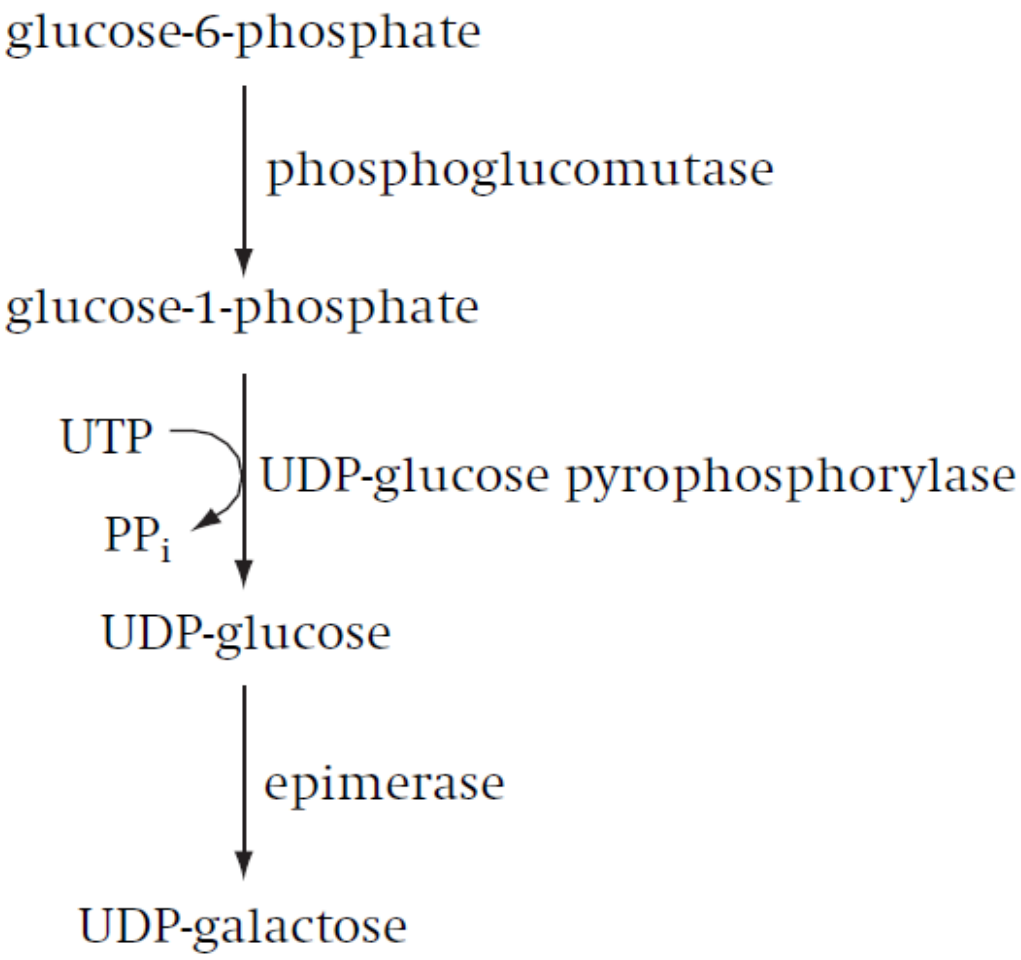


Fig 15.1: Synthesis of isopentenyl pyrophosphate (IPP), the precursor of isoprenoids through the mevalonate pathway or through the mevalonate-independent pathway. (Mol. Microbiol. 37:703–716, 2000)

1, hydroxylmethylglutaryl-CoA synthase; 2, hydroxymethylglutarate reductase; 3, mevalonate kinase; 4, phosphomevalonate kinase;5, diphosphomevalonate decarboxylase; 6, 1-deoxy-D-xylulose-5-phosphate synthase; 7, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; 8, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase; 9, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase;10, 2C-methyl-D-erythritol-2,4-cyclodiphosphate synthase

