

# Enzymes, Photosynthesis, Metabolism and Bioenergetics

## INTRODUCTION

Biochemical reactions in living beings are critical for survival and reproduction of the species. Enzymes are biological catalysts that drive these reactions in an energy efficient and rapid manner. At the end of the reaction, enzymes emerge unmodified, thereby allowing them to be recycled for the next round of reaction. Enzymes have wide applications not only in living organisms but also in industry.

Photosynthesis is a process in which plants and some bacteria use carbon dioxide and water in the presence of sunlight to produce and store sugars. In these reactions, oxygen is also a product. The sugars are oxidized, when required to form ATP (Adenosine-5'-Triphosphate). The energy rich ATP is used to drive forward energetically unfavorable biochemical reactions. The oxygen produced in the photosynthesis reaction is used by all aerobic organisms. Hence, sunlight mediated photosynthesis produces oxygen and sugars, two products crucial for all living beings on earth.

Energy is fundamental to life and is of critical importance to species survival and propagation. Bioenergetics is the energy required to build or break chemical bonds of the molecules in living organisms in order for the organism to store and utilize energy for growth, development, and metabolism. The chemical reactions either produce energy or consume energy to constitute catabolic or anabolic reactions. These chemical reactions occur in multiple biochemical processes such as glycolysis, tricarboxylic acid cycle (also known as citric acid cycle and Kreb's cycle), and electron transport chain with the crucial contribution from enzymes that make the reactions proceed with less activation energy.

Protein synthesis is a critical process in the body in which a cell manufactures proteins. These proteins are essential for a variety of functions in the body ranging from molecular level such as cell signaling

to the macro level such as muscle development. At the cellular level, protein synthesis is a multi-step process through which appropriate protein molecules are produced from amino acids and they are stored or exported out of the cell to meet the body's requirements. Various cellular components including the nucleus, endoplasmic reticulum, and Golgi bodies are involved in the process of protein synthesis. Deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and ribosomes are the central players in this process.

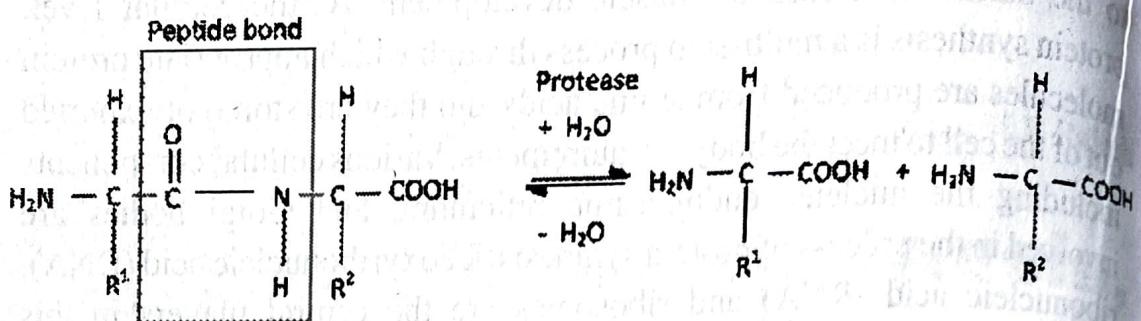
---

### **3.1 ENZYMES AS BIOLOGICAL CATALYSTS**

Consider a biochemical reaction under physiological temperature, pH, and aqueous conditions, which left to itself is likely to take a relatively long duration, say hours or even days to reach equilibrium. Such a reaction might not be useful to a microorganism that needs to conduct metabolism fast for survival and reproduction. Also, considering the diversity of biochemicals and pathways involved in organisms, many wasteful side reactions are possible making cellular metabolism an energy inefficient process. Nature has evolved biological catalysts termed as "enzymes" to regulate the biochemical reactions of cells and organisms. As the definition of catalyst suggests, enzymes facilitate biochemical reactions, but are not consumed in the reaction and emerge unmodified, thereby allowing them to be recycled for the next round of reaction. In these biochemical reactions, the reactants on which these enzymes act are termed as "substrates". The enzyme facilitation involves regulating (increasing) the rate or velocity of the biochemical reactions as well as channelizing the substrates through useful pathways and preventing unwanted side reactions. This helps the cells and organisms to prioritize their needs for survival and reproduction as well as conserve energy and precious biochemicals.

Most enzymes are proteins, with the exception of non-proteinaceous enzymes such as ribozymes. Proteins comprising of amino acid residues linked through peptide bonds have complex tertiary and quaternary structures and this provides them with the structural features necessary to act as a catalyst. They work in aqueous solutions under mild pH and temperature conditions and are capable of increasing the reaction rates when compared to uncatalyzed reactions. They work in tandem with each other to catalyze and regulate key biochemical processes.

A classic example of enzymes are proteases, which catalyze the reverse of peptide synthesis. An example is shown below which depicts the reversible hydrolysis of peptide bond between two amino acid residues of a dipeptide



**Fig. 3.1 Protease-catalyzed hydrolysis of dipeptides to constituent amino acids**

(Fig. 3.1). In this case, the dipeptide is the substrate whereas; the resulting free amino acids are the products. The equilibrium of the reaction exists far to the right, with the products dominating the reaction mixture.

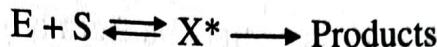
**Naming and classification of enzymes:** Most enzymes are named after their substrate they act on and have their names ending with “ase”. For e.g. urease catalyzes the hydrolysis of urea. Some enzymes have their names based on either their broad functionality or the source of the enzyme. For e.g. proteases, papain and bromelain are named after their source, papaya (*Carica papaya*) and pineapple (*bromeliaceae* family) respectively.

To prevent any ambiguities, the Enzyme Commission of the IUBMB (International Union of Biochemistry and Molecular Biology) has adopted a classification system. In this system, each enzyme is provided with a systematic name and divided into 6 classes: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. They are further sub-classified and provided with a unique 4 number Enzyme Commission number (E.C. number) that indicates the class, sub-class etc. that go onto uniquely identify each enzyme in the database. For e.g., the E.C. number for papain is “3.4.22.2” with first number, “3” denoting “hydrolases” class and the second number, “4” sub-classifying it as a hydrolases that specifically acts on peptide bonds (peptidases). The third number, “22” denotes a peptidases that act with a cysteine endopeptidase mechanism and the last number, “2” denotes the specific cysteine endopeptidase, papain. Enzymes are located in cell cytoplasm, mitochondria, tissues and body fluids. Enzymes can be also classified in many ways: intra and extracellular, soluble and insoluble etc.

