Indira Gandhi
National Open University
School of Sciences

BBYCT-137
PLANT PHYSIOLOGY AND METABOLISM

Block

3

ENZYMES AND RESPIRATION

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BLOCK 3 : ENZYMES AND RESPIRATION

You have read about the processes and mechanisms of photosynthesis operative in plants for the manufacture of food in Units 5, 6 and 7 of Block II. Also, the various pathways for translocation of these photo assimilates have been described in Unit 8.

Block III is devoted to the mechanisms of utilization of these photo assimilates by the complex machinery of cellular respiration. This block comprises three Units 9,10 and 11.

Unit 9 introduces you to the fascinating world of biocatalysts that catalyse biochemical reactions needed for the life processes. This unit deals with the aspects of discovery, structure and modern classifications of enzymes.

The concept of activation energy of enzymes has been discussed in detail, along with a description of isozymes, abzymes, ribozymes and deoxy ribozymes. Regulation of enzyme action is explained through allosteric enzymes highlighting negative feedback mechanisms. Modern aspects of active site, mechanism of enzyme action and various types of enzyme inhibitions also find a detailed treatment in this Unit.

Units 10 and 11 are devoted exclusively to respiration and its mechanism. Unit 10 introduces you to the nature of the respiratory process comparing it with combustion, and deals with the nature of respiratory substrates, and the concept of RQ (respiratory quotient).

In addition, the processes of aerobic and anaerobic respiration have also been compared for their energy outputs. The location of the respiratory reactions within the powerhouse of a cell – the mitochondria, have been explained with the help of its ultrastructural features.

A detailed description of the respiration mechanism follows in Unit 11, which forms an in-depth study of glycolysis. The preparatory phase and the payoff phase of glycolysis have been dealt with in details along with a balance sheet of ATP yield. Various steps of Krebs cycle along with its regulation are also discussed.

The alternative pathways like Pentose Phosphate Pathway (PPP) have also been explained. In addition, the shuttle-mechanisms involved in the utilization of cytosolic NADH have also been described. This will make you familiar with various options present in the cellular machinery to utilize NADH molecules in the electron transport chain. The chemiosmotic model and ATP synthesis has been explained in the light of recent discoveries in the field.

Objectives

After studying the block, you will be able to:

- describe the role of biocatalysts in cell metabolism;
- appreciate the classification and nomenclature of enzymes;
- describe the mechanism of action and regulation of enzyme activity;
- describe the main features of anaerobic and aerobic respiration;
- familiarize with the concept of respiratory quotient of various substrates;
- get in-depth information of glycolysis including its variations in plants;
- describe the mechanism of Krebs cycle, electron transport chain and current knowledge on ATP synthesis, along with net yield in aerobic respiration; and
- appreciate the alternative shuttle mechanisms operative across the mitochondrial membrane.

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UNIT 9

ENZYMES

Structure

9.1 INTRODUCTION

You have studied about the structure of cell membranes and the process of transport across these membranes in the previous units. Membrane proteins act as transporting molecules. The other important function of cellular proteins is to act as enzymes and catalyse biochemical reactions at a rate appropriate to the needs of the cell.

In this unit you will study about the nature of enzymes and their functional aspects. Enzymes control the overall metabolic functions of the cell. They do not affect the equilibrium constant and remain unaffected at the end of the reaction. The reactants bind to a specific site on the surface of enzyme molecule called **active site**. Enzymes show specificity for their substrate as well as for the reactions. Various factors such as temperature, pH, concentrations of enzymes and substrate affect the rate of enzyme-catalysed reactions.

All the living cells carry out huge variety of biochemical reactions, yet they are able to rapidly construct very large and complicated molecules, or regulate the flow of materials through complex metabolic pathways with absolute precision and accuracy. Thus, enzymes are biological catalysts and make possible the conversion of substrate molecules to products, but they themselves are not permanently changed by the reaction .Cells contain thousands of enzymes and each one catalyzing a particular reaction.

Nearly all known enzymes are proteins. Broadly, enzymes carry out two important functions in a living organism. They are able to accelerate or retard or bring about chemical reactions. They are also able to regulate a number of different reactions at the same time because of their specificity.

Objectives

After studying this unit, you would be able to:

- describe the role of enzymes in lowering the activation energy and in coupled reactions;
- list the type of enzymes and describe cofactors and discuss the mechanism of enzyme action;
- discuss the effects of temperature, pH and enzyme concentration on the rate of enzyme action;
- list the essential features of allosteric enzymes and inhibitors;
- describe the need for the regulation of enzyme activity; explain the genetic control of enzyme activity; and
- explain the process of feedback regulation, allosteric regulation, covalent modulation of enzyme activity, and hormonal regulation of enzyme activity.

9.2 STRUCTURE AND FUNCTION

It would be interesting to know about how the enzymes were discovered. You will study about fascinating discoveries of various enzymes in detail.

9.2.1 Discovery

The history of enzymes is intimately connected to the early studies made on the process of digestion. Till the beginning of 18th century, digestion was

considered to be primarily a mechanical process. As early as in 1752, **Rene-Antoine Reaumur** fed meat pieces enclosed in a perforated metal tube to his pet falcon, only to find that the meat got digested in the bird's stomach but the metal tube was intact. This, along with other experiments proved the existence of some chemical reactions during the process of digestion. **Theodore** (1735) isolated a non-acid digestive substance from gastric juice and named it ***pepsin*** (*Gr*: to digest). This was later shown to be an enzyme. **Dubrunfaut** (1830) prepared malt extract from germinating barley seeds and found that the this extract hydrolysed starch into sugar. Later, **Payen and Persoz** (1833) extracted a substance diastase from the malt extract (again, an enzyme). One of the oldest studied chemical reaction-alcoholic fermentation was thought to be a spontaneous decomposition of matter till **Louis Pasteur** (1857) demonstrated that the living yeast cells were responsible for the process and they digested sugar for their own metabolic needs. Pasteur used the term "ferments" for such biocatalysts. This "vitalistic" view prevailed for many decades. **Kühne**, a German physiologist (1837-1900) later proposed the term **enzyme** (in yeast).

The modern era in study of the enzymes began when **Buchner** (1897) accidentally discovered that even a juice extracted from yeast cell brought about fermentation of sugar like that of the living yeast cells.

$$C_6H_{12}O_6 \longrightarrow 2\ CH_3CH_2OH + 2\ CO_2$$

When the juice was boiled it lost its power of fermentation. The cell-free extract was found to be a complex enzyme which he named as ***'zymase'***. His work thus demonstrated that the catalyst had the ability to function even when removed from the cells. This study refuted the vitalistic views held earlier.

The real breakthrough in the study on enzymes was accomplished by **Sumner** (1926) who was the first to isolate and crystallize the enzyme ***urease*** in a purified form from the jack bean (*Canavalis enciformis*). He found urease enzyme crystals to be proteins and postulated that all enzymes are proteins. The nature of enzymes was later firmly established by **Northrop and Kunitz** (1936) who crystallized *pepsin*, *trypsin* and *chymotrypsin*. **J.B.S. Haldane**, in his book "Enzymes" suggested that weak bonding between the substrate and the enzyme formed the basis of catalysis. All enzymes were regarded as proteins for more than half a century till 1982 when **Cech and Altmann** discovered ***ribozymes*** (RNA molecules with enzyme like properties). In some cases, even DNA can act as an enzyme (***deoxyribozyme***).

9.2.2 Structure

Enzymes are classified either on the basis of their site of action or their structure.

Classification on the basis of their site of action :

i) Intracellular (Function inside the cell); and

ii) Extracellular (Function outside the cell).

Their site of synthesis is different from the site of action. They are also called **Exoenzymes**. Such enzymes are present in bacteria for synthesis of mucilage

cover or for breaking down of complex substances in the extracellular medium for absorption.

Enzymes can possess either only one (monomeric) or many (oligomeric) subunits of proteins. Monomeric enzymes have a single polypeptide chain usually composed of 100-300 amino acids, and are called **simple** enzymes.

They are mostly synthesized as ***zymogens***. Most of these enzymes belong to the class of ***hydrolases*** (Table 9.1)

Table 9.1: Members of monomeric enzymes

Enzyme type	Examples
Protease	*Chymotrypsin* *Trypsin* *Elastase* *Thrombin* *Subtilisin* *Pepsin* *Ficin* *Carboxypeptidase A* *Carboxypeptidase B*
Nuclease	*Ribonuclease*
Glycosidase	*Lysozyme*

Oligomeric enzymes are made up of two or more polypeptide chains called **subunits**. The subunits may or may not be identical. Identical subunits are called **protomers**. All regulatory enzymes are oligomeric enzymes and are controlled by feedback or allosteric regulation.

Some of the oligomeric enzymes are listed in Table 9.2

Table 9.2: Members of oligomeric enzymes

Oligomeric enzyme	Number of subunits present
Lactate dehydrogenase	Four
Lactose synthase	Two
Tryptophan synthase	Two
Pyruvate dehydrogenase	Multienzyme complex

Many enzymes have an attached non-protein group called **conjugated** enzymes. The protein part of a conjugated enzyme is called **Apoenzyme** while **cofactor** is the non-protein part. Together, they constitute a fully functional enzyme, the **Holozyme**.

Apoenzyme + cofactor = Holoenzyme (Holozyme)

The cofactor could be in the form of small molecular weight organic molecule or metallic ions. The nature of binding of the cofactor with the apoenzyme forms the basis of another form of classification:

a) **Prosthetic group :** These are organic compounds which are very tightly bound (sometimes even covalent) and are almost a permanent part of the holozyme e.g., Haem is the prosthetic group in the peroxisomal enzyme *catalase*.

b) **Coenzymes :** are non-protein organic molecules bound to the protein molecules and metal activated enzymes are loosely bound to the apoenzyme.

c) **Metallozymes :** When inorganic metal ions are tightly bound to the enzyme, it is called a "**metallozyme**".

The transition state metal ions bind to various groups (ligands) of apoenzymes to enhance the reaction rate. Some of the common ligands are free amino groups, free carboxyl groups and thiol group of cysteine residues which interact with the metal ion to form a stable complex thereby converting the protein into its active conformation (Fig. 9.1).

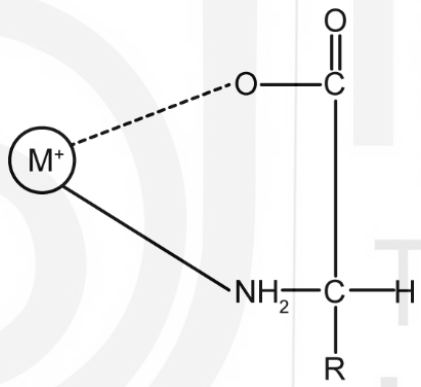


Fig.9.1: Stable complex formed due to interaction of metal ion (M^+) with the amino group and carboxyl group of apoenzyme.

Metals are generally called **activators** and play a vital role in plant nutrition. Some enzymes requiring metal activators are shown in Table 9.3.

Table 9.3: Some metal activator requiring enzymes

Metal	**Enzyme/s**
Zinc	*Carbonic anhydrase; Carbo peptidases,* various *dehydrogenases; Alkaline phosphatase*.
Copper	*Ascorbic acid oxidase; Cytochrome oxidase; Tyrosinase; Phenol oxidase; Superoxide dismutase* (SOD)
Iron	*Catalase; Peroxidase; Nitrogenase; Cytochrome oxidase*
Manganese	*Peptidases; SOD; Glycosyl transferase; Carboxylase; Pyruvate carboxylase; Isocitrate dehydrogenase*.
Magnesium	*Pyruvate kinase; ATPase; phosphatases Adenylate cyclase*
Cobalt	*Nitrate reductase; Nitrogenase; Xanthin oxidase, Glutathione peroxidase*;

Molybdenum	*Nitrogenase; Xanthinoxidase; Formate dehydrogenase*; *Sulphite oxidase*
Calcium	*ATPase; Succinate dehydrogenase; Phosphorylase kinase*
Potassium	*Fructokinase; Phosphopyruvate transphosphorylase*
Nickel	*Arginase; Carboxylase; Trypsin, Urease*
Chloride	*Salivary amaylase*
Selenium	*Glutathione peroxidase*
Vanadium	*Nitrate reductase*
Fluorine	*Adenylate cyclase*

9.2.3 Prosthetic Group and Coenzymes

Coenzymes are non-protein, low molecular weight, organic cofactors. They are also called **cosubstrates**. Coenzymes are loosely bound to the proteinaceous apoenzyme and function to transfer the chemical groups between enzymes.

Coenzymes are different from prosthetic groups in not being a permanent part of enzymes structure. Coenzymes act as carriers of specific functional groups during biochemical reactions. Unlike the prosthetic group, the coenzymes get consumed and are continuously recycled in metabolism. It means that one set of enzymes add a chemical group to the coenzyme while another set removes it.

SAQ 1

a) Fill in the blanks with appropriate words/ terms.

i) The term enzyme was coined by ……..

ii) The protein part apoenzyme of an enzyme combines with …….. to form a fully functional …….

iii) Tightly bound and almost permanent non-protein organic components are called…….. .

iv) Metallozymes contain tightly bound…….. .

b) Match the contents of Column **A** and those of Column **B**.

Column A	**Column B**
i) Sumner	a. Non-vitamin derived conenzyme
ii) Coenzyme A	b. *Nitrate reductase*
iii) Haem	c. Vitamin derived coenzyme
iv) Coenzyme Q_{10}	d. *Catalase*
v) Cobalt	e. *Urease*

9.3 CLASSIFICATION AND NOMENCLATURE

Enzymes can be classified in a number of ways depending on various factors like the composition of enzyme, type of substrate on which the enzyme acts, type of reaction catalyzed etc. We will discuss about trivial system of enzyme classification briefly with suitable examples as well as enzyme classification given by Enzyme Commission in details. The latter classification is more uniform in nature and followed universally.

Trivial System of Enzyme Classification

Classifying enzymes simply for the sake of convenience and understanding falls under this scheme. It can be done in many ways, like classification based on enzyme composition, nature of substrate on which the enzyme acts or end product of reaction and so on. Let us study some of the ways of classifying enzymes under trivial system.

Enzymes have been named in an arbitrary manner in the past giving no indication of the substrate or the type of reaction. For example, *catalase, pepsin* or *trypsin* do not indicate much.

Type of substrate on which the enzyme acts: In this system of classification, enzymes are generally named by simply adding the suffix -***ase*** to the name of the substrate catalyzed. For example *carbohydrases* act upon carbohydrate, *proteinases* on proteins, *lipases* on lipids, *maltase* on maltose, *urease* on urea and so on. This trivial system does not take into account the type of reaction catalyzed.

Type of reaction catalyzed: Enzymes have also been classified depending on the **type of reaction** and simply by adding the suffix –*ases.* For example, *hydrolases* (catalyzing hydrolysis), i*somerases* (isomerization), *oxidases* (oxidation), *dehydrogenases* (dehydrogenation*), transaminases* (transamination), *transaldolases* (transaldolation), *transketolases* (transketolation), *phosphorylases* (phosphorylation) and so on. Unfortunately, this system also does not indicate the nature of the substrate acted upon.

Type of Substrate and Type of reaction catalyzed:

A slightly better system combines both the above mentioned parameters. For example, the enzyme *succinate dehydrogenase* indicates the substrate and the nature of the reaction.

IUBMB Classification of Enzymes - Enzyme Commission Code (EC)

Thus, in order to bring some consistency to the classification of enzymes, and to remove ambiguities associated with the use of trivial nomenclature, in 1964, the International Union of Biochemistry and Molecular Biology (IUBMB) established an Enzyme Commission (EC) to develop a nomenclature for enzymes which is uniform and universally accepted.

The Enzyme Commission has grouped enzymes into **six** main **classes** on the basis of their functional specificity i.e., the type of reaction catalyzed (Tables 9.4 and 9.5).

Each major class is divided into **sub-class** (Table 9.4) and each sub-class is further subdivided into many sub-subclasses.

Table 9.4: Classification of enzymes

Class	Subclass	Name of the reaction catalyzed	Specific reaction catalyzed	Example
Oxidoreductase	Dehydrogenase	Redox	Removal of 2 hydrogen atoms with double bond formation	*Lactate dehydrogenase*, *Malate dehydrogenase*
	Oxidase	Redox	Reduction of oxygen	*Cytochrome oxidase*
	Peroxidase	Redox	Reduction of H_2O_2	*Catalase*
	Hydroxylase	Redox	Introduction of OH groups	*Phenylalanine-4-hydroxylase*
	Oxygenase	Redox	Incorporation of molecular oxygen	*Tryptophan oxygenase*
	Oxidative deaminase	Redox	Oxidation of amino acid with the liberation of NH_3	*Amino acid oxidase*
Transferase	One carbon transferase	Group transfer between two substrates	Transfer of one-carbon group	*Methyl transferase , Hydroxymethyl transferase, Formyl transferase*
	Aldehyde and ketone transferase	Group transfer between two substrates	Transfer of aldehyde/keto group	*Acetaldehyde transferase*
	Acyl transferase	Group transfer between two substrates	Transfer of acyl/acetyl group to a suitable acceptor	*Aminoacyl transferase*
	Glycosyl transferase	Group transfer between two substrates	Transfer of glycosyl group	*Hexosyl transferase*
	Alkyl transferase	Group transfer between two substrates	Transfer of alkyl other than methyl	*Ethyl transferase*
	Nitrotransferase	Group transfer between two substrates	Transfer of amino group	*Amino transferase*

	Phospho transferase	Group transfer between two substrates	Transfer of phosphoryl group	*Kinase*
	Sulpho transferase	Group transfer between two substrates	Transfer of sulphur containing group	*Dehydro epiandrosterone , Sulphotransferase*
Hydrolase	Esterase	Hydrolytic bond cleavage	Hydrolysis of ester	*Acetylcholine esterase*
	Peptidase	Hydrolytic bond cleavage	Hydrolysis of peptide bond	*Trypsin*
	Glycosidase	Hydrolytic bond cleavage	Hydrolysis of glycosidic bond	*Lysozyme*
	Phosphatase	Hydrolytic bond cleavage	Hydrolysis of phosphoric acid ester	*Alkaline phosphatase*
	Deaminase	Hydrolytic bond cleavage	Hydrolysis of amines	*Glucosamine-6-p-deaminase, Glutaminase*
	Deamidase	Hydrolytic bond cleavage	Hydrolysis of amides	*Nicotinamide deamidase*
Lyase (Desmolase)	C=C lyase	Non-hydrolytic bond cleavage and generation of double bonds	Cleavage of carbon-carbon acid ester	*Aldolase*
	C=O lyase	Non-hydrolytic bond cleavage and generation of double bonds	Cleavage of carbon-oxygen bond	*DNA lyase*
	C = N lyase	Non-hydrolytic bond cleavage and generation of double bonds	Cleavage of carbon nitrogen bond	*Ammonia lyase*

Isomerase	Racemase	Intramolecular rearrangement of groups	Interconversion of optical isomers	*Alanine racemase*
	Epimerase	Intramolecular rearrangement of groups	Interconversion of epimers	*Maltose epimerase*
	Cis-trans Isomerase	Intramolecular rearrangement of groups	Interconversion of geometrical isomers	*Fumarase*
Ligase (Synthetase)	C = O ligase	Bond formation at the expense of ATP*	Formaton of C – O bond	*Tyrosine-RNA ligase*
	C = S ligase	Bond formation at the expense of ATP*	Formation of C-S bond	*Acetate CoA ligase*
	C = N ligase	Bond formation at the expense of ATP*	Formaton of C – N bond	*NAD^+ synthase Tryptophan synthease*
	C = C ligase	Bond formation at the expense of ATP*	Formation of C – C bond	*Pyruvate carboxylase*

*An enzyme that catalyzes bond formation without the expense of ATP is called ***synthase***.

Reactions catalyzed by different classes of enzymes are summarized in Table 9.5.

Table: 9.5: Reaction catalyzed by different classes of enzymes

Class	**Model reaction catalyzed by the specific class of enzyme**
Oxidoreductase	$A_{Red} + B_{oxd} \leftrightarrow A_{Oxd} + B_{red}$ $A^- + B^+ \leftrightarrow A^+ + B^-$
Transferase	$A\text{-}B + C \leftrightarrow A^+ + B^-$
Hydrolase	$A\text{-}B + H_2O \leftrightarrow A\text{-}H + B\text{-}O$
Lyase (Desmolase)	A X-BY↔ A=X -Y
Isomerase	A X-BY ↔ AY – BY
Ligase (Synthetase)	A + B + ATP ↔ A – B + ADP + Pi

Each enzyme has been assigned a specific **code** by the Enzyme Commission. This is called as the **enzyme code**. The code consists of **four** digits:

- The first digit or integer represents the **major class**,
- The second digit or integer represents the **sub-class**,
- The third digit or integer represents the **sub sub-class**, and

- The fourth digit or integer represents the individual serial number. Thus, a series of four digits specifies a particular enzyme.

An example illustrates the EC code nomenclature:

The enzyme *D-Glucose-6-phosphotransferase* catalyses the phosphorylation of D-glucose by ATP.

$$ATP + D-Glucose \xrightarrow{D\text{-}glucose\ 6\text{-}phosphotransferase} ADP + D-Glucose-6-phospate$$

CLASS : Above reaction shows that the enzyme is a ***transferase*** (Class 2) as it catalyses the transfer of phosphate group from ATP to the –OH group of the sixth carbon of glucose (Entry 1 = **2**).

SUBCLASS : Since the phosphorus-containing groups are being transferred, the transferase enzymes are called ***phospho transferases*** which are grouped under the seventh subclass of transferases (Entry 2 =**7**).

SUB-SUB CLASS : The sub-sub class is of category 1 as an alcohol group is the acceptor of phosphate group. This makes the Entry 3 = 1.The 4^{th} entry within this sub-sub class is ATP which is designated the category (Entry 4 = **2**).

Therefore, the Enzyme code (EC) number of the enzyme *D-glucose-6-phosphotransferase* is **2.7.1.2.** The position of the classes, sub-classes and the sub-sub classes is fixed and thus it makes a universally accepted and understood enzyme language.

EC classification system of enzyme brings out clarity and complexities of the reaction catalyzed by the enzyme but at the same time it makes the names lengthy and relatively cumbersome. So, common names of the enzymes are still being used in many cases. Some examples of enzymes with their EC number, systematic and common names are given in Table 9.6:

Table 9.6: IUBMB System of Enzyme Classification

EC Number	Systematic name	Common or trivial name
1.1.1.1	*Alcohol : NAD oxidoreductase*	*Alcohol dehydrogenase*
1.11.1.6	*H_2O_2: H_2O_2 oxidoreductase*	*Catalase*
2.7.1.1	*ATP : D-hexose-6-phosphotransferase*	*Hexokinase*
3.1.1.3	*Glycerol ester hydrolase*	*Lipase*
4.1.2.7	*Ketose-1-phosphate aldehyde-lyase*	*Aldolase*
4.2.1.2	*L-malate hydro-lyase*	*Fumarase*
5.3.1.9	*D-glucose-6-phosphate keto-isomerase*	*Glucosephosphate isomerase*
6.3.1.2	*L-glutamate : ammonia ligase (ADP)*	*Glutamine synthetase*

SAQ 2

a) Match the contents of Column A and those of Column B.

	Column A		Column B
i)	*Malate dehydrogenase*	a.	*Isomerase*
ii)	*Acetylcholinesterase*	b.	*Transferase*
iii)	*Phosphoglucomutase*	c.	*Oxidoreductase*
iv)	*Rubisco*	d.	*Hydrolase*
v)	*Hexokinase*	e.	*Lyases*

b) State whether the following statements are True or False?

i) The Enzyme Commission has grouped enzymes into eight main classes. (T/F)

ii) Adding the suffix-*ase* to the name of the substrate is the most acceptable system of enzyme classification. (T/F)

iii) The position of classes and subclasses in IUBMB system of enzyme classification are fixed. (T/F)

iv) The EC Code consists of 5 digits. (T/F)

9.4 PROPERTIES OF ENZYMES

By now you have been acquainted with the fact that enzymes act as catalysts and influence the rate of reaction in all the living organisms. In fact, essentially all chemical reactions in the living beings are catalysed by enzymes. Almost all the enzymes known till now are proteins with a unique three dimensional structure that provides an active site for binding the other molecules, i.e., substrates to their surface.

Each enzyme normally catalyses a few reactions but most often only one type of reaction. They are required in minute quantity/concentration to convert the substrates into products. The enzyme remains unaffected at the end of the reaction. The enzymes molecule is much larger than the molecule of its substrate. The molecular weights of enzymes range from thousands to millions, whereas the molecular weights of substrates are usually in few hundreds. Some enzymes are purely proteins, whereas other requires non-protein assistant/additional compounds for their catalytic activity.

9.4.1 Activation Energy

All the chemical reactions in a biological system have an energy barrier which prevents reactions from proceeding in an uncontrolled and spontaneous manner. The input of energy required to break this energy barrier or to start a reaction is called the **activation energy**. In biochemical reactions, the substrate (S) gets converted to the product (P) by passing through a high energy transition state(X^{++}).

$$S + X^{++} \longrightarrow P$$

This $X^{++}$is intermediate in structure between the S and P. Energy required for the formation of $X^{++}$is called **activation energy (binding energy).**

For example, a mixture of hydrogen and oxygen will not react with each other until they receive enough energy from a heat source to achieve the activation energy. You may have noticed that a tin of petrol or kerosene oil kept exposed to air at room temperature would not catch fire unless kindled by a spark. This tiny spark is adequate to supply the activation energy for few molecules to react. The amount of energy released as a result of the conversion of the first few molecules of reactant to product is sufficient to activate other molecules. Similarly, chemical reactions taking place in living organisms also require activation energy.

In biological systems, enzymes are important components which enhance the chemical reactions by lowering the activation energy and help the reactions to occur at a rate appropriate to the needs of the cell. The enzymes speed up the reactions by lowering activation energy. Enzymes combine with different types of substrates in such a way that they reduce the amount of energy required for a particular reaction (see Fig. 9.9). In the absence of enzymes in your gut, it would take many years instead of few hours for your last meal to be digested.

9.4.2 Isoenzymes

Several enzymes exist in their multiple forms termed **isoenzymes (isozymes** or **isoforms)**.These isoenzymes are **not** isomers. All isoenzyme forms of a given enzyme catalyse the same reaction but are chemically distinct molecules. They may differ in amino acid composition, amino acid sequence, charge, molecular weight, etc. They usually differ in one or more kinetic properties such as K_m and V_{max} for their substrate. In addition, they also vary in their regulatory properties and electrophoretic mobility. Since isozymes are multiple forms of a single enzyme and obtained from different genes, they infact, represent different loci or product of different alleles of the same gene. Thus, they are also called **allozymes**.

Isoenzymes are found in all vertebrates, insects, plants and unicellular organisms. Different tissues may contain different isoenzymes which may differ in their affinity for the substrates.

9.4.3 Ribozymes

The discovery of ribozymes has forced the biologists to reconsider the sequence of events leading to the origin of life on earth. According to them, RNA molecules were perhaps the first catalysts rather than the proteins – since the former can function both as a catalyst and replicating systems which can transfer genetic information from one generation to the other. This forms the basis of the theme of **RNA world** hypothesis proposed by Carl Woese.

Carl Woese *et al* first suggested that RNA molecules, on account of their complex secondary structures and hairpin active centers could be potential candidates to act as enzymes.

9.4.4 Deoxyribozymes

DNA fragments can also possess catalytic activity. **Ronald Breaker** discovered first such **deoxyribozyme**. This enzyme catalyzed a Pb^{++}-dependent RNA cleavage. Some DNA enzymes catalyze ATP-dependent capping and may use light to repair thymine dimers. Deoxyribozymes or

DNAzymes catalyze a variety of reactions and are considered to be cost-effective in synthesizing chiral molecules.

Both ribozymes and deoxyribozymes are used to tailor defined RNA sequences with many pharmaceutical applications like killing of invading pathogenic viruses, and cleaving the HIV RNA. They are now also used extenstively in functional genomics and gene discovery.

9.4.5 Abzymes

Our immune system produces proteinaceous antibodies which can bind to the foreign antigen molecule to produce an immune response. **Abzymes** are artificial constructs of catalytic antibodies which can bind to the transition state of an enzyme-catalyzed reaction to bring about enhancement in reaction rate. Abzymes are usually obtained from human and animal serum. The natural abzymes can break down proteins (protabzymes) or hydrolyze DNA (DNA-abzymes)

9.4.6 Allosteric Enzymes

Some enzymes may have two functionally different binding sites. One of the site, the active site, binds the substrate and catalyses the reaction. The other site, known as **allosteric** or **regulatory site** binds another molecule which is called **effector** or **modulator**. Such enzymes are known as **allosteric enzymes**. These enzymes are oligomeric in nature, i.e., they have more than one subunit. The active site and allosteric site may be located on the same subunit or on different subunits of the enzyme and the conformational changes caused by binding are transmitted between subunits. Effector molecules are of two types: positive effectors, i.e., **activators** that enhance enzyme activity and negative effectors or **inhibitors** which inhibit enzyme activity. Binding of effectors causes conformational changes in the allosteric enzymes which influence their catalytic activity (see Fig. 9.2 a, b).

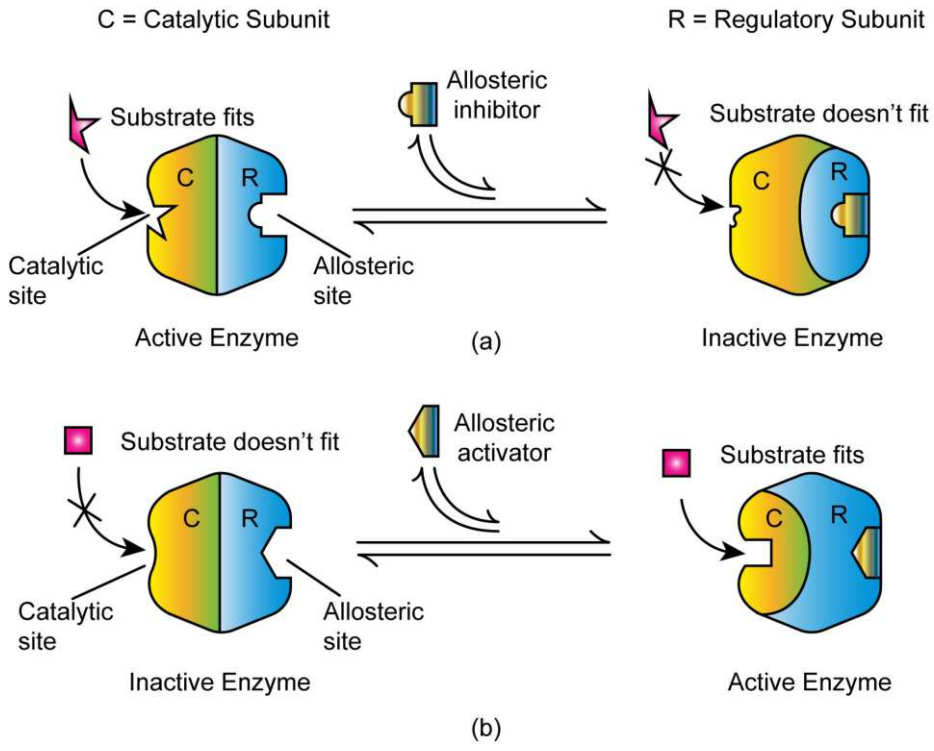


Fig. 9.2: Schematic diagrams showing the functioning of allosteric enzymes in a) inactive and b) active form.

We can also say that allosteric enzyme action is regulated by effector molecules. These effectors molecules can bind at the same allosteric site or at different allosteric sites. Allosteric enzymes exist in two forms: **T form** (Tensed or inactive form) and **R form** (Relaxed or active form). Enzymes can exist in one of the two forms but not in both together.

In an enzyme with more than one active site, it is observed that binding of a substrate to one active site may influence subsequent bindings to other active sites. The behavior of enzymes is known as **cooperativity**. When binding of the first substrate molecule promotes binding of subsequent substrate molecules, it is called **positive cooperativity**. Actually binding with the substrate, the conformation of the enzyme shifts from T form to R form. **Negative cooperativity** is when, after the binding of the first substrate molecule subsequent substrate binding occurs less readily. Allosteric enzymes and enzymes that show cooperativity do not obey Michaelis-Menten Kinetics. Such enzymes which are composed of more than one subunit and more than one active site show a sigmoidal curve when reaction velocity is plotted against substrate concentration (Fig. 9.3) and not a hyperbola as shown by non-allosteric enzymes.

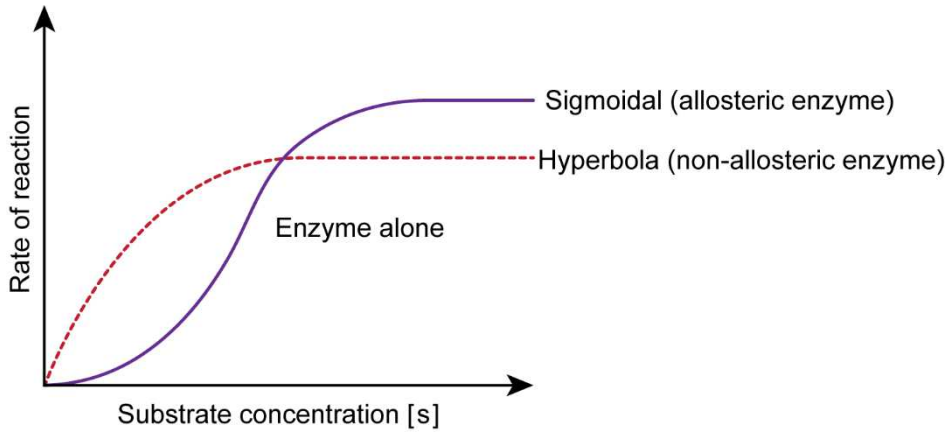


Fig. 9.3: Allosteric enzymes show sigmoidal curve when reaction rate is plotted against substrate concentration.

Many allosteric enzymes function at the beginning of the metabolic sequence pathway and are inhibited whenever end product of the reaction pathway accumulates. Such inhibition is described as the **feedback inhibition**. Several important metabolic pathways are regulated by feedback inhibition both in prokaryotes and eukaryotes.

SAQ 3

a) Match the contents of Column A and those of Column B.

	Column A		**Column B**
i)	Ribozyme	a.	Inhibitors
ii)	Abzymes	b.	Artificial constructs
iii)	LDH	c.	*RNAase P*
iv)	Dexyribozymes	d.	Repair of Thymine dimers
v)	Negative effectors	e.	Isoenzyme

b) State whether the following statements are True or False?

i) Substrate is the reactant of an enzyme catalyzed reaction [ ]

ii) All enzyme-catalyzed reactions produce energy for ATP synthesis. [ ]

iii) Exorgonic and endergonic reactions are coupled in all living organisms. [ ]

iv) All the enzyme- catalyzed reactions have higher activation energy. [ ]

9.5 MECHANISM OF ENZYME ACTION

Now you are going to study the mechanism of enzyme catalysis. There is a small site known as active site or active centre to which the substrate binds. We will discuss how an **enzyme-substrate complex** is formed and also study various hypothesis related to it.

9.5.1 Concept of Active Site

As you have studied earlier, the specificity of an enzyme is due to the proteinaceous structure/character of its apoenzyme. Within a large protein molecule, a very small region is infact involved in the binding of substrate and subsequent catalysis of the reaction. The region of specific molecular conformation is called "**active site**" or "**active centre**". The active site contains certain amino acid residues whose side chains are in specific conformation and participate in the catalyzed reaction. The properties and positions of side chains of the enzymes exposed at the active site (Fig. 9.4) determine which substrates will bind to it.