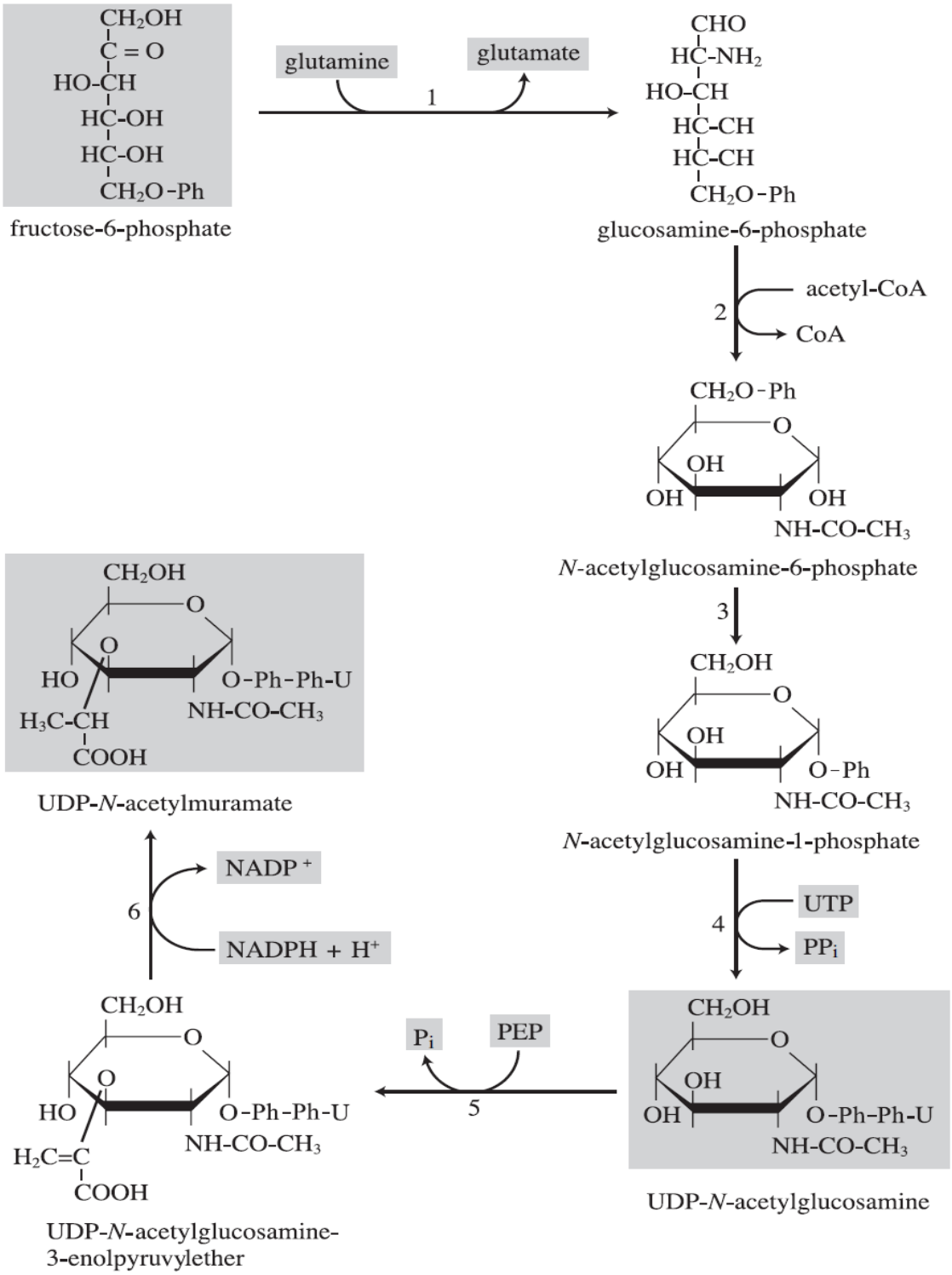


Fig 15.2: Synthesis of murein monomers, UDP-Nacetylglucosamine and UDPN-acetylmuramate. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.24. Springer, New York)

1, glutamine: fructose-6-phosphate aminotransferase; 2, glucosamine phosphate transacetylase; 3, Nacetylglucosamine phosphomutase;4, UDP-N-acetylglucosamine pyrophosphorylase; 5, UDP-Nacetylglucosamine-3-enolpyruvylether synthase; 6, UDPN-acetylenolpyruvyl glucosaminereductase.

15.3. MONOMERS OF MUREIN:

Fructose-6-phosphate is used to synthesize the murein monomers, uridine diphosphate (UDP)-N-acetylglucosamine and UDP-Nacetylmuramate. The precursor is aminated to glucosamine-6-phosphate using glutamate as the amine group donor before being acetylated to N-acetylglucosamine-6-phosphate. The latter is activated to UDP-N-acetylglucosamine condensing with UTP. PEP is used to add enolpyruvate to this intermediate before being reduced to UDP-N-acetylmuramate consuming NADPH (Figure 15.3).

Amino acids are added to UDP-N-acetylmuramate to synthesize UDP-N-acetylmuramylpentapeptide. L-alanine, D-glutamate, mesodiaminopimelate and D-alanyl-D-alanine form a peptide on the lactyl Synthesis of murein monomers, UDP-N acetylglucosamine and UDPN- acetylmuramate. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.24.Springer, New York)

1, glutamine: fructose-6-phosphate aminotransferase; 2, glucosamine phosphate transacetylase; 3, Nacetylglucosamine phosphomutase;4, UDP-N-acetylglucosamine pyrophosphorylase; 5, UDP-Nacetylglucosamine-3-enolpyruvylether synthase; 6, UDPN-acetylenolpyruvylglucosamine reductase