### **3.2 SIGNIFICANCE OF ENZYMES**

**(i) Catalytic power (reaction rate):** An important question in chemical reactions is: What is the driving force for a reaction to proceed from reactants to products? From a chemical thermodynamics point of view, as long as the free energy of products is lower than that of the reactants, i.e., the change in

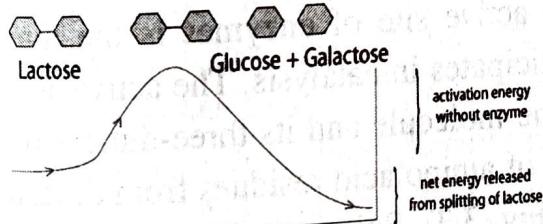
free energy,  $\Delta G$  ( $= G_{\text{products}} - G_{\text{reactants}}$ ) is negative, the reaction is feasible.  $\Delta G$  is a thermodynamic concept that defines the usable energy available from a chemical process and has the units of joules/mole. However, thermodynamics does not take into account the transition states the reactants have to pass through before they form the products.



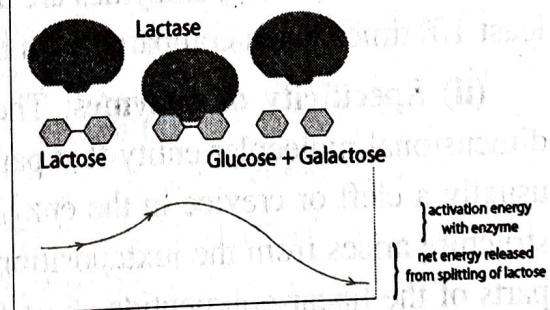
The above equation shows that substrate, S in the presence of enzyme, E has to go thorough an activated high energy unstable transition state,  $X^*$  before it forms the products.

A better example is the splitting of disaccharide lactose (substrate) into its constituent monosaccharides, glucose and galactose (products) by the enzyme lactase (Fig. 3.2a). The reaction coordinate is a measure of the progress of the reaction from the reactants to the product through various transition states. Figure 3.2a shows that the conversion of reactant to products proceeds with the formation of at least one high energy transition state which imposes an "energy barrier" for the reactants. To surmount this barrier, the reactants have to acquire a critical energy, called "activation energy" and form an energy rich transition state before forming products. Activation energy (joules per mole) can be defined as the minimum energy required for initiating a chemical reaction.

Without Enzyme



With Enzyme



**Fig. 3.2** A schematic representation of the free energy vs. reaction coordinate plot for hydrolysis of lactose to glucose and galactose in the absence (a) and presence (b) of the enzyme, lactase. The plots show that the enzymatic-catalysis lowers the activation energy and facilitates the formation of the transition complex needed to drive the reaction to product formation.

At any given instant, only a fraction of the reactant molecules in a chemical reaction are likely to have sufficient activation energy to surmount the barrier. Hence, the reaction rate would be relatively slow. In the case of

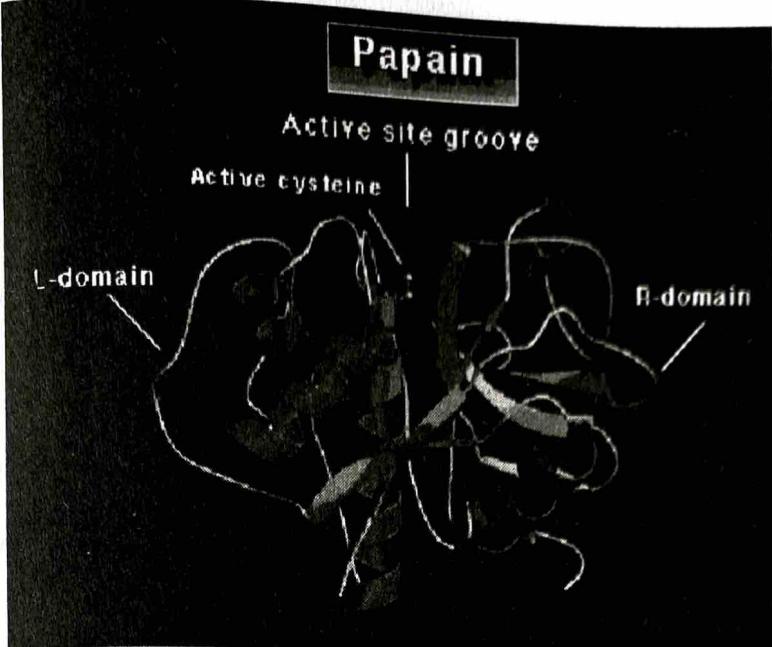
more than one reactants, the collisions between the reactants need to be sufficiently energetic enough and the reactants should also possess the right orientation (for the reacting parts of the molecules) to form the transition state.

In the case of uncatalyzed biochemical reactions at physiological conditions, most of the reactions might be thermodynamically favorable, but are too slow as only a limited number of substrate molecules might possess sufficient activation energy. As shown in figure 3.2b, enzymes by virtue of their property to bind selectively with substrates are able to lower the activation energy requirement. This lowering of the activation energy barrier increases the fraction of reactant molecules that can attain the transition state with concomitant increase of the reaction rate.

Enzymes are able to reduce the activation energy by two methods. Firstly, by interacting (or reacting) with the reactant molecules and forcing them to take a particular strained or distorted state or a particular orientation that resembles the transition state, it can facilitate the transition. Secondly, in the case of single reactant molecule reactions, the enzyme can bring together different parts of the same reactant molecule or in the case of more than one reactant, it can bring the different reactant molecules together in such a way so as to orient them in the proper direction and this will allow the transition state to be reached easily.

Hence, enzyme is a biocatalyst that produces the optimum structural conformation and orientation for reactants to reduce the activation energy and favor the reaction. Enzymes are able to accelerate the reaction rate by at least  $10^6$  times when compared with the uncatalyzed reaction.

**(ii) Specificity of enzymes:** The active site of enzymes is the three-dimensional molecular entity that participates in catalysis. The active site is usually a cleft or crevice in the enzyme molecule and its three-dimensional structure arises from the juxtaposition of amino acid residues from different parts of the linear polypeptide chain (Fig. 3.3). Substrates bind to the group of residues at the active site with combination of covalent, ionic, hydrogen bond, hydrophobic and Van der Waals forces. The active site has a combination of polar and hydrophobic amino acid residues, which play a major role in the catalysis. The polar residues are necessary to bind the substrate with ionic interactions, donate and accept protons and the hydrophobic residues enhance the binding of the substrate and anchor them with the right orientation in the site. The precisely arranged three-dimensional active site structure endows the enzyme with the ability to accommodate specific substrates and form an enzyme-substrate complex. This specificity is extended to their stereo- and