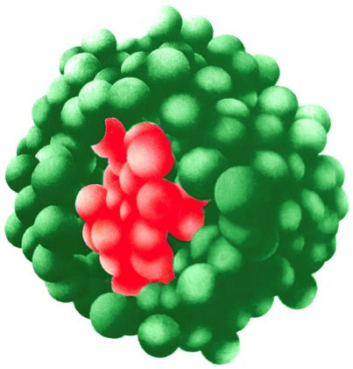


Fig 9.4: A space–filing model of the tertiary structure of an enzyme showing the active site deep in the molecule

The specific amino acid side chains at the active site are not necessarily close to each other in the linear chain. Infact, they are most often widely separated from each other in their location at the level of primary structure of protein. Folding of the protein chain, however, brings these groups together. The side chains of these amino acids form a part of the pocket located either on the surface of the enzyme molecule (Fig. 9.5 a) or by forming deep opening in the enzyme 3-D structure (Fig. 9.5 b).

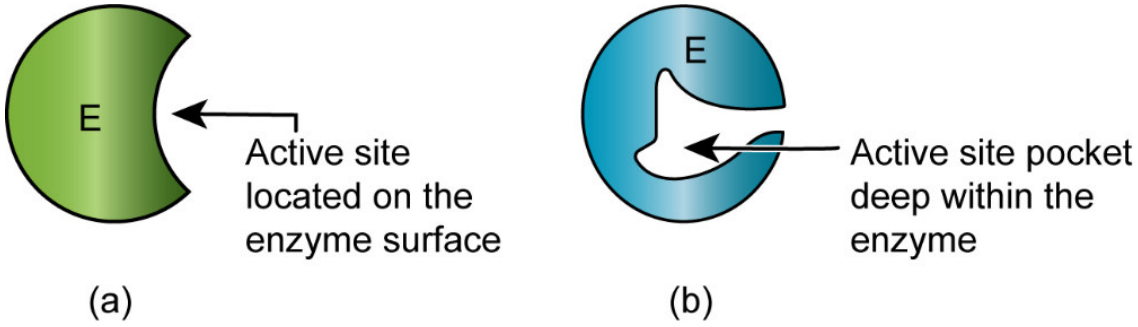


Fig. 9.5: Location of active sites in an enzyme a) on enzyme surface; b) Deep within the enzyme molecule.

An active site is functionally divided into two subsites:

a) **Substrate binding subsite** (specificity subsite) which is responsible for recognition of the substrate.

b) **Reaction subsite** where the actual chemical reaction takes place.

These far located groups are however, brought in close proximity by folding of the protein chain. For example, negatively charged side chain groups, like those of **aspartate** and glutamate, can be forced together, due to folding of the enzyme protein molecules in spite of their tendency to repel each other. This leads to an increase in affinity for the positively charged groups of the substrate.

The negatively charged substrate is steered into the binding site by the electrostatic forces torques. Sometimes, salt links between groups on the protein surface prevent the solvent to enter the binding sites. These gates are first disrupted by the right substrate in order to enter the binding site. This is called **Gate Binding**. The affinity and specificity of binding and orientation of the ligand in the binding site depend on the contribution of electrostatic interactions between the ligand the protein (Fig. 9.6).

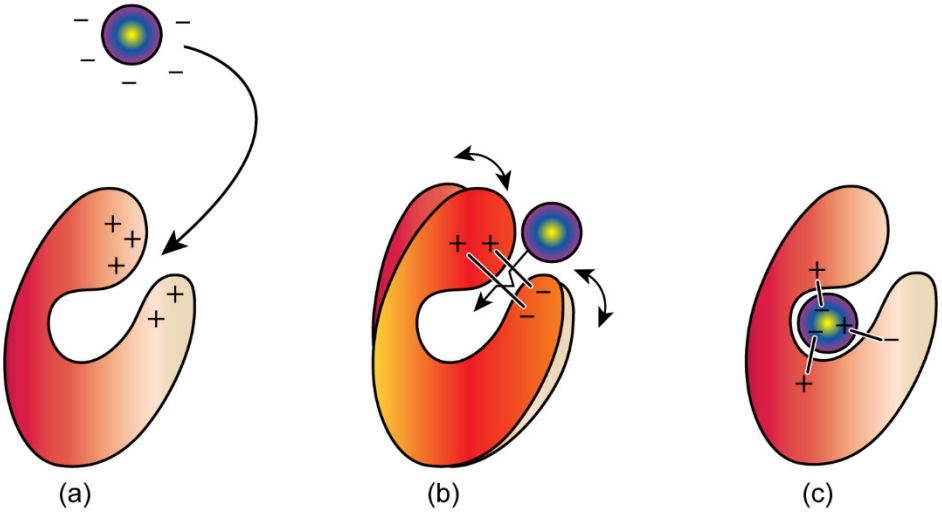


Fig. 9.6 (a-c): Steps in Enzyme-substrate binding steered by electrostatic forces-gated binding.

9.5.2 Enzyme-Substrate Complex

The enzyme recognizes and binds to the specific substrate to its active site and form an **enzyme-substrate complex** (ESC) (Fig 9.7). The binding is

facilitated by the flexible nature of the active site. The amino acids making up the active site are brought together in just the right conformation by the specific three-dimensional folding of the polypeptide chain. The cofactors or coenzymes also facilitate the formation of this complex. This complex is now converted into a high-energy state or activated enzyme-substrate complex, also called **transition state complex** (ES $^{++}$). The enzyme acts upon the specific bonds within the substrate resulting in the formation of the product. The events in enzyme catalysed reaction are represented by the equation.

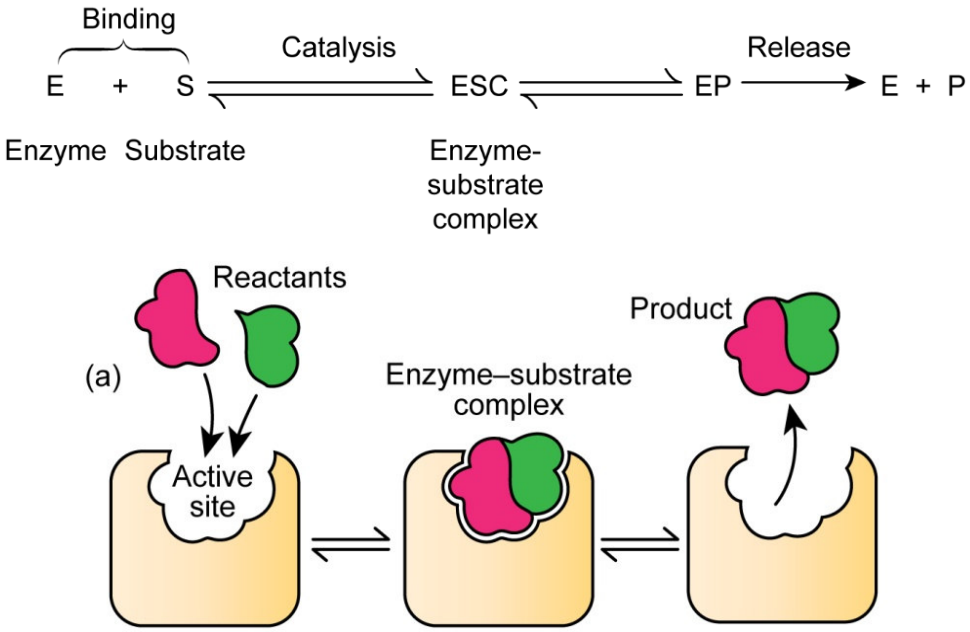


Fig 9.7: Formation of enzyme-substrate complex (ESC).

The enzymes lower the energy barrier and allow the organism to carry out reactions faster. Although enzymes differ widely in structure, specificity and mode of catalysis, various mechanisms operate at the active site of enzymes, each contributing to the lowering of activation energy.

9.5.3 Lock and Key Hypothesis

For a long time the enzymologists considered the active site to be a rigid structure. The German biochemist **Emil Fischer** (1894) suggested a **lock and key hypothesis**. According to this hypothesis, an enzyme is a rigid molecule with predetermined structure. It is complimentary to the substrate as the later fits into the active site as the key fits into a lock (Fig. 9.8). This results in the formation of transient enzyme-substrate complex which breaks down into the enzyme and the product/s.

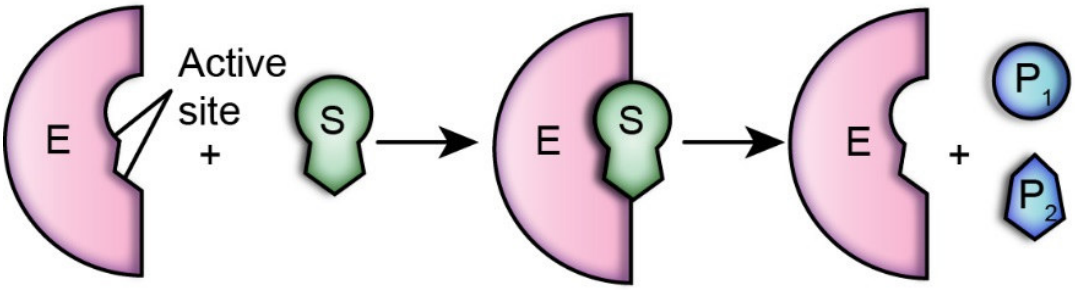


Fig. 9.8: Lock and Key Model with a rigid structure of active site. The substrate 'Fits' into the active site.

This model however, was not able to explain some questions that were subsequently raised. Many a times, some other compounds other than the substrate may bind to the enzyme. The reaction however fails to takes place in such cases.

9.5.4 Induced - Fit Model

A more useful model called as the induced-fit model was proposed in 1958 by **Daniel Koshland**. This model assumes that the active site of an enzyme does not initially exist in a shape complimentary to the substrate. The active site is actually flexible and the complimentarily is induced only after the binding of the substrate (Fig. 9.9).

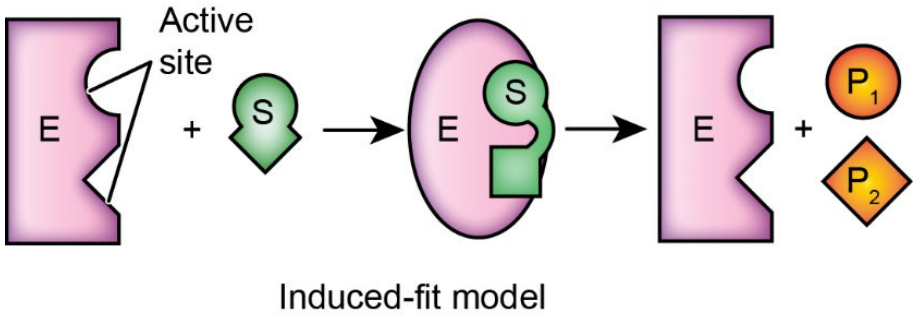


Fig. 9.9: Induced-fit Model. The substrate induces a conformational change in the active site conformation.

Initial binding of the substrate may distort the enzyme and substrate and the stressed covalent bonds are more susceptible to catalytic attack which favors the reaction. The alterations in the enzyme involve a conformational change in the shape of the enzyme molecule and ultimately the configuration of the active site.

Both the above models account for monosubstrate reactions. There are other models (viz., Cleland notation, Random Sequential Model and Ordered Sequential Model) which try to explain the mechanism of bisubstrate reactions. The multisubstrate reactions have been explained on the basis of a Ping-Pong Model.

SAQ 4

Complete the following statements with appropriate words.

i) The substrate binds on the …… of the enzyme and forms …….. .

ii) Enzymes do not alter the …….. of the reactions.

iii) According to …….. active site assumes the complementary shape after the …….. is bound.

iv) …… of the polypeptide chains bring the side chains together in the active site region.

v) …… amino acids create a water free zone for ……. reactants.

vi) Transfer of protons to and from the reactants is …… catalysis.

9.6 ENZYME INHIBITION

Enzyme activity can be inhibited by certain chemical substances called **inhibitors**. Enzyme inhibitors have provided information about substrate specificity of enzymes, the nature of functional groups at active site and the mechanisms of the catalytic activity. Some drugs considered to be useful in medicine function by inhibiting certain enzymes. For example, the inhibitions of bacterial enzymes which affects the bacterial metabolism and in turn their growth and multiplication.

Enzyme inhibitors are of two types: Irreversible and reversible.

9.6.1 Irreversible Inhibitors

These inhibitors bind covalently to the enzymes causing a permanent loss of their catalytic activity by altering or destroying a functional group on the enzyme molecules. The inhibitor does not bear any similarity with the substrate and binds on a site other than the active site. The binding is either with the enzyme or the ESC. Change in conformation of the enzyme induced by this binding prevents the reaction to proceed.

9.6.2 Reversible Inhibitors

These inhibitors bind non-covalently to enzymes and their effects can be reversed. Important information on the structure of the active sites of various enzymes has been obtained with the help of reversible inhibitors. The two main types of reversible inhibitors are competitive and non-competitive inhibitors.

a) **Competitive inhibitors:** Competitive inhibitors compete with the real substrate for binding to the active site of the enzyme because they resemble the substrate molecules. The catalytic ability of the enzyme is not affected by this binding but the ability of the substrate to bind to the enzyme is reduced. However, these inhibitors cannot be transformed into products (Fig. 9.10). Competitive inhibition is reversed simply by increasing the substrate concentration. For example, *succinate dehydrogenase*, which catalyses the removal of 2 hydrogen atoms from succinate is inhibited by malonate which resembles succinate structurally.

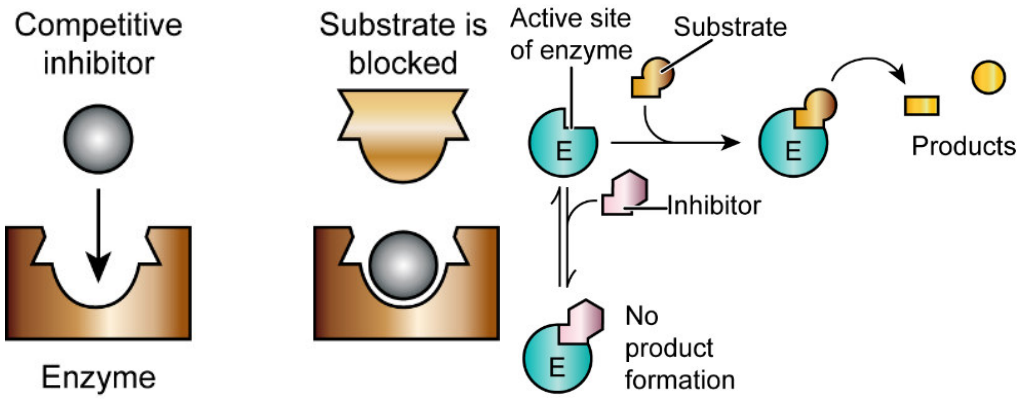


Fig. 9.10: Competitive inhibition. Both substrate and the inhibitor compete for the same active site (From Becker).

b) Non-competitive inhibitors: These inhibitors bind at a site on the enzyme other than the catalytic site, i.e., substrate binding site, altering the conformation of the enzyme molecule and leading to the distortion and inactivation of the catalytic site. Non-competitive inhibitors bind reversibly to both the free enzyme and the ES complex to form the inactive complexes (Fig. 9.11).

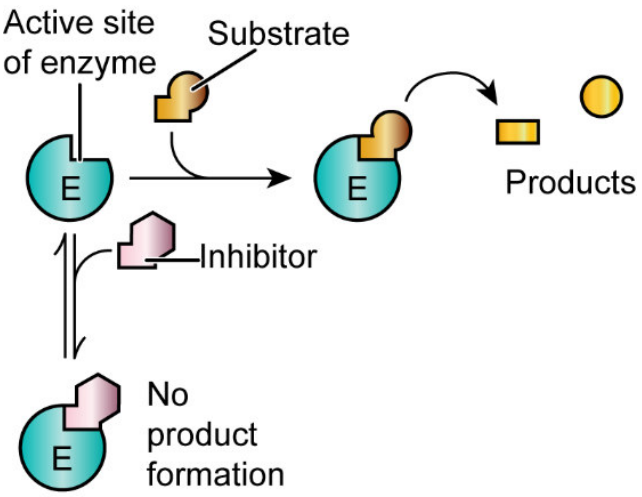


Fig. 9.11: Non-competitive inhibition. The substrate and inhibitor bind to different sites.

Addition of more substrate will not restore the previous rate of reaction in contrast to competitive inhibitors. Important non-competitive inhibitors are the naturally occurring metabolic intermediates. For example *L-threonine dehydratase is* inhibited by L-isoleucine. Other examples are: Heavy metal ions, EDTA, fluoride, H_2S and cyanide. Competitive and non-competitive inhibitors are not necessarily harmful. Both are used extensively by cells for metabolic regulation.

9.6.3 Uncompetitive Inhibition

This inhibition is somewhat similar to the non-competitive inhibition as it does not bind to the active site. But uncompetitive inhibitors bind only to the enzyme substrate complex (Fig. 9.12).

$$E + S \rightarrow ES + I \rightarrow ESI \rightarrow \text{No Reaction}$$

Inactive

Uncompetitive inhibition is common in bisubstrate reactions.

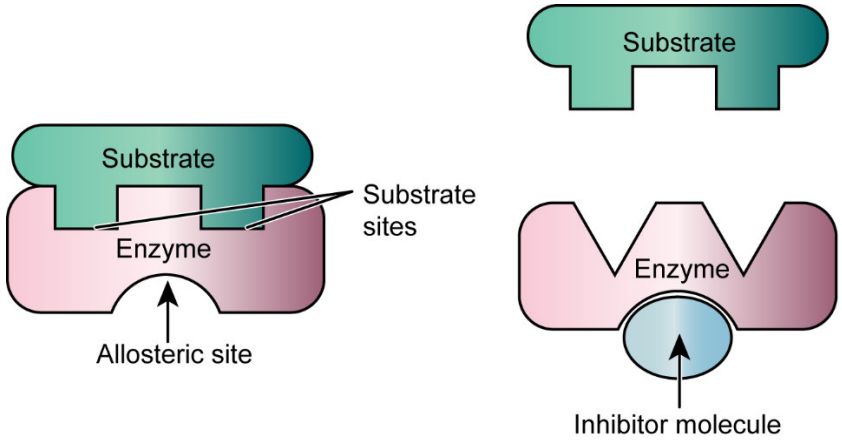


Fig. 9.12: Uncompetitive inhibition.

9.7 REGULATION OF ENZYME ACTIVITY

All chemical reactions must proceed at rates in accordance with the requirements of the cell or organism. All the enzymes within the cell do not exhibit their maximum activity at all times. Some may be functioning at greatly reduced rates while others may be completely inactive when not required as the related metabolic reactions stop according to the requirement of the cell. Life processes like ATP production, synthesis of molecules and macromolecules, transport, secretion, absorption, etc., are regulated to respond to the changes in the internal as well as external environment of the cell, organ or organism. Since the metabolic reactions are enzyme catalysed, regulation is achieved by altering the enzyme activity. Regulation depends mainly on 'specificity'. If an enzyme is inactive or not available, no other enzyme can take its place and the reaction will stop. However, reactions unrelated to this particular enzyme will not be affected.

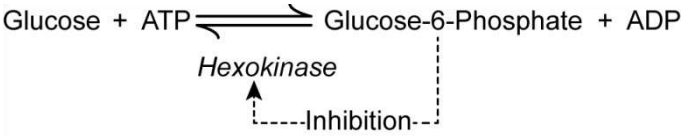
Immediate regulation of enzyme activity is achieved by changes in pH, temperature, enzyme and substrate concentration. Regulation based on interactions of substrates and products with the enzymes is called **substrate-level regulation**. Some enzymes are synthesized in an inactive or less active from called **proenzymes** or **zymogens**. These proenzymes or zymogens are converted to the active enzymes by removal of a specific amino acid or peptide in the protein molecule.

This type of regulation, however, cannot account for most reaction sequences occurring in metabolism. Other mechanisms operate through **allosteric regulation** and covalent modification by modulating enzyme activities through precise on or off switches. This helps to fine tune the reaction rates according to the metabolic requirements of a cell.

The mechanism of control of catalysis involves a change in enzyme activity without a change in the total amount of enzyme synthesized. Important mechanisms of this type are **feedback inhibition, allosteric regulation** and **covalent modulation**. Let us study in detail about these mechanisms.

9.7.1 Feedback Regulation

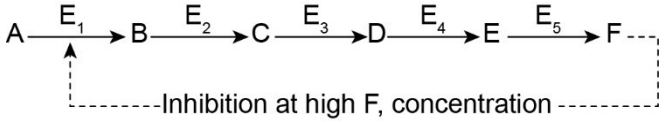
Feedback regulation means that the product of a reaction or a multienzyme reaction controls its own production. Let us take the example of inhibition of the first-reaction of glycolysis. During the initial stages of glycolysis, glucose in the presence of enzyme *hexokinase* combines with ATP to produce glucose-6-phosphate and ADP. Increased concentration of glucose-6-phosphate inhibits the enzyme *hexokinase*.



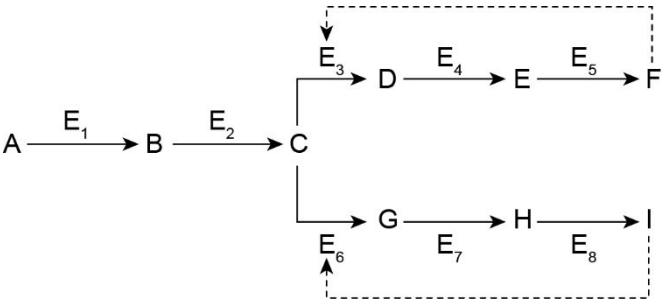
As the concentration of glucose-6-phosphate decreases, enzyme *hexokinase* becomes active again.

As you have read above, that metabolism involves multi-enzyme systems in which the product released by one enzyme is the substrate for the next enzyme. Often the products formed at the end of a pathway regulate the activity of the earlier enzymes in the metabolic pathway and affect the whole

pathway. For example, in a pathway of five enzyme catalysed reactions, substrate A is converted into the end-product F. Also, E_1, E_2, E_3, E_4 and E_5 are the enzymes involved in the pathway. Synthesis of product F can be regulated if it can combine with enzyme E_1. In such a case, at high concentration of F, E_1 will be inhibited, whereas at low concentrations of F the metabolic pathway will proceed towards the formation of F. The first irreversible reaction in a biosynthetic pathway is called the **committed step** and final product usually inhibits the enzyme which catalyses the committed step. The process in which an intermediate metabolite or an end-product inhibits its own production is called as **feedback inhibition** or **negative feedback control**.



Some of the metabolic pathways are branched at certain points. For example, substrate A may be converted either to end-product F or end-product I. In such a case, the feedback inhibition would occur at the branch point, i.e., end-product F will inhibit enzyme E_3, whereas end-product I will inhibit enzyme E_6. The inhibition of enzyme E_3 will not in any way affect the formation of end-product I and vice versa. However, feedback inhibition before the branch point will stop the synthesis of both the end-products, i.e., F and I.



End –product regulation at the initial stages of a reaction pathway has its own significance. Thus the accumulation of intermediate compounds, which may not be required by the cell or organism, is avoided. Also, since all the reactions dissipate some energy into a useless form, less energy is wasted if intermediate reactions are inhibited.

9.7.2 Allosteric Enzyme Regulation

As you have read above, the feedback inhibition is one of the ways in which enzyme activity can be regulated. Not only the end-products of the reaction pathways but other metabolites also may bind with enzyme and alter the enzyme activity.

The binding can occur at the **active site** or at the **regulatory site** on the enzyme surface as in case of **allosteric enzymes**. You have read about allosteric enzymes and their mode of functioning in section 9.4.6. As you know, allosteric enzymes have a regulatory binding site apart from the active site. The active site binds with the substrate and converts an **effector**, which influences the enzyme activity. It is now known that enzymes at the initial stages of metabolic pathways are usually allosteric enzymes. The regulatory sites of these enzymes are specific to the product of the metabolic pathway.

As the metabolism proceeds, the concentration of the end-product increases. The end-product molecules act as negative effectors and bind to the regulatory sites of the allosteric enzyme resulting in conformational changes in the active site, thus inactivating the enzyme. The effectors bind non-covalently to the regulatory site of the enzyme. Therefore, inactivation of enzymes is reversible. Unbinding of the negative effector converts the inactive enzymes to their active form.

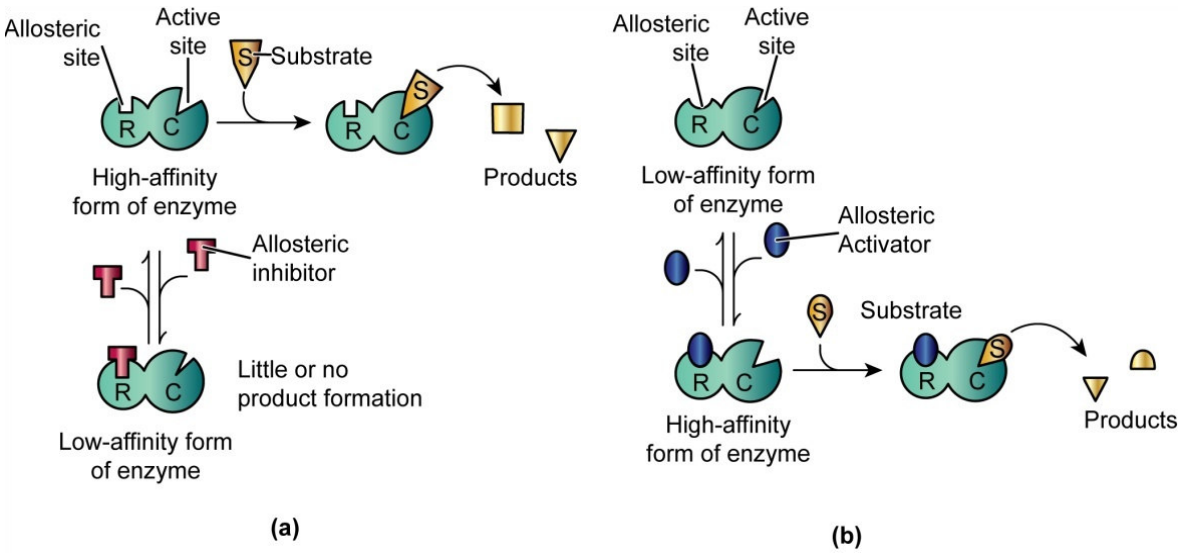


Fig. 9.13: a) Inhibition- Active enzyme in an uncomplexed form is transformed into its inactive form by binding of an allosteric inhibitor. b) Allosteric Activation-Inactive uncomplexed enzyme with low affinity for the substrate gets stabilized into a high-affinity formed after an allosteric activator binds to it.

You have read section 9.4.6 that binding of a positive effector molecule to the regulatory site of the allosteric enzymes converts the inactive enzyme to its active form. This is brought about by changes in the conformational structure of the enzymes. In case of isoleucine synthesis pathway, *threonine deaminase* is the allosteric enzyme and isoleucine is the allosteric effector. The effector can bind to one of the two interconvertible forms of the enzyme. An effector may be either an **allosteric inhibitor** or an **allosteric activator** depending upon the effect it would have after binding to the allosteric site. Fig. 9.13 a, b depicts the mechanisms of allosteric inhibition and activation.

9.7.3 Covalent Modulation

Enzyme activity is also controlled by covalent modulations of the enzymes. Covalent modifications cause conformational changes in the enzymes. These conformational changes are brought about by covalent bonding of a phosphate group (**phosphorylation**) to the polypeptide chain or by removal of a small polypeptide chain by a process known as **proteolysis**. These covalent modifications stimulate the enzyme activity. For example the conversion of glycogen into glucose-l-phosphate is catalysed by an enzyme *glycogen phosphorylase*. This enzyme occurs in two forms: *phosphorylase a*, the active form and *phosphorylase b***,** the relatively inactive form. These two forms are interconvertible. *Phosphorylase b* is converted into active *phosphorylase a* by covalent binding of phosphate groups. The enzyme involved in this conversion is *phosphorylase kinase*.

$$2\ \text{ATP} + phosphorylase\ b \xrightarrow{Phosphorylasekinase} 2ADP + phosphorylase$$

The process is reversible as the phosphate groups can be removed hydrolytically (dephosphorylation) by an enzyme called *phosphorylase phosphatase* (Fig. 9.14).

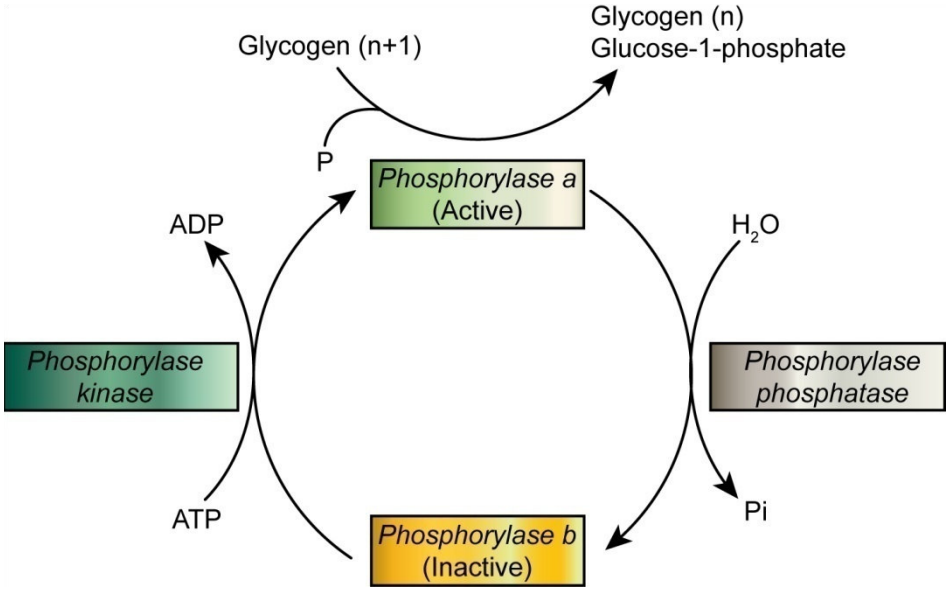


Fig. 9.14: Activation and inactivation of enzyme *phosphorylase*.

Thus the cell regulates the rate of glycogen utilization according to the requirement of the organism. Covalent bonds are strong and as a result covalent modifications are fairly stable.

SAQ 5

a) Fill in the blanks with appropriate words.

i) Allosteric enzymes are …….. proteins.

ii) Effectors induce ……. changes in allosteric enzymes.

iii) Multiple forms of an enzyme are known as…….. .

iv) The …….. of the isoenzymes may occur in different combinations.

v) Metabolites are the ……. products in a pathway.

vi) Regulation mainly depends upon the ……. of the enzyme.

vii) Increased concentration of the end-product…….. its own …… .

viii) Inhibition ……. the branching point of a pathway will stop the synthesis of all the end-products.

ix) Often the enzymes at the initial stages of metabolic pathways are …… .

x) Effectors bind to the ……. site of the allosteric enzyme.

b) Differentiate between competitive and non-competitive inhibitors. Answer briefly in the space provided.

9.8 FACTORS AFFECTING THE RATE OF ENZYME ACTION

Enzymes are capable of enhancing the reaction rate, sometimes a million times compared to uncatalysed reactions. The rates of enzyme catalysed reactions are expressed as the "**turnover number**". The turnover number ($\mathbf{k_{cat}}$) is the maximum number of substrate molecules transformed into product in unit time by a single enzyme molecule or by single catalytic site, and depends upon the enzyme concentration. It is also called **Molecular Activity**. So if the enzyme concentration is known, the turnover number can be calculated.

$$\text{Turnover number} = \frac{\text{moles of products formed per unit time}}{\text{moles of enzymes}}$$

Turnover number of some enzymes is depicted in Table 9.7.

An **International Unit** (**IU**) is the amount of enzyme that catalyzes the formation of one micromole of product in one minute under optimal conditions of pH, temperature and ionic strength.

Specific activity (**U/mg**) is the number of units of enzyme activity per milligram of total protein present. Enzyme concentration [**E**] is units/mg protein x mg protein/mL.

Table 9.7: Turnover Number of Some Enzymes

Enzyme	Turnover Number
Carbonic anhydrase	3.6×10^6
Acetylcholinesterase	1.4×10^4
Urease	1.0×10^6
Amylase	1.0×10^5
Lactic dehydrogenase	6.0×10^4
Chymotrypsin	6.0×10^3
Lysozyme	3.0×10^1
Catalase	40,000,000

Rates of enzyme catalysed reactions depend on several factors like reaction temperature, pH, concentration of enzyme and substrate, oxidation and radiation exposure.

9.8.1 Effect of Temperature

Within certain limits temperature influences the enzyme catalysed reactions in the same way as it affects ordinary uncatalysed chemical reactions. As the temperature increases, the rate of a chemical reaction increases owing to an increase in the number of activated molecules. But when the temperature rises above a certain limit, it destroys the tertiary structure of the enzyme causing the loss of its activity. Similarly, low temperature, such as freezing temperatures,

generally inactivates the enzyme. It, therefore, follows the rule that for every enzyme under a given set of conditions, there is an optimum temperature at which the activity of the enzyme is at its maximum (see Fig. 9.15).

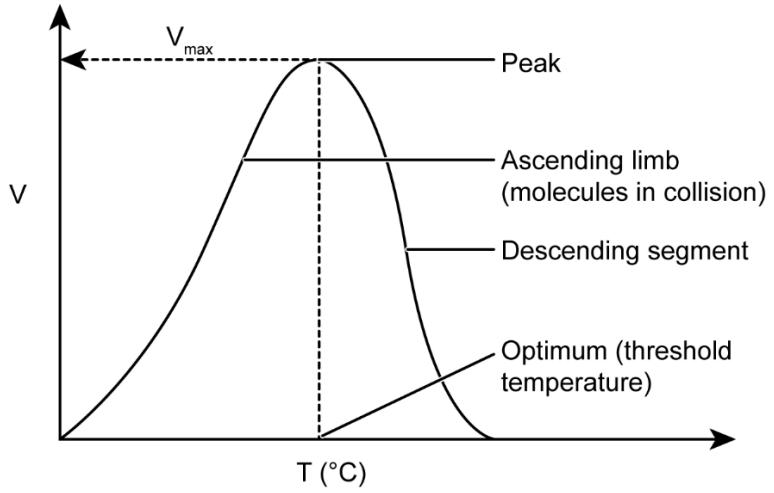


Fig. 9.15: Effect of temperature on the rate of enzyme catalyzed reactions. The optimum temperature varies in different enzymes and in different species.

Buffers are the combination of substances that resist change in pH when acid or bases are added. It consists of a weak acid and its conjugate base or a weak base and its conjugate acid, for example acetic acid (CH_3COOH) and acetate (CH_3COO^-) can serve as a buffer when pH of the solution has to be adjusted around pH 1.

The plot of velocity *vs* temperature is **bell-shaped**. Most enzymes are denatured above 50º C. Optimum temperature for mammals and birds is about 35-40ºC whereas in plants and in other animals it is between 20-35º C. Some prokaryotes and certain fishes found in Antarctica region are exceptional in that they are adapted to life in hot spring and at freezing cold temperatures. They have optimum temperature at over 80º C and below freezing point respectively.