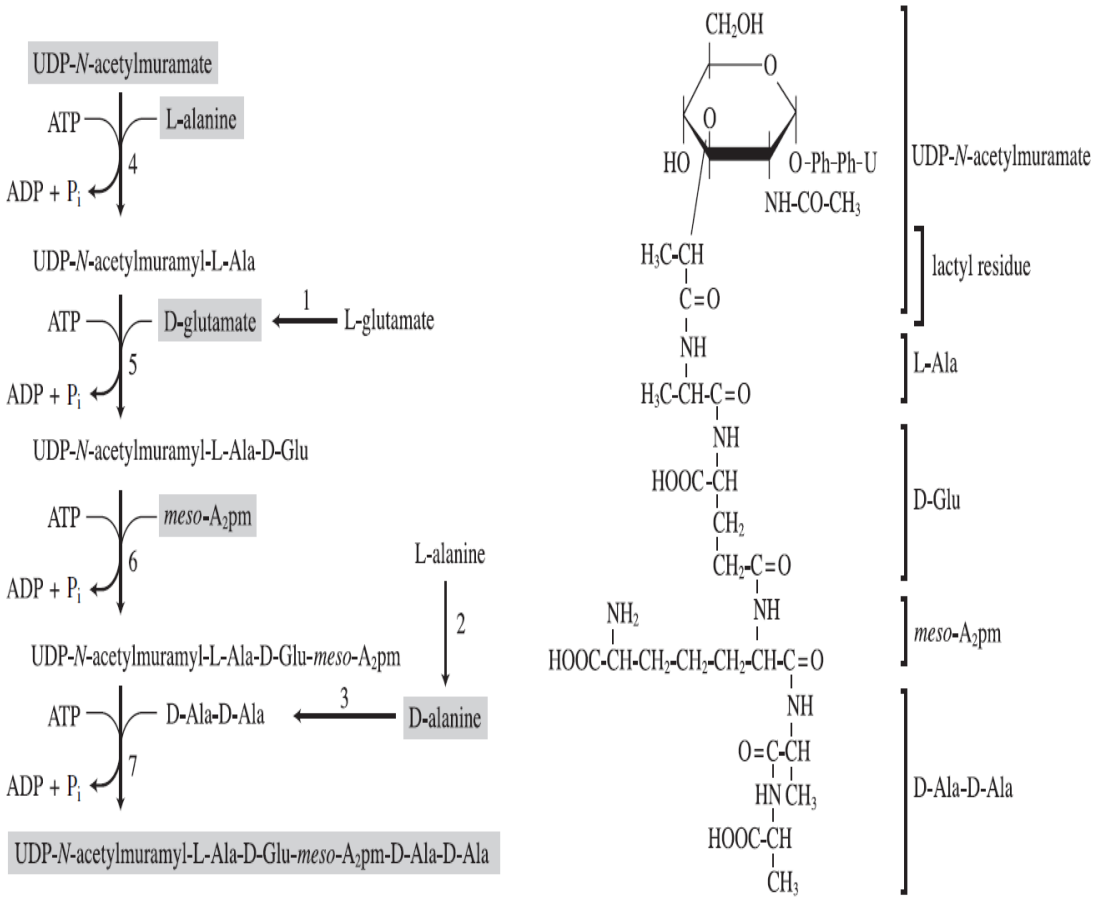


Fig 15.3: Synthesis of UDP-N-acetylmuramylpentapeptide through a non-ribosomal peptide synthesis process adding amino acids to the lactyl group of UDP-N-acetylmuramate. (Microbiol. Mol. Biol. Rev. 63:174–229, 1999)

The precursor for the bacterial cell wall murein synthesis, UDP-N-acetylmuramylpentapeptide, is made through a non-ribosomal peptide synthesis process. Amino acids are not activated and mRNA is not required. The amino acid sequence is determined by the enzyme specificity, and ATP is consumed to provide energy needed for the formation of peptide bonds. Different amino acids are found in the second and third positions depending on the bacterial species.

1, glutamate racemase; 2, alanine racemase; 3, D-alanine-D-alanine ligase; 4, UDP-N-acetylmuramate-alanine ligase; 5, UDPN-acetylmuramyl-alanine-D-glutamate ligase; 6, UDP-N-acetylmuramyl-alanyl-D-glutamate-2,6-diaminopimelate ligase; 7, D-alanyl-D-alanine adding enzyme group of UDP-N-acetylmuramate consuming ATP (Figure 15.4).

This reaction is a non-ribosomal peptide synthesis process independent from mRNA and the ribosomes. The amino acid sequence is determined by the enzyme specificity. Murein monomers are synthesized in the cytoplasm.