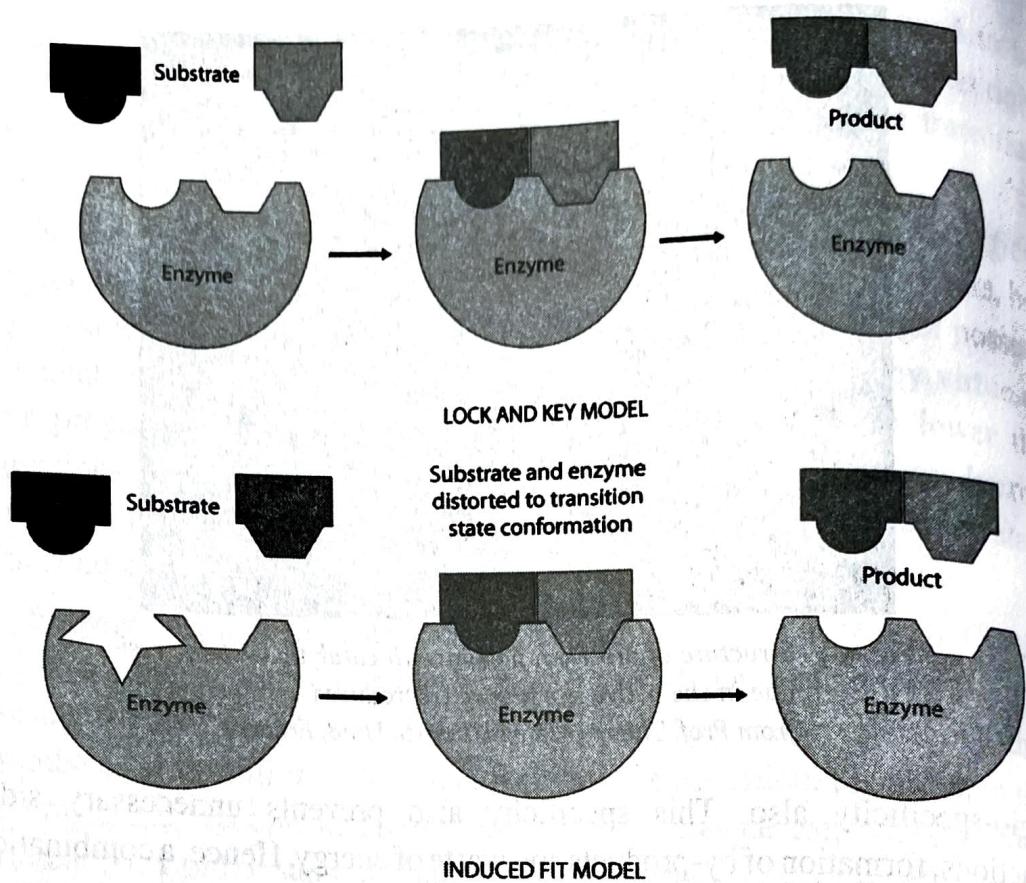


**Fig. 3.3** Structure of protease, papain with catalytic cysteine residue in the active site groove (reproduced with permission from Prof. Thierry Moreau, Inserm U618, France)

regio-specificity also. This specificity also prevents unnecessary side-reactions, formation of by-products and waste of energy. Hence, a combination of steric complementarity, covalent and non-covalent interactions serve to provide a concerted effect that optimizes the orientation of specific substrates and lowers the activation energy to produce high reaction rates.

The above discussion suggests that a key requirement for enzyme catalysis is ability of the substrate molecule to complement the shape of the cleft on the active site. This led to the postulation of the "lock-and-key" hypothesis in 1894 (figure 3.4A). According to this hypothesis, enzyme specificity arises most probably by the ability of the enzyme to accommodate the substrate in the same way that a lock accommodates the key specific for it. This hypothesis explained the specificity part, but did not explain much about how this aids the formation of the intermediate transition state complex. It was until 1958 that the "induced-fit model" was used to explain this phenomenon (Fig. 3.4B). This model postulated that the merely fitting the rigid key in the lock by itself was not sufficient and that the substrate needs to induce a structural reconfiguration of itself as well as the enzyme so as to form an enzyme-substrate complex. This enzyme-substrate complex would distort or orient the substrate to closely approximate the structure of the transition state complex.

The specificity of enzymes varies. Some enzymes have absolute specificity and can catalyze only one substrate such as protease Lys-C which cleaves peptides at the carboxy terminal of lysine residues. Some enzymes



**Fig. 3.4** (A) In the lock-and-key model, the substrate fits precisely into the active site of the enzyme. (B) In the induced-fit model, substrate binding distorts the conformations of both substrate and enzyme. This distortion brings the substrate closer to the conformation of the transition state, thereby accelerating the reaction.

can catalyze substrates with similar functional groups, side chains etc., such as protease trypsin which cleaves peptides at the carboxy terminal of basic amino acid residues. Some enzymes have very poor specificity such as protease chymotrypsin which cleaves at carboxy terminus of hydrophobic amino acid residues.

Protein chemists have devoted much research work to gaining an understanding of the nature of the active site in enzymes. The catalytic residues as well as the amino acid residues adjacent to it in the active site are important to understand their role in the action of enzymes. For example, in papain, cysteine has been determined to be the catalytic residue responsible for its ability to hydrolyze peptide bonds. In papain active site, an adjacent histidine residue with its imidazole side chain capable of accepting and donating protons is reported to a key player in its proteolytic action.

**(iii) Thermodynamics of enzymatic reactions:** Catalysts such as enzymes lower the activation barrier thereby enabling more molecules to attain the transition state. Once the transition state is attained, the activated

reactants can either move back to form reactants again or move forward to form the products. The products can also utilize the catalytic power of the enzyme to surmount the activation barrier and form reactants. Thus, enzyme-catalyzed reaction rate enhancement is valid for both directions in a reversible reaction.

This brings us to the important concept of difference between reaction rate and reaction equilibrium. Equilibrium is the steady state at which the concentrations of the reactants and product species in a reaction mixture remain unchanged with time. This occurs when the rate of the forward and backward reactions are equal. An uncatalyzed reaction left to itself, when provided with enough time will eventually attain equilibrium. Reaction rate is the rate of (dis)appearance of a species in a reaction mixture.

In a chemical reaction, if the free energy of the products is higher than that of the reactants, the net change in free energy,  $\Delta G$  is positive making it thermodynamically unfavorable (Fig. 3.5). Enzymes cannot make such reactions favorable. On the contrary, if the  $\Delta G$  is negative for a reaction, enzymes cannot convert this thermodynamically favourable reaction to an unfavorable one. Enzymes cannot alter the thermodynamically dictated feasibility and the final equilibrium established in biochemical reactions. Enzymes can lower the activation energy and enhance the reaction kinetics, i.e. reaction rate at which the equilibrium is attained.