9.8.2 Effect of pH

The hydrogen ion concentration of the pH has a marked influence on the rate of enzyme reaction. Characteristically, each enzyme has a pH value at which the reaction rate is optimum usually within the pH range 7.0±1.5. This is known as optimum pH at which a certain enzyme causes a reaction to progress most rapidly (Fig. 9.16). On each side of this pH value the reaction is decreased and at certain pH values on both sides an enzyme may be inactivated or even denatured. Therefore, while studying enzymes, buffers are used to keep the enzyme at an optimum pH.

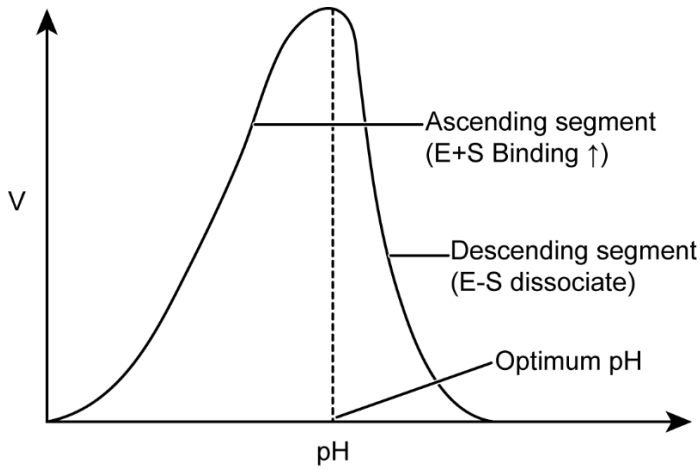


Fig. 9.16: Effect of pH on the rate of enzyme catalysed reaction.

While majority of enzymes function optimally around neutral pH values, some enzymes like pepsin in stomach has a usually low optimum pH. This is an adaptation to the acidic conditions of stomach which has pH ranging from 1.5 to 2.5 due to the presence of HCl in the gastric juice. Pancreatic enzymes have their optimum pH in highly alkaline range.

Enzyme	Optimum pH
Pepsin	2
Chymotrypsin	7.8
Papain	7-8
Lipase	5-8
α-glucosidase (maltase)	6.6
β-amylase	5.2
Invertase	4.5

9.8.3 Effect of Enzyme Concentration

As you know, enzymes increase the reaction rate through catalysis. Under constant conditions of temperature, pH and substrate concentration, the velocity of the reaction is directly proportional to the amount of enzymes present, i.e., the reaction rate is increased with the increase in enzyme concentration. However, the equilibrium constant for the reaction is not affected by the presence of enzymes. A chemical reaction in the cell can be started or stopped by making or destroying a particular enzyme. As you have read, turnover number is a useful parameter to study the effect of enzyme concentration.

Enzymes not only speed up the reactions, they also have a marked effect on the kinetics of the reaction. Substrate concentration plays an important role in enzyme kinetics about which you will read in the following section.

9.8.4 Effect of Substrate Concentration – K_m

The concentration of substrate also influences the rate of enzyme catalysed reaction. At low substrate concentrations, the numbers of substrate molecules are too low to occupy all the active sites of the enzyme molecules. The initial velocity of the reaction is directly proportional to the substrate concentration. With the increase in substrate concentration more and more active sites are occupied by many more molecules of substrate. A stage will finally be reached when the substrate concentration is high enough to occupy all the active sites of the enzymes. At this saturation point the maximum velocity of the reaction is attained and is called V_{max}. The maximal velocity is not affected by further increase in substrate concentration. The curve of the rate of enzyme reaction against substrate concentration shows the shape of a rectangular hyperbola for enzymes composed of single polypeptide chain with one active site (see Fig. 9.17). The hyperbola shows two distinct regions:

i) **Linear region:** In the initial stage of the reaction the velocity of the enzyme catalysed reaction increases **linearly** with an increase in

substrate concentration. At this stage the substrate concentration is low and the reaction follows the **first order** kinetics.

ii) **Plateau region:** As the substrate concentration is raised gradually, the maximum velocity (V_{max}) is attained after which the velocity is constant even when substrate concentration is increased (plateau). The point in the graph where the linear plot gets transformed into a plateau is called **saturation point**. At this point the active sites of the enzyme are completely saturated with the substrate.

At a particular concentration of substrate where half the enzyme molecules are saturated with substrate molecules, the velocity (V) of the reaction is half the maximal velocity ($\boldsymbol{V_{max}/2}$). This substrate concentration at half the maximal velocity is termed as $\boldsymbol{K_m}$ or **Michaelis constant**.

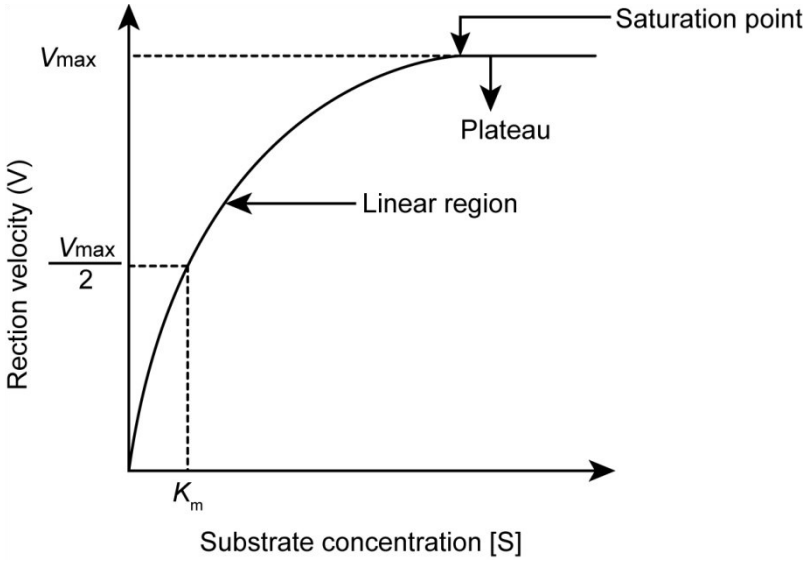


Fig. 9.17: Relationship between reaction rate (V) and substrate concentration (S). The initial velocity of the reaction increases with the substrate concentration. At high substrate concentration V becomes independent of S. Michaelis constant K_m is equal to substrate concentration when reaction rate is half its maximum value ($V_{max}/2$). All such curves are hyperbola.

Box: 9.1: Michaelis-Menton Kinetics

In 1913 Leonor Michaelis and Maud Menton proposed a simple yet convincing algebraic expression to explain the kinetics of enzyme reaction, highlighting the quantitative relationship between substrate concentration [S] and the initial velocity (V_o).

The Michaelis – Menton equation in its most familiar form is expressed as:

$$Vo = \frac{V\,max[S]}{K\,m + [S]}$$

Where V_o = initial velocity

V_{max} = maximum velocity

[S] = initial substrate concentration

K_m = Michaelis constant

When the initial velocity Vo is exactly half of V_{max} (maximum velocity), as shown in Fig. 9.17, the equation can be numerically expressed as :

$$\frac{V\max}{2} = \frac{V\max[S]}{K\ m + [S]}$$

Dividing by V_{max}, the equation becomes

$$\frac{1}{2} = \frac{[S]}{K\ m + [S]}$$

Which can be further solved for K_m to get

$K_m + [S] = 2\ [S]$

or $K_m = S$, when $V_o = \frac{1}{2} V_{max}$, which leads to practical definition of K_m being equivalent to the substrate concentration at which V_o is one half of V_{max} .K_m is usually used to express the affinity of an enzyme for the substrate. Since K_m is numerically equal to the substrate concentration at which the reaction velocity attains half of its maximum value, we can easily consider K_m as a measure of the substrate concentration required for effective catalysis.

A lower value of K_m would mean a higher affinity of an enzyme for the substrate and *vice versa*.

SAQ 6

Match the statements in column I with those in column II.

	Column I		**Column II**
i)	Temperature influences enzyme catalysed reaction	a.	It is active only in acidic medium.
ii)	Mammals and birds also have optimum temperature.	b.	Enzyme concentration has its affect on reaction rate.
iii)	Enzyme pepsin acts on peptide bonds in the stomach.	c.	Enzymes are inactivated at freezing temperatures.
iv)	Reaction rate is directly proportional to the amount of enzyme.	d.	It is between 35ºC to 40ºC.

9.9 SUMMARY

In this unit you have studied that:

- Enzymes are proteins that catalyse the biochemical reactions by lowering the activation energy. Enzymes increase the rate of the reactions but do not alter the equilibrium point.
- Enzymes facilitate the coupling of energy releasing exergonic reactions to energy requiring endergonic reactions.
- Enzymes are highly specific with respect to the nature of reaction as well as to their substrate. Enzymes substrate specificity has been explained by lock and key and induced fit hypothesis.

- Reactions by the enzyme are facilitated on the basis of proximity and orientation of side chains of the substrate and enzyme, acid-base catalysis by the side chain of charged amino acids of active site and by covalent interactions between enzymes and substrates.
- Most enzyme catalysed reactions follow a hyperbolic substrate saturation curve which is explained by the Michaelis-Menten equation for enzyme kinetics. Substrate concentration at half the maximum velocity of reaction rate is called Michaelis-Menten constant (K_m).
- Changes in the concentration of enzymes, substrates, pH and temperature influence the rate of enzyme catalysed reaction.
- Some enzymes require additional factors called cofactors for their catalytic activity. Prosthetic groups are organic cofactors and are permanently bound to the enzymes. Coenzymes are also organic compounds but their association is transient. Some enzymes require metal ions for their activity.
- Allosteric enzymes are oligomeric and have more than one active site where the allosteric effector molecules bind and influence enzyme activity. Influence of one substrate binding on subsequent substrate bindings is known as cooperativity. The graphs of kinetics of allosteric enzymes and enzyme cooperativity are sigmoidal.
- Enzyme activity is also regulated by inhibitors. Inhibitor actions can be reversible or irreversible. Reversible inhibitors may be competitive or non-competitive.
- All enzyme catalysed reactions involved in the metabolic processes of the cell are controlled by regulating enzyme activity.
- Feedback regulation is the process in which the end-product or an intermediate metabolite of a metabolic pathway binds with the enzyme and regulates its activity at the initial stage of the reaction pathway.
- The effector or regulator metabolite binds to the regulatory site of the allosteric enzyme producing conformational changes in the structure of the enzyme.
- Covalent modifications by phosphorylation or by proteolysis cause conformational changes in the enzyme. Such types of modification covert the inactive form of enzymes into active forms. Localisation of metabolic pathways into membrane bound cellular compartment also help to regulate enzyme activity.
- Enzyme activity in higher organisms is influenced by the hormones produced in their body.

9.10 TERMINAL QUESTIONS

1. What do you understand by activation energy?
2. Discuss briefly the variations in the degree of enzyme activity.

3. Explain briefly the functioning of allosteric enzymes.
4. Discuss briefly what you understand by feedback inhibition.

9.11 ANSWERS

Self-Assessment Questions

1. a) i) Kühne
   ii) Apoenzyme, cofactor, holoenzyme
   iii) Prosthetic group
   iv) inorganic metal ion
   b) i) *Urease*
   ii) Vitamin derived coenzyme
   iii) *Catalase*
   iv) Non-vitamin derived conenzyme
   v) Non-vitamin derived conenzyme
2. a) i) *Oxidoreductase*
   ii) *Hydrolase*
   iii) *Isomerase*
   iv) *Lyases*
   v) *Transferase*
   b) i) False; ii) False; iii) False; iv) False
3. a) i) *RNAase P*
   ii) Artificial constructs
   iii) Isoenzyme
   iv) Repair of Thymine dimmers
   v) Inhibitors
   b) i) True; ii) False; iii) True; iv) False
4. i) active site, enzyme-substrate complex
   ii) equilibrium state
   iii) induced fit hypothesis, substrate
   iv) Folding
   v) Hydrophobic, non-polar
   vi) acid-base

5. a) i) Oligomeric

   ii) conformational

   iii) isoenzymes

   iv) subunits

   v) transformation

   vi) specificity

   vii) inhibits, production

   viii) before

   xi) allosteric enzymes

   x) regulatory

   b) Competitive inhibitors resemble the substrate and compete for the active site. These do not affect the catalytic ability of an enzyme. Non-competitive inhibitors do not resemble the substrate and reduce the catalytic properties of enzymes.

6. i) Enzymes are inactivated at freezing temperatures

   ii) It is between 35º C to 40º C

   iii) It is active only in acidic medium

   iv) Enzyme concentration has its effect on reaction rate

Terminal Questions

1. The energy required to start a reaction is called the activation energy. In biological systems activation energy is lowered with the help of enzymes.

2. Enzymes exhibit specificity for the substrate as well for the reactions they catalyse. There is also variation in specificity within the particular groups of enzymes. Example of proteolytic enzymes can be given.

3. Allosteric enzymes are regulated by other molecules called as effectors. These effectors bind to the allosteric or regulatory site of the enzyme and may act as activator or inhibitor of enzyme action.

4. Feedback regulation is a process in which the product of a reaction or a pathway inhibits its own production. In the branched metabolic pathways, the end-product can inhibit its own production or the production of all the other products.

UNIT 10

RESPIRATION

Structure

10.1 INTRODUCTION

Respiration is one of the fundamental characteristics of all living organisms. Respiration provides energy for growth, maintenance, and reproduction. You have already read in Units 5-7, that the photoautotrophs trap solar energy and transform it into chemical energy and synthesize organic compounds.

A series of anabolic and catabolic reactions are required to utilize these organic substances obtained from external sources to generate energy. Respiration provides energy through a multistep, enzyme-controlled breakdown of organic substances. Metabolic energy released during the process is conserved in the form of ATP (energy currency of a cell). ATP is utilized in different cellular activities like cell division, cell elongation, ion transport, cytoplasmic movements and many more. This oxidation process ensures that energy is released in small steps for its immediate use or else for temporary storage. Besides ATP generation, intermediates of the respiratory pathway also serve as precursors for the biosynthesis of different biomolecules in the cells. Respiratory pathway can occur in the presence or absence of O_2, depending upon the environmental conditions.

Unlike animals, plants do not have specialized respiratory organs like lungs and nostrils. Instead, the function of gaseous exchange is carried out by stomates and lenticels (present in leaves, stems, and fruits respectively).

Leaves with tiny pores or stomata are the main sties of gaseous exchange in a plant. The number, distribution, and structure of the stomates varies in dicots and monocots as does the structure of guard cells. Stomates are also present on the stems of herbaceous plants but not in the woody stems and developing fruits. The gaseous exchange is carried out through lenticels. Oxygen in the interspaces of soil diffuses into the root hair. Similarly, CO_2 also escapes the root hairs by diffusion.

In the present unit you will be made familiar with concepts of cellular energetics, i.e., the concept of ATP, Gibbs free energy, endergonic and exergonic reactions. In addition, a detailed discussion on the respiratory quotient (RQ) will help you to appreciate the nature of different respiratory substrates. An overview of the energy releasing pathways will highlight the various alternatives present in plants to breakdown food to obtain energy under diverse physiological and environmental conditions.

Objectives

After studying this unit, you would be able to :

- differentiate between respiration in plants and animals;
- appreciate the concept of free energy with reference to the respiratory process and compare it with combustion;
- describe the ultrastructure and organization of mitochondria – the "Powerhouses" of a cell;
- familiarize with different membrane domains of the mitochondria and the location of the elementary/F_1 particles;
- describe respiration under aerobic and anaerobic conditions;
- explain the respiratory quotient and its significance ,and
- to prepare an overview of the different alternative pathways in respiration.

10.2 RESPIRATION *V/S* COMBUSTION

Since the term respiration was used by animal physiologists to describe the breathing movements of animals, the two terms respiration and breathing appear to be synonymous. This is not true. You will agree that breathing refers to a type of muscular movement involved in taking in of oxygen and expulsion of CO_2 from the lungs. Respiration is somewhat more than this. It includes a series of multi step, controlled chemical reactions, where the complex organic compounds like carbohydrates, fats and proteins are broken down using O_2 to produce CO_2 and H_2O, and releasing energy is small steps, a part of which is stored in a biologically useful form (ATP).So, in animals, breathing is in fact, a part of respiratory process.

Some major differences between combustion and respiration are presented in Table 10.1.

Table 10.1: Comparison between Combustion and Respiration

Combustion	Respiration
1. It is a physiochemical process	1. It is a biochemical cellular process.
2. Many chemical bonds break down simultaneously. Oxygen combines directly with the substrates to yield energy.	2. The bonds break in a sequence thereby releasing energy step by step and in a controlled manner. Moreover, O_2 only indirectly combines with substrate. It binds only at the terminal steps.
3. Occurs at high temperature (200-300° C).	3. Temperatures remain normal.
4. The entire energy is released as heat.	4. Some energy is stored in a biologically useful form (ATP). Rest is lost as heat.
5. One step process	5. Multistep; Each step catalyzed by a specific enzyme.
6. Emits light	6. No emission of light.
7. No intermediates formed.	7. Many intermediates are formed during the process.

Can we really compare cellular respiration to burning of wood? Well, the processes of respiration and combustion are comparable in a thermodynamic sense. Whether it is metabolic oxidation of glucose, combustion of glucose in calorimeter, or burning of wood (cellulose – a polymer of glucose), same amount of free energy is liberated.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \quad \Delta G' = -2870 \text{ kJ/mol } (= 686 \text{ kcal/mol}) \quad \text{(i)}$$

Stoichiometry is the relationship between reactants and products in a reaction .It represents the quantitative relation between the number of moles (and therefore, mass) of various products and reactants in a chemical reaction.

The biological oxidation of glucose during respiration is a far more complex process than combustion. This is because in combustion, the entire energy is released in the form of heat and a device is needed to convert this heat into useful work, e.g., a steam engine). In contrast, biological systems are characterized by not allowing a large fluctuation (increase) in temperature, and a large part of this free energy is converted into ATP. This energy currency of the cell will be utilized to provide driving force to various endergonic reactions with $+\Delta G$ (energy requiring). However, energy is released in small instalments in biological systems to avoid sudden surge in temperature.

A series of coupled oxidation– reduction reactions occur with the help of intermediate electron carriers like NAD^+ and FAD^+ before the electrons are finally transferred from a reduced substrate to oxygen.

Although the net oxidation processes of combustion and biological oxidation are identical (see equation i), the biological oxidation of glucose is more accurately represented by the following coupled reaction.

$$C_6H_{12}O_6 + 10\ NAD^+ + 2FAD^+ + 6H_2O \longrightarrow 6CO_2 + 10\ NADH + 10\ H^+ + 2FADH_2$$

$$10\ NADH + 10\ H^+ + 2FADH_2 + 6O_2 \longrightarrow 10\ NAD^+ + 2FAD^+ + 12H_2O$$

$$\text{Net } C_6H_{12}O_6 + 6O_2 \longrightarrow 6\ CO_2 + 6H_2O \quad \text{(ii)}$$

In the section11.7, we will conclude that the oxidation of 1 mole of glucose during respiration is coupled to phosphorylation of ~32 moles of ADP, resulting in the generation of 32 ATP.

$C_6H_{12}O_6 + 6O_2 + 32ADP + 32Pi \longrightarrow 6CO_2 + 38H_2O + 32\ ATP$ (iii)

The coupling stoichiometric properties of ATP are a result of adaptation i.e., a compromise, and are therefore, different from the ones observed in equation (i) and (ii). As to the evolutionary settlement on a stoichiometry of ~32 ATP, you should appreciate that the free energy changes of coupled reactions are **additive**. Thus, if the above equation (iii) is reviewed in terms of free energy yielding (exergonic = - ΔG) and free energy requiring(endergonic = + ΔG) reactions, the following representation can be made.

Gibbs free energy (*G*) is a thermodynamic potential which is used to calculate the maximum reversible work that may be performed by a thermodynamic system at a constant temperature and pressure.
The concept was developed by the American scientist Josiah Willard Gibbs in1873. The Gibbs free energy $\Delta G = \Delta H - T\Delta S$ (measured in Joules in SI units).

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$ ΔG (kJ/mol glucose) = -2900

$32ADP + 32\ Pi \longrightarrow 32\ H_2O + 32\ ATP$ ΔG = + 50 kJ/mol ×32 mol ATP = +1600

Sum :

$C_6H_{12}O_6 + 6O_2 + 32\ ADP + 32Pi \longrightarrow 6CO_2 + 38H_2O + 32\ ATP$

ΔG = -2900 + 1600 = - 1300 kJ/mol glucose

Gibbs free energy change (ΔG) for glucose oxidation has been estimated to be equal to – 2900 kJ/mol. Similarly, synthesis of each mol of ATP requires +50kJ/mol glucose. If we follow the stoichiometry of 32 ATP with a net ΔG of -1300 kJ/mol glucose, it is clear that immense driving force exists which ensures a favorable respiratory process under almost all physiological conditions.

10.3 MITOCHONDRIA - ULTRASTRUCTURE AND ORGANIZATION

Mitochondria (singular, mitochondrion) are double membrane-bounded organelles present in eukaryotes. They can be seen as tiny granules or as rods or cylinders even under the light microscope. The number of mitochondria varies in accordance with the metabolic status of cell. For example, there are thousands of mitochondria in meristematic cells and in other cells active in metabolism. They are dynamic organelles which can change their shape. Mitochondria are usually nicknamed as "**Powerhouses**" of a cell, as they are the sites of aerobic respiration and generate energy by biological oxidation of various substrates.

Ultrastructure of mitochondria reveals a double membrane system .The outer and inner membranes are separated by an intermembrane/peripheral space. The outer membrane is smooth, acting as a boundary and contains dynamic, specialized, protein- bounded channels called **porins** which makes it freely permeable to ATP, NAD^+ and CoA (compounds which play an important role in respiration). The inner mitochondrial membrane is more complex and consists of **two** distinct, interconnected domains.

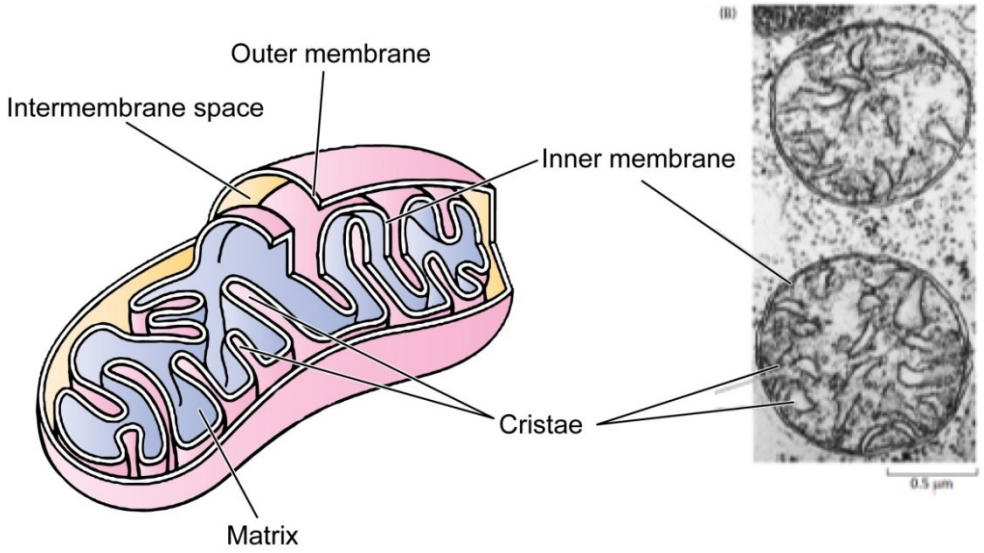


Fig. 10.1: a) A three-dimensional representation of mitochondrion highlighting the invaginations (cristae) of the inner membrane, along with inter membrane space/peripheral space and matrix; b) Electron micrograph of mitochondrion from the mesophyll cell of *Vicia faba* (From Gunning & Steer, 1996).

Just inside the outer mitochondrial membrane resides the inner boundary membrane (Fig. 10.1a). Since this membrane is impermeable, special membrane transporters are located which help to carry the molecules to the matrix.

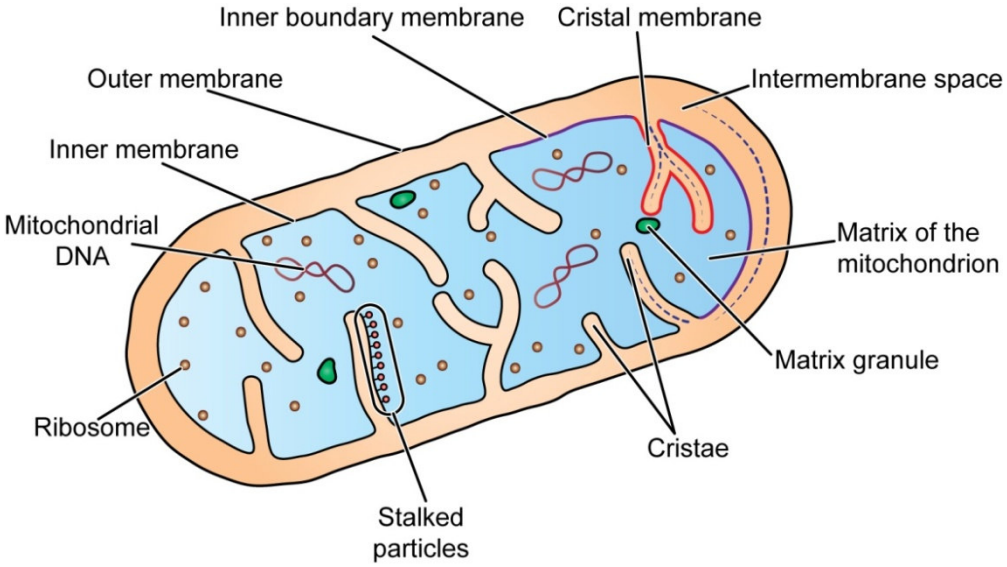


Fig 10.2: A detailed schematic diagram of the mitochondrion showing the various components.

The other domain of the inner membrane is formed by a series of infoldings or membranous sheets called *cristae* (sin. crista). The cristae amplify the surface area for the ATP-synthesizing machinery (Figs. 10.1b, 10.2). Both, the membranes of cristae, and the inner boundary membrane are joined by narrow **cristae junctions.**

The inner face of the inner mitochondrial membrane which faces the matrix is designated as the M-face. Elementary particles/oxysomes /Fi particles are present on this face of the membrane at regular intervals. These racquet-shaped particles are the sites for ATP synthesis through the processes of chemiosmotic coupling, and oxidative phosphorylation.

The space between the two cristae membranes is called **intracristal space**. (Fig. 10.2) Thus, mitochondria are divided into two distinct aqueous compartments - the **intermembrane space** between the outer and the inner membranes, and another within the interior of the organelle- the jelly filled **matrix**. The matrix contains various enzymes (nearly 70% of a cell's enzymes reside in the mitochondria!) including the ones needed for Krebs cycle, which operates here, along with mitochondrial DNA and free ribosomes (70S). The non-chromosomal mitochondrial DNA encodes number of polypeptides and ribosomal RNAs.

Mitochondria are semi-genetically autonomous organelles and are thought to have originated as endosymbionts from prokaryote-like ancestors.

SAQ 1

a) In the following sentences choose the right alternative word given in the parenthesis.

i) The mitochondrial cristae are extensions of the (outer/inner) membrane which (shorten/increase) the membrane area.

ii) The outer membrane of mitochondria contains (porins/pore complex) which are (carbohydrate/protein) bounded channels.

iii) Most of the mitochondrial enzymes are present in its (outer space/matrix).

iv) Elementary particles or oxysomes are located on the (M/C) face of the inner mitochondrial membrane.

v) Ribosomes present within the mitochondria are of (70S/80S) type.

vi) Mitochondrial DNA resembles that of (eukaryotes/prokaryotes).

b) Match the content of column I corresponding to those of column II.

	Column I		**Column II**
i)	Matrix	a)	Oxysomes
ii)	ATP synthesis	b)	Combustion
iii)	Aerobic respiration	c)	32 ATP
iv)	Physiochemical process	d)	Krebs cycle
v)	Proton channels	e)	F_1 particles

10.4 TYPES OF RESPIRATION

Depending upon the utilization of molecular oxygen in the process of oxidation, respiration can be classified into two types, viz., aerobic, and anaerobic respiration.

10.4.1 Aerobic and Anaerobic Respiration

Majority of the living organisms, including plants, both terrestrial and aquatic utilize oxygen for breaking down the organic food to liberate chemical or potential energy. This method of respiration is called **aerobic** respiration. This oxyrespiration results in complete oxidation of the respiratory substrate, thereby releasing CO_2, generating water and release of energy. This type of respiration involves **five** main steps viz., glycolysis, pyruvate oxidation, Krebs cycle, electron transfer chain and chemiosmotic ATP synthesis. It occurs in the cytosol and mitochondria.

Complete oxidation of one hexose sugar molecule can be represented as:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 686 \text{ kcal/mol}$$

The reaction is exothermic ($-\Delta H$) as well as exergonic ($-\Delta G$).

Oxygen does not combine directly with sugars but functions as a terminal acceptor of electrons and protons combining with hydrogen to form water (**metabolic water**).

The equation for aerobic respiration is better expressed as:

$$C_6H_{12}O_6+6H_2O+6O_2 \longrightarrow 6CO_2 + 12H_2O + 686 \text{ kcal (2870.22 kJ)}$$

Respiration occurring in the absence of oxygen is called **anaerobic** respiration. It occurs in many bacteria, fungi, oxygen-starved roots of water-logged soils, germinating seeds, and deep-seated tissue of plants. In the absence of oxygen, only incomplete oxidation of respiratory substrate takes place. Since there is no terminal oxidation of electrons, water is not formed and less energy is released (only 5% of energy available in glucose). Moreover, this mode of respiration yields many products, usually ethanol or lactic acid. Anaerobic respiration includes just two steps: glycolysis and incomplete breakdown of pyruvate, both of which occur in the cytosol. Mitochondria do not participate in this process.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + 56 \text{ kcal (234.3 kJ)}$$

or

$$C_6H_{12}O_6 \longrightarrow 2C_3H_6O_3 + \text{Energy}$$

This type of respiration is not an efficient method in terms of energy yield.

ATP molecule is related to a ribonucleotide as it has an adenine base, a ribose sugar, and three phosphate groups (Fig. 10.3).Two high energy bonds (phosphoester bonds)denoted by a sign ~ are present

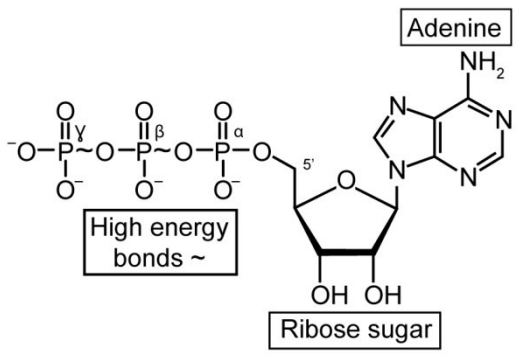


Fig. 10.3: Structure of ATP molecule.

The ɣ bond with the outermost phosphate group is prone to hydrolysis first.

$$ATP \xrightarrow[H_2O]{ATPase} ADP + Pi + 7.3 \text{ kcal (30.5 kJ/mol)}$$

Box 10.1 : Efficiency of Respiration

ATP ADP + Pi $\Delta\acute{G}$ = - 32.2 kJ/mol (7.69 kcal)

ADP AMP + Pi $\Delta\acute{G}$ = -32.4 kJ/mol (7.74 kcal)

AMP Adenosine + Pi $\Delta\acute{G}$ = -13.8 kJ/mol (3.29 kcal)

For complete oxidation of a glucose molecule, a maximum of 32 molecules of ATP are generated. Since one ATP stores 7.3 kcal, the 32 ATP will store 7.3 × 32 = 233.6 kcal or 977.38 kJ.

Thus, efficiency of aerobic respiration is $\frac{32 \times 7.3}{686} \times 100 =$ **#34**%

Rest of the energy is lost as heat.

In case of anaerobic respiration, only 2 ATP molecules are released. So, efficiency is $\frac{2 \times 7.3}{686} \times 100 =$ **2.12**%

The overall equation for aerobic respiration can also be represented as

$C_6H_{12}O_6 + 6O_2 + 10\ H_2O \longrightarrow 6O_2 + 16H_2O$ + 686 kcal (2870.22 kJ)

180g 192g 264g 108g ($6H_2O$) **32 ATP**

and, for incomplete oxidation of substrates:

$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2C_2H_5OH$ + 56 kcal (234.3kJ)

180g 88g 92g **2 ATP**

According to the old classical concept the net gain of ATP for each glucose molecule oxidized was thought to be 36. Thus, according to the old concept, the efficiency of aerobic respiration is $\frac{36 \times 7.3}{686} \times 100 =$ **8.30** %

Obligate and facultative aerobes

Obligate aerobes are organisms which necessarily require O_2 for cellular respiration. Higher plants are obligate aerobes, their individual tissue and organs may switch to anaerobic respiration if oxygen is not available. A facultative aerobe is an organism which makes ATP by aerobic respiration in the presence of oxygen but can switch to anaerobic respiration on fermentation when oxygen is absent e.g., Baker's yeast and many bacteria like *E.coli*, *Salmonella*, *Staphylococcus* and *Listeria*.

Many higher plants have the capability to survive without oxygen from a few hours to a few days. This O_2-free condition is termed **anaerobiosis**. There are various physiological situations of oxygen stress ranging from seeds immersed in water during germination to water- logged roots.

Facultative anaerobes are normally aerobic, but organisms could change their metabolism to an anaerobic one for a short period during oxygen shortage.

Obligate and Facultative anaerobes

Obligate anaerobes carry out respiration strictly under anaerobic conditions, i.e., in an oxygen free atmosphere. Oxygen is toxic to them as they lack the oxygen- scavenging enzyme *superoxide dismutase*. Yeast is an example of facultative anaerobe. This unicellular Ascomycete fungus carries out complete reactions of glycolysis ,Krebs cycle and ETC(when oxygen is readily available).If the O_2 supply falls below a certain level, this microbe undertakes only glycolysis as the source of ATP by Substrate level phosphorylation (while the TCA cycle and ETC are switched off).

10.4.2 Respiratory Substrates

Any organic reserve food material which can be oxidized to yield energy is a **respiratory substrate.** There are many respiratory substrates which act as raw materials for respiration.

Carbohydrates: Sugars are the most important and readily mobilized respiratory substrates. Starch, sucrose, inulin, glucose, and fructose are metabolized by plants. The complex carbohydrates first get hydrolyzed to simple hexose sugars and then enter the respiratory cycles. Sugars in the form of sucrose are being continuously made available to the plant tissue through translocation.

Fats: Fats serve as respiratory substrates in seeds of majority of angiosperms. A large proportion of fats is converted into sugars in glyoxylate cycle at the time of seed germination. Fats do not enter the respiratory channel directly but are first hydrolyzed into fatty acids and glycerol. Glycerol can convert into glyceraldehyde to enter the respiratory pathway, while the fatty acids are converted to acetyl coenzyme A by β-oxidation before entering the Krebs cycle.

Interestingly, since fats are poorer in oxygen as compared to carbohydrates, their respiratory quotient (RQ) is < 1 while carbohydrates have a RQ = 1. (We shall discuss RQ in details in the coming sections).Despite a lower RQ, fats have a higher calorific value than carbohydrates and will liberate more energy per gram than sugars. One gram of fat yields 9.1 kcal (38.07kJ) as against just 3.8 Kcal (15.89 kJ) produced by carbohydrates.