15.4. MONOMERS OF TEICHOIC ACID:

Ribose-phosphate and glycerol-phosphate are activated to CDP-ribitol and CDP-glycerol, as the precursors of ribitol teichoic acid and glycerol teichoic acid through a similar reaction as in the formation of UDP-sugars, consuming CTP instead of UTP. 6.8.4 Precursor of lipopolysaccharide, O-antigen Lipopolysaccharide consists of lipid A, core polysaccharide and O-antigen. O-antigen has a structure based on repeating oligosaccharide. Sugar-nucleotides are added to

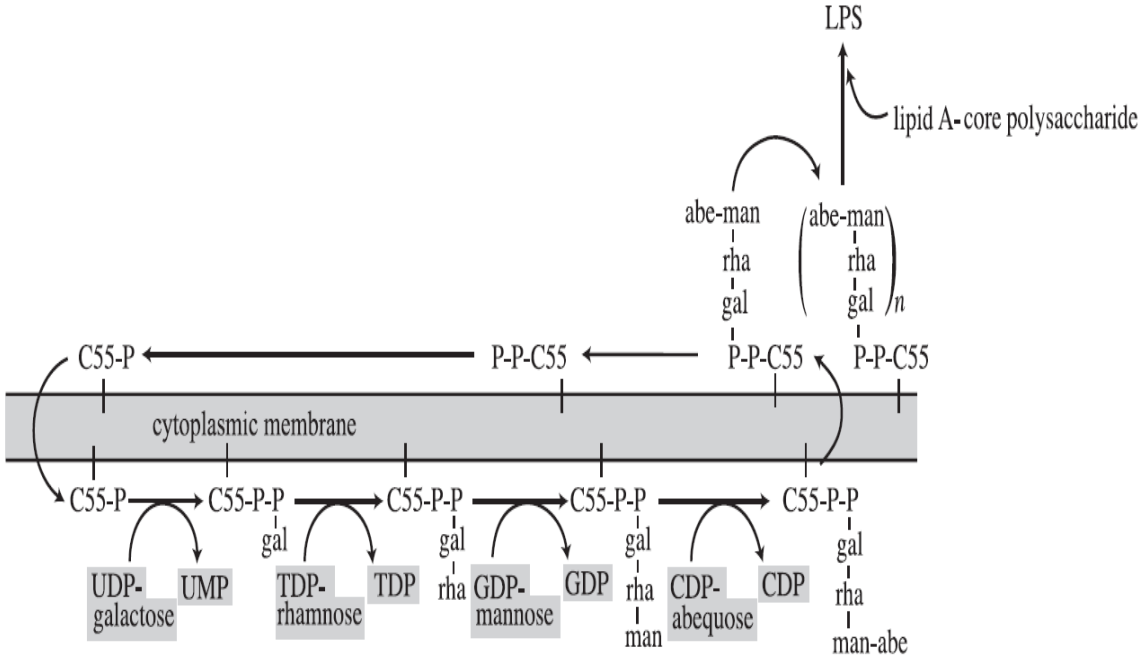


Fig. 15.4: Synthesis of O-antigen and lipopolysaccharide in Salmonella enterica. The O-antigen of LPS has a structure of repeating oligosaccharide. The unit oligosaccharide is

synthesized onto undecaprenyl phosphate embedded in the cytoplasmic membrane, which receives the sugar moiety from sugar-nucleotides. Undecaprenyl phosphate carries the oligosaccharide across the membrane. LPS is synthesized by the addition of the oligosaccharide to the core polysaccharide that has been transported to the periplasm bound to lipid A. LPS is transported to the outer membrane by the LPS exporter, a member of the ATP-binding cassette (ABC) family.

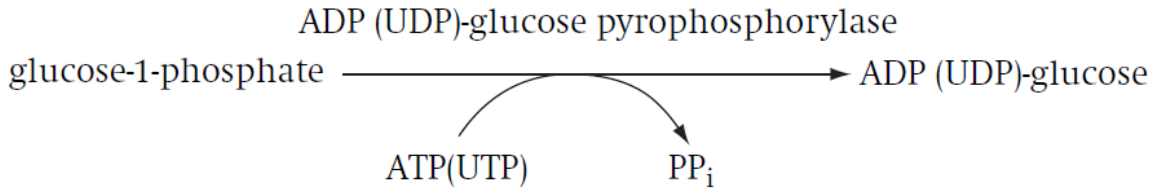
C55-P, undecaprenyl phosphate. undecaprenyl (bactoprenol) phosphate embedded in the cytoplasmic membrane (Figure 15.3). Undecaprenyl phosphate transports the oligosaccharide across the membrane. O-antigen ligase transfers the oligosaccharide to the core polysaccharide-lipid A in the periplasm to synthesize LPS. Core polysaccharide-lipid A is synthesized in the cytoplasm, and crosses the cytoplasmic membrane. The hydrophobic lipid A is the carrier of the core polysaccharide.

15.5. POLYSACCHARIDE BIOSYNTHESIS AND THE ASSEMBLY OF CELL SURFACE STRUCTURES:

Polysaccharides in bacterial cells include glycogen, a storage material, and structural polymers such as murein and teichoic acid in the cell wall, and LPS in the outer membrane. The precursors are synthesized in the cytoplasm and murein and LPS are synthesized after the precursors are transported across the cytoplasmic membrane. For this reason, the synthesis and assembly of the cell wall and the outer membrane are closely related to the transport of their precursors.