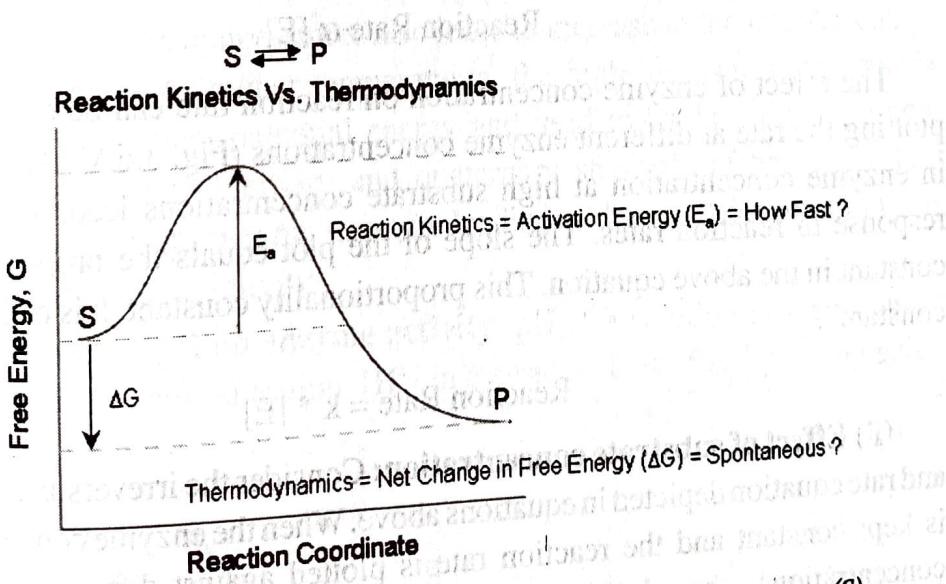


Fig. 3.5 The free energy vs. reaction coordinate diagram for substrate (S) leading to product (P). Thermodynamics determine the feasibility of a reaction. Reactions are feasible only when  $\Delta G$  (Gproducts - Greactants) is negative. In the absence of an activation barrier, this reaction will be spontaneous. Catalysts (enzymes) lower the activation barrier and determine the reaction rate.

### 3.3 FACTORS AFFECTING ENZYME ACTIVITY

Factors that affect the catalytic activity of enzymes are: enzyme and substrate concentrations, temperature, pH, inhibitors and activators.

#### (i) Effect of enzyme concentration

Consider an irreversible enzyme (E) catalyzed transformation of substrate, S to products.



The reaction rate is directly proportional to the enzyme and substrate concentrations.

$$\text{Reaction Rate} \propto [E] [S]$$

Where the square brackets refer to the concentrations of the species.

When substrate concentration is in far in excess of the enzyme concentration, only a small fraction of the substrate molecules would go onto saturate the enzyme active site, while the rest of the substrate molecules would still be in solution. Under these conditions, the substrate concentration can be considered to be constant.

The reaction rate would then be independent of the substrate concentration in the equation above and it would dependent only on the enzyme concentration.

$$\text{Reaction Rate} \propto [E]$$

The effect of enzyme concentration on reaction rate can be obtained by plotting the rate at different enzyme concentrations (Fig. 3.6A). Any change in enzyme concentration at high substrate concentrations leads to a linear response in reaction rates. The slope of the plot equals the proportionality constant in the above equation. This proportionality constant, k is called "rate constant".

$$\text{Reaction Rate} = k * [E]$$

**(ii) Effect of substrate concentration:** Consider the irreversible reaction and rate equation depicted in equations above. When the enzyme concentration is kept constant and the reaction rate is plotted against different substrate concentrations, the plot in figure 3.6B is obtained. At low substrate concentrations, the enzyme concentration is relatively high and all enzyme active sites are not saturated with substrate molecules. Any increase in substrate concentration, leads to more number of active sites being occupied by the substrate with concomitant increase in reaction rate. However, beyond

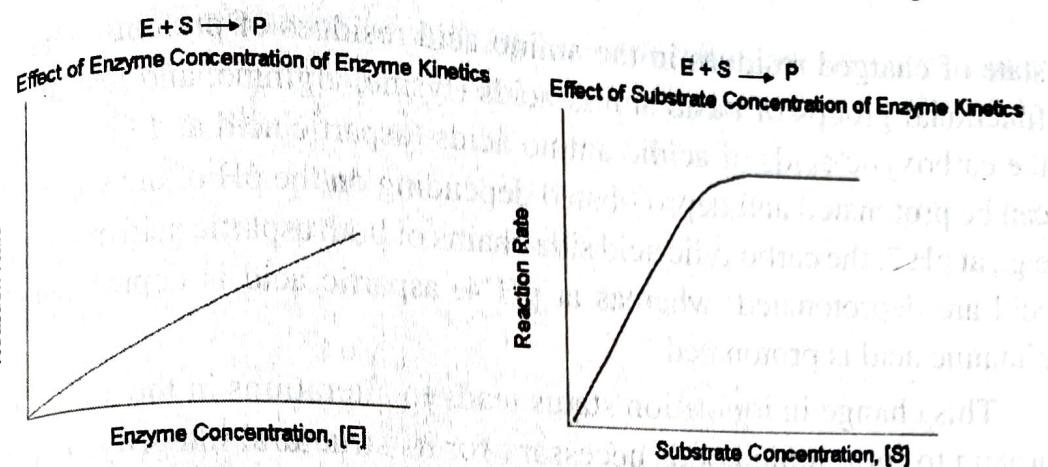


Fig. 3.6 Effect of Enzyme (A) and Substrate (B) concentrations of reaction rate

a certain point, increase in substrate concentration leads to saturation of the all the enzyme active sites with no further increase in reaction rate.

(iii) **Effect of temperature on enzyme activity:** When the temperature increases in a system, it leads to the increase in the kinetic energy and internal energy of the molecules (Fig. 3.7). In an enzyme-catalyzed system, this leads to more energetic collisions as well as increased frequency of collisions between substrates and enzyme molecules. The energy from such collisions can in turn be converted to chemical potential energy and used to offset the activation energy needed to attain the transition states. Hence a moderate increase in temperature leads to increase in the reaction rates as shown in figure 3.7A until a maximum is reached.

This increase in temperatures also leads to increase in the internal energy of the molecules. At higher temperatures, the high internal energy can be converted to chemical potential energy and used to break the fragile non-covalent bonds in the tertiary and quaternary structure of enzymes. This would destabilize the enzyme structure leading to thermal “denaturation” i.e. loss of the enzymatic activity.