This is on account of the presence of more bonds present in fats. To appreciate how much net energy can be extracted from nutrients, let us consider a specific chemical reaction – the complete oxidation of 1mole of fatty acid, palmitic acid.

$CH_3(CH_2)_{14}$ COOH (solid) + $23O_2$(gas) $\longrightarrow$ $16CO_2$ (gas) + 16 H_2O liquid.

Large amount of energy is released in the form of heat. Change in enthalpy (ΔH) = -9970.6 kJ/mol (2383 kcal) shows that it is an exergonic reaction ($-\Delta H$).

The combustion of fat provides much more heat energy than combustion of sugars of the equivalent mass. Thus, fat is said to have a higher caloric content than carbohydrates (estimated as the heat output/enthalpy by using a calorimeter).

Oxidation of a typical saturated fatty acid, palmitic acid yields

$C_{16}H_{32}O_2+23O_2 \longrightarrow 16CO_2 + 16H_2O$
$\Delta G' = -9.30$ kcal/g = -38.90 kJ/g

as against glucose

$C_6H_{12}O_6+6O_2 \longrightarrow 6CO_2 + 6H_2O$
$\Delta G' = -3.74$ kcal/g = -15.64 kJ/g

Organic acids

Organic acids are rich in oxygen compared to carbohydrates; their respiratory quotient is >1.Large amounts of organic acids are present in members of the family Crassulaceae. In addition, anthocyanin rich leaves also accumulate large quantities of organic acids. These acids are formed by partial oxidation of carbohydrates in the dark.

$2C_6H_{12}O_6 + 3O_2 \longrightarrow 2C_6H_{18}O_7 + 4H_2O$
Citric acid

$2\ C_6H_{12}O_6 \longrightarrow 3C_4H_6O_5 + 3H_2O$
Malic acid

Dark acidification accumulates those organic acids which serve as source of carbon o most preferred mode whereas the protoplasmic respiration is resorted to either in the absence of sugars and fats (starvation).

Proteins

Proteins are the respiratory substrates in protein rich seeds like legumes hemp, pumpkin, pistachios, sunflower, flax, sesame, chia, hazelnuts, pinenuts and many more. In other plants, proteins will be broken down only when the carbohydrates and fats are not available. Proteins are first broken down into amino acids, which undergo deamination to give organic acids. The latter enter the Krebs cycle.

Based on respiratory substrates utilized, two types of respiration have been recognized.

Floating respiration

It is the common mode of respiration where carbohydrates and fats are oxidized. Direct oxidation is through **Pentose phosphate pathway** while normal respiration or Dark respiration involves indirect oxidation of the carbohydrate respiration substrates.

Protoplasmic respiration

When proteins act as respiratory substrates, they are said to undergo protoplasmic respiration. This occurs in starved plants and the proteins are first hydrolyzed to form amino acids, which later form different organic acids. These acids enter the Krebs cycle which operates in the mitochondrial matrix.

Floating respiration is the most preferred mode whereas the protoplasmic respiration is resorted to either in the absence of sugars and fats (starvation)

Stress condition of injury may induce a higher rate of respiration. This **induced respiration** involves oxidation of damaged lipids of cell membranes (Freshly sliced discs of carrot roots, potato tubers, or sweet potatoes).

10.4.3 Respiratory Quotient (RQ)

As you have learnt from the above paragraphs, the respiratory substrates include carbohydrates, fats, protein, and organic acids. Among these, sugars (glucose) are the most common respiratory substrates and are consumed first. Proteins get consumed only during starvation. Oxidation of fats yields maximum energy while the organic acids give minimum energy output. Interestingly, fats are first broken down to acetyl CoA prior to entering the Krebs cycle.

Since the process of respiration involves exchange of gases it is also essential to know the ratio between two gases involved viz., O_2 and CO_2 under variable conditions.

Respiratory quotient (RQ) or **Respiratory ratio** (**RR**) is the ratio of the volume of CO_2 evolved to the O_2 consumed during respiratory oxidation/process.

$$\text{RQ} = \frac{\text{Volume of } CO_2 \text{ evolved}}{\text{Volume of } O_2 \text{ consumed}} = \frac{CO_2}{O_2} \text{ v/v}$$

Please remember that RQ is different from PQ (Photosynthetic Quotient) which is $\frac{\text{Volume of } O_2 \text{ evolved}}{\text{Volume of } CO_2 \text{ absorbed}}$ during photosynthesis.

RQ is measured with the help of an apparatus called Ganong's respirometer.

The value of RQ is variable and depends upon the following parameters:

i) Nature of the respiratory substrate: The proportion of oxygen as compared to carbon in the substrate. The extent of oxygen richness will decide RQ value.

ii) The extent of breakdown of the respiratory substrate i.e., complete, or incomplete oxidation.

Thus, RQ represents a function of the oxidation state of the substrate being respired. Richer a compound in oxygen, less oxygen will be required to break it down further. As a result, higher will be the value of RQ. Reverse will be the case with RQ where the respiratory substrate is incompletely oxidized. The values of RQ may range from zero, less than unity (<1), unity (1), more than unity(>1) to infinity(∞).

Let us examine each situation in detail.

RQ = 1 (unity)

Breakdown of carbohydrates like sugars and starch as respiratory substrates (e.g., germinating cereal grains, sprouting potatoes, carbohydrate-rich flowers and fruits).

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$

$$RQ = \frac{6CO_2}{6O_2} = 1.0$$

RQ = <1

Fats and proteins are poor in oxygen and hence require more oxygen for breakdown.

$C_{16}H_{32}O_2 + 11O_2 \longrightarrow C_{12}H_{22}O_{11} + 4CO_2 + 5H_2O$

Palmitic acid $RQ = \frac{4CO_2}{11O_2} = 0.36$

$2(C_{51}H_{98}O_6) + 145O_2 \longrightarrow 102CO_2 + 98H_2O + \text{energy}$

Tripalmitin $RQ = \frac{102CO_2}{145O_2} = 0.7$

As mentioned above, fats have a higher calorific value than carbohydrates. This shows that RQ depends on the proportion of C and O and is independent of the calorific value which depends on the number of bonds present.

Similarly, proteins (germinating seeds of soybean, gram, pea, Brazil nut) are also poor in oxygen. Therefore, their RQ value varies from 0.8-0.9 (Av. 0.84). Proteins are first hydrolyzed into amino acids by the enzymes *peptidases* and *proteases*. The amino acids are subsequently broken down evolving less O_2 than the CO_2 consumed.

RQ = 0

Cacti and succulents (showing CAM cycle) during night incompletely oxidise carbohydrates into organic acids (usually malate) by the process of acidification or dark fixation. Since their scotoactive stomates open during night, CO_2 is not released but fixed. Incomplete oxidation of carbohydrates results in evolution of small amounts of CO_2.

$2C_6H_{12}O_6 + 6O_2 \longrightarrow 2C_4H_6O_5 + 4CO_2$

$$RQ = \frac{4CO_2}{6O_2} = 0.67$$

This CO_2, however, is not released but taken back to the chloroplast for dark fixation. So ultimately no CO_2 is released, and the final equation is

$2C_6H_{12}O_6 + 3O_2 \longrightarrow 2C_4H_6O_5 + 3H_2O$

$$RQ = \frac{0CO_2}{3O_2} = 0$$

RQ = >1

After prolonged darkness, the cacti, and succulents (CAM plants) show deacidification, i.e., oxidation of organic acids to release CO_2. Being rich in O_2, the organic acids require less O_2 for oxidation. The value of RQ, therefore, is greater than one.

1 Kilocalorie (Kcal) = 4.184 kilojoule(KJ)

$C_4H_6O_5 + 3O_2 \longrightarrow 4CO_2 + 3H_2O$

Malic acid

$$RQ = \frac{4CO_2}{3O_2} = 1.33$$

$2\ C_2H_2O_4 + O_2 \longrightarrow 4CO_2 + 2H_2O$
Oxalic acid

$$RQ = \frac{4CO_2}{1O_2} = 4$$

RQ = ∞

In anaerobic respiration, O_2 is not consumed at all. Since CO_2 is produced in most cases, the value of RQ becomes incalculable or infinite.

$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + \text{Energy}$

$$RQ = \frac{2CO_2}{0} = \infty$$

Box 10.2: RQ of maturing seeds

RQ of maturing fatty seeds is >1 as oxygen-rich sugars get converted in relatively O_2-poor fatty acids and oxygen may be produced during cell metabolism

$19C_6H_{12}O_6 \longrightarrow 2C_{57}H_{104}O_6 + 10H_2O + 46O_2$

RQ of maturing cereals is 1 as the sugars are respiratory substrates. RQ of starved leaves is <1 because it is only after the sugars have been consumed that the proteins are oxidized (protoplasmic respiration).Succulents show a RQ = O in dark and > 1 during daytime.

If whole of the O_2 is consumed internally in respiration, the value of RQ would apparently be ∞.

SAQ 2

a) Match the terms related to respiration (given in Column I) with the names/examples (given in Column II) used for this process.

	Column I		Column II
i)	Anaerobic respiration	a)	Condition of O_2 stress
ii)	Protoplasmic respiration	b)	Substrate rich in O_2
iii)	Aerobic respiration	c)	RQ = ∞
iv)	Anaerobiosis	d)	Carbohydrates and Fats
v)	Floating respiration	e)	Incomplete breakdown of pyruvate
vi)	RQ >1	f)	Protein

b) i) One hexose sugar molecule on complete oxidation yields ……..... kcal energy.

ii) Anaerobic respiration normally results in the formation of ……. or ……. .

iii) Direct oxidation of sugars are through ……. pathway.

iv) Respiratory quotient of maturing fatty seeds is ……. 1.

v) A fat molecule upon oxidation yields …… energy than carbohydrates. However, the RQ of sugars is …… than that of fats.

10.5 MECHANISM : AN OVERVIEW

As you have studied in the above pages, glucose molecule possesses a high potential energy. Its complete oxidation results in a standard free energy change of -2,840 kJ/mol. The unutilized, excess glucose gets stored in the form of starch. Interestingly, starch has been chosen as the storage form as its presence does not increase the osmolarity of the cell, whereas glucose or sucrose would do so.

In the wake of energy demands, the intracellular storage carbohydrates are made available to be used as energy sources to produce ATP, which can be either through aerobic or anaerobic pathways.

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \quad \Delta\dot{G}' = -2{,}840$ kJ/mol (-686 kcal/mol).

Glucose can be oxidized by **two** major pathways :

a) Glucose undergoes a series of reactions of **glycolysis** – an initial pathway of glucose metabolism taking place in the cytosol. This does not involve molecular oxygen and some part of the potential energy stored in the hexose sugar is utilized to synthesize ATP. Glycolysis results in the formation of 2 molecules of a three-carbon compound pyruvate (CH_3COCOO^-) from one hexose sugar molecule. Glycolysis is the central pathway for most organisms and can proceed under both, the aerobic as well as anaerobic conditions.

   Anaerobes and microbes derive their entire metabolic energy from glycolysis as there are no further ATP-yielding steps. Under anaerobic or hypoxic (low O_2) conditions, the organisms convert pyruvate into ethanol and CO_2 by fermentation:

   $C_6H_{12}O_6 + 2\ ADP + 2Pi + 4H^+ \longrightarrow 2C_2H_5OH + 2ATP + 2H_2O + 2CO_2$

   Alternatively, some microbes may convert pyruvate into lactate. Similar reaction occurs in vigorously contracting muscles and red blood cells.

   $C_6H_{12}O_6 + 2\ ADP + 2P_i + 2H^+ \longrightarrow 2\ Lactate + 2ATP + 2H_2O$

   In the **presence of oxygen**, pyruvate formed in glycolysis is transported into the mitochondria where it undergoes three steps.

   Oxidation of pyruvate into CO_2 and water within mitochondria generates an additional 28 ATP molecules. Bulk of these ATP are generated via chemiosmotic coupling at the F_1 particles.

   Thus, the energy yield of aerobic respiration (28+2= 30) far exceeds that of anaerobic respiration (2 ATP).

   The energy yield of aerobic respiration can be represented as:

   $C_6H_{12}O_6 + 6O_2 + 30P_1 + 30\ ADP \longrightarrow 6CO_2 + 30\ ATP + 6H_2O$

b) Glucose can also get oxidized by an alternate pathway which is independent to glycolysis the **Pentose Phosphate Pathway (PPP)** or Hexose Monophosphate Pathway/Shuttle (HMP/HMS). This pathway operates in rapidly dividing cells in both plants and animals. This alternative uses NAD^+ as used in glycolysis.

c) Some microorganisms substitute glycolysis by utilizing a series of enzyme-catalysed chemical reactions collectively called as the **Entner-Doudoroff Pathway** (ED Pathway), which is distinct from both glycolysis and the PPP. This pathway occurs exclusively in prokaryotes and uses enzymes like *6-phosphogluconate dehydratase* and *2-keto-3-deoxyphosphogluconate aldolase* to catabolize glucose into pyruvate.

Many microbes can also use hydrocarbons as an energy source under aerobic conditions. These microbes are popularly called **"HUM bugs**" and can survive in jet fuel and diesel. By virtue of their hydrocarbon consuming capability, many of them are being exploited as effective biosurfactants for solubilizing hydrocarbon contaminants like oil spills. Bioremediation through the removal of environmentally hazardous hydrocarbons is a very important aspect of the respiratory substrate preference of these microbes.

Some other microbes are also capable of utilizing organic acids like amino acids, fatty acids, and lactates as their sole source of energy. Figure 10.4 gives an overview of the different alternatives of aerobic and anaerobic respiration.

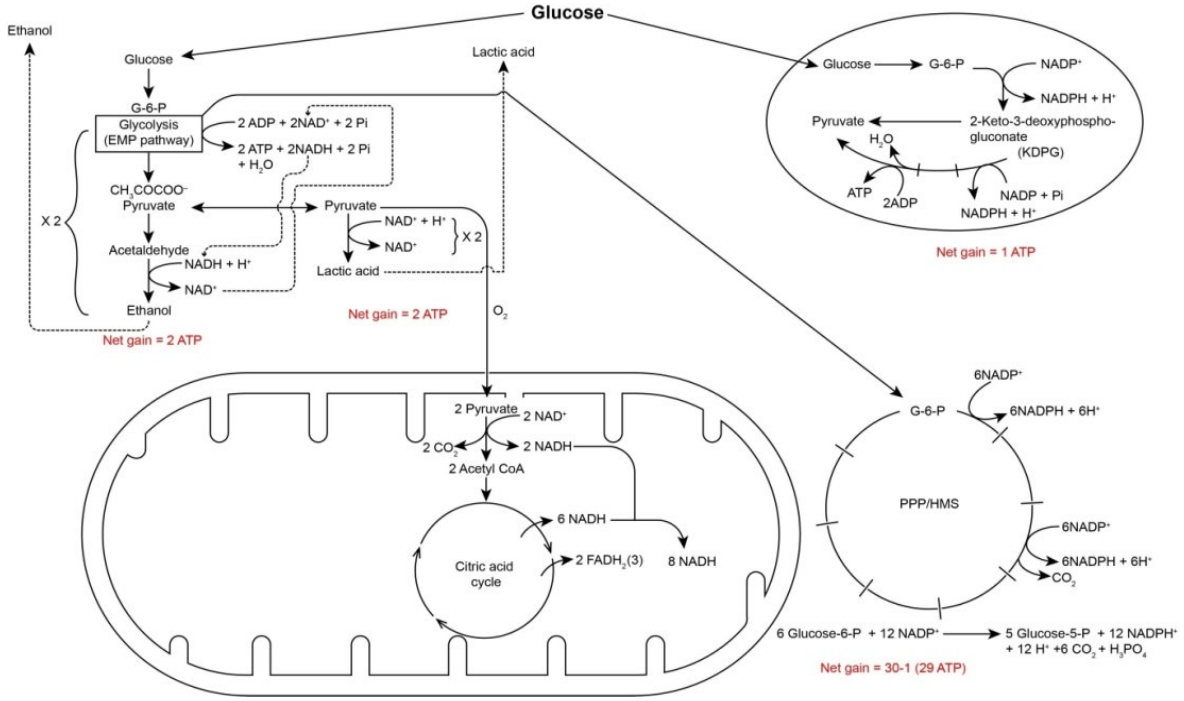


Fig. 10.4: Overview of aerobic and anaerobic respiration.

SAQ 3

Match the content of column I corresponding to those in column II.

	Column I		**Column II**
i)	$NADP^+$	a)	Ethanol
ii)	Acetyl CoA	b)	ED pathway
iii)	Cytosol	c)	Pentose phosphate pathway
iv)	Anaerobic conditions	d)	Krebs Cycle
v)	Oxygen	e)	Oxidative decarboxylation
vi)	Bacteria	f)	Glycolysis

10.6 SUMMARY

- All living organisms generate energy from complex organic compounds by a coordinated, multistep, process called respiration.
- Respiration differs from combustion as it occurs inside a cell under constant temperature and pressure conditions not allowing the heat to be released at one go.
- The living organisms are rightly called isothermal chemical engines as a part of energy is stored in the form of ATP (called as the energy currency of a cell).
- Certain respiratory substrates are preferred over others to be readily metabolized.
- Respiratory quotient (RQ) values vary with different substrates and are indicative of the oxidation state of the substrate being respired.
- Respiration takes place either in the presence or absence of oxygen. Glucose gets oxidized either by glycolysis or by the pentose phosphate pathway.

10.7 TERMINAL QUESTIONS

1. Define the following terms :
   - i) Floating respiration
   - ii) Exergonic reaction
   - iii) Respiratory quotient
   - iv) Facultative anaerobes
   - v) $\Delta G'$ of aerobic respiration
2. Answer the following:
   - i) Water is formed only in aerobic and not in anaerobic respiration. Explain.
   - ii) What is the role of porins?
   - iii) Write a note on overview of mechanism of respiration.
3. Differentiate between
   - i) Respiration and combustion
   - ii) Anaerobic and aerobic respiration
   - iii) Floating and protoplasmic respiration
   - iv) RQ of fats and organic acids
   - v) Obligate aerobes and facultative anaerobes.

10.8 ANSWERS

Self-Assessment Questions

1. a) i) inner; increase
   ii) porins, protein
   iii) matrix;
   iv) M
   v) 70S
   vi) prokaryotes

   b) i) Krebs cycle
   ii) F_1 particles
   iii) 32 ATP
   iv) Combustion
   v) Oxysomes

2. a) i) RQ = ∞
   ii) Protein
   iii) Incomplete breakdown of pyruvate
   iv) Condition of O_2 stress
   v) Carbohydrates and Fats
   vi) Substrate rich in O_2

   b) i) 686 kcal
   ii) ethanol, lactic acid
   iii) Pentose phosphate pathway
   iv) greater than(>)
   v) greater/higher; more/higher

3. i) Pentose phosphate pathway
   ii) Oxidative decarboxylation
   iii) Glycolysis
   iv) Ethanol
   v) Krebs Cycle
   vi) ED pathway

Terminal Questions

1. i) Refer to Subsection 10.4.2.
   ii) Refer to Sections 10.2 and 10.4.
   iii) Refer to Subsection 10.4.3.
   iv) Refer to Subsection 10.4.1.
   v) Refer to Section 10.4.
2. i) Refer to Subsection 10.4.1.
   ii) Refer to Section 10.3.
   iii) Refer to Section 10.5.
3. i) Refer to Section 10.2.
   ii) Refer to Subsection 10.4.1.
   iii) Refer to Subsection 10.4.2.
   iv) Refer to Subsection 10.4.1.
   v) Refer to Subsection 10.4.1.

Acknowledgements

Fig.10.2 : https://i.pinimg.com/originals/4d/4b/83/4d4b834510f3cb07577783449765b373.png

Fig. 10.3 : https://i.pinimg.com/originals/17/0d/b1/170db1464e036a6d7dfc0b6806d86029.png

UNIT 11

RESPIRATION MECHANISM

Structure

11.1 INTRODUCTION

In the previous unit 10, you were made familiar with the basic concepts of respiration, and the organelles involved. A comprehensive account of the RQ helped you to understand the characteristics of different respiratory substrates.

This unit will make you familiar with the mechanism of the respiratory process both aerobic and anaerobic. Details of glycolysis being the common step in the presence or absence of O_2 have been discussed. Direct oxidation of sugars is also possible via the oxidative pentose pathway, which is located both in the cytosol and plastids. Complete oxidation of pyruvate takes place in the citric

acid cycle within the mitochondrial matrix. Both glycolysis and Krebs cycle are regulated by enzymes and products. NADH and $FADH_2$ molecules produced in the above-mentioned process pass through the electron transport chain which contains various electron carriers and transmembrane multiprotein complexes. Transport of electrons to oxygen viz. complexes I, III and IV are coupled to ATP synthesis.

Objectives

After studying this unit, you would be able to:

- describe various steps of the EMP pathway and the gain of ATP during the process;
- appreciate the differences between plant and non-plant glycolysis;
- have a detailed understanding of Krebs cycle along with some unique variation in plants;
- trace the path of electrons along the ETC and their terminal oxidation to generate H_2O;
- appraise yourself to the latest findings regarding ATP energetics to calculate the net gain of ATP through the shuttles;
- familiar with the direct oxidative pathway; and
- appreciate the role of environmental and internal factors in modulating respiration.

11.2 GLYCOLYSIS - EMP Pathway

The term glycolysis is derived from the Greek ***glykys***= "sweet" and ***lysis***, meaning "splitting". During this process one molecule of glucose (hexose) is degraded through a series of enzyme – catalyzed reactions to form two molecules of pyruvate – CH_3COCOO^- (a three-carbon compound). The sequence of reactions of glycolysis was perhaps the first biochemical pathway to have been studied in detail and has an interesting history.

Box 11.1: Historical Perspective – Elucidation of Glycolysis

- **Louis Pasteur** (1854-1864) – demonstrated that fermentation is caused by microorganisms and is thus a vital force. He also observed that aerobic growth of microbes requires less glucose than in the aerobic conditions (**Pasteur effect**).
- **Eduard Buchner** (1897) – discovered that fermentation can be carried out in broken yeast cells and even in cell-free extracts. This contradicted Pasteur's ideas of fermentation being a vital force.
- **Arthur Harden** and **W.J. Young** (1905) were the first to demonstrate that an inorganic phosphate- Fructose 1, 6-bisphosphate was required in the process of fermentation. (Fructose 1, 6-bisphosphate = *Harden & Young's ester*). Similarly, the other glycolytic intermediates are also named after their discoverers, viz., glucose 6-phosphate = *Robinson's ester* and fructose 6-phosphate as *Neuberg's ester*. Harden and Young also gave the concept of *Zymase* (heat labile protein

fraction) and cozymase (heat insensitive part-consisting of ATP, ADP, metal ions and other non-protein components like NAD^+. Harden won the Nobel Prize in 1929.

- **Otto Warburg** and **Hansvon Euler-Chelpin** elucidated the whole glycolytic pathway in yeast. Warburg purified and crystallized seven of the enzymes of glycolysis and won the Nobel Prize.

Works of **Gustav Embden**, **Otto Meyerhof,** and **Jakub Karol Parnas** resulted in formulation of a complete set of reactions occurring in the extracts of muscle leading to the pathway which we know as GLYCOLYSIS or EMP (Embden-Meyerhof and Parnas) pathway. Meyerhof won the Nobel Prize in Physiology and Medicine in 1922.

Glycolysis constitutes the initial stage of sugar breakdown. This pathway takes place in the cytosol and is independent of oxygen. In fact, it functions more efficiently under anaerobic conditions (Pasteur effect). Glycolysis seems to be one of the most ancient pathways to have been evolved in prokaryotes living in an atmosphere without free molecular oxygen. Today, it is the common metabolic pathway of most organisms – both aerobic and anaerobic.

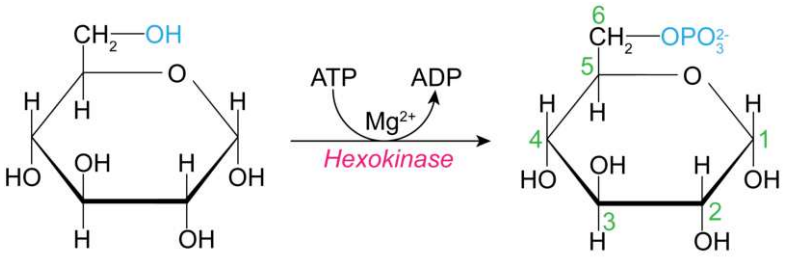
11.2.1 Reactions

Glycolysis begins with glucose in animals and non-plants. In contrast, sucrose is, the major translocated sugar in most plants, and is therefore, the substrate for plant respiration.

Since the basic reactions associated with glycolytic and fermentation pathways in plants are almost same with those of animal cells, we begin with glucose as the starting sugar. We shall describe the variation in glycolysis in plant respiration later in this chapter.

The first five reactions constitute the **preparatory phase**, while the remaining five form the **payoff phase**.

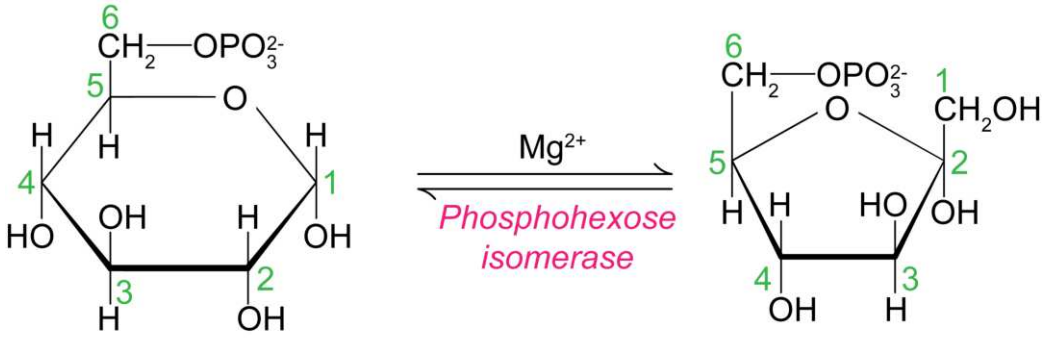
1. **Phosphorylation of hexoses** (mainly glucose)**:** The initial step is also called ***hexokinase* reaction**. Glucose is first activated by phosphorylation to yield Glucose 6-phosphate. ATP is the donor of phosphoryl and this irreversible reaction is catalyzed by the enzyme *hexokinase*. This enzyme requires Mg^{2+}-ATP complex as its substrate. Infact, uncoupled ATP may act as a potent competitive inhibitor of the enzyme. *Hexokinase* itself undergoes large conformational changes upon binding with glucose. The enzyme *hexokinase* is inhibited allosterically by glucose-6-phosphate.



Glucose Glucose 6-phosphate

$\Delta G'^{\circ} = -16.7$ kJ/mol

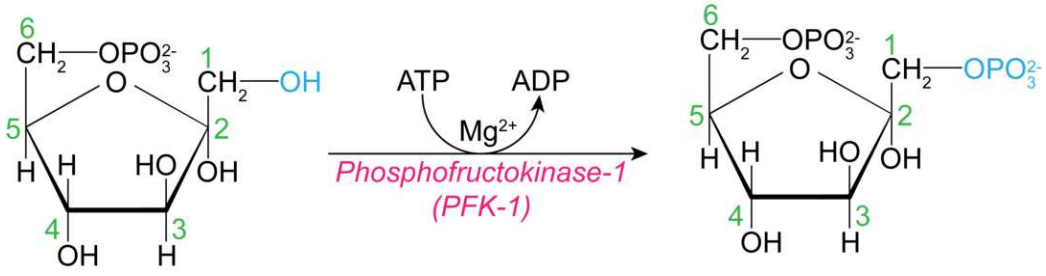
2. **Isomerization of Glucose 6-phosphate to Fructose 6-phosphate:**The enzyme *phosphohexose isomerase* (*phosphoglucose isomerase*) catalyses the reversible isomerization of glucose 6-phosphate (an aldohexose) to fructose 6-phosphate (a ketohexose). This enzyme is specific for these two hexoses.



Glucose 6-phosphate — Fructose 6-phosphate

$\Delta G'^{\circ} = -1.7$ kJ/mol

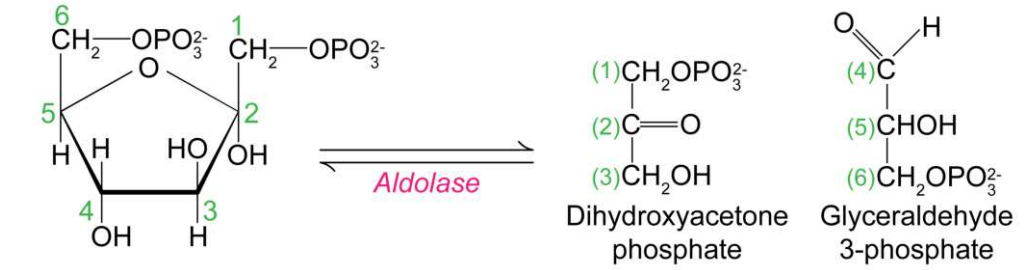
3. **Phosphorylation of Furctose 6-phosphate to produce Fructose 1, 6-bisphosphate:** This is the second priming reaction of glycolysis which involves the transfer of a phosphoryl group from ATP to form fructose 1, 6-bisphosphate. The reaction is catalyzed by the enzyme *Phosphofructokinase -1* (PFK-1). This is an irreversible step committed to target this bisphosphate towards glycolysis. **In addition, this is also a regulatory step which is rate-limiting**. The enzyme in question is the most complex regulatory enzyme with various allosteric inhibitors and activators. Since ATP is an allosteric inhibitor of this step, the activity of PFK-1 increases whenever the cell's ATP supply is depleted, or when ADP and AMP are in excess. In some organisms, fructose 2, 6-bisphosphate in an allosteric activator of PFK-1.



Fructose 6-phosphate — Fructose 1,6-bisphosphate

$\Delta G'^{\circ} = -14.2$ kJ/mol

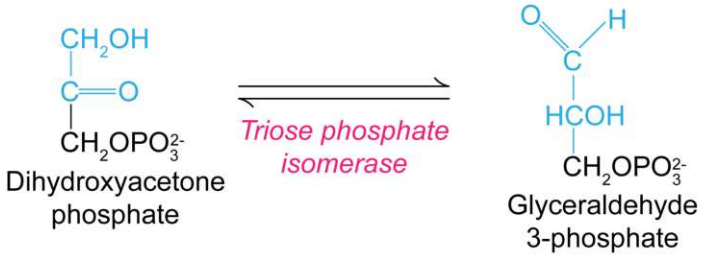
4. ***Aldolase* reaction-Cleavage of Fructose 1, 6-bisphosphate:**The enzyme *aldolase* (*fructose 1, 6 –bisphosphate aldolase*), catalyzes a reaction reverse of aldol condensation reaction, where the newly formed fructose 1, 6-bisphosphate is broken down to give two triose phosphates viz. an aldose,glyceraldehyde 3-phosphate (3C) and a ketose, dihydroxyacetone phosphate (DHAP-3C). The enzyme also requires Zn^{2+} as an activator. The 3C products in the reaction have their C atoms renumbered.



Fructose 1,6-bisphosphate

$\Delta G'^{\circ}$ = 23.8 kJ/mol

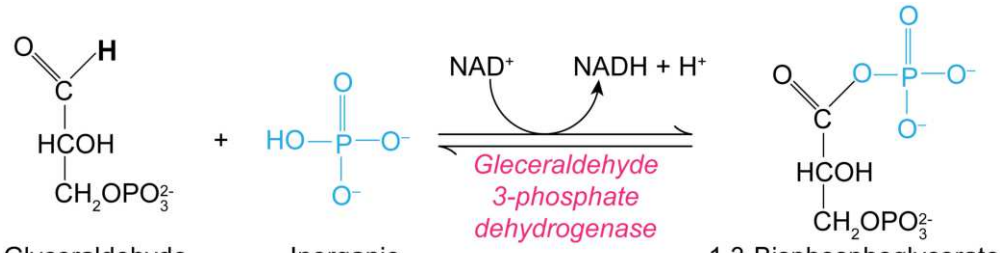
5. **Interconversion of Triose Phosphates:**Since only one of the triose phosphates formed by aldolase, viz., glyceraldehyde 3-phosphate can be directly used up in the subsequent steps of glycolysis, a molecule of DHAP rapidly converts into glyceraldehyde 3-phosphate. This is a reversible reaction catalyzed by the enzyme *triose phosphate isomerase*.



$\Delta G'^{\circ}$ = 7.5 kJ/mol

As a result, the glycolytic sequence has **two molecules** of glyceraldehyde 3-phosphate now.

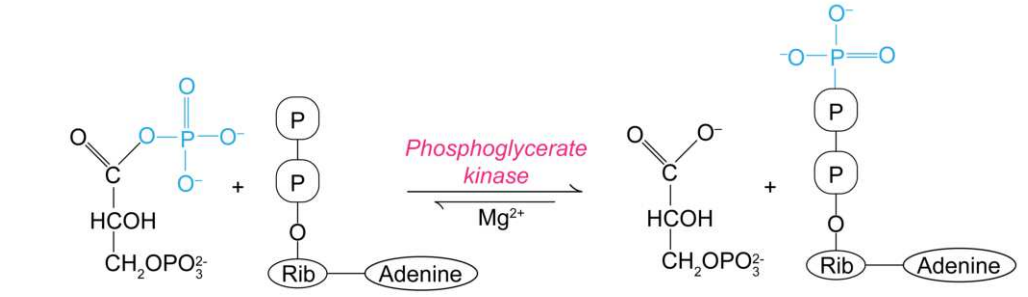
6. The first reaction of the **payoff phase** of glycolysis is an energy yielding step wherein an inorganic phosphate is incorporated without any expense of ATP. This energy conserving step involves oxidation of glyceraldehyde 3-phosphate (2mols) to 1, 3-bisphosphoglycerate. The reaction is catalyzed by the enzyme *glyceraldehyde 3-phosphatedehydrogenase*. NAD^+ is the cofactor in this reaction and acts as an oxidizing agent. Reduction of NAD^+ yields the reduced coenzyme NADH. The other hydrogen atom is released as H^+ into the solution.



$\Delta G'^{\circ}$ = 6.3 kJ/mol

The NADH so generated needs to be continuously reoxidized, so as to maintain levels of NAD^+.

7. ***Phosphoglycerate kinase* reaction:** The high energy phosphoryl group from 1, 3-bisphosphoglycerate is transferred to ADP by the enzyme *phosphoglycerate kinase* resulting in the formation of ATP. The enzyme can catalyze the reaction in both directions.



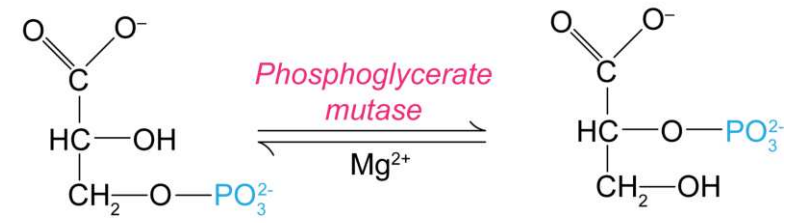
$\Delta G'^{\circ}$ = -18.5 kJ/mol

This step, along with the previous one (6), together constitutes the energy-coupling process with 1, 3-bisphosphoglycerate as a common intermediate. The overall reaction is exergonic. The two reactions can be summed up as:

Glyceraldehyde 3-phosphate + ADP + Pi + NAD^+ ⟶ 3-phosphoglycerate + ATP + NADH + H^+ $\Delta G'^{o}$ = 12.5kJ/mol

Since the energy released on oxidation is conserved by the coupled formation of ATP directly from ADP and Pi, it is called **substrate-level phosphorylation**. This process does not require either membrane-bound enzymes or proton gradients in membranes - a feature essential for respiration-linked phosphorylation.