15.5.1. Glycogen Synthesis:

UDP-glucose is the precursor for glycogen synthesis in eukaryotes. Glucose-1-phosphate is activated to ADP-glucose in prokaryotes before being polymerized to glycogen by glycogen synthase and glycosyl (4-6) transferase.



Glycogen synthase transfers the glucose moiety of ADP (UDP)-glucose to the non-reducing end of the existing glycogen to form #-1,4-glucoside (Figure 15.5).

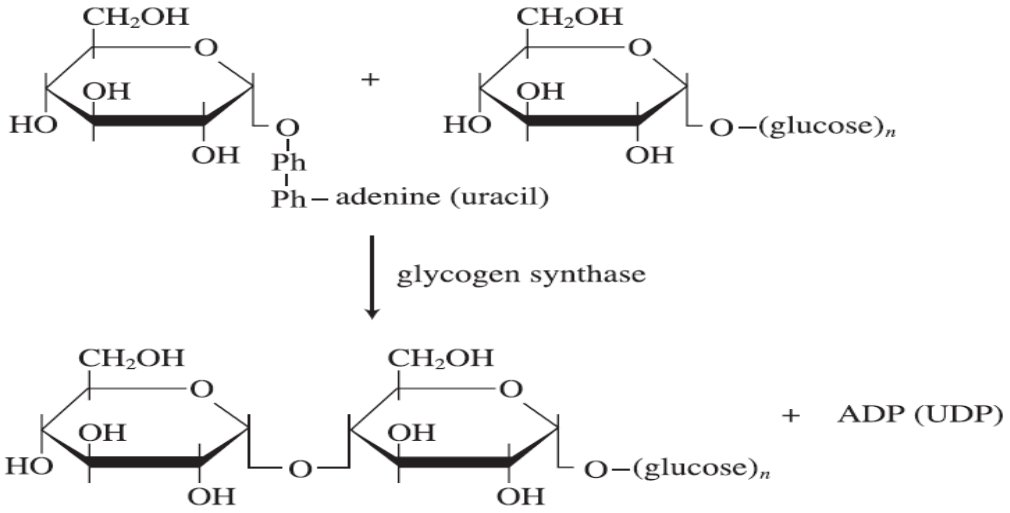


Fig 15.5: Formation of the a-1,4 linkage in glycogen by glycogen synthase. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.28.Springer, New York)

When the-β1,4 chain reaches a certain length, glycosyl (4!6) transferase catalyzes a transglycosylation reaction, transferring the β-1,4 chain to form an α1,6 linkage (Figure 15.6).