(iv) **Effect of pH on enzyme activity:** pH of a solution is a measure of the hydrogen ion concentration,  $[H^+]$  in a system. This affects the ionization

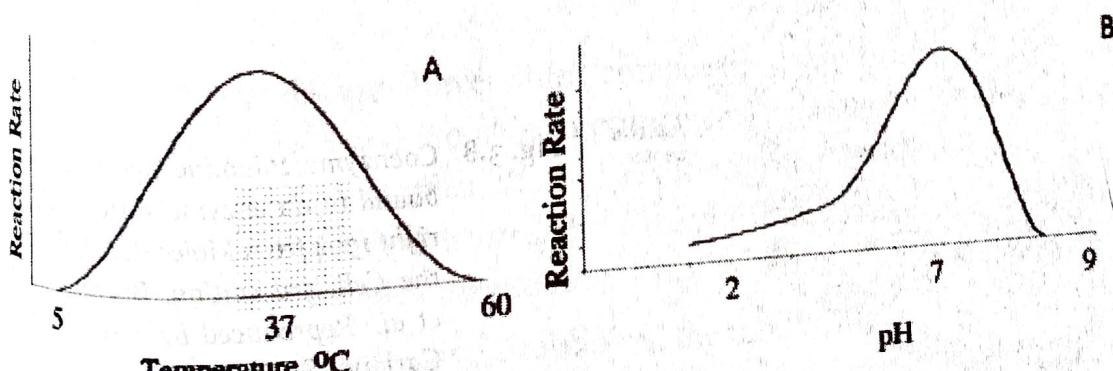
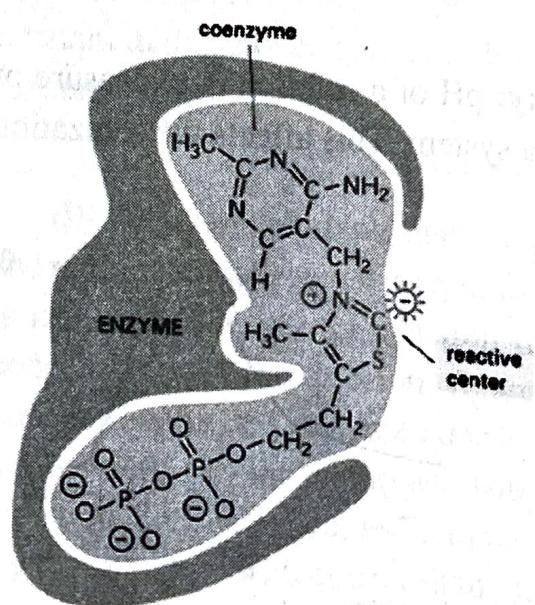


Fig. 3.7 Effect of Temperature (A) and pH (B) on reaction rate

state of charged residues in the amino acid residues of proteins. The amine functional groups of basic amino acids (lysine, arginine, and histidine) and the carboxylic acids of acidic amino acids (aspartic acid and glutamic acid) can be protonated and deprotonated depending on the pH of the solution. For e.g., at pH 7, the carboxylic acid side chains of both aspartic acid and glutamic acid are deprotonated, whereas at pH 4, aspartic acid is deprotonated and glutamic acid is protonated.

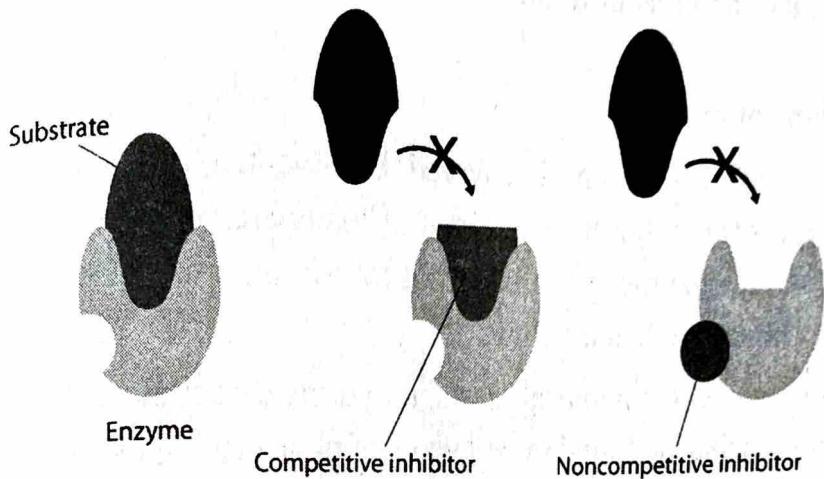
This change in ionization status leads to alterations in the ability of the protein to form ionic bonds necessary for its structural integrity. This leads to altered specificity, poor binding with its natural substrate or even complete denaturation of the protein. The pH can also similarly affect the shape and/or ionization status of the substrate leading to its poor binding with the enzyme. Each set of enzyme and substrates have a unique pH optimum and moving away from this pH values results in loss of activity as shown in figure 3.7B.

**(v) Effect of cofactors:** Cofactors are non-proteinaceous compounds that are needed by enzymes for their activity. Cofactors could be either ions (inorganic) or small organic molecules. Ions used as cofactors are usually heavy metals  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $Mn^{2+}$ ,  $Zn^{2+}$  etc. Small organic molecules used as cofactors are called coenzymes. Most coenzymes are derived from vitamins. Examples of coenzymes are thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD), pyridoxyl phosphate, coenzyme A (CoA) etc. Coenzymes actively participate in the catalysis and play a role in the active site of enzymes. They are used as carriers of electrons or functional groups during catalysis (Fig. 3.8).



**Fig. 3.8** Coenzyme thiamine pyrophosphate bound to an enzyme surface. Copyright 1994 from *Molecular Biology of the Cell*, 3rd edition, Bruce Alberts et al., Reproduced by permission of Garland Science/Taylor & Francis Books, Inc.

(vi) Effect of inhibitors on enzyme activity: Inhibitors are molecules or ions that hinder or terminate the catalytic activity of enzymes (Fig. 3.9). Inhibition can be classified into two types: *irreversible* and *reversible*. *Reversible* inhibition can be further subdivided into *competitive*, *uncompetitive*, and *non-competitive* inhibitors.



**Fig. 3.9** Enzyme-substrate complex in the top panel. In the middle is an example of competitive inhibition where the inhibitor binds to the active site preventing access to the substrate and in the bottom panel is an illustration of a noncompetitive inhibition where the inhibitor can bind to the enzyme and prevents the substrate from accessing the active site.

**Irreversible inhibition:** Inhibitors in this category resemble the substrate structurally with similar molecular geometry providing them the ability to interact with the enzyme active site. The interaction can be covalent or non-covalent and is strong enough to bind irreversibly with the enzyme and destroy its catalytic properties. An interesting example is the action of antibiotic penicillin. Penicillin is an irreversible inhibitor of transpeptidase enzyme involved in bacterial cell wall synthesis. Penicillin mimics the natural D-alanine-D-alanine dipeptide substrate binding covalently to the active site of transpeptidase. This prevents synthesis of rigid cell walls and promotes bacterial cell lysis. Penicillin and its analogs are therefore important anti-bacterial drugs.