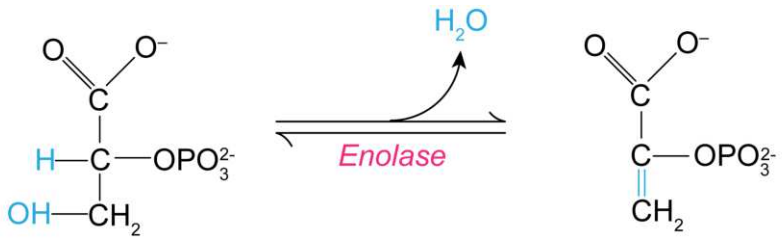
8. ***Phosphoglycerate mutase* reaction:** The enzyme *phosphoglycerate mutase* catalyzes the reversible conversion of 3-phosphoglycerate to 2-phosphoglycerate by shifting of the phosphorylgroup between C-2 and C-3 of glycerate.



3-Phosphoglycerate 2-Phosphoglycerate

$\Delta G'^{\circ}$ = 4.4 kJ/mol

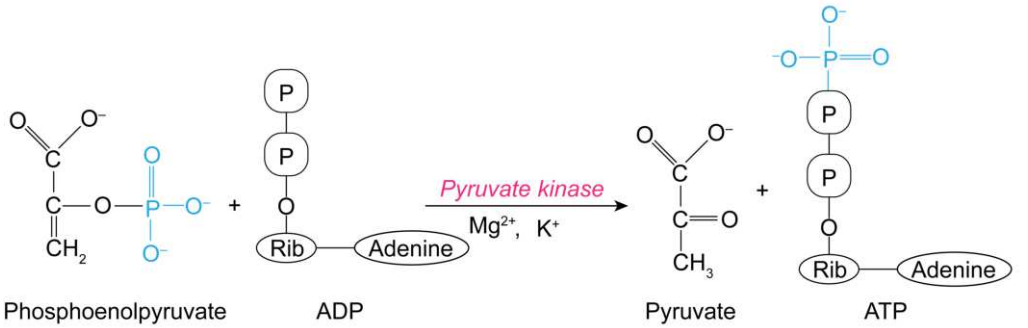
9. ***Enolase* Reaction**: This reaction involves dehydration (removal of water) from 2-phosphologycerate to form phosphoenol pyruvate (PEP).



2-Phosphoglycerate Phosphoenolpyruvate

$\Delta G'^{\circ}$ = 7.5 kJ/mol

10. ***Pyruvate kinase* Reaction:** involves transfer of the phosphoryl group from Phosphoenolpyruvate to ADP generating ATP and pyruvate. This last step requires the enzyme *pyruvate kinase* and is also an example of substrate-level phosphorylation. The enzyme requires Mg^{2+} and K^{+} for its function.



$\Delta G'^{\circ}$ = -31.4 kJ/mol

Since this step is an irreversible one, it constitutes and important site of regulation. Like *PFK*, *pyruvate kinase* acts as a positive modulator. Its activity slows down when ATP is in excess, and reverse happens when a cell is ATP deficient.

The reactions of glycolysis are graphically summarized in Fig.11.1.

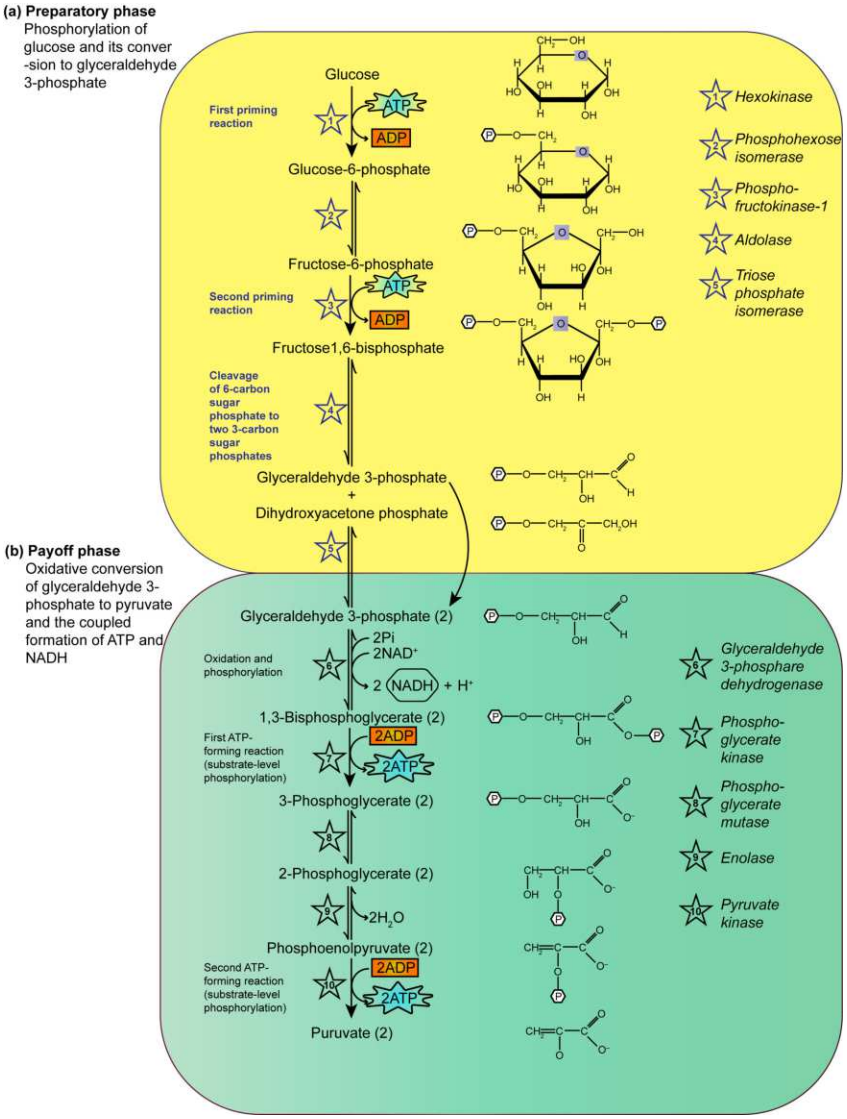


Fig.11.1: A summary of reactions in glycolysis. (After Nelson & Cox, 2017).

Box.11.2: ATP Yield and Balance Sheet

Since one molecule of glucose yields **two** molecules of pyruvate (3C), the balance sheet for glycolysis is:

$Glucose + 2NAD^+ + 4ADP + 2Pi \longrightarrow 2\ Pyruvate + 2\ ADP + 2NADH + 2H^+ + 4\ ATP + 2H_2O$.

In the preparatory phase 2 molecules of ATP are consumed while in the payoff phase 4 ATP molecules are generated, in addition to reduction of $2NAD^+$ to 2 NADH.

So the net reaction of glycolysis under aerobic conditions can be represented as:

$Glucose + 2NAD^+ + 2ADP + 2Pi \longrightarrow 2\ Pyruvate + 2NADH + 2H^+ + 2ATP + 2H_2O$

$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2Pi \longrightarrow 2CH_3COCOO^- + 2NADH + 2H^+ + 2ATP + 2H_2O$

Thus, there is a **net gain of two ATP** molecules in addition to generation of **2 NADH**.

11.2.2 Alternative Glycolytic Reactions in Plants

Interestingly, glycolysis in plants takes place both in the cytosol and plastids. There are distinct nucleus-coded isoenzymes for catalysis. There are two distinctly alternate reactions of glycolysis occurring in the cytosol that enhance the ATP yield of this pathway in plants. These alternate reactions help the plants in their acclimation to environmental stress. Plant mitochondria normally do not respire fatty acids whereas animal mitochondria do. The latter may even respire pyruvate derived from glycolysis. A large portion of carbon entering the plant glycolysis and Krebs cycle may not get oxidized to CO_2. Instead, it gets utilized in the biosynthesis of various other compounds like amino acids, fatty acids, nucleic acids, and many secondary metabolites (See Plaxton, 1996).

In contrast to the non-plant classical glycolysis which begins with glucose and consists of a sequence of ten enzymatic reactions, culminating in the formation of pyruvate, higher plants are known to utilize sucrose and starch as the principal substrates. Also hexose phosphates and triose phosphates from starch breakdown in plastids can enter the cytosolic glycolysis. In many cases organic acids, proteins and lipids can also act as a source of carbon.

Thus, plant respiration can be better expressed as:

$C_{12}H_{22}O_{11} + 13H_2O \longrightarrow 12CO_2 + 48H^+ + + 48e^-$

$12O_2 + 48H^+ + 48e^- \longrightarrow 24H_2O$

Hence, the net reaction can be expressed as:

$C_{12}H_{22}O_{11} + 12O_2 \longrightarrow 12CO_2 + 11H_2O$

Plant glycolysis pathway operating in the cytosol shows three parallel enzymatic reactions which provide more metabolic options to plants helping them to respond physiologically to change in a single environmental factor, O_2 deprivation or P_i (inorganic phosphate) starvation. The glycolytic reactions occurring in the plant cytosol and plastids are summarized in Fig. 11.2.

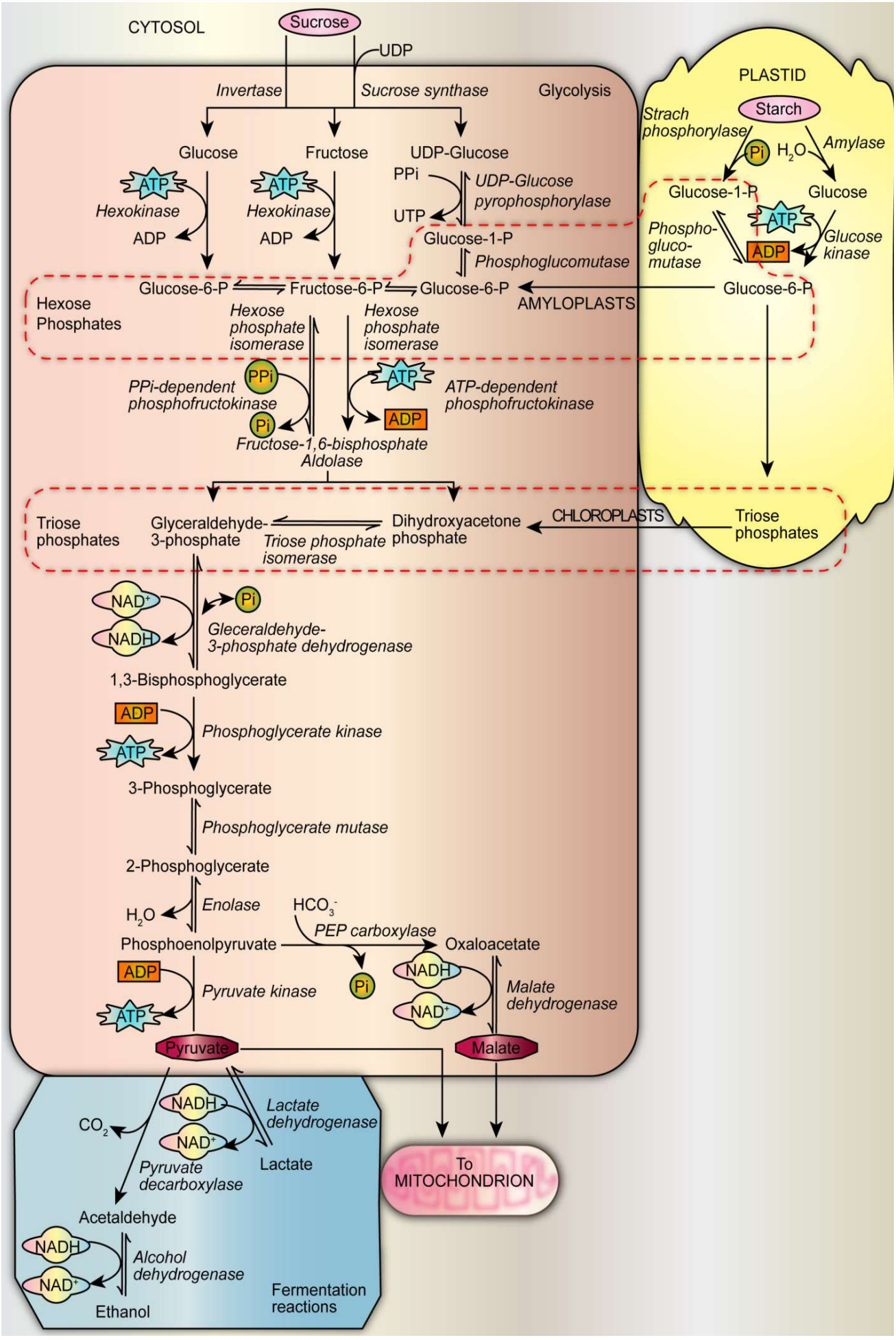


Fig. 11.2: An overview of plant glycolysis (After Taiz & Zeiger, 2010).

Recent evidence suggests that many plants like rice under anoxic (lack of oxygen) conditions show such bypass pathways, and since no ATP is needed in sucrose metabolism via sucrose synthase pathway, these bypasses result in a net yield of 8 ATP per sucrose as compared to 4 ATP obtained via the non plant glycolytic pathway.

11.2.3 Regulation of Glycolysis

Glycolysis serves two important functions

a) Generation/Production of ATP, and

b) Synthesis of intermediates.

Control of glycolysis in plants is unique in the sense that primary regulation is exercised at the level of PEP metabolism by *pyruvate kinase* and *PEP carboxylase*. Secondary regulation is exerted by PEP at the site of conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate. This is akin to “bottom up” regulation which perhaps permits plants to control net glycolytic flux to pyruvate independently of Calvin cycle.

In order to avoid wasteful production of these intermediates, a precise control is needed to regulate the rate of these reactions. Since most of the reactions of glycolysis are reversible, it is the steps 1, 3 and 10 which are irreversible and the potential sites of control and regulation.

These control points are :

Step 1 : Conversion of glucose to glucose -6 phosphate by *hexokinase* (HK)

Step 3 : Conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate by *phosphofructokinase* (PFK), and

Step 10 : Conversion of phosphoenol pyruvate to pyruvate by *pyruvate kinase*.

Depending upon the cell’s need for ATP, rate of glucose oxidation in glycoysis is controlled by either inhibiting or stimulating various enzymes.

Hexokinase enzyme (step 1) in inhibited by its reaction product glucose 6-phosphate. Thus, a high concentration of this product would indicate less need for glucose. The allosteric control is restored once the product gets consumed.

Phosphofructokinase (Step 3) is the principal rate-limiting enzyme of the glycolytic pathway. The enzyme is inhibited by high ATP concentration. In addition, PFK-1 is allosterically activated by AMP (Fig. 11.3). Thus, the rate of glycolysis is extremely sensitive to a change in cell’s ATP: AMP ratio. The enzyme constitutes the most important control point and acts as a valve to control the passage of glucose into the glycolytic pathway (nicknamed as **pacemaker of glycolysis**).

Pyruvate kinase (Step 10) is also allosterically inhibited by ATP and in presence of excess ATP, the rate of glycolysis slows down. The enzyme *pyruvate kinase* controls the exit point of glycolysis and is inhibited in the presence of excess ATP and acetyl coenzyme A.

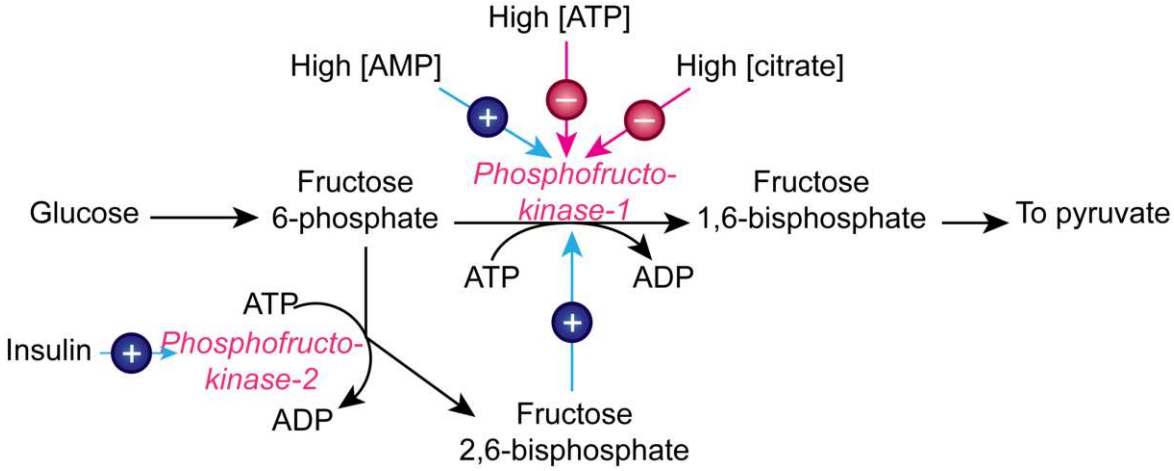


Fig. 11.3: Allosteric control of glycolysis at the level of fructose-6-phosphate (from Lodish).

SAQ 1

a) In the following sentences, choose the right alternative word given in the parenthesis.

i) According to E. Buchner, fermentation could be carried out by (cell-free extracts/living microbes alone).

ii) Glycolysis constitutes the (common step/intermediate step) between aerobic and anaerobic respiration.

iii) The intermediates of glycolysis need to be (carboxylated/ phosphorylated) inorder to facilitate catalysis.

iv) Glycolysis in plants begins with (glucose/sucrose), whereas (glucose/ sucrose) is the starting point in animal glycolysis.

v) The glycolytic pathway comprises (nine/ten) set of reactions which are grouped into (two/three) phases.

b) Which among the following statements are true ? Write T for true and F for false in given boxes.

i) The preparatory and the payoff phases comprise six reactions each. [ ]

ii) Formation of fructose 1, 6-bisphosphate is an irreversible and a regulatory step in glycolysis. [ ]

iii) Net gain of ATP in glycolysis under aerobic conditions is 4ATP. [ ]

iv) Fe^{2+} ions are needed for the functioning of most of the enzymes of glycolysis. [ ]

v) Glycolysis in plants takes place both in the cytosol and plastid. [ ]

vi) One hexose sugar at the end of glycolysis yields 2 molecules of 3-carbon compound-- pyruvate. [ ]

11.3 AEROBIC OXIDATION OF PYRUVATE - OXIDATIVE DECARBOXYLATION

Under aerobic conditions pyruvate (3C) enters the mitochondrial matrix, where its oxidation takes place in three stages:

i) Oxidative decarboxylation to from Acetyl CoA (2C) and CO_2 [oxidation of pyruvate which loses a pair of electrons; and loss of CO_2 (decarboxylation)].

ii) Krebs cycle/Tricarboxylic acid cycle.

iii) Electron transport chain and oxidative phosphorylation; generation of ATP.

Although the outer mitochondrial membrane is permeable to metabolites (presence of porin channels), the inner mitochondrial membrane is a major permeability barrier. Pyruvate enters the matrix via a proteinaceous transporter called pyruvate transporter/mitochondrial pyruvate carrier, which is embedded within the inner membrane (Fig. 11.4), along with the influx of H^+ (contransport).

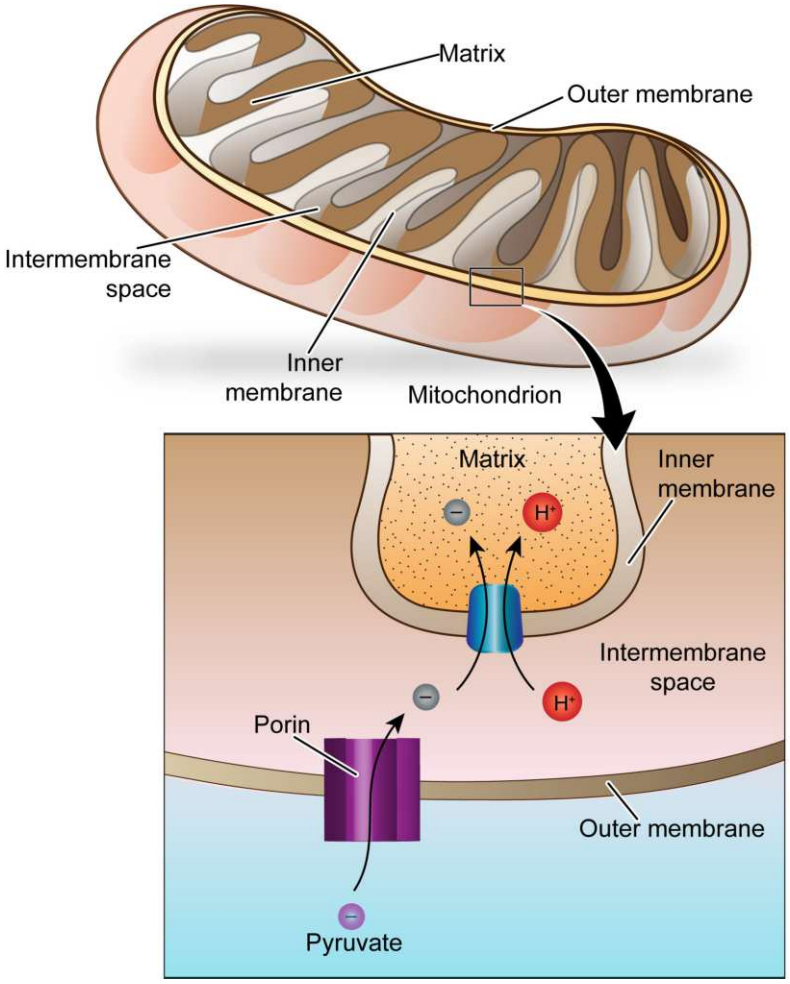


Fig. 11.4: Transport of pyruvate into the mitochondria through porins. (from Ochs, 2012).

In the matrix, pyruvateis oxidized to acetyl-CoA and CO_2 by a cluster of enzymes, collectively called as *Pyruvate dehydrogenase complex* (PDH).

These enzymes are present in the mitochondrial matrix of eukaryotes and in the cytosol of prokaryotes. This enzyme complex is a group of enzymes that catalyzes a metabolic sequence without releasing intermediates. Acetyl - CoA is infact a thioester of acetate with CoA which has a free -SH group at the end of the molecule (Fig.11.5). So, it is often written as CoA -SH.

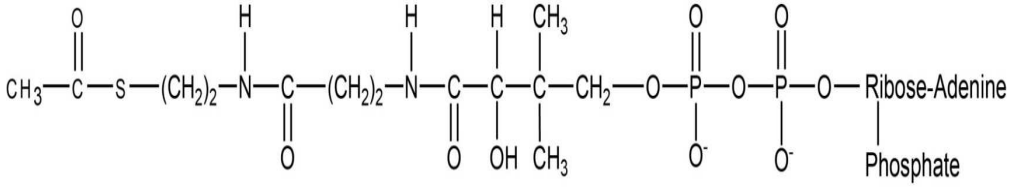


Fig.11.5: A CoA molecule.

Formation of acetyl CoA is a metabolically irreversible conversion which involves three separate enzymes viz., *pyruvate dehydrogenase* (E1), *acetyl transferase* (E2) and *lipoamide dehydrogenase* (E3). In addition, five cofactors viz., thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), Coenzyme A (CoA-SH), nicotinamide adenine dinucleotide (NAD) andlipoamide (Fig. 11.6). The reaction of oxidative decarboxylation is called variously as link reaction, transition reaction, connecting link or the **gateway step** - simply because it links glycolysis and Krebs cycle.

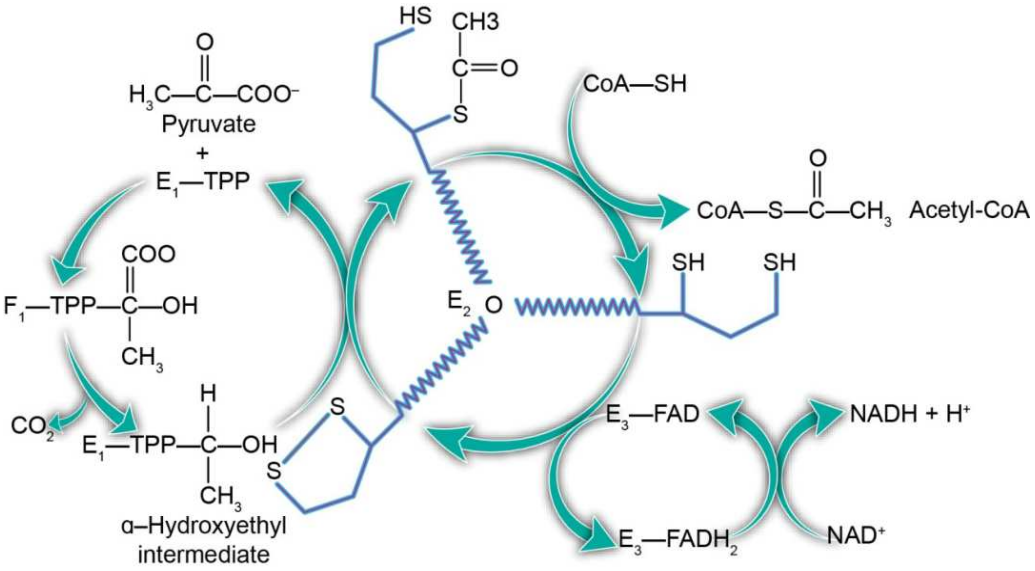


Fig. 11.6: Formation of acetyl CoA (From: Nelson&Cox, 2017).

The details of this complex reaction involving decarboxylation, transacetylation transesterification and dehydrogenation are summarized below.

$$\text{Pyruvic acid + TPP} \xrightarrow{Mg^{2+}} \text{TPP complex + } CO_2$$

$$\text{TPP Complex + Lipoic acid} \longrightarrow \underset{\text{(oxidized form)}}{\text{Acetyl lipoic acid complex}} + \text{TPP}$$

$$\text{Acetyl lipoic acid complex + CoA-SH} \longrightarrow \underset{\text{(Reduced form)}}{\text{Acety CoA. SH + Lipoic acid}}$$

$$\underset{\text{(Reduced form)}}{\text{Lipoic acid}} + NAD^+ \longrightarrow \underset{\text{(Oxidized form)}}{\text{Lipoic acid}} + NADH + H^+$$

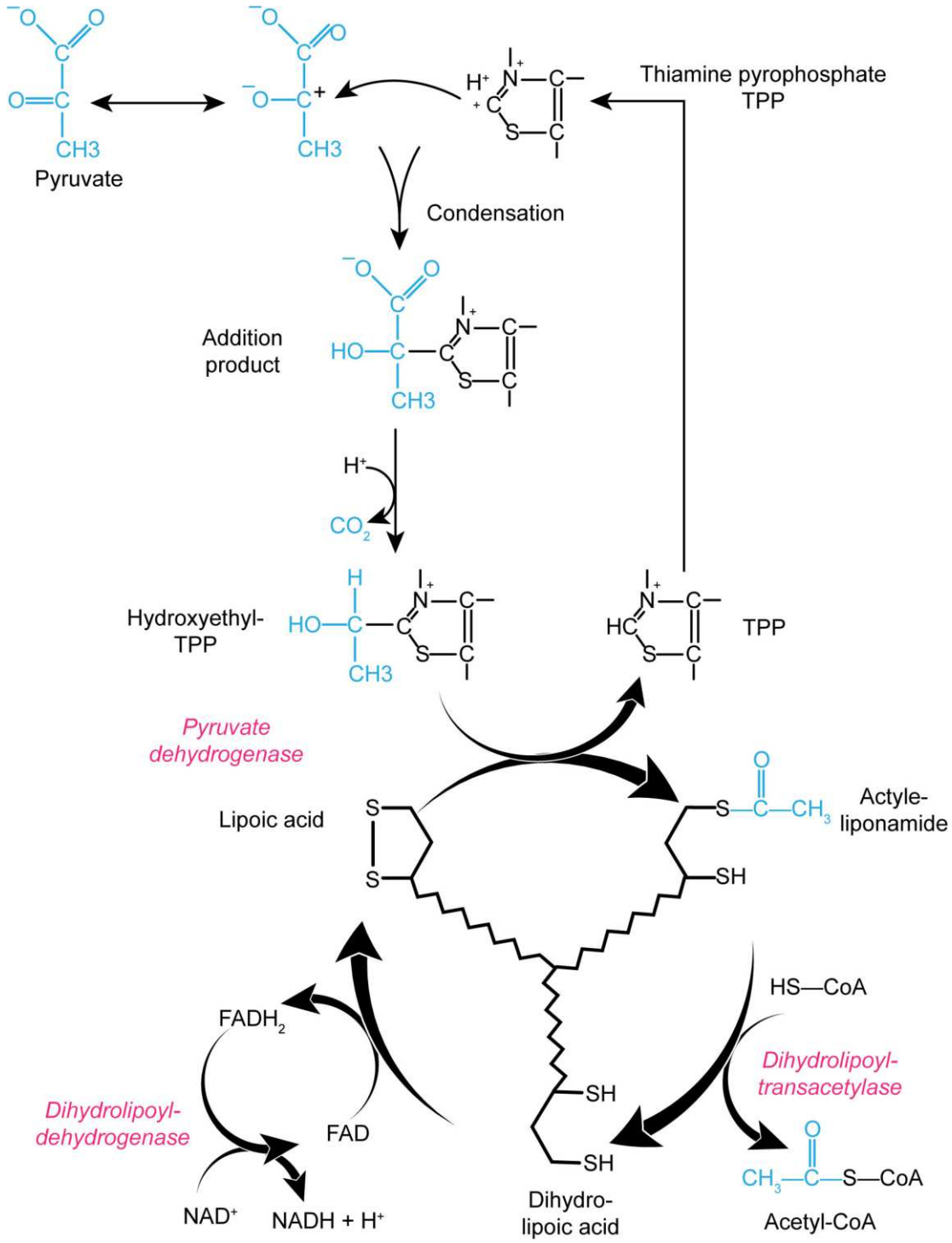


Fig. 11.7: Structure and function of the Pyruvate dehydrogenase complex (After Nelson & Cox, 2017).

$2CH_3CO.COOH + 2CoA\text{-}SH + 2NAD^+ \xrightarrow{PDH\ Complex}$ 2 (CH_3. CO-SH. CoA) + 2 NADH

Acetyl CoA

$+ 2H^+ + 2CO_2$

$\Delta G'^0 = -33.4kJ/mol$

Acetyl CoA is an important intermediate in the aerobic oxidation of pyruvate, fatty acids, and many amino acids. Several biosynthetic pathways derive their acetyl groups from acetyl CoA (e.g., lysine residues from histones). Acetyl CoA is also a biosynthetic precursor of cholesterol and other steroids.

11.4 KREBS CYCLE (TCA CYCLE)

Discovery of Citric acid cycle

Most of the initial work leading to the elucidation of this pathway was based on studies on liver, kidneys, and muscles. The German biochemist **Hans Krebs** proposed in 1937 that organic fuels like sugars are oxidized via a cyclic pathway. Krebs found out in 1932 that citrate, succinate, fumarate, malate and acetate got readily oxidized in liver and kidney slices. A Hungarian biochemist **Albert Szent-Györgyi** found that oxygen consumption was much greater than the expected results when dicarboxylic acids like succinate, fumarate or malate were added to minced pigeon breast muscles (tissue with a very high respiration rate). This led Krebs to infer that these acids were somehow acting catalytically rather than being consumed as substrates. Meanwhile **Carl Martins** and **Franz Knoop** discovered that citrate is converted to α-ketoglutarate. Krebs discovered the other intermediates to finally propose the sequence of reactions of this cyclic pathway. However, Krebs was not aware that pyruvate first combined with Coenzyme A to form acetyl CoA, which subsequently reacted with oxaloacetate to form citrate. Coenzyme A was discovered by **Fritz Lipmann** in 1947.

Later, Hans Krebs and Fritz Lipmann shared the Nobel Prize 1953 for their contributions in the TCA cycle**. Eugene Kennedy** and **Albert Lehninger** demonstrated in 1948 that the entire set of Krebs cycle reactions take place within the mitochondrial matrix, where all the necessary proteins, enzymes and factors are present.

Strategy of Krebs Cycle

The strategy of Krebs cycle in essentially based on the oxidation - reduction of organic compounds and harnessing the energy from electron carriers to generate ATP.

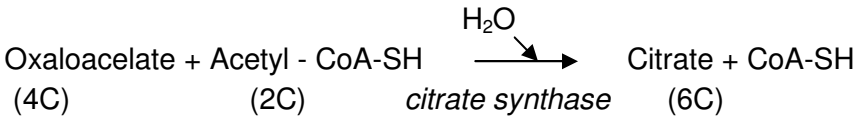
Carbon from metabolic fuels is incorporated into acetyl CoA. In doing so, the electrons are released during oxidation. Since these free electrons cannot exist in a cell, they are immediately transferred to specialized electron carriers like NAD^+ or FAD.The energy of various oxidation steps is conserved in the form of reduced coenzymes NADH and $FADH_2$. As the oxidized substrate and the electron acceptor have different affinities for electrons, this drives an exergonic electron flow, releasing free energy. The free energy is finally captured by the cell - in the form of ATP. This is done by reoxidizing the reduced electron carriers during the electron transport chain. Krebs cycle oxidizes acetyl - CoA to produce such reduced electron-carriers (NADH and $FADH_2$), which are subsequently re oxidized to yield additional ATPs.

Krebs cycle is also called as citric acid cycle or tricarboxylic acid cycle. Krebs cycle has **eight** successive reaction steps. It is customary to begin TCA cycle with acetyl-CoA, since it gives input to this pathway.

1. Condensation of acetyl - CoA to Citrate

The acetyl group of acetyl CoA-SH joins to the carbonyl group of the primary acceptor oxaloacetate (4C) to produce a 6C compound, **citrate**. This reaction is metabolically irreversible, with no allosteric modulation and is catalyzed by the enzyme *citrate synthase*. During this reaction a water molecule is utilized

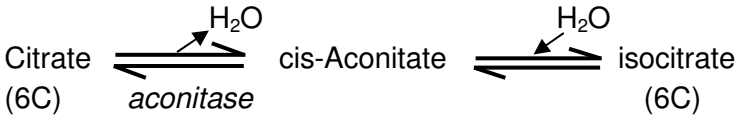
and CoA-SH is regenerated, which is recycled to participate in the oxidative decarboxylation of another molecule of pyruvate by the *Pyruvate Dehydrogenase Complex.*



ΔG^{-0}= - 32.2 kJ/mol

2. Formation of isocitrate via cis-aconitate (Isomerization of citrate)

Citrate is transformed to isocitrate via a reversible reaction catalyzed by the enzyme *aconitase* (*aconitate hydratase*). This reaction also involves an intermediate step of cis-aconitate formation. Citrate is first dehydrated (elimination of H_2O) to form an enzyme- bound C=C bond of cis-aconitate (6C), which is rehydrated (addition of H_2O) to form **isocitrate** (6C).