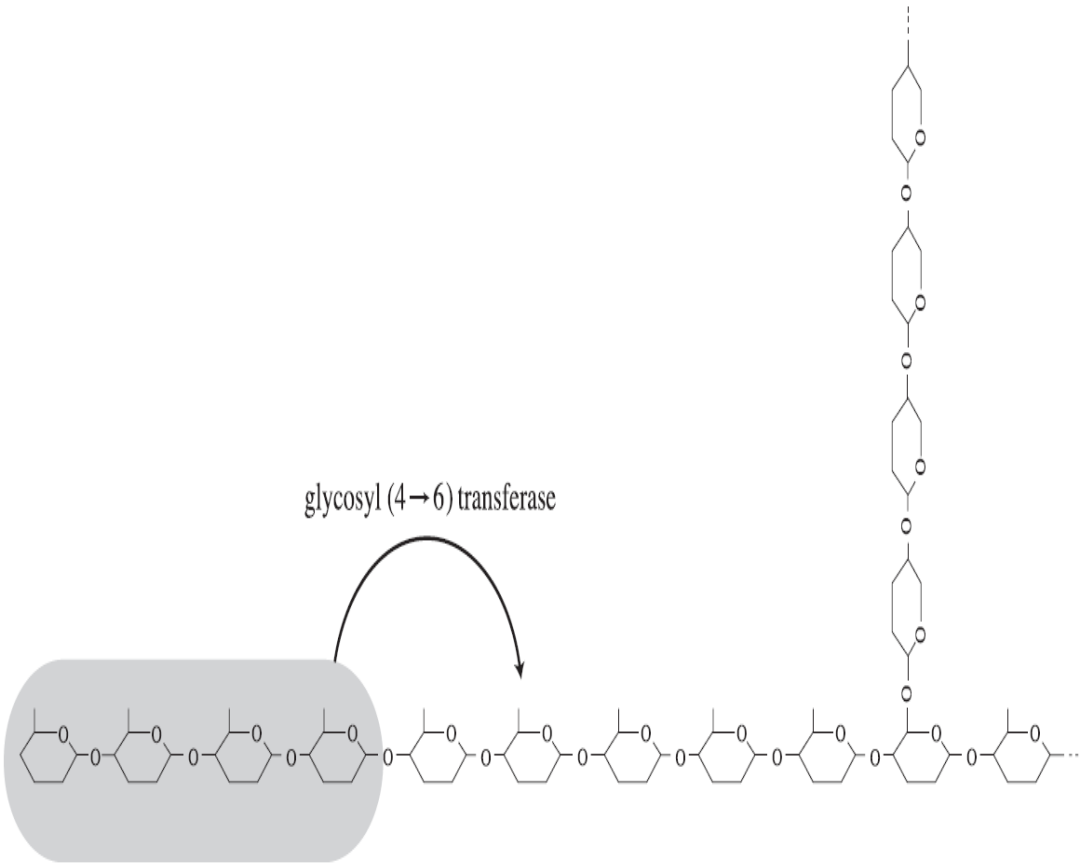


Fig 15.6: Formation of the a-1,6 side chain in glycogen through the transglycosylation reaction catalyzed by glycosyl (4-6) transferase. (Gottschalk, G. 1986, Bacterial Metabolism, 2nd edn., Figure 3.29. Springer, New York)

15.5.2. Murein Synthesis and Cell Wall Assembly:

Transport of Cell Wall Precursor Components through the Membrane:

The cell wall consists of murein and teichoic acid in Gram-positive bacteria, and of murein in Gram-negative bacteria. Various proteins are also associated with the cell wall, especially in Gram-positive bacteria. Monomers of the polymeric compounds are synthesized in the cytoplasm before being transported to the periplasm to be polymerized.

They are hydrophilic in nature, and cannot diffuse through the cytoplasmic membrane. To overcome this, the hydrophobic membrane compound, undecaprenyl phosphate (Figure 15.7), transports them through the membrane.

Murein Synthesis:

Phospho-N-acetylmuramylpentapeptide (phospho-MurNAc-pentapeptide) is transferred to the undecaprenyl phosphate embedded in the membrane from UDP-MurNAc-pentapeptide (lipid I) separating uridine 50-monophosphate (UMP). N-acetylglucosamine (GlcNAc) transferase forms undecaprenyl-GlcNAc-MurNAc-pentapeptide pyrophosphate (lipid II) separating UDP from UDP-GlcNAc. In Gram-positive bacteria, lipid II is further modified by the addition of amino acids to the third amino acid position, which is lysine in the pentapeptide. Different amino acids are added depending on the species. Glycyl-tRNA is consumed to add five glycyl units in Staphylococcus aureus. Undecaprenyl-GlcNAc-MurNAc-pentapeptide-(gly)5 pyrophosphate crosses the membrane to transfer GlcNAc-N-acetylmuramylpentapeptide-(gly) 5 to the existing murein, liberating undecaprenyl pyrophosphate through the action of transglycosylase, and a transpeptidase cross-links the neighbouring chains. A phosphatase converts undecaprenyl pyrophosphate to undecaprenyl phosphate, which starts another round of the same series of reactions (Figure 15.7). Inhibitors of murein synthesis, ristocetin and vancomycin, inhibit transglycosylase, and bacitracin interferes with the dephosphorylation of undecaprenyl pyrophosphate. !-lactam antibiotics inhibit transpeptidation and carboxypeptidation reactions.

15.5.3. Teichoic Acid Synthesis:

Teichoic acid is synthesized in a similar way as murein in Grampositive bacteria (Figure 15.8). GlcNAc is transferred from UDPGlcNAc to undecaprenyl phosphate before taking glycerol-phosphate from CDP-glyceride to form glycerol-P-N-acetylglucosamine-P-Pundecaprenyl.

Glycerol-P-N-acetylglucosamine-P is transferred to the existing teichoic acid separating undecaprenyl phosphate. Teichoic acid synthesis is not inhibited by bacitracin (which inhibits dephosphorylation of undecaprenyl pyrophosphate in murein synthesis).

Subsequently, GlcNAc is transferred to MurNAc-pentapeptide undecaprenyl pyrophosphate from UDP-GlcNAc, separating UDP to form undecaprenyl-GlcNAc-MurNAc-pentapeptide pyrophosphate, also known as lipid II (2). Five glycyl groups bind the third amino acid, lysine, in the pentapeptide, consuming glycyl-tRNAs before being translocated to the outer leaflet of the membrane (3). GlcNAc-

MurNAc-pentapeptide-(gly)5 forms a!-1,4-glucoside with the existing murein by a transglycosylation reaction (5), and a transpeptidase cross-link (6). Inhibitors of murein

synthesis, ristocetin and vancomycin, inhibit transglycosylase, and bacitracin interferes with the dephosphorylation of undecaprenyl pyrophosphate (4). !-lactam antibiotics inhibit (6) transpeptidation and (7) carboxypeptidation reactions.