**Reversible inhibition:** Reversible “competitive” inhibitors have similar geometry as substrates and are able to compete with them for formation of the enzyme-substrate complex. The formation of the enzyme-inhibitor complex is reversible but it lowers the catalytic rate as the proportion of substrates bound to enzymes is reduced.

In reversible “uncompetitive” inhibition, the inhibitor is unable to bind to free enzyme by itself, but can bind once the enzyme-substrate complex is

formed. This binding hinders the ability of the enzyme-substrate complex to move forward to form products, reducing the reaction rate.

In reversible “noncompetitive” inhibition, the inhibitor binding site is different from the substrate binding site. The inhibitor can bind to the free enzyme as well as enzyme substrate complex. This binding lowers the enzyme turnover number and reaction rate.

### **Applications of enzymes**

Many enzymes have been discovered by scientist and some of their functions are known, but the functions of many are yet to be ascertained. However, many enzymes have been put to use in industries. Some of the important classes are listed below.

- (i) **Cellulase:** As the name suggests, it hydrolyzes cellulose. Cellulose is a polysaccharide found in cell walls of plants and trees and is the raw material used to make paper, cotton, and other textiles.
- (ii) **Hemicellulase:** This enzyme hydrolyzes another plant cell wall polysaccharide called hemicellulose.
- (iii) **Xylanase:** This is an important industrial enzyme that breaks down Xylan, a linear polysaccharide present in the cells walls of plants. It finds applications in the pulp and paper industry.
- (iv) **Amylase:** Enzyme that hydrolyzes starch to sugars and finds wide application in food industry, especially in brewing industry.
- (v) **Protease:** As explained earlier, this a common name used to denote enzymes that hydrolyze peptide bonds. It is widely used in food, brewing, detergent, and leather industries.
- (vi) **Lipases:** These classes of enzymes break down triglycerides to glycerol and fatty acids and are used in food industries.

## **3.4 MECHANISMS OF ENZYME ACTION**

The binding of the substrate to the active site is aided by covalent and non-covalent interactions. The covalent interactions involve cleavage and formation of bonds between the substrate and the amino acid residues in the active site cleft. The chemistry behind this can be divided into the following 4 types:

- (i) **General acid-base catalysis:** The intermediates formed in chemical reactions may be charged making them unstable and revert back to form

reactants. Stabilizing these charged intermediates by donating or withdrawing protons (from molecules other than water) will facilitate their transformation to products. In enzyme-catalyzed reactions, this role is played by precisely placed active site amino acid residues which can donate and accept protons. Glutamic acid, aspartic acid, lysine, arginine, cysteine, histidine, serine and tyrosine side-chains with their near neutral pKa values are known to play this role in enzymes. This plays a major role in defining enzyme specificity and catalytic activity.

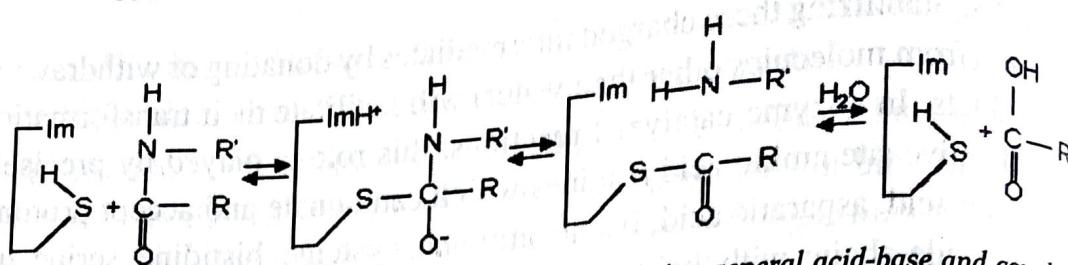
(ii) **Covalent catalysis:** This involves the formation of a temporary but unstable covalent bond between the enzyme and the active site residues. This lowers the activation energy leading to further reactions which releases the product and regenerates the enzyme. Amino acid residues that can form a nucleophile are usually used in enzyme catalysis. Papain which utilizes a nucleophilic thiolate ion to form a covalent bond with the peptide backbone is an example of this type of mechanism.

(iii) **Metal ion catalyst:** Metals attached to substrates or active site residues can play a role in enzyme catalysis. With ionic interactions they stabilize charged transition states. They can also mediate oxidation-reduction reactions by changing the oxidation states of the metal ions. Some of the ions used are  $Zn^{+2}$ ,  $Mg^{+2}$ ,  $Mn^{+2}$ ,  $Fe^{+2}$ ,  $Cu^{+2}$ ,  $K^+$ , and  $Na^+$ .

### 3.5 STRATEGIES UTILIZED BY ENZYMES TO EFFECT CATALYSIS

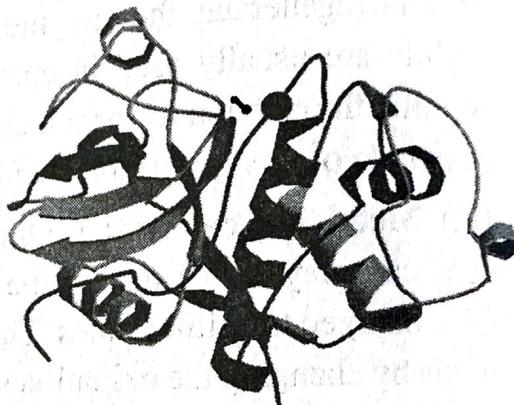
Enzymes usually use a combination of the mechanisms described above for catalysis. These are explained further with 4 specific examples described below:

(i) **Proteases:** As explained earlier, proteases are enzymes that catalyze the hydrolytic cleavage of peptide bonds in proteins and peptides. Although, peptide bond hydrolysis is thermodynamically favored, the reaction is too slow for quick turnover. Turnover refers to the rate at which unwanted proteins are degraded to constituent amino acids and recycled to form new proteins. A quick turnover is necessary to match the fast metabolic requirements. Proteases are also needed to break down proteins and peptides in food before they are absorbed in the intestines. They also play a role in regulating the activity of many other enzymes. For e.g., inactive precursor proinsulin is processed by proteases to form mature insulin. Proteases are able to ensure all these by lowering the activation energy to catalyze the formation product peptides.



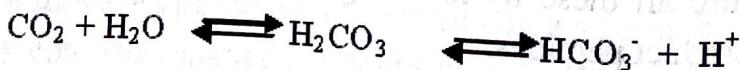
**Fig. 3.10** Papain-catalyzed peptide bond hydrolysis utilizing general acid-base and covalent catalysis mechanisms. "Im" refers to the imidazole group of histidine residue.