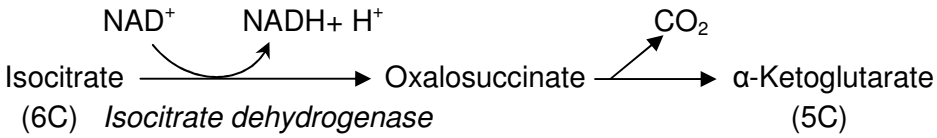


ΔG^{-0}= 13.3 kJ/mol

The enzyme *aconitase* requires Fe^{2+} as cofactors. Enzymes in the steps 1 and 2 are inhibited by fluoroacetate poisoning.

3. Oxidation of Isocitrate to α-Ketoglutarate and CO_2(conservation of energy released by an oxidative decarboxylation in the reduced electron carrier NADH**)**.

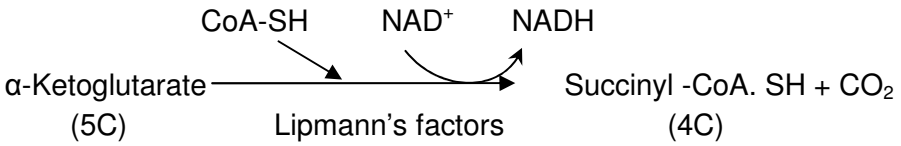
In this step, isocitrate (6C) loses one carbon by oxidative decarboxylation to release CO_2 and form α-Ketoglutarate (5C). This oxidation step is irreversible and electrons are accepted by NAD^+. The reaction is catalyzed by the enzyme *isocitrate dehydrogenase* which requires Mg^{2+} and Mn^{2+}. Oxalosuccinate (6C) is formed as an intermediate compound. The overall reaction is summarized as



This reaction constitutes first of the four oxidation- reduction reactions of Krebs cycle.

4. Oxidation of α-Ketoglutarate to Succinyl CoA and CO_2

This is the second of the four redox reactions. In this oxidative decarboxylation step, α-ketoglutarate is converted to Succinyl-CoA-SH(4C) and CO_2 by the action of the enzyme complex- the *α-ketoglutarate dehydrogenase complex*. Interestingly, this is nearly identical to the action of the *pyruvate dehydrogenase complex* described in step 1. The reaction also involves the three enzymes *E1, E2, E3*, same cofactors and follows identical mechanism.



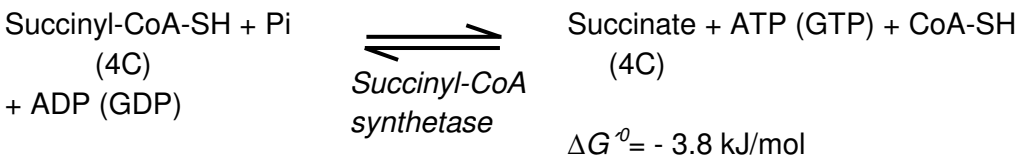
α-ketoglutarate dehydrogenase complex

$\Delta G'^{0}$= - 33.5 kJ/mol

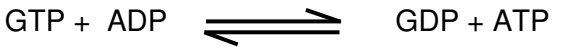
Since the reaction is irreversible, it constitutes an important regulatory site of the Krebs cycle. You should not forget that two carbon atoms had entered the Krebs cycle as acetyl-CoA. Two carbon atoms are subsequently lost as CO_2 in the oxidative decarboxylations in steps 3 and 4. Also, a total of 3 molecules of CO_2 are generated per molecule of pyruvate that entered the mitochondrion. On this account, each molecule of sucrose (1sucrose = 2 glucose = 4 pyruvate) oxidized would yield 3×4=12 CO_2 molecules.

5. Conversion of Succinyl- CoA-SH to Succinate (substrate-level Phosphorylation)

This is the only reaction of Krebs cycle in which a high energy phosphate (ATP/GTP) is directly formed (substrate-level phosphorylation). Free energy in the thioester bond of succinyl CoA is conserved in the form of ATP or GTP in higher animals. In plants, mammalian brain cells and some bacteria-ATP is generated. This reversible reaction constitutes a step of substrate-level phosphorylation, which is catalyzed by the enzyme *succinyl-CoA synthetase* or *succinic thiokinase*. A similar reaction utilizing the free energy available in the thioester bond of acetyl CoA involved citrate synthase enzyme is step 1.



$\Delta G'^{0}$= - 3.8 kJ/mol



Nucleoside diphosphate kinase

$\Delta G'^{0}$= - 0 kJ/mol

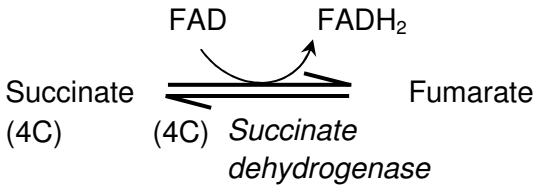
As ATP and GTP are energetically equivalent, no energy is lost in their conversion.

6. Oxidation of Succinate to Fumarate/A Flavin-dependent Dehydrogenation.

The Succinate (4C) formed from succinyl-CoA is eventually oxidized (dehydrogenated) to Fumarate (4C) by the enzyme complex *succinate dehydrogenase.* This enzyme is a complex (like *PDH and α-Ketoglutarate* complexes) containing three different iron-sulfur clusters and a prosthetic group in the form of FAD (Flavin adenine dinucleotide), which is much stronger

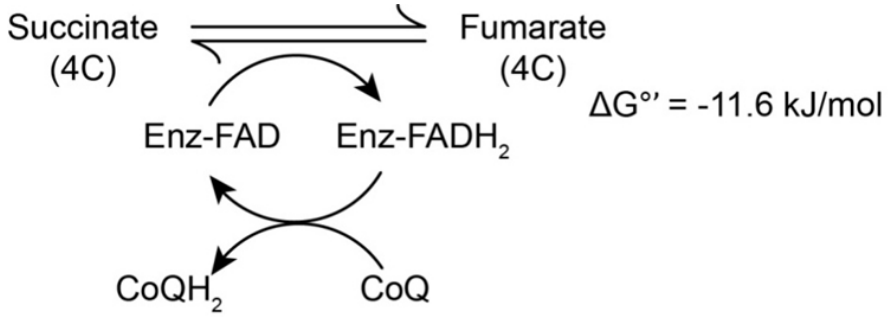
oxidant than NAD^+ as it must oxidize a single C-C bond of succinate rather than the C-O bonds usually oxidized by NAD^+.

The enzyme *succinate dehydrogenase* is the **only** enzyme of Krebs cycle which is tightly bound to the inner mitochondrial membrane as it is coupled to other electron carriers of the respiratory chain. It is also called complex II. All other Krebs cycle enzymes exist freely in the mitochondrial matrix.



$\Delta G'^{0}$= - 0 kJ/mol

Since reduced flavin ($FADH_2$) must be reoxidized for the enzyme to act again, the two electrons from $FADH_2$ are transferred through three iron-sulfur centers of SDH, to conenzyme Q (another electron carrier of the mitochondrial electron transport system.

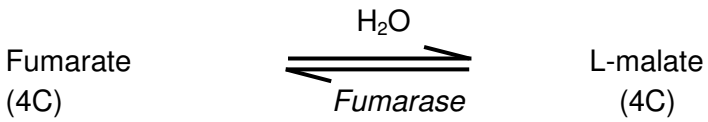


The electrons reach the final acceptor O_2 and the coupling generates about 1.5 ATP per pair of electrons via respiration-linked phosphorylation (details are given in the subsequent pages).

Succinate is competitively inhibited by malonate, which is a structural analogue of succinate. Although malonate is not normally present in cells, its use to block the activity of citric acid cycle led Krebs to elucidate the cyclic nature of this pathway!

7. Hydration of a carbon-carbon double bond/Hydration of Fumarate to Malate

There is a stereospecific ***trans*** addition of water to the double bond of fumerate to form L-malate (4C). This reversible hydration reaction is catalyzed by the enzyme *fumarase* (earlier called *fumarate hydratase*). The enzyme is highly stereospecific and only L-isomer of malate is formed.



$\Delta G'^{0}$= -3.8kJ/mol

8. Regeneration of Oxaloacetate by oxidation/Oxidation of malate to oxaloacetate

In the final reaction of Krebs cycle, oxidation of L-malate to oxaloacetate is catalyzed by the NAD-linked enzyme *α-malate dehydrogenase*.

NAD^+ $NADH + H^+$

Malate ⇌ Oxaloacetate

(4C) *Malate dehydrogenase* (4C)

$\Delta G'^0$= 29.7 kJ/mol

Events occurring in the Tricarboxylic acid cycle are diagrammatically represented in the Fig.11.8.

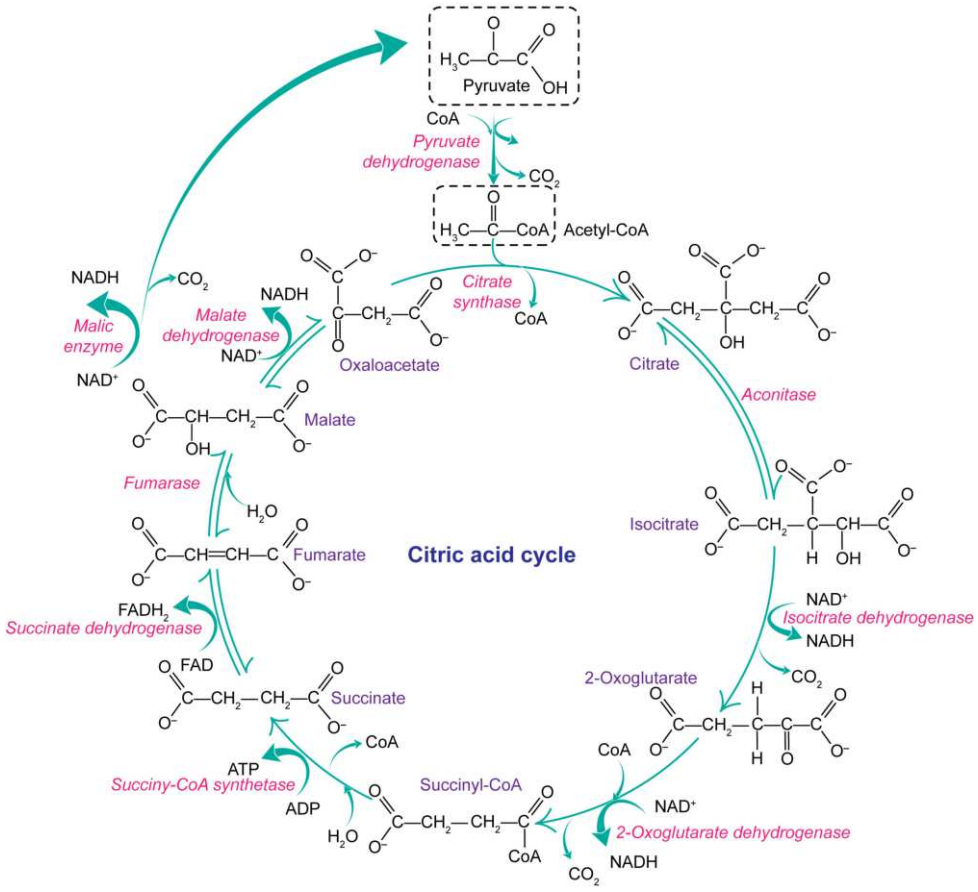


Fig. 11.8: Reactions of the Krebs cycle (After Nelson & Cox, 2017).

One molecule of pyruvate passing through stepwise oxidation in the mitochondrion generates **3** molecules of CO_2, free energy released in conserved in the form of **4** NADH and **one** $FADH_2$. In addition,**one** ATP molecule is produced as a result of substrate-level phosphorylation.

With respect to Krebs cycle :

a) Two carbon atoms enter the cycle in the form of acetyl-CoA.

b) Two carbon atoms leave the cycle in the form of CO_2.

c) One turn of Krebs cycle generates **one** high energy phosphate (ATP) through substrate-level phosphorylation.

d) **Four pairs** of hydrogen atoms leave the Krebs cycle during **four** oxidation reactions. As a result,**three** molecules of NAD^+ and **one** molecule of FAD get reduced to **3** NADH and **1**$FADH_2$ respectively.

e) Energy released by the reoxidation of these electron carriers in the electron transfer chain is utilized to synthesize ATP from ADP + Pi.

A sum of eight reactions involved in one turn of the Krebs cycle can be represented as:

$$\text{Acetyl-CoA-SH} + 2H_2O + 3NAD^+ + FAD + ADP + Pi \longrightarrow 2CO_2 + 3NADH + 3H^+ + FADH_2 + \text{CoA-SH} + ATP.$$

If we also include glycolysis (2ATP + 2NADH + $2H^+$) and the gateway step of *pyruvate dehydrogenase* reaction (NADH + H^+ +CO_2), then It is worth recalling that each glucose molecule generates **two** molecules of pyruvate. The following equation represents the pathway of glucose via glycolysis and citric acid cycle.

$$\text{Glucose} + 2H_2O + 10NAD^+ + 4ADP + 4Pi + 2\ FAD \longrightarrow 6CO_2 + 10NADH + 10H^+ + 2FADH_2 + 4ATP$$

Glycolysis	2	Glycolysis	2
PDH	2	Krebs Cycle	2
Krebs Cycle	6		

PDH	2
Krebs Cycle	4

Form the above equation, it becomes very clear that most of the ATP is not generated by the substrate-level phosphorylations, but rather by indirect means, i.e., by the reoxidation of the reduced electron carriers (NADH and $FADH_2$) in the mitochondrial electron transport chain. As the oxidation of these carriers a highly exergonic process, their transference to molecular oxygen via stepwise electron transport chain releases energy which drives ATP synthesis.

It has been calculated that reoxidation of each NADH generates about 2.5 moles of ATP whereas 1.5 moles of ATP are generated per $FADH_2$ reoxidation.

11.4.1 TCA Cycle as an Amphibolic Pathway

In addition its primary function of oxidation of acetyl-CoA to CO_2, the Citric acid cycle also behaves as a metabolic hub where one or more of its enzymes play a vital role in other pathways. TCA cycle provides a number of metabolic intermediates which themselves serve as precursors of several biosynthetic processes. Krebs cycle is considered to be **Amphibolic pathway** as it serves both as a catabolic as well as an anabolic pathway. TCA cycle plays a vital role in the oxidative catabolism of sugars, fatty acids and amino acids. It also provides precursors for many biosynthetic pathways viz., synthesis of fatty acid, sterols, chlorophylls, porphyrins, amino acids and nucleic acid bases purines and pyrimidines.

The relationship between the TCA cycle and other biosynthetic processes is depicted in Fig.11.9.

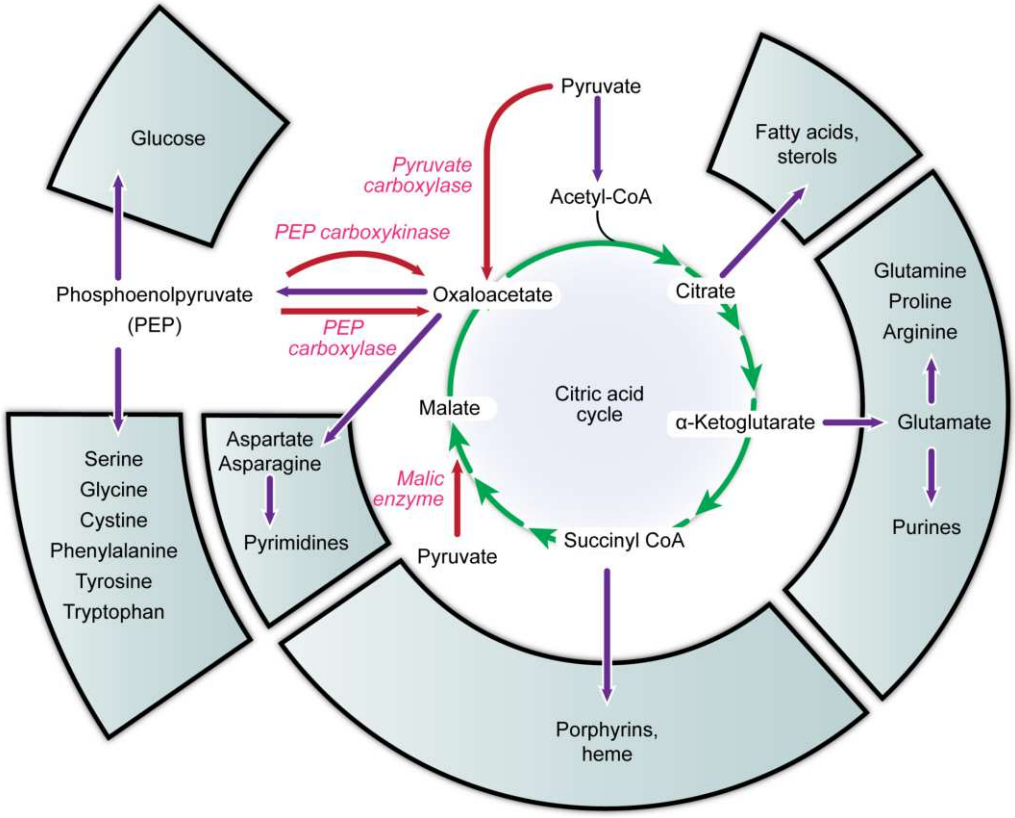


Fig.11.9: Relationship between TCA cycle and different biosynthetic pathways (from Nelson & Cox, 2017).

11.4.2 Regulation of TCA Cycle

As we discussed a little earlier in glycolysis, the rates of production of intermediates is regulated by modulation of certain key enzymes to ensure constant supply, but at the same time also avoid wasteful overproduction. In addition, many allosteric effectors and covalent modifications of cycle enzymes perform the role of regulation.

SAQ 2

State whether the following statements are true or false and indicate by putting **T** for True and **F** for False in the given brackets.

i) Krebs demonstrated that the entire set of citric acid cycle reactions take place within the mitochondrial matrix. [ ]

ii) Krebs cycle involves eight successive reactions steps. [ ]

iii) α- Ketoglutarate is the only 5C intermediate in the entire Krebs cycle. [ ]

iv) There are three decarboxylation steps in the TCA cycle. [ ]

v) Oxidation of succinate to fumerate involves FAD instead of NAD^+. [ ]

vi) One molecule of ATP is produced per molecule of pyruvate in Krebs cycle by substrate level phosphorylation. [ ]

vii) Reaction involving the enzyme *succinyl – CoA synthetase* produces GTP in plants instead of ATP in animals. [ ]

viii) Krebs cycle is an amphibolic pathway as it serves only as a catabolic pathway. [ ]

ix) Aerobic conditions result in decrease in glycolysis. [ ]

11.5 SHUTTLE MECHANISMS

Utilization of Cytosolic NADH- Shuttle Mechanisms

The NADH molecules (2 molecules of NADH per glucose) produced during glycolysis are extra- mitochondrial as they are generated in the cytosol. The NADH *dehydrogenase* of the inner mitochondrial membrane of animal cells can accept electrons only from NADH in the matrix. And the inner membrane is not permeable to NADH.Thus, the NADH generated by glycolysis in the cytosol cannot be deoxidized to NAD^+ by O_2 via the respiratory chain since there is no mitochondrial transporter which can directly transport NADH into the mitochondrial matrix for their reoxidation via the electron transport chain located at the M face of the inner mitochondrial membrane.Thus, there are mechanisms or **shuttles** which can transport NADH in the form of its reducing equivalents (electrons from cytosolic NADH) to a molecule that can pass through the inner mitochondrial membrane. These amounts to indirect route for oxidation of cytosolic NADH as the mitochondrial shuttle is subsequently oxidized generate as NADH or $FADH_2$ in the matrix which can now freely enter the electron transport chain (Fig.11.10). It is the ratio of $NADH/NAD^+$ which determines the direction of NADH transfer across the mitochondrial membrane. A lower $NADH/NAD^+$ ratio prompt translocation of the NADH equivalents into the matrix. A higher ratio induces NADH equivalent transfer in the reverse direction.

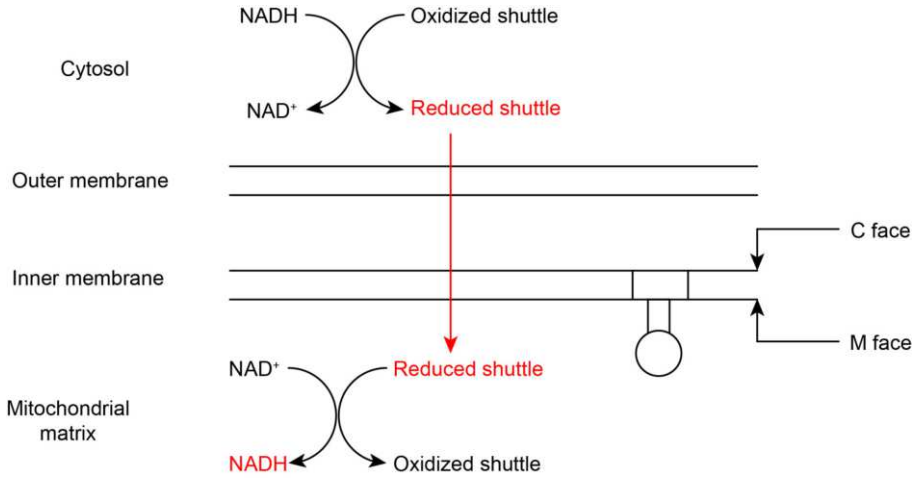


Fig. 11.10: The mode of action of a shuttle in mitochondria. C face= cytosolic face; M face= matrix face.

Even though there is no physical movement of NADH, its reduced equivalent passes into the mitochondria, gets reoxidized inside the matrix, and the oxidized substrate is transported back into the cytosol to repeat the cycle. Two NADH shuttle systems operate in animals and plants.

11.5.1 Glycerol 3-phosphate Shuttle

This shuttle comprises two redox reactions which utilize the same pathway intermediates in different cellular compartments. However, these reactions take place in opposite directions. First dihydroxyacetone phosphate (3C) gets reduced to glycerol 3-phosphate (3C) by utilizing NADH. This reaction is catalyzed by the enzyme *glycerol 3-phosphate dehydrogenase*. Electrons are thus transferred from NADH.

Dihydroxyacetone phosphate + NADH $\xrightarrow[\text{Glycerol phosphate dehydrogenase}]{}$ Glycerol 3-phosphate + NAD^+

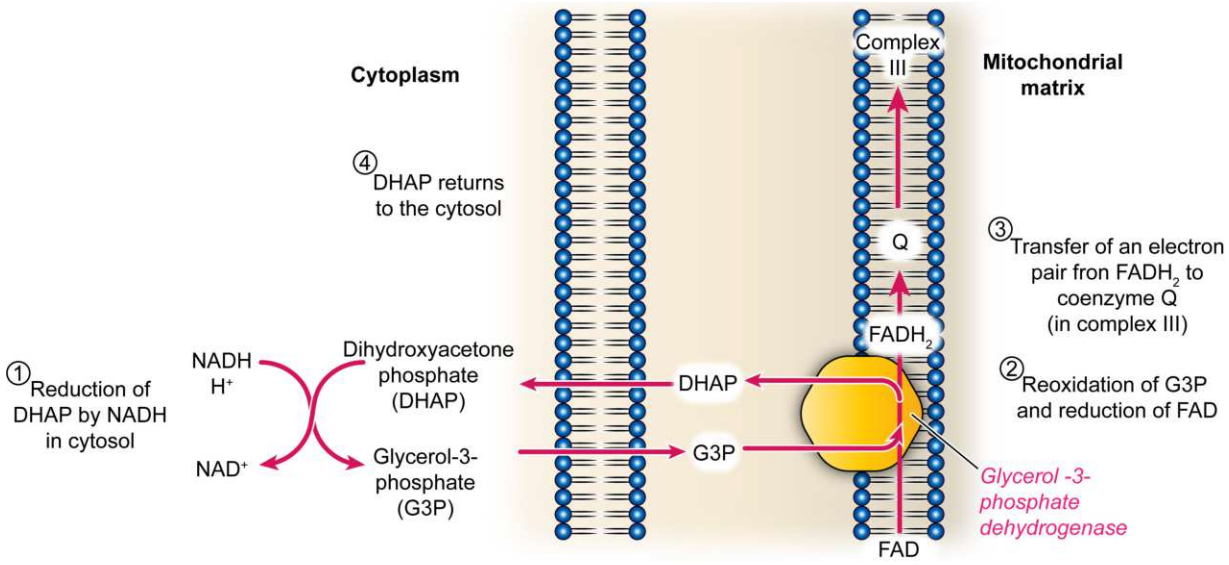


Fig. 11.11: The glycerol phosphate shuttle (From Appling)

Interestingly, the glycerol phosphate does not require a membrane transport protein as the *mitochondrial glycerol 3-phosphate dehydrogenase* is located on the C face (outer face) of the inner membrane. Dihydroxyacetone phosphate is regenerated in the process while the electrons are transferred to the enzyme linked FAD group (Fig. 11.11). The resulting $FADH_2$ now enters mobile carrier QH_2. The *glycerol phosphate oxidase* complex is quite similar to *succinate dehydrogenase* and is located on the C-face (cytosolic face)of the inner membrane. As a result of the action of *glycerol phosphate oxidase* on glycerol phosphate, the two electrons are transferred to FAD^+ resulting in the formation of $FADH_2$ which enters the electrons transport chain at Complex II until electrons are ultimately captured in QH_2. Since both, the complex II and *glycerol phosphate oxidase* do not contribute to the proton gradient, the oxidation of cytosolic NADH by this shuttle bypasses complex I and delivers the reducing equivalents to ubiquinone–CoQ and the complex III, the energy released is not sufficient to transfer H^+ ions across the inner mitochondrial membrane. As a result the yield from this transfer is capable of synthesizing only 1.5 ATP molecules per pair of electrons. The $FADH_2$ oxidation can extract only about ⅔ of the energy of NADH (2ATP) due to incapability of complex II to pump electrons across the membrane.

Glycerol 3-phosphate + Q $\longrightarrow$ Dihydroxyacetone phosphate + QH_2

Since there is less ATP produced, the residual energy is released as heat. Heat generated by this method contributes to the protective role of adipose

tissue in cold sensitive parts of the body of animals during hibernation. Reactions of this shuttle are summarized below:

1. Reduction of DHAP by NADH in cytosol.
2. Reoxidation of glycerol 3-phosphate (G3-P) and reduction of FAD.
3. Transfer of an electron pair from $FADH_2$ to Fe-S clusters of QH_2 (Coenzyme Q) in Complex II before Complex III.
4. Returning of DHAP back into the cytosol.

11.5.2 Malate-Aspartate Shuttle

This shuttle involves malate, aspartate and oxaloacetate. The cytosolic NADH is utilized by the enzyme *malate dehydrogenase* to reduce oxaloacetate to malate. The latter is then transported across the inner mitochondrial membrane by a specific carrier protein (*malate – α-ketoglutarate carrier protein*). Once inside the matrix, malate gets reoxidized to OAA by the mitochondrial *malate dehydrogenase*. This reaction is coupled to the formation of NADH from NAD^+. NADH enters the electron transport chain at Complex I to yield 2.5 of ATP.

Reactions of the shuttle have been summarized in Fig 11.12.

1. Reduction of OAA to malate in cytosol.
2. Entry of malate into the matrix.
3. Reoxidation of malate in the matrix.
4. Oxidation of NADH by Complex I.
5. Transport of aspartate back to cytosol.

Since OAA cannot cross the inner membrane, it is transaminated by the enzyme *aspartate aminotransferase* to aspartate which now can be easily translocated out into the cytosol via a specific aspartate/glutamate carrier protein located at the inner membrane. Aspartate is reconverted in cytosol to OAA, again by transamination to continue the cycle. The two transamination reactions require a continuous influx of glutamate and outflux of α-ketoglutarate.

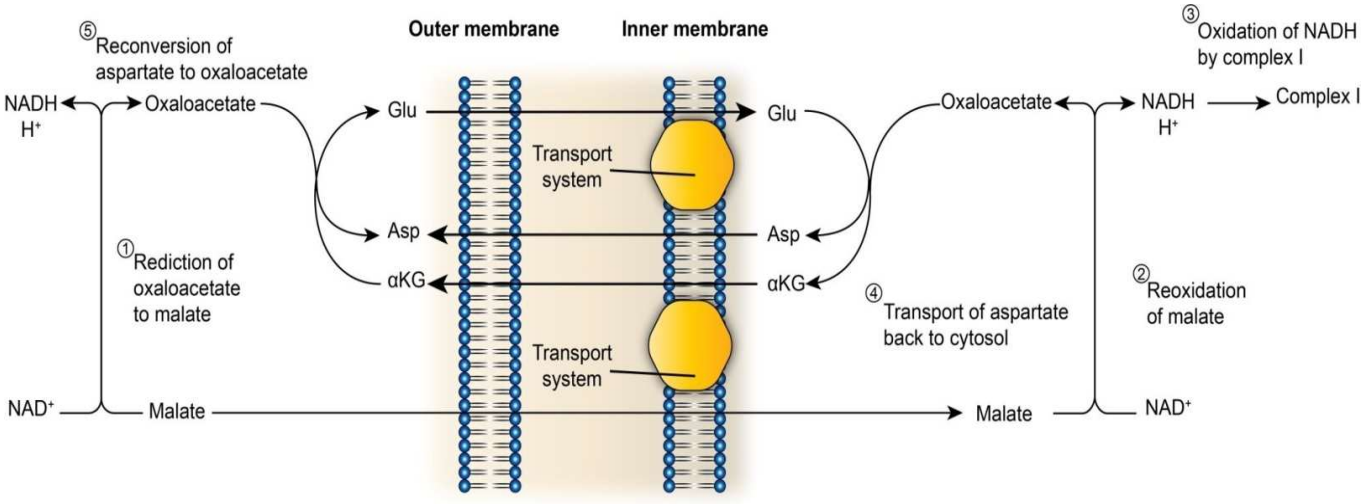


Fig. 11.12: The Malate-Aspartate Shuttle (From Appling).

Transamination in the mitochondria utilizes an amino group from glutamate, to convert it to α-ketoglutarate which is now transported back into the cytoplasm where it acts as an amino acceptor for regeneration of OAA (Fig. 11.13).

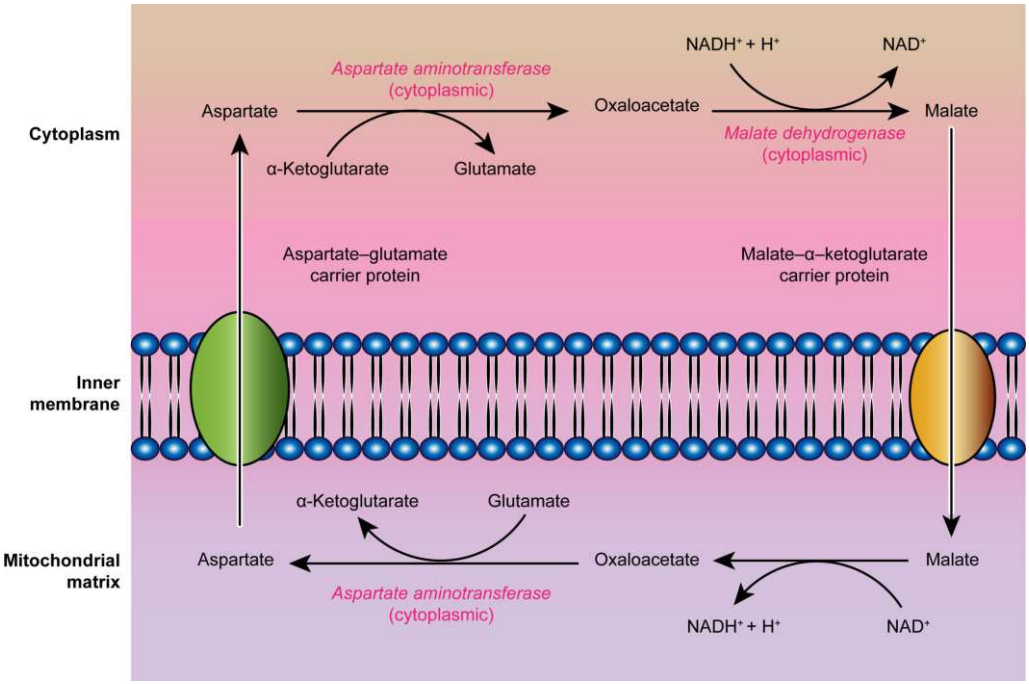


Fig. 11.13: Regeneration of OAA in mitochondria by transamination.

The mitochondria of plants have an external *NADH dehydrogenase*, which is capable of transferring electrons directly from the cytosolic NADH into the electron transport chain at the level of ubiquinone. Since the process bypasses the complex I and the associated proton movement, the yield of ATP from cytosolic NADH is about 1.5 moles per two electrons.

The mitochondria of plants show some unique features.

1. There is an alternative *NADH dehydrogenase* enzyme in the matrix which transfers NADH directly to ubiquinone, thereby bypassing the complex I and its associated proton movement.

   Unlike the other dehydrogenases, the enzyme is rotenone-insensitive and its activity would result in lower ATP yields (1.5 ATP) instead of the normal 2.5 moles released per pair of electrons.

2. An external *NAD (P) H dehydrogenase* enzyme present on the C-face of the mitochondrial inner membrane, which is capable of transferring electrons from NADH or NADPH) in the intermembrane space to ubiquinone, thereby bypassing the complex I. As a result the ATP yield is less than that from matrix-generated NADH (1.5 moles of ATP instead of 2.5 moles).

3. Certain plants show an alternative pathway called **cyanide resistant respiration**. This unique variant involves an *alternative cyanide resistant oxidase* enzyme that transfers electrons from the ubiquinone pool directly to oxygen. In this manner the proton-translocating steps of Complexes III and IV are bypassed. Since no terminal cytochrome a_3 of complex IV is involved there is no sensitivity to cyanides. But at the same time when the complex III and IV are bypassed the ATP

production is substantially reduced (Fig. 11.14) Instead of being conserved as ATP, energy is released as heat which gives many plants their thermogenic properties.

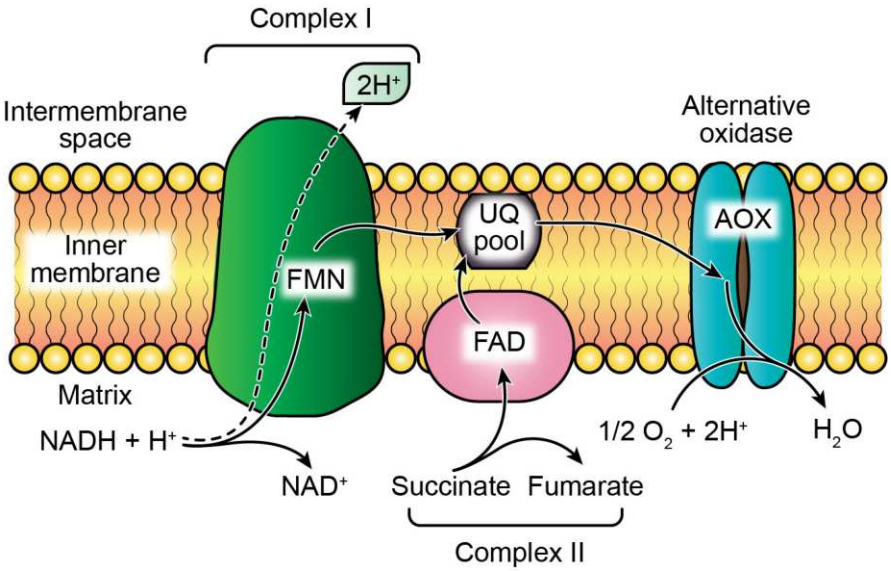


Fig. 11.14: Cyanide resistant respiration with the help of an *alternative oxidase* (AOX) which intercepts the electrons, allowing them to pass through one or no phosphorylating site (From Hopkins and Hüner).