1, phospho-N-acetylmuramoyl pentapeptide transferase; 2, N-acetylglucosamine transferase; 3, glycyl transferase; 4, undecaprenyl diphosphatase; 5, transglycosylase; 6, transpeptidase; 7, carboxypeptidase.

C55–P, undecaprenylphosphate.

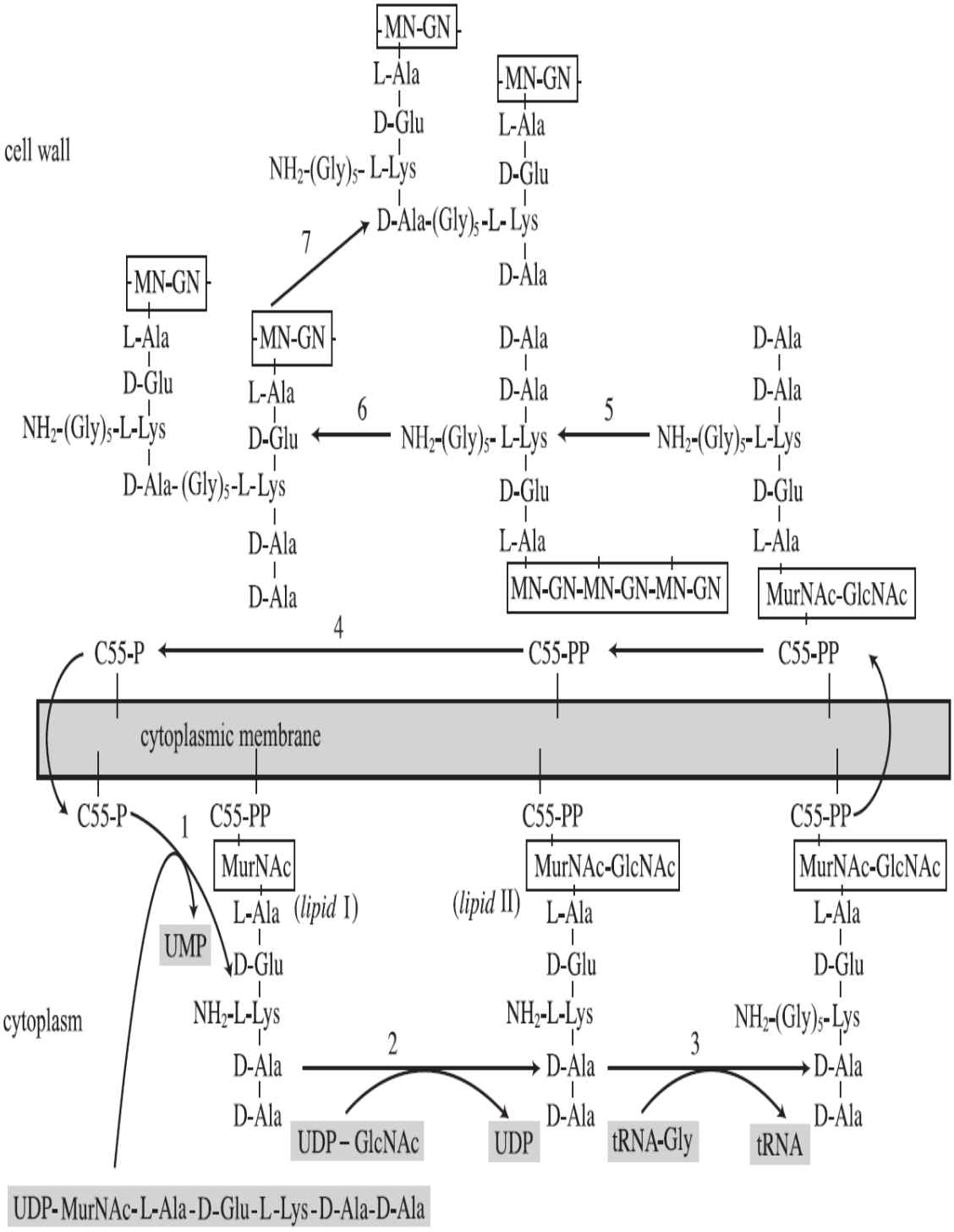


Fig 15.7: Murein synthesis in a Gram-positive bacterium, Staphylococcus aureus. (Microbiol. Mol. Biol. Rev. 63:174–229, 1999) Murein precursors, N-acetylglucosamine-UDP (GlcNAc) and UDP-N-acetylmuramate (MurNAc)-pentapeptide are synthesized in the cytoplasm (Figures 6.28 and 6.29). UMP is separated from UDP-MurNAc-pentapeptide, transferring phospho-MurNAcpentapeptide to undecaprenyl phosphate to form MurNAc-pentapeptide undecaprenyl pyrophosphate, which is known as lipid I (1).