In papain, during peptide bond hydrolysis, a proton is donated from the sulfhydryl group of a catalytically active cysteine residue to an adjacent imidazole group of a histidine residue in the active site (general acid-base catalysis; Figs. 3.10 and 3.11). This is followed by a nucleophilic attack by the thiolate anion on the carbonoyl group carbon of the peptide bond (covalent catalysis) to form an unstable tetrahedral intermediate. The intermediate breaks down followed by hydrolysis to release the peptides and donate the proton back to the cysteine and regenerating the enzyme. Hence, protease papain uses two mechanisms: (i) acid-base and (ii) covalent catalysis. This example also shows how enzyme-substrate complex leads to the intermediate formation that can lower activation energy to drive the reaction forward.



**Fig. 3.11** Structure of papain. Image from the RCSB PDB ([www.pdb.org](http://www.pdb.org)) of PDB ID 1PPN, R. W. Pickersgill, R.W. Harris, E. Garman (1992), Structure of Monoclinic Papain at 1.6 Angstroms Resolution, *Acta Crystallogr.*, Sect. B 48: 59-67.

(ii) Carbonic anhydrase: In mammals, aerobic metabolism in tissues results in the formation of carbon dioxide, a major end product. This carbon dioxide needs to be removed rapidly from the system to prevent harmful accumulation. This is accomplished by transferring the carbon dioxide to the blood, where it is converted to a soluble form and transported to the lungs. In the lungs it is exhaled in exchange for oxygen. The solubilization of carbon dioxide in blood occurs due to its conversion to carbonic acid. Carbonic acid is a relatively weak acid and is formed by the reversible hydration of carbon dioxide as shown below:

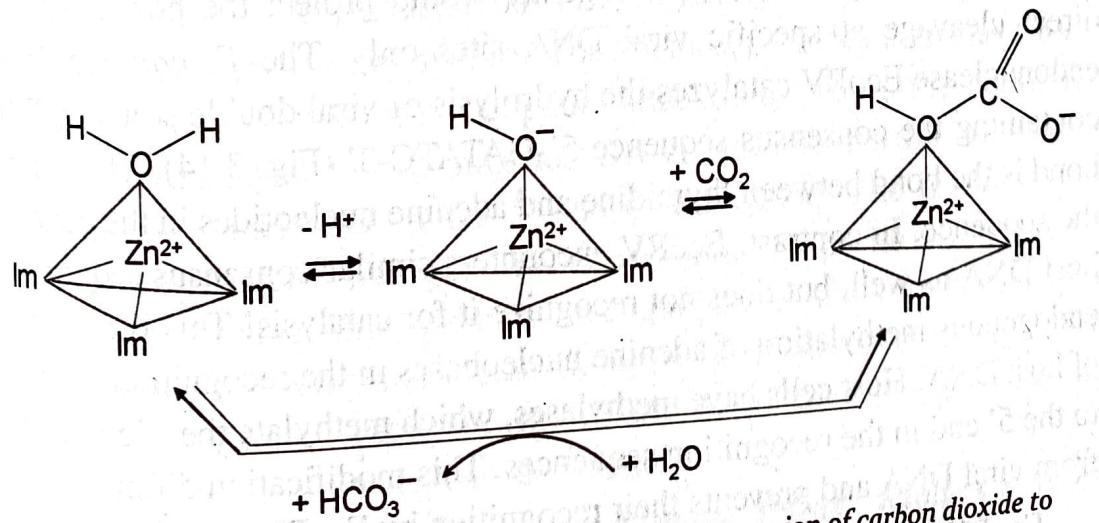


However, the carbonic acid can be reversibly deprotonated to form bicarbonate ion. These steps are coupled to facilitate the transport in blood and exhalation of carbon dioxide as the blood passes through the lungs. This reversible reaction is catalyzed rapidly to rates as high as million times per second per enzyme molecule by carbonic anhydrase. Carbonic anhydrase uses a zinc cofactor coordinated to imidazole side chains of 3 histidines in the active site to catalyze this reaction. (Figs. 3.12 and 3.13).

The zinc atom coordinated with imidazole side groups of 3 histidine residues in carbonic anhydrase is able to bind with a water molecule (Figs. 3.12 and 3.13). This leads to polarization of the water molecule and development of a slight negative charge on the oxygen atom. This facilitates the deprotonation of the water molecule and generation of a negatively charged hydroxyl ion coordinated to the positively charged zinc atom. The



**Fig. 3.12** Structure of human carbonic anhydrase II. Image from the RCSB PDB ([www.pdb.org](http://www.pdb.org)) of PDB ID 1CA2, A.E. Eriksson, T.A. Jones, A. Liljas (1988), Refined structure of human carbonic anhydrase II at 2.0 Å resolution, *Proteins* 4:274-282.



**Fig. 3.13** Role of zinc atom in carbonic acid catalyzed conversion of carbon dioxide to carbonic acid. "Im" refers to the imidazole group of histidine residues. This catalysis utilizes 2 mechanisms: metal-ion and general acid-base.

deprotonation step is an example of general acid-base catalysis step where the proton is donated to another histidine group. The presence of perfectly positioned carbon dioxide molecule in the active site of carbonic anhydrase helps it react with the nucleophilic hydroxyl ion and form a bicarbonate ion. The carbonic anhydrase is regenerated when it reacts with a water molecule to release the bicarbonate anion. This bicarbonate anion is soluble in blood and is transported to the lungs. In the lungs, carbonic anhydrase catalyzes the reverse reaction releasing carbon dioxide for exhalation. Carbonic anhydrase is an example of an enzyme that utilizes acid-base as well as metal-ion catalysis mechanism. This enzyme is an example of (i) metal-ion catalysis, (ii) acid-base catalysis, (iii) fast enzymatic conversions that meet metabolic requirements to transform substrate to a soluble form that can be transported and reconverted.

**(iii) Restriction enzymes:** Viruses can infect host cells, hijack their cell machinery, and replicate themselves. This may eventually cause the lysis of the host cells. Bacteria have evolved mechanisms to counter these viruses. They produce hydrolytic enzymes called restriction enzymes that can identify and cleave viral DNA at specific sequences and thereby, neutralize the effects of these infectious agents. These specific sequences are termed recognition sequences. The most well studied restriction enzyme is “type II restriction endonuclease”.

These enzymes should be very specific with respect to

- (i) Recognition sequences so that miscleavages are minimized and
- (ii) Host DNA should not be degraded in contrast to viral DNA which needs to be cleaved at the recognition sequences.

Both these specificities put together would protect the host DNA and direct cleavage at specific viral DNA sites only. The *E. coli* restriction endonuclease EcoRV catalyzes the hydrolysis of viral double stranded DNA containing the consensus sequence 5'-GATATC-3' (Fig. 3.14). The scissile bond is the bond between thymidine and adenine nucleotides in the center of the sequence. In contrast, EcoRV encounters similar consensus sequence in host DNA as well, but does not recognize it for catalysis! This is due to the endogenous methylation of adenine nucleobases in the recognition sequence of host DNA. Host cells have methylases, which methylate the adenine close to the 5' end in the recognition sequences. This modification differentiates it from viral DNA and prevents their recognition by EcoRV for degradation.