SAQ 3

i) The NADH molecules generated during …….. can not directly enter the …….. as there is no transporter for this.

ii) The …….shuttle comprises two redox reactions in two different cellular compartments.

iii) The Malate shuttle involves ……. and aspartate.

iv) The ……. shuttle operates chiefly in brain, skeletal muscles, and brown adipose tissue, whereas ……. shuttle is active in liver, kidney, and heart.

v) The cyanide resistant respiration involves an alternative ……enzyme. Due to …….. ATP production, the plants show thermogenic properties where energy is released as heat.

11.6 ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

A closer look at the processes of glycolysis and Krebs cycle help us to reach the following conclusions:

Aerobic oxidation of one molecule of hexose sugar via the above mentioned pathways yield energy which is sufficient to synthesize 4 ATP, 10 NADH and 2 $FADH_2$ molecules. Eight of the ten NADH molecules are generated within the mitochondria while the remaining two are produced in the cytoplasm (extra mitochondrial). These extra mitochondrial NADH enter the mitochondrial

transport chain in the form of their reducing equivalents via the Glycerol phosphate or Malate-aspartate shuttles (as discussed above).

In general, ATP is synthesized by **three** different ways by the mitochondrial electron transport chain.

1. From NADH present with the mitochondrial matrix,
2. From extra mitochondrial NADH (in the cytosol), and
3. From $FADH_2$ from the mitochondrial matrix.

In living cells it is these oxidation-reduction couples which are grouped in a sequence and participate in forming an electron transport chain. Each redox couple possesses a variable affinity for the electrons which determines their relative capacity to donate electrons. Movement of electrons from one redox couple to another will be determined by their **redox potential**. Redox potential refers to the affinity of a compound (in the present case, NADH or $FADH_2$) for the electrons. It is measured relative to the redox potential of hydrogen. It means that electrons move from compounds having less affinity for electrons (reducing compounds) to the oxidizing compounds that have more affinity for electrons. Thus, if more than one redox couple is present in the medium, electrons from one donor of a redox couple will be easily accepted by the acceptor of another redox couple.

Structure and Function of the Mitochondrial Electron Transport Chain

Components of the electron transport chain are located in the form of four transmembrane multiprotein complexes in the inner mitochondrial membrane,

These complexes are named by Roman numerals I, II, III and IV (Fig.11.15). Three of these viz., I, III, and IV are involved in proton pumping across the membrane. As mentioned above while discussing the free energy change and redox potential, transfer of two electrons from NADH (or $FADH_2$) to oxygen is catalyzed by the electron transfer chain.

$$NADH + H^+ + \tfrac{1}{2}O_2 \longrightarrow NAD^+ + H_2O$$

The standard free energy change during the overall reaction is calculated to be about 220 kJ $mole^{-1}$ of NADH by comparing the reduction potentials for NADH-NAD^+ pair (-0.320mV = -0.32 eV) and H_2O-$\frac{1}{2}O_2$ pair (+810mV = +0.81eV). In contrast, the reduction potential of succinate-fumarate is much higher (+30mV), only 152 kJ mol of succinate is released. Some of the free energy released is utilized by the ETC to generate an electrochemical proton gradient across the inner mitochondrial membrane.

NADH → Complex I →CoQ→ Complex III →Cytc→ Complex IV

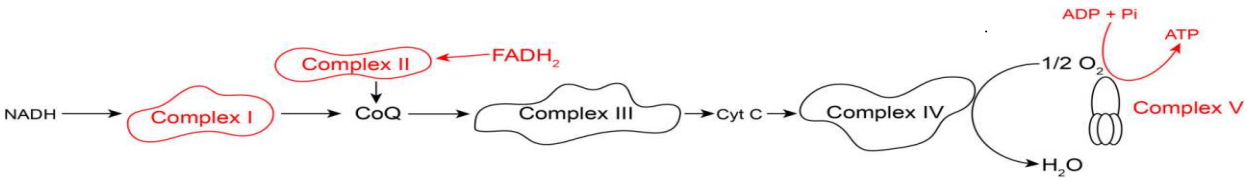


Fig. 11.15: A sequential arrangement of ETC complexes on the inner mitochondrial membrane.

One should remember that since the membrane is considered to be a fluid mosaic of lipids and proteins, these complexes and intermediate carriers must also be floating independent of each other. This would definitely reduce the efficiency of electron transport chain functioning. Recent studies indicate that instead of moving freely in the membrane, these complexes are often co-located on lipid rafts as supercomplexes now named **respirasomes**. A respirasome comprises complexes I, III, and IV along with the intermediate carriers CoQ and cytochrome $_c$ placed in a specific orientation. Components of the respirasome are stabilized by cardiolipin present in the inner mitochondrial membrane. This phospholipid constitutes nearly 20% of the mitochondrial membrane and helps in energy conversion and membrane dynamics.

Same set of electron carriers are found in mitochondria of plants and animals except that plant ETC possesses some exclusive multiple rotenone insensitive *NADP*(*H*) *dehydrogenases* and an alternate *oxidase*. In addition, a transmembrane complex V containing the enzyme F_0F_1 *–ATP synthase* is also present which utilizes the energy of the proton gradient to synthesize ATP.

Electrons from NADH enter the ETC via Complex I. Electrons from $FADH_2$ enter the chain via Complex II. An intermediate carrier in the form of ubiquinone or coenzyme Q (Co*Q*) is required for the electron transfer from Complex I or II to Complex III Fig. 11.15). Transfer of electrons from Complex III to IV is mediated by Cyt_c. Electrons are transferred to oxygen from Complex IV.

Complex I (*NADH Dehydrogenase*) 1000kDa 45 polypeptides

This complex is also called *NADH ubiquinone oxidoreductase*. It is the largest complex with 44 polypeptide subunits. It also contains flavin mononucleotide (FMN) and 9 Iron-sulfur or FeS clusters. A polypeptide containing FeS cluster is called FeS protein. Complex I is the site of oxidation of electrons which are generated from NADH in the mitochondrial matrix. The electrons are passed to FMN thereby reducing it to $FMNH_2$.

Each electron transfer is accompanied by uptake of a proton from the mitochondrial matrix into the intermembrane space. This is a case of **vectorial transport** i.e., movement from one face of the membrane to the other. The electrons are transferred from Complex I to ubiquinone (Co*Q*-a small lipid soluble electron and proton carrier), yielding its reduced form-ubiquinol ($CoQH_2$) from the mitochondrial matrix into the intermembrane space for every electron pair passing through the complex Fig. 11.16).

$$NADH + H^+ + CoQ \longrightarrow NAD^+ + CoQH_2$$

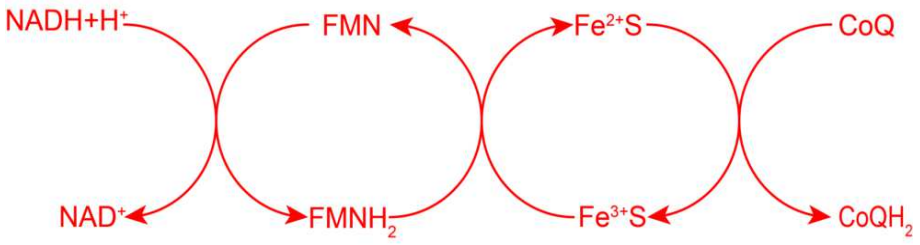


Fig. 11.16: Transfer of electrons from Complex I to Ubiquinol (Co*Q*H_2).

The reaction has a Δ° = -69.5 kJ mol^{-1}, which is ulitized to transfer four H^+ions vectorially (from one face side of the membrane to another) from mitochondrial matrix into the intermembrane space for every electron pair passing through the complex. Thus, complex I behave as a **PROTON PUMP**.

Complex II (*Succinate Dehydrogenase/Succinate-CoQ Reductase* Complex) 140 kDa 4 polypeptides

Oxidation of succinate to fumarate is catalyzed by the enzyme *succinate dehydrogenase*(SDH) in step 6 to the TCA cycle. The SDH is closely linked to the complex II in the inner mitochondrial membrane. The reducing equivalents in the form of two electrons generate $FADH_2$ which immediately transfers them to the FeS cluster of Complex II, from where they pass to Co*Q* and thereafter to Complexes III and IV (Fig. 11.17).

Succinate + Co*Q* ⟶ Fumarate + Co*Q*H_2

This reaction does not release enough energy to pump protons and thus yields lesser number of ATP per molecule of $FADH_2$ as compared to NADH.

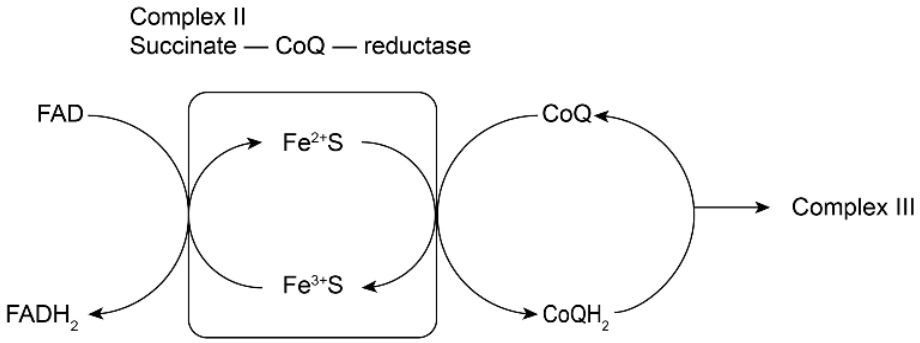


Fig. 11.17: Complex II *Succinate –CoQreductase*.

Complex III (Cytochrome *bc* complex/*$CoQH_2$ – cytochrome c reductase* complex) 250 kDa 10-11 polypeptides.

This complex is in the form of a dimer with two identical monomer units. Each monomer possesses 11 different subunits. Each monomer has cytochromes (b_{565} and b_{560}), hemes b_H and b_2, Rieske iron-sulfur proteins and cyt_{C1}, as its functional core. Since Co*Q*H_2 is lipid soluble, it can easily move laterally from one site to the other within the inner mitochondrial membrane. Complex III oxidizes reduced ubiquinone (ubiquinol) and transfers the electrons to cytochrome *C*. Electrons are first transferred to heme groups of cytb_{562}. As a result the Fe^{3+} ion in the haem gets reduced to Fe^{2+}. Electrons now flow to cytochrome b_{566} and pass through the FeS cluster, heme group of cyt_{C1} towards Cyt. *C*.

CO*Q*H_2 + 2 cyt_c^{3+} ⟶ Co*Q* + 2H^+ + 2 cyt_c^{2+}

cyt_c^{3+} and cyt_c^{2+} are oxidized and reduced forms of cyt_c respectively.

The energy released during this step is sufficient to pump 4 protons out of the matrix per electron pair. This proton pumping across the inner mitochondrial membrane involves a mechanism called the Q-cycle

Transfer of electrons between complexes III and IV is mediated by Cytochrome $_C$. This cytochrome is a mobile carrier and is loosely attached to the C face (outer surface) of the inner mitochondrial membrane.

Complex IV (*Cytochrome $_c$Oxidase Complex*) 200kDa 13 polypeptides

Cytochrome $_c$ forms a link between Complex III and IV by transferring electrons from complex III to molecular oxygen which leads) to its final reduction into H_2O. It is large complex (mol wt = 204,000) and contains two copper centres –C_{4A} and C_{4B} along with two cytochromes *cyt$_a$* and *cyt$_{a3}$*.

The electrons are first transferred to C_{4A} a ion, which reduces them from Cu^{2+} (cupric) to Cu^{+} (cuprous) state. Thereafter, the electrons move via the heme of *cyt*$_{a3}$ to the C_{4B} ion, and via heme of cytochrome $_{a3}$ reach oxygen, to finally form water.

$$2\ cyt_c^{2+} + 2H^+ + \tfrac{1}{2}O_2 \longrightarrow 2\ cyt_c^{3+} + H_2O$$

The overall scheme of the mitochondrial electron chain is depicted in Fig. 11.18.

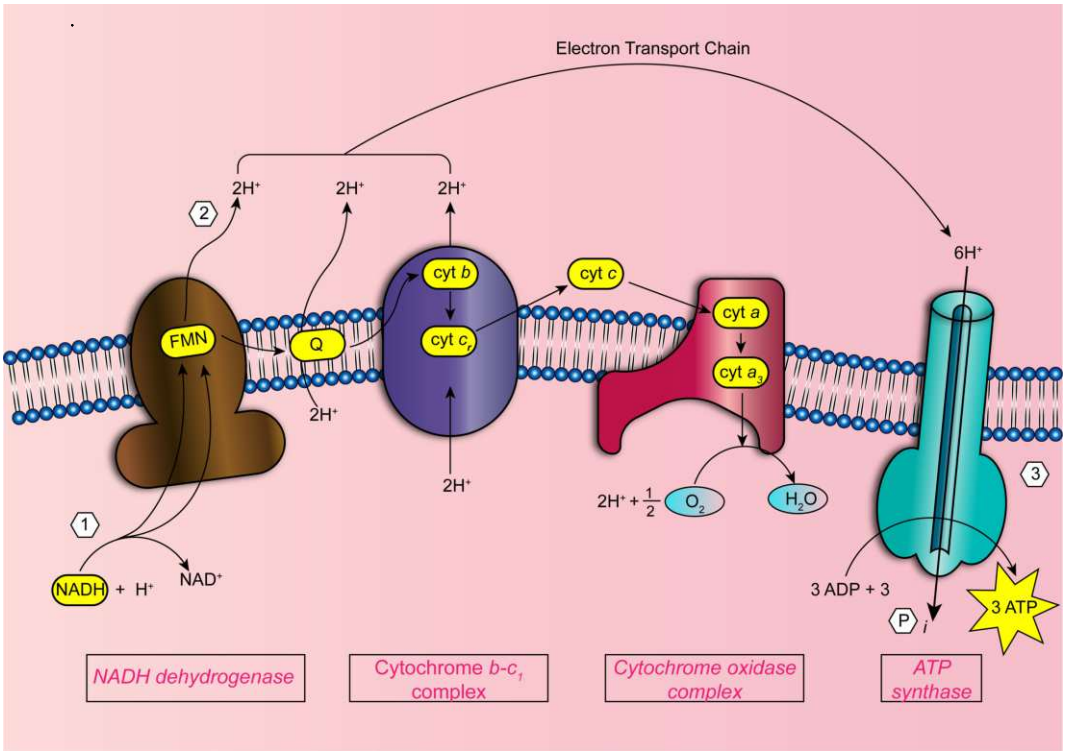


Fig. 11.18: Overall view of the mitochondrial electron transport chain.

Overall, four electrons are required to reduce one oxygen molecule to form 2 molecules of water. Two protons are pumped out of the matrix per electron pair and this completes the oxidation of NADH molecules.

$$O_2 + 4^{e^-} \longrightarrow 2(O_2^-)$$

$$2(O_2^-) + 4(H^+) \longrightarrow 2H_2O$$

Out of the 220 kJ/mol released by oxidation of one mole of NADH, about 20 kJ/mole in consumed in outward pumping of H^+. Remaining about 200kJ is conserved in the proton gradient. There is far greater concentration of H^+ions in the intermitochondrial space (outer membrane space) compared with that in the mitochondrial matrix (Fig.11.19).

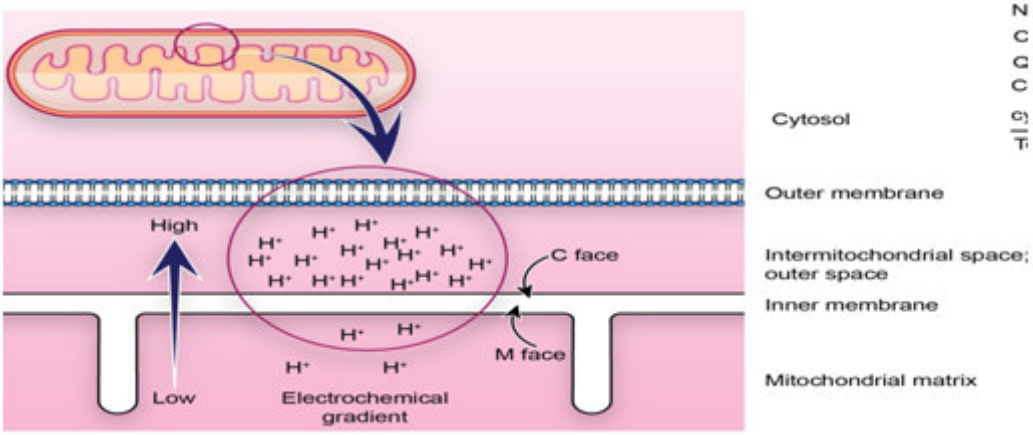


Fig. 11.19: An electrochemical gradient set up across the inner mitochondrial membrane due to proton pumping.

An uneven distribution of charged ions at one location as compared to the other one results in a charge difference between these two locations. Proton pumping along the electron transport chain sets up an electrochemical gradient across the inner mitochondrial membrane (Fig. 11.19). Energy stored in such a gradient is called **Proton-motive force** (pmf) which has two components: a) chemical potential energy difference in concentration of protons on either side of the inner membrane, and b) electrical potential energy : due to differential charge distribution across the membrane.

SAQ 4

In the following sentences choose the right alternate word given in the parenthesis.

i) ATP is synthesized by…….. (two/three) different ways by the mitochondrial electron transport chain.

ii) Redox potential refers to the affinity of a compound for …….. (electrons/protons).

iii) The redox potential E is expressed in ……. (mhos/volts) and measured under standard conditions of pH (5/7).

iv) There is ……. (increase/decrease) in electron affinity from NADH to the terminal oxidation step of the electron transport chain.

v) Respirasomes are ……. (multiprotein/lipid) complexes located on the inner mitochondrial membrane.

vi) Out of all complexes, complex …….. (III/IV) are involved in proton pumping across the membrane.

vii) Cytochrome $_{bc}$, complex is the complex ……. (II/III), whereas the complex I is the …….. (*Succinate dehydrogenase/NADH dehydrogenase*) complex.

viii) Overall……. (two/four) electrons are needed to reduce ……. (one/two) oxygen molecules to form ……. (one/two) molecules of water.

11.7 CHEMIOSMOTIC MODEL AND ATP SYNTHESIS

Since the electrochemical gradients have a natural tendency to revert to an equilibrium, the surplus protons present in the outer space between the two membranes must move back into the mitochondrial matrix so as the equalize the charge on either side of the inner membrane. For this re-entry, the protons pass through another multiunit protein called the *F_0F_1, ATPase* (*ATP synthase*) which constitutes the Complex V. This complex includes a passageway for protons along with active sites responsible for the synthesis of ATP from ADP and Pi. The pumping out of protons out of the mitochondrial matrix keeps pace with the passing of electrons down the electron transport chain from NADH and $FADH_2$. Thus, a gradient is maintained which ensures the flow of protons back into the matrix via the *ATPase* (Fig. 11.20).

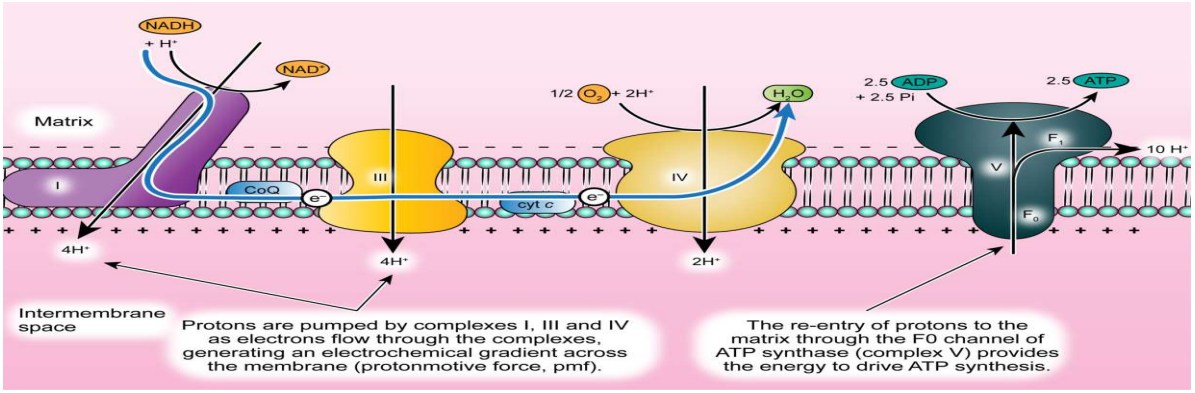


Fig. 11.20: Chemiosmotic coupling of electron transport and ATP synthesis (After Appling).

So ATP is generated in direct response to the oxidation of NADH and $FADH_2$.Use of electron transport blockers and artificial electron donors have confirmed that there are three coupling sites which are capable of driving ATP synthesis. These are complexes I, II, III and IV through which the electrons flow and protons and pumped across the membrane. This process indirectly drives ATP synthesis.

A summary of the **Net Yield of ATP** during aerobic oxidation of glucose and sucrose via different shuttle mechanismsis presented below:

GLYCOLYSIS	**ATP yield**
Glucose ⟶ Glucose 6-phosphate	-1ATP
Fructose 6-phosphate ⟶ Fructose 1,6-bisphosphate	-1ATP
2 Glyceraldehyde 3-phosphate ⟶ **2** 1,3-bisphosphoglycerate **2 × 1.5 2NADH (ext)**	+ 3ATP

If the $NADH+H^+$ produced in glycolysis is oxidized via Malate-aspartate shuttle, the NADH + H^+ generated within the mitochondrial matrix will yield 2.5 ATP instead of 2 ATP. Thus the net yield of this step of glycolysis will be 5 instead of 3 and total yield of ATP per glucose will be 32 instead of 30.

2 1,3-bisphosphoglycerate ⟶ **2** 3-phosphoglycerate + 2ATP
(Substrate Level Phosphoryation)

2 Phospho 3-enol pyruvate ⟶ **2** Pyruvate + 2ATP
(Substrate Level Phosphoryation)

MITOCHONDRIA

2 Pyruvate ⟶ **2**Acetyl CoA-SH	2NADH	2×2.5	+5ATP

KREBS CYCLE

2 Isocitrate ⟶ **2** α-ketoglutarate	2NADH	2×2.5	+5ATP
2 α-ketoglutarate ⟶ **2** Succinyl CoA	**2** NADH	2×2.5	+5ATP
2 Succinyl-CoA ⟶ **2** Succinate (Substrate Level Phosphorylation)	**2** GTP		+2ATP
2 Succinate ⟶ **2** Fumarate	**2** $FADH_2$	2×1.5	+ 3ATP
2 Malate ⟶ **2** Oxaloacelate	**2** NADH	2×2.5	+5ATP

Net Yield per glucose Molecule **32-2=30ATP**

Net Yield per sucrose Molecule **64-4=60ATP**

A model to explain oxidative phosphorylation involving chemiosmotic coupling was proposed in 1961 by British biochemist Peter Mitchell, who received a Nobel Prize in 1978. The model, in simple terms can be explained as:

i) Free energy from exergonic reactions of electron transport chain drives an active transport system.

ii) This system pumps protons from the mitochondrial matrix into the intermembrane space.

iii) An electrochemical gradient for protons is generated.

iv) A high concentration of protons in the intermembrane space prompts them to flow back into the matrix along their electrochemical gradient.

v) Maintenance of proton gradient requires expenditure.

vi) Energy is dissipated when protons flow back from the intermembrane space into the matrix.

vii) A part of this energy is channelized/utilized for the synthesis of ATP (see Fig. 11.18).

Box 11.3: How many ATP per NADH and $FADH_2$?

What is the number of ATP molecules generated during the electron transport Chain? Some clues have been found by working on the isolated mitochondria:

a) Calculation of the P/O ratio(number of ATP molecules synthesized per pair of electrons carried through the electron transport chain to oxygen).

b) Phosphate incorporation into ADP forms the basis to calculate ATP synthesis, and

c) In addition, oxygen uptake is taken as a measure to quantify electron pairs.

Experiments with isolated plant mitochondria demonstrate that when NADH is oxidized the ADP: O ratio is experimentally equivalent to 2.4-2.7 (Theoretical value 2.5). On the other hand, the oxidation of succinate proceeds with a P/O ratio of 1.6-1.8 (Theoretical value 1.5). The NADH (external) also has the same value (1.5). The P/O values were earlier thought to be integers (not fractions) and the P/O ratios were 3 for NADH and 2 for $FADH_2$. The non integer P/O values clearly indicate that phosphorylation and oxidation are not directly coupled.

The following equation summarizes the oxidation of NADH coupled to the synthesis of ATP.

$NADH + H^+ + ½O_2 + 2.5\ ADP + 2.5\ Pi \longrightarrow NAD^+ + H_2O + 2.5\ ATP$

$FADH_2 + ½O_2 + 1.5\ ADP + 1.5\ Pi \longrightarrow FAD^+ + H_2O + 1.5\ ATP$

SAQ 5

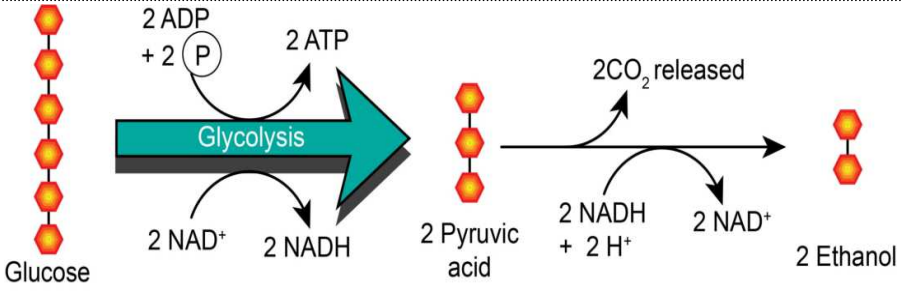
Match the content of **Column I** corresponding with those of **Column II**.

	Column I		**Column II**
i)	Proton passage way	a.	ATP *synthase*
ii)	Electron transport inhibitors	b.	F_1 particles
iii)	1.5 ATP	c.	2 Malate $\longrightarrow$ 2 Oxaloacetate
iv)	Rotary motor	d.	Complex V
v)	2 PEP $\longrightarrow$ 2 Pyruvate	e.	β-subunits of stationary ball
vi)	ATP synthesis	f.	2.5 ATP
vii)	E. Racker	g.	α-unit
viii)	5 ATP	h.	2 ATP
ix)	F_0F_1*ATPase*	i.	$FADH_2$
x)	NADH	j.	Coupling sites

11.8 FERMENTATION

The process of oxidative phosphorylation cannot operate in the absence of oxygen. Since the supply of NAD^+ is limited under anaerobic conditions, glycolysis cannot continue. How can pyruvate be metabolized under such conditions? Plants and microorganisms regenerate NADH from glycolysis under anaerobic conditions by a two-step process called alcoholic fermentation.

Pyruvate is first decarboxylated to acetaldehyde by the enzyme *pyruvate decarboxylase*. This enzyme requires Mg^{2+} and the coenzyme TPP. The second step involves the reduction of acetaldehyde to ethanol by the enzyme *alcohol dehydrogenase*. NADH provides reducing power to the reaction.

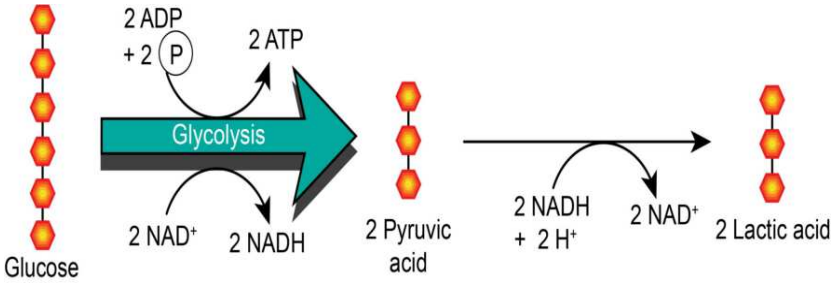


The overall equation of ethanol fermentation is

Glucose + 2 ADP + 2Pi $\longrightarrow$ 2 Ethanol + $2CO_2$ + 2ATP + $2H_2O$

Baker's and brewer's yeast (*Saccharomyces cerevisiae*) and many plants contain the enzyme *pyruvate decarboxylase*. Use of yeast for alcoholic fermentation is perhaps the earliest example of prehistoric biotechnology (about 9000 years ago) where different natural products were used to produce wine (from grapes) or beer (from barley) .This was also an ancient practice by early Egyptians and later the Greeks who practiced the use of CO_2 produced during fermentation for leavening of bread even 2500 years ago.

Another mechanism to regenerate NAD^+ during anaerobic glycolysis is through lactic acid fermentation.This type of fermentation takes place mostly in animal tissues like active skeletal muscles and erythrocytes. Pyruvate is converted to lactate by the enzyme *lactate dehydrogenase*. A molecule of NAD^+ is regenerated from NADH. Since this reaction has a large negative standard free energy change, the equilibrium strongly favors the forward reaction.



In addition to lactate and ethanol production of the fermentation process also yields other products like butyric acid, propionic acid, butanol, acetone, lactic acid (yogurt), which are also exploited at the commercial scale.

11.9 PENTOSE PHOSPHATE PATHWAY (PPP)

In 1935, Warburg *et al* and later in 1938, Dickens observed that direct oxidation of glucose 6-phosphate was possible by a pathway which was independent of glycolysis.

The pentose phosphate pathway (PPP) is one of the alternatives to glycolysis. By a series of reactions Glucose -6-phosphate converts into NADPH and 5-carbon sugars (pentoses). The pathway is also called hexose monophosphate shunt (HMS) or 6- Phosphogluconate pathway. NADPH (the reduced form of $NADP^+$) and the pentose sugars are useful raw materials for many downstream biological processes. In plants most of the reactions of PPP occur with the help of soluble enzymes present in the plastids. Some reactions of this pathway occur in the cytosol. On the contrary, the entire PPP take place in the cytosol alone especially in those tissues which are actively synthesizing

steroid hormones or fatty acids (e.g., adipose tissue, adrenal cortex and mammary glands). Details of HMS were elucidated by Horecker and Racker.

Pentose Phosphate Pathway is completed in two Phases – the **oxidative phase**, which starts with oxidation of glucose -6-phosphate. During this process, a CO_2 mol is lost and two molecules of NADPH are produced along with ribulose-5-phosphate (5C), and the **non-oxidative** or synthetic phase which comprises synthesis of different sugars with 3-7 carbons that are used in a variety of biological processes. (Some of the intermediates are glyceraldehyde-3-phosphate (3C), erythrose-4-phosphate (4C), xylulose-5 phosphate (5C); fructose-6-phosphate (6C) and sedoheptulose- 7-phosphate (7C).

We will now describe various steps of both the phases in details:

Oxidative Phase

Step 1: Glucose-6-phosphate gets oxidized to 6-phosphoglucone-δ-lactone. The reaction is catalyzed by a NADP-depedent enzyme *glucose 6-phosphate dehydrogenese*. A molecule of NADPH is produced in this reaction.

Step 2: 6-phosphoglucono-δ-lactone is hydrolyzed to 6-phosphogluconate by the enzyme *lactonase***.**

Step 3: 6-phosphogluconate (6C) undergoes oxidative decarboxylation to ribulose 6-phosphate (5C). This reaction is catalyzed by the enzyme *6-phosphogluconate dehydrogenase*. During this process, another molecule of NADPH is released.

Step 4: Ribulose 5-phosphate isomerizes to ribose 5-phosphate by the enzyme *phosphopentose isomerase*.

The oxidative phase can be summarized as follows:

Glusose 6-phosphate (6C)+2NADP$^+$+H_2O ⟶ Ribose 5-phosphate (5C)+CO_2+2NADPH+2H$^+$+Pi

Non-Oxidative Phase

The second phase includes a series of reactions involved in the conversion of ribulose 5-phosphate into glycolytic intermediates like glyceraldehyde 3-phosphate and fructose 6-phosphate. Ribose 5-phosphate may be used in the synthesis of nucleotides and amino acids. The products can also get metabolized in glycolysis to yield pyruvate (3C). Alternatively, the glycolytic enzymes can also catalyze reactions which regenerate glucose 6-phosphate from fructose 6-phosphate (6C) and glyceraldelyde 3-phosphate (3C).

Step 5: Ribulose 5-phosphate from Step 3 is converted to its isomer xylulose 5-phosphate. This reaction is catalyzed by the enzyme *phosphopentose epimerase* (*isomerase*).

Step 6: Two carbons and the attached side groups from xylulose 5-phosphate is transferred to ribose 5-phosphate to yield two products viz., a 3-carbon glyceraldehyde 3-phosphate (3C) and a 7C sugar i.e., sedoheptulose 7-phosphate. *Transketolose* enzyme catalyzes this reaction.

Step 7: Glyceraldehyde 3-phosphate and the 7-carbon sugar sedoheptulose 7-phosphate undergo another intermolecular transfer to yield two products viz., fructose 6-phosphate (6c) and erythrose 4-phosphate (4c). The enzyme *transaldolase* catalyzes this reaction.

Step 8: Finally erythrose 4-phosphate combines with another molecule of Xylulose 5 phosphate (from Step 5) to produce another molecule of fructose 6-phosphate (6C) by the enzyme *transketolase*. A molecule of glyceraldehyde 3-phosphate is regenerated during this process.