Subsequently, GlcNAc is transferred to MurNAc-pentapeptide undecaprenyl pyrophosphate from UDP-GlcNAc, separating UDP to form undecaprenyl-GlcNAc-MurNAc-pentapeptide pyrophosphate, also known as lipid II (2). Five glycyl groups bind the third amino acid, lysine, in the pentapeptide, consuming glycyl-tRNAs before being translocated to the outer leaflet of the membrane (3). GlcNAc-

MurNAc-pentapeptide-(gly)5 forms a!-1,4-glucoside with the existing murein by a transglycosylation reaction (5), and a transpeptidase cross-link (6). Inhibitors of murein synthesis, ristocetin and vancomycin, inhibit transglycosylase, and bacitracin interferes with the dephosphorylation of undecaprenyl pyrophosphate (4). !-lactam antibiotics inhibit (6) transpeptidation and (7) carboxypeptidation reactions.

1, phospho-N-acetylmuramoyl pentapeptide transferase; 2, N-acetylglucosamine transferase; 3, glycyl transferase; 4, undecaprenyl diphosphatase; 5, transglycosylase; 6, transpeptidase; 7, carboxypeptidase.

C55–P, undecaprenylphosphate.

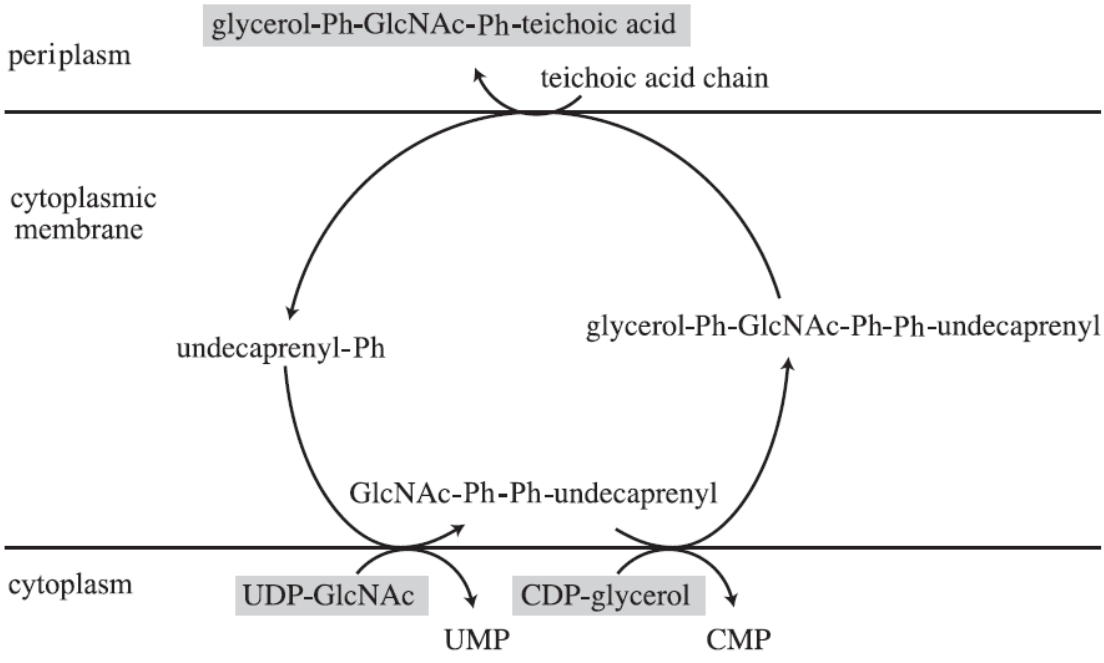


Fig 15.8: Synthesis of glycerol teichoic acid. Undecaprenyl-phosphate translocates the teichoic acid precursors across the cytoplasmic membrane as in murein synthesis. UDP-N-acetylglucosamine and CDP-glyceride react with undecaprenyl-phosphate at the cytoplasmic side of the membrane to form glycerol-P-Nacetylglucosamine- P-Pundecaprenyl,which crosses the membrane before glycerol-P-Nacetylglucosamine-P is transferred to the existing teichoic acid separating undecaprenylphosphate.

15.6. SUMMARY:

Bacterial cell wall synthesis, primarily peptidoglycan (PG) biosynthesis, builds a protective layer outside the cell membrane, crucial for shape and preventing osmotic lysis, involving precursor (NAG/NAM-peptide) synthesis in the cytoplasm, transport via a lipid carrier (bactoprenol) across the membrane, and final polymerization/cross-linking by enzymes (like transpeptidases) on the outer surface, a process targeted by antibiotics like penicillin.

15.7. SELF-ASSESSMENT:

1) Hexose phosphate and UDP-sugar
2) Monomers of Murein
3) Monomers of Teichoic Acid
4) Polysaccharide Biosynthesis and The Assembly of Cell Surface Structures
5) Glycogen Synthesis
6) Murein Synthesis and Cell Wall Assembly
7) Teichoic Acid Synthesis

15.8. REFERENCES:

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Dr. J. Madhavi