Reactions of PPP are outlined in Fig.11.21:

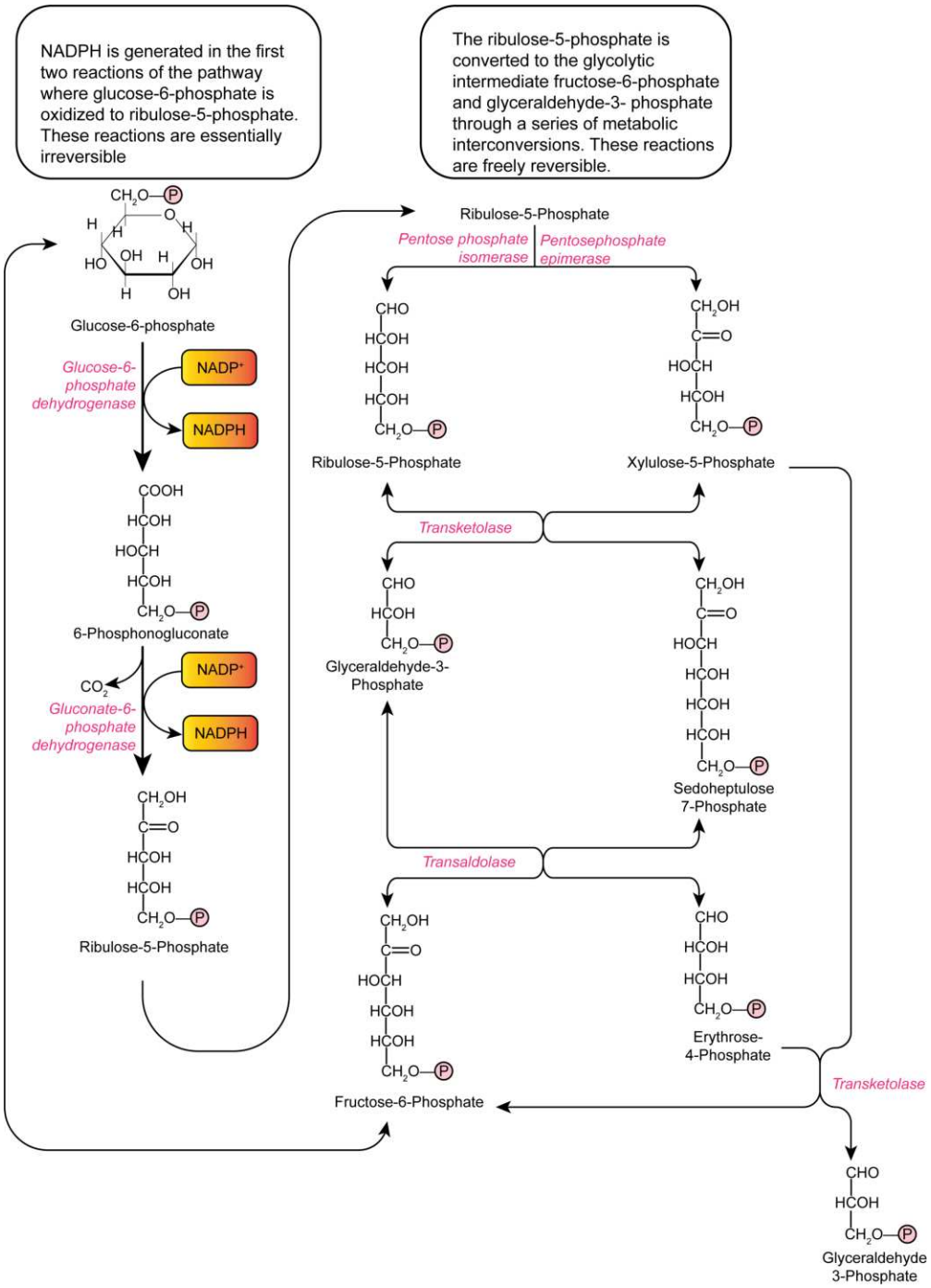


Fig. 11.21: A Schematic view of PPP (From Taiz & Zeiger).

The overall reaction can be depicted as

6 Glucose 6-phosphate ⟶ **5** glucose 6-phosphate+ **6**CO_2 + Pi + + **12** $NADP^+$+**7**H_2O **2** NADPH + **12**H^+

Metabolic Importance of PPP

It has been estimated that nearly 15-25% of glucose breakdown occurs via PPP and the remaining via glycolysis. However, depending upon the change in physiological conditions and developmental stage, the contribution of PPP to overall metabolism is variable.

1. PPP contributes to cellular energy metabolism by producing NADPH which takes part in various biosynthetic reactions. In addition this reduced coenzyme II plays an important role in various reactions in the cytosol which are connected to the removal of reactive oxygen species
2. This pathway occurs whenever there are limitations of oxygen supply, and NAD^+. Availability of a higher volume of NADPH prompts more of PPP which in turn, produces different substrates like ribose 5-phosphate, erythrose 4-phosphate that act as substrates for ribose, deoxyribose sugars for nucleic acids. Erythrose 4-phosphate helps in synthesis of lignins and plant phenolic compounds.
3. Under stress conditions like wounding PPP is the principal metabolic route.

11.10 FATTY ACID BREAKDOWN – GLYOXYLATE CYCLE

Seeds of many plants, e.g., peanut, castor and mustard contain fats and oils as their principal carbon reserve. The reserve triglycerides stored in seeds need to be converted into sugars at the time of germination to provide energy resource to the germinating seedling, till photosynthesis begins. Moreover, fats cannot be transported from the cotyledons to other parts of the germinating seedling. So it is imperative to first convert the stored fats into more mobile sucrose so that structural polysaccharides like cellulose can be synthesized. This conversion is brought about by the **GLYOXYLATE CYCLE**.This cycle is infact, a bypass of the TCA cycle and operates in the microorganisms and higher plants.

First described by Kornberg and Krebs in 1957, glyoxylate cycle is a multistep process and involves different compartments viz., oil bodies or spherosomes, glyoxysomes, mitochondria, and enzyme complexes in the cytosol.

Triggered by germination, the glyoxylate cycle is completed in the following steps.

1. First, the triglycerides stored in reserve fats and oils of spherosomes are hydrolyzed into free fatty acids and glycerol by the enzyme *lipase*.
2. Fatty acids enter the single membrane bounded glyoxysomes and undergo a sequence of reactions comprising β-oxidation to yield a 2C compound acetyl CoA.
3. Acetyl CoA is metabolized in the glyoxysome and cytosol via glyoxylate cycle. During the sequence of these reactions, one succinate (4C) is generated by two molecules of acetyl-CoA (2C).
4. Succinate is transported from the glyoxysomes into the mitochondria where it gets converted first into fumerate and then into malate.

5. Malate is transported into the cytosol where enzymes (isozymes) convert malate into oxaloacete. Thereafter, by the process of gluconeogenesis, the malate/OAA gets converted to glucose and finally to sucrose. It has been estimated that just 30% of the acetyl-CoA is utilized for energy production, while the rest gets converted to sucrose.

   Interestingly, different isozymes forms of the same enzyme *malate dehydrogenase* for the conversion of malate to OAA are located in cytosol, mitochondria and the glyoxysomes. OAA can be reimported into the glyoxysome where it combines with another molecule of acetyl CoA (2C) to keep the cycle going.

   The Fig.11.22 outlines the events of glyoxylate cycle.

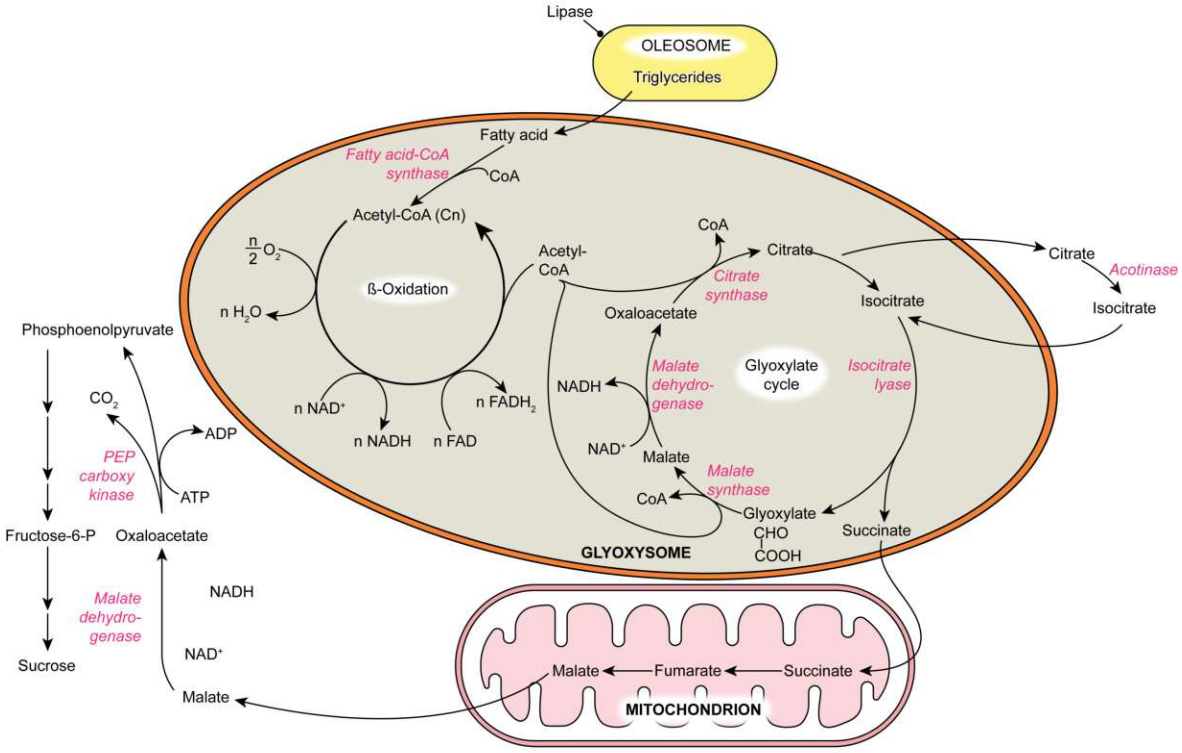


Fig. 11.22: Events of Glyoxylate cycle (From Taiz *et al*).

11.11 FACTORS AFFECTING RESPIRATION

Several environmental factors are known to affect the rate of respiration in plants. These are external as well as internal. External factors include temperature, light, CO_2concentration, O_2 concentration, mineral salts, organic substances, mechanical stimulation, moisture content, injury, wounding, and atmospheric pollutants. Among the internal factors, the notable ones are concentration of the respiratory substrate, age of the plant and protoplasmic factors. The influence of these factors is briefly discussed below.

External factors

a) **Temperature**: Like all other physiological processes, respiration also occurs in a narrow range of temperature. The range however, varies on the type of species and the environmental conditions. The optimum temperature for respiration in most plants is around 35ºC. However, this

may vary in temperate and tropical species. Whereas the plants growing in Arctics may grow normally at around OºC, the tropical plants grow well between 25-35º C. For most of the plants, the freezing temperatures results in zero respiration, whereas some conifers do respire very slowly at -35º C.

You must have observed that potato tubers grown on hills are much larger in size than the ones grown on plains. It is interesting to note that optimum temperature for photosynthesis is 25º C, which is much lower than that for respiration. At lower temperatures in hills, respiration rate slows down while photosynthesis prevails. Thus, there is more accumulation of starches at low temperatures, Generally, photosynthesis rate is about ten times greater than that of respiration. In addition, there is an abrupt spurt in respiration near the freezing point. This is the reason why the temperature in cold storages is kept well above the freezing point (around 10º C).

b) **Light**: Unlike photosynthesis, the process of respiration is not directly affected by light. It is the indirect effect of light which influences respiration by providing increased supply of organic foods, and wider opening of stomata. In addition, there is speedier gaseous exchange. C_3 plants, under high light conditions also show photorespiration, in addition to normal respiration.

c) **Oxygen:** Oxygen is the terminal oxidant in aerobic respiration as it is the ultimate electron and proton acceptor in the ETS. Anaerobic respiration proceeds under low O_2conditions. Interestingly CO_2 is released in both anaerobic as well as aerobic conditions. The minimum oxygen concentration upto which aerobic respiration is possible is called **extinction point**. The value varies between 3-10% of oxygen. Under conditions lower than extinction point, both aerobic as well anaerobic respiration continue simultaneously. This is called **transition period** (Fig. 11.23). During this period, a stage would be reached where anaerobic respiration is absent whereas aerobic respiration is minimum. The consumption of organic food and evolution of CO_2 are minimum. This is called **Pasteur Effect**.

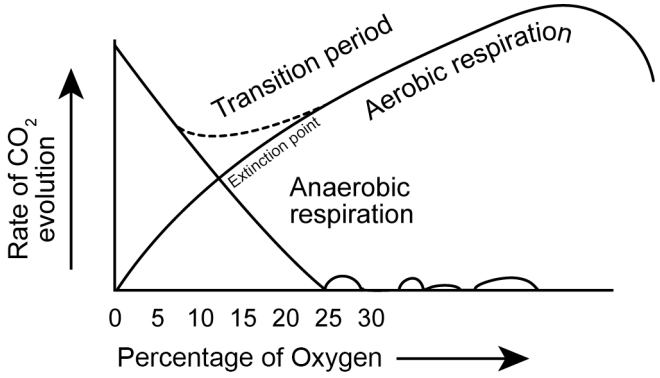


Fig. 11.23: Rate of respiration as affected by oxygen concentration.

Increased O_2 concentration also leads to increased photorespiration in C_3 and CAM plants. Prolonged exposure to high O_2 may also induce free radicals which are harmful. Lack of oxygen induces anaerobic conditions which may result in accumulation of toxic ethanol.

d) **Carbon-dixoide concentration:** Increase in CO_2 concentration around the plant may briefly result in an increase in respiration on account of an increased availability of photosynthate. However, excessive amount of CO_2 reduces respiration rate as stomata close. Thus, many types of climacteric fruits can be stored at higher concentration of CO_2 to keep them fresh (gas storage).

e) **Mineral salts:** You have studied in Unit 3 that various mineral ions are needed for the functioning of enzymes. Many of them are either activators or constituents of enzymes themselves. Deficiency of micro and macro elements results in decrease in the respiratory rate. On the other hand, there is an increase in the rate of respiration during the mineral ion absorption. This is called **salt respiration**, in contrast to normal respiration.

f) **Organic substances:** Many chemicals like cyanides, azides and CO are known to inhibit respiration. These chemicals may block the ETC at different steps or may act as catalytic poisons. Small quantities of anaesthetics like ether and chloroform may initially result in an increase in respiratory rate. Excess of these chemicals is however, toxic (inhibitory). Many bacteria, algae and higher plants show cyanide resistant respiration as they possess an *alternate oxidase* enzyme system.

g) **Injury and Wounding of tissues:** Rate of respiration shoots up in tissues after mechanical injury. Wounding results in disruption of the membranes, whose repair requires an increased ATP? More energy is required for the meristematic activity or 'wound callus' to repair the injured tissue. Interestingly, the temperature also increases during the injury and wound healing process. This is called "**fever reaction**".

h) **Mechanical stimulation: When** plants are subjected to mechanical shock by bending or rubbing, there is a sudden many fold rise in respiratory rate. This is also called **induced respiration**.

i) **Air pollutants:** Low concentration (below 0.2ppm) of atmospheric pollutants increase respiration in many plants. Higher concentration of SO_2, NO_2, and O_3 lowers the pH of cells resulting in membrane damage and a fall in rate of respiration. Heavy metals like Pb and Cd act as enzymatic poisons thereby inhibiting respiration.

j) **Moisture content in leaf**: Rate of respiration is found to be directly proportional to the water content of cell as water hydrates and activates the enzymes needed for the process. Dry seeds show very low respiratory rates. Also under conditions of water stress, the stomata close, hampering the gaseous exchange.

Internal Factors

i) **Concentration of the respiratory substrate**: Within certain limits, there exists a linear relationship between the rate of respiration and the availability of respiratory substrate. External supply of sugars further increases the respiratory rate. An excess concentration of soluble sugars results in an increased osmotic potential which may hinder the rate of gaseous diffusion as cells become fully turgid.

ii) **Age of the plant**: Respiration rate is particularly high in the younger plants and it decreases with age. An early burst in respiration rate is observed at the time of seed germination. The rate slows down a bit during vegetative phase. The flower formation again shows a rise in respiration rate. During fruit ripening there is a burst in respiration in climacteric fruits (e.g., Mango, banana, and apple). This is called '**climacteric rise**'in respiration.Some non-climacteric fruits are lemons, watermelon, grapes, and strawberries. In general, more normal respiration (indirect oxidative pathway) occurs in young meristematic tissue while more mature and senescent tissue resort to direct oxidative pathway in the form of pentose phosphate pathway.

SAQ 6

State whether the statements are true or false and indicate by putting **T**, for True and **F** for False in the given brackets.

i) Glycolysis can continue even when the supply of NAD^+ is limited. [ ]

ii) *Saccharomyces cerevisiae* contains the enzyme *pyruvate decarboxylase* [ ]

iii) Fermentation (alcoholic and lactic acid) can regenerate NAD^+ during anaerobic glycolysis. [ ]

iv) Details of PPP were worked out by Racker and Krebs. [ ]

v) The PPP comprises two phases : 1) non-oxidative phase and 2) oxidative phase. [ ]

vi) PPP begins with glucose 6-phosphate and the first intermediate is 6-phosphogluconate. [ ]

vii) PEP generates NADPH in place of NADP. [ ]

viii) The enzyme *lipase* first breaks the triglycerides in reserve fats during the glyoxylate cycle. [ ]

ix) PPP is the principal metabolic route of young, meristematic aerial parts of a plant. [ ]

11.12 SUMMARY

- Glycolysis is the pathway which helps the oxidation of a hexose sugar molecule into two molecules of pyruvate (3C).
- Glycolysis is the common pathway between aerobic and anaerobic respiration and results in the formation of pyruvate.
- The fate of pyruvate under anaerobic conditions leads to the formation of either ethanol or lactic acid with a net gain of 2 ATP.
- During the EMP pathway ten enzymes participate, and all of them operate in the cytosol. Two phases of glycolysis viz., **preparatory phase**

(expenditure of ATP for conversion of glucose to fructose 1,6-bisphosphate) and the **pay off phase** (oxidation of glyceraldehyde 3-phosphate to yield pyruvate) are associated with two mobile cofactor pairs viz. ATP/ADP and NAD^+/NADH. Glycolysis is a highly regulated process with the *phosphofructokinase*-catalyzed reaction being the most important regulatory step. The regulation ensures adequate supply of metabolic intermediates. Plant glycolysis exhibits several enzymes at different steps which utilize alternate substrates and yield different products.

- The oxidative pentose phosphate pathway oxidizes carbohydrates directly to provide the reducing power as NADPH.
- Pyruvate enters the mitochondrial matrix and gets oxidized by the Krebs cycle. In the process reducing equivalents in the form of NADH and $FADH_2$ are generated. ATP/GTP is generated only at one step (substrate level phosphorylation). NADH and $FADH_2$ pass through the electron transport chain at the inner mitochondrial membrane. This process is completed by various enzyme complexes to proton transport thereby generating an electrochemical proton gradient resulting in chemiosmotic synthesis of ATP at the F_1 particles.
- Recent findings on ATP energetics show that each NADH passing through the ETC generates 2.5 ATP while each $FADH_2$ generates 1.5 ATP. By this account, 30-32 molecules of ATP are produced per molecule of glucose (depending upon the nature of the shuttle).

11.13 TERMINAL QUESTIONS

1. Write short notes on:
   i) Fermentation
   ii) Glycolysis
   iii) Amphibolic pathway
   iv) Substrate level phosphorylation
   v) F_0 F_1*ATP synthase*
2. With the help of suitable flow diagram, describe the process of glycolysis.
3. How is the plant glycolysis different from the non-plant one? Explain.
4. How is the process of glycolysis regulated?
5. Describe the aerobic oxidation of pyruvate, with the help of a flow chart.
6. Give an account of Krebs cycle indicating the metabolic intermediates and sites for production of NADH, $FADH_2$ and ATP.
7. Give an account of the electron transport chain indicating the sequential arrangement of complexes.

8. In the light of new ideas on ATP energetics, make a summary chart of the number of ATP molecules generated at each step of the aerobic oxidation of a) glucose and b) sucrose, highlighting the net gain of ATP.

9. Give an account of the internal and external factors affecting respiration.

10. Give a schematic account of glyoxylate cycle.

11.14 ANSWERS

Self-Assessment Questions

1. a) i) cell-free extracts
   ii) common step
   iii) phosphorylated
   iv) Sucrose, glucose
   v) ten, two

   b) i) False; ii) True; iii) False; iv) False; v) True; vi) True

2. i) False; ii) True; iii) True; iv) False; v) True
   vi) True; vii) False; viii) False; ix) True

3. i) glycolysis, mitochondrial matrix
   ii) glycerol 3-phosphate shuttle
   iii) oxaloacetate
   iv) glycerol 3-phosphate,malate-aspartate shuttle
   v) *Oxidase*, reduced

4. i) three
   ii) electrons
   iii) volts, 7
   iv) increase
   v) multiprotein
   vi) three
   vii) III, *NADH dehydrogenase*
   viii) four, one, two

5. i) Complex V
   ii) Coupling sites
   iii) $FADH_2$

iv) α-unit

v) 2 ATP

vi) β-subunits of stationary ball

vii) F_1 particles

viii) 2 Malate $\longrightarrow$ 2 Oxaloacetate

ix) ATP *synthase*

x) 2.5 ATP

6. i) False; ii) True; iii) True; iv) False; v) True; vi) True; vii) True; viii) True; ix) False

Terminal Questions

1. i) Refer to Section 11.8

   ii) Refer to Section 11.2

   iii) Refer to Section 11.4.

   iv) Refer to Section 11.2.

   v) Refer to Section 11.7.

2. Refer to Section 11.2.
3. Refer to Section 11.2.
4. Refer to Section 11.2.
5. Refer to Section 11.3.
6. Refer to Section t 11.4
7. Refer to Section 11.6.
8. Refer to Section 11.7.
9. Refer to Section 11.11.
10. Refer to Section 11.10

Annexure 1 : Krebs cycle in plants - Some Variations

In plants some variations are observed in the reactions outlined in section 11.4.

1. Step no. 5 involving the enzyme *succinyl-CoA synthetase* produces ATP in plants instead of GTP, in animals.
2. *Malic enzyme* is present in the mitochondrial matrix of plants which provides alternative pathways for the metabolism of PEP, which is derived from glycolysis.

Malic enzyme catalyzes the oxidative decarboxylation of malate

$$\underset{(4C)}{\text{Malate}} + NAD^+ \longrightarrow \underset{(3C)}{\text{Pyruvate}} + CO_2 + NADH$$

Malate can be synthesized from phospho-enol pyruvate in the cytosol via the enzymes *PEP carboxylase* and *malate dehydrogenase* (Fig.1).

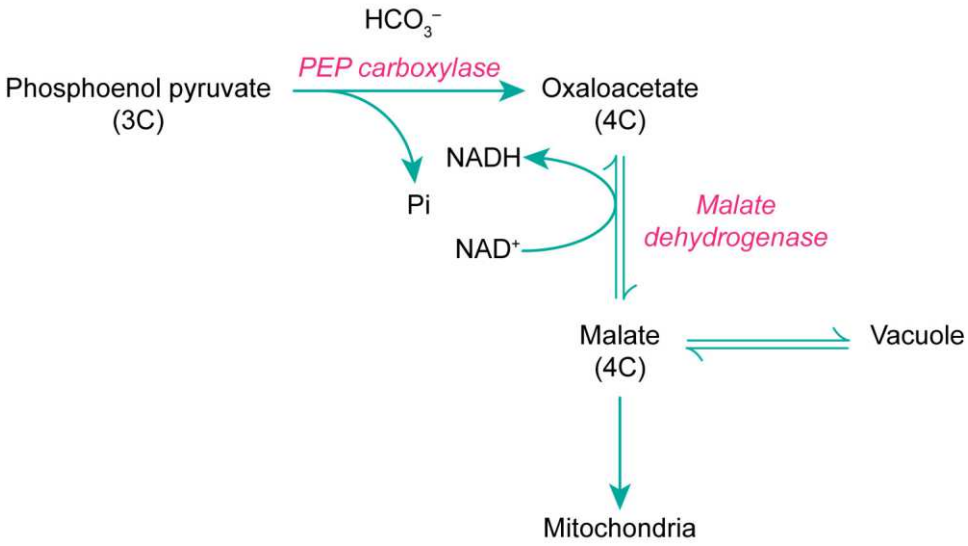
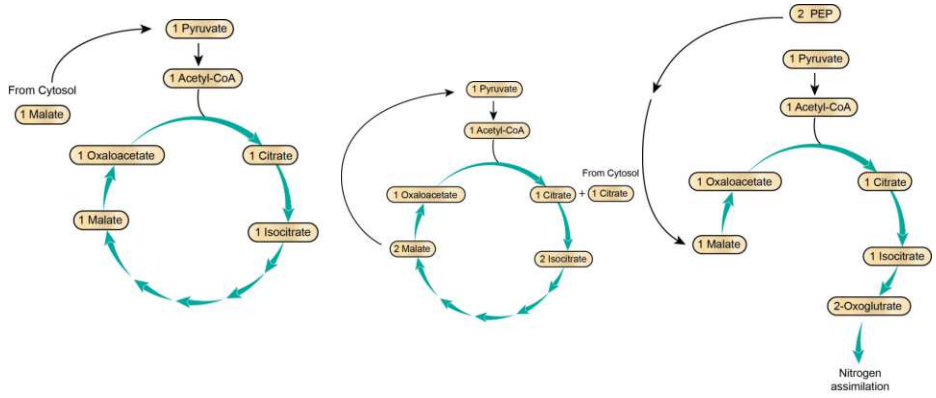


Fig. 1: Synthesis of malate from PEP.

The two key enzymes viz., *malic enzyme* and *PEP carboxylase* give plants the necessary "flexibility" for metabolizing PEP and pyruvate. Three options are possible :

a) Malate can be converted into pyruvate by the *malic enzyme*. The plant mitochondria could easily oxidize malate (Fig. 2a).



a) b) c)

Figs. 2: a) Oxidation of malate by plant mitochondria; b)Oxidation of citrate; c) Conversion of PEP to 2-Oxoglutatate for nitrogen assimilation (From Taiz et al).

b) Plant mitochondria can also oxidize citrate to CO_2 without involving the pyruvate from glycolysis (Fig. 2b).

c) Plants can also add *PEP carboxylase* to the pathway because of which the PEP from glycolysis can be converted to 2-oxogluterate (α-ketoglutarate), which can be used/or nitrogen assimilation.

As you have learnt in the unit of photosynthesis (Unit 7), plant tissue especially with CAM cycle), do store large concentrations of malate and other organic acids in their vacuoles. The levels of these acids are maintained by the mitochondrial *malic enzyme*.

Interestingly in the option depicted in Fig. 2c, malic acid can be replenished by the activity of *PEP carboxylase.* Export of α-ketoglutarate towards nitrogen assimilation in the chloroplast will result in a shortage of malate. This can be compensated by the reaction. Such reactions that involve replenishing of intermediates in any metabolic cycle, e.g., Krebs cycle, are called ANAPLEROTIC. Such typesof reactions help in maintaining the concentrations of citric acid cycle intermediates by replenishing the intermediates that have got consumed into other pathways.

Thus, in addition to energy generation, the TCA cycle also constitutes an important starting point for various biosynthetic pathways

- Citrate is used as a source of Acetyl CoA needed for fatty acid synthesis
- α-ketoglutarate is the substrate for glutamate, other amino acids, and purines
- Succinyl CoA is the starting point for the synthesis of porphyrins, haem and chlorophyll.
- Oxaloacetate is the precursor to produce aspartate, other and other amino acids.

Annexure 2 : *ATP Synthase* and Mechanism of ATP Synthesis

Electron microscopic studies using special staining techniques have shown the mitochondrial cristae to be covered with knoblike (racquet shaped- ball and stick) structures. Each of these knobs is attached to the inner membrane by a short stalk and projects into the matrix

The protons re enter the mitochondrial matrix by passing through these knob-like structures representing a large complex multi subunit protein (400kDa) called the *F_0F_1ATP synthase* or *coupling factor* (*CF_0-CF_1*). This enzyme is also called *ATPase* and is responsible for the formation of ATP from ADP and Pi in response to proton flow.

Each of the two components of ATPase viz., F_0 and F_1 comprise multiple protein subunits. The 0 Part of F indicates that this portion makes the component sensitive to oligomycin (Oxidative phosphorylation inhibitor) The F_0 component comprises an oligomer of 8-14 C subunits, that form a barrel spanning the inner mitochondrial membrane. This barrel has the ability to rotate within the membrane. A single copy of another subunit *a* is present alongside this barrel. The subunit *a*forms the channel through which the protons pass from the outer space into the matrix. The central stalk of the c ring the F_0 complex makes the “rotor” of *ATP synthase*.(Fig.1). In addition, the other components of F_0 complex, viz., a, b, d, F_6 and OSCP constitute the “stator”, (or peripheral stalk) which prevents the rotation of the three α β

dimers of F_1. The F_1 component of *ATP synthase* is in the form of ball-and-stick structure. The stick of F_1 fits into the F_0 barrel. The ball protrudes into the matrix. The F_1 complex consists of five proteins, α, β, ɣ, δ and Σ. A total of nine subunits are present which can be represented as α_3, β_3, ɣδe. The ball of F_1 is made up of six subunits (three each of α and β). The ball is attached to one α unit, which forms the stick that holds the F_1 part into the F_0 barrel. The ɣ unit rotates along with the rotating F_0 rotor. However, the α and β ball cannot rotate as they are held tight by a structure made up of *b* (two copies) and δ (one copy).

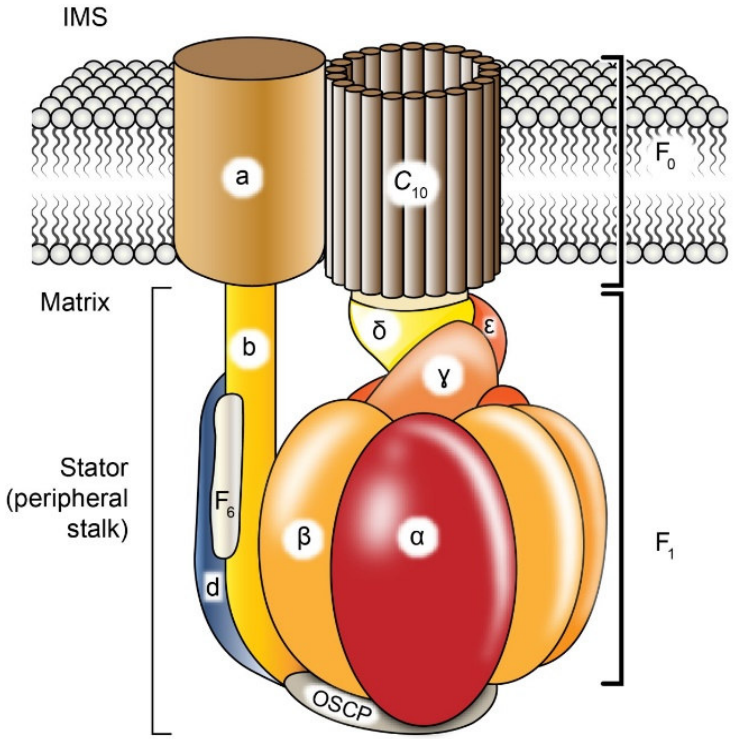


Fig. 1: *ATP synthase* (F_0F1 complex) (From Appling).

The Austrian biochemist Efraim Racker was the first to isolate F_1 particles by sonication of mitochondria.

Since these spheres, when detached from the internal membrane, could catalyze the hydrolysis of ATP, they were designated as *ATPase*. Actually the spheres represent just the F_1 component of the larger multiprotein complex. They could synthesize ATP only when attached to the F_0 component, hence the name *ATP synthase*.

Studies of x-ray crystallography of *F_0-F_1-ATP synthase* complex were carried out by John E. Walker and helped us to understand the spatial arrangement of various components of Complex V and the mechanism of passage of protons through the F_0-F_1 complex.

ATP Synthesis

The essential feature of *ATP synthase* is that its stick rotates, but not the ball. This feature of the *ATP synthase complex* supports the idea given many years earlier by Paul Boyer which suggested a Binding Change Mechanism for ATP synthesis. According to this idea of rotational catalysis, the *ATP synthasecomplex* functions as a molecular motor or a three cylinder engine. The basic premise of the model (Fig. 2) is summarized below:

1. Passage of protons through the channels (subunit a) in F_0 subunit causes the barrel of C subunits, and the stalk of ɣ attached to the barrel to rotate by (120°). The α, β dimer assemblies of the ball remain stationary as they are held in place by the stator of F_0 (a, b, d, F_6 and OSCP).

2. The rotation of the ɣ subunit interacts sequentially with the α-β assemblies so that the latter adopt a cycle of three different conformational changes (in the β-subunits) resulting in a repeated series of a) ADP binding, and b) phosphorylation and release of ATP. One ATP can be generated by each of the three β subunits in the stationary ball structure subunit. Thus three ATP molecules can be generated per turn of the ɣ subunit. The ɣ -unit is thought to rotate about 700 revolutions per second (and nearly 100 revolutions per second *in vivo*).

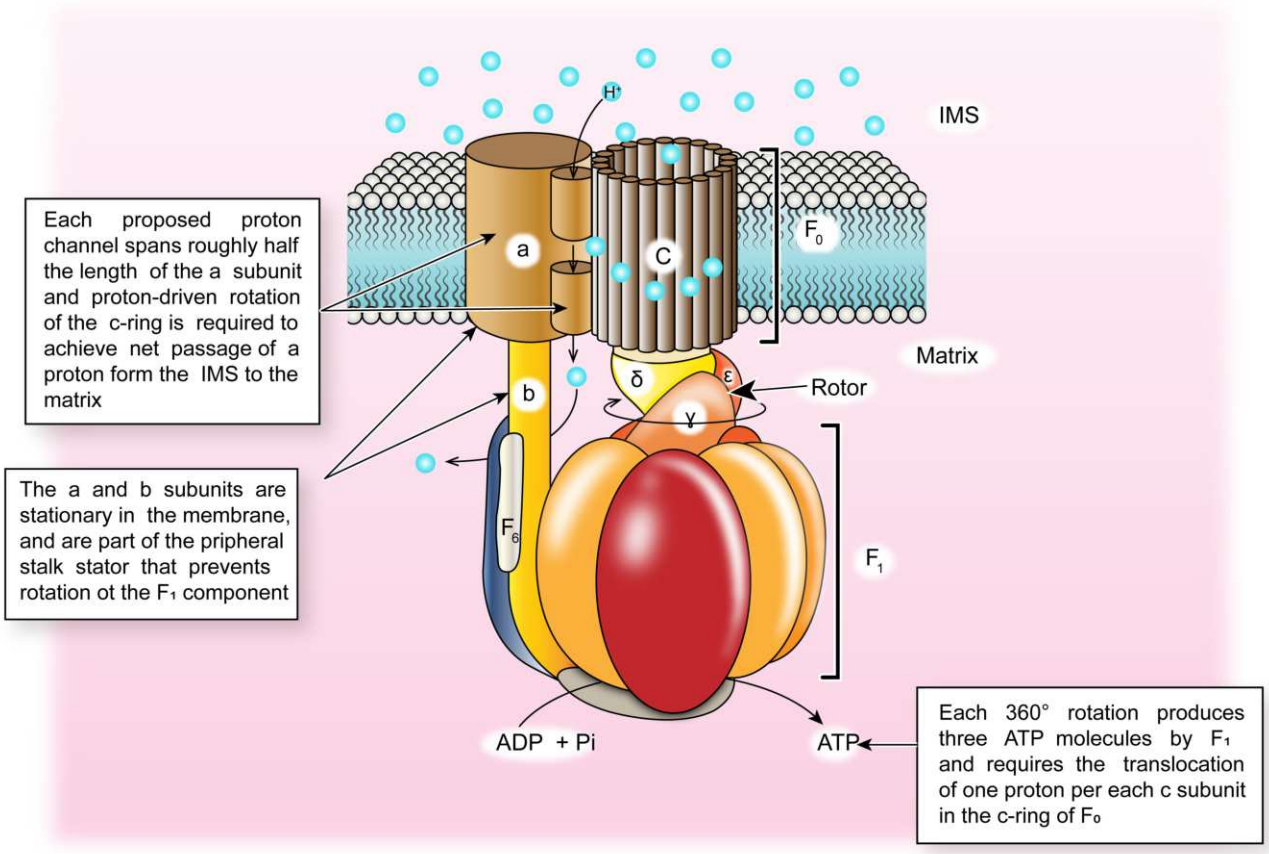


Fig. 2: F_0 component of F_0F_1 *ATP synthase* showing proton-driven rotation (From Appling.)

Thus, the ATP-generating molecular motor the rotary engine is driven by the passage of protons through channels in F_0, thereby releasing energy in a readily usable form. Walker and Boyer shared the 1977 Nobel Prize in Chemistry for their contributions to the structure of F_0, F_1 complex and mechanism of ATP synthesis.