EcoRV catalyzes the hydrolysis of the phosphodiester bond in the center of the recognition sequence, 5'-GATATC-3'. As shown in figure 3.15, the

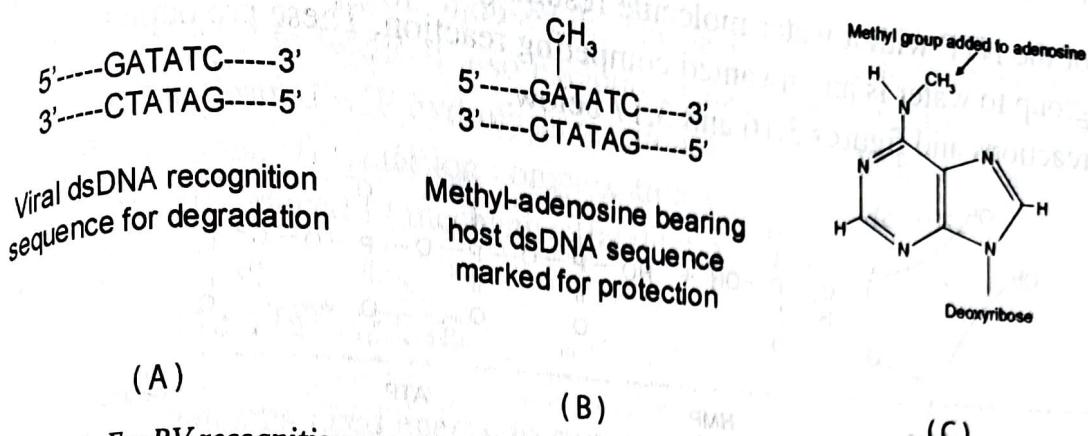


Fig. 3.14 EcoRV recognition sequence in DNA. (A) The 5'-TA-3' sequence in the center of the viral dsDNA recognition sequence is cleaved in viral DNA. (B and C) In host DNA, the presence of a methyl group on the adenine nucleotide at the 5' end of the recognition sequence protects the DNA from cleavage.

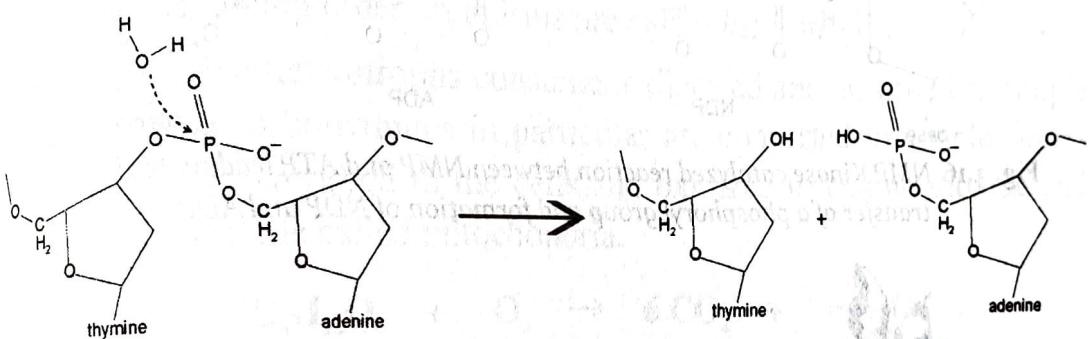
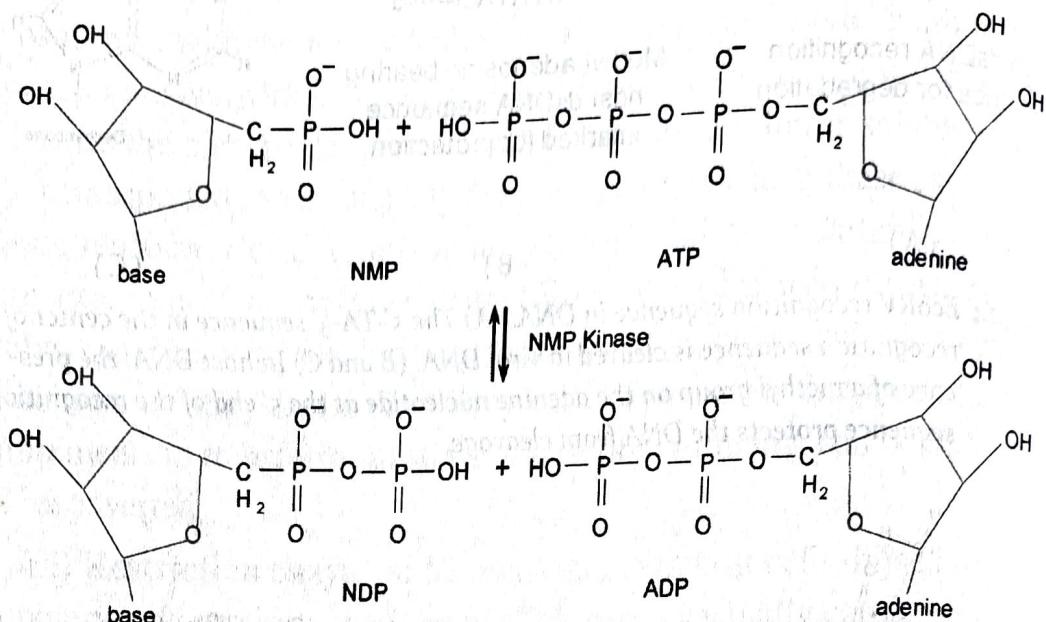


Fig. 3.15 EcoRV catalyzed single displacement mechanism involved in hydrolytic cleavage of phosphodiester bond linking 5'-thymidine and 3'-adenosine in 5'-GATATC-3' recognition sequences of viral DNA. In the recognition sequence, the bond between the 5'-TA-3' dinucleotide in the center of the sequence is cleaved by the enzyme.

bond between the 3'-oxygen atom of the deoxythymidine and 5'-phosphoryl moiety attached to deoxyadenosine is cleaved by the hydrolytic action of a water molecule. A magnesium ion is known to be essential for the catalysis, but its role is not clearly understood. The magnesium is likely to play a role similar to zinc in carbonic anhydrase. The ion most probably activates a water molecule that can launch a nucleophilic attack and cleaves the bond. The inability of the enzyme to recognize host DNA is attributed to the methylation of the adenine nucleotide at the 5' end of the strand. The methyl group prevents the hydrogen bonding of the adenine amino group with an asparagine residue in the enzyme. This hinders the recognition necessary for the catalytic action. Restriction endonuclease is an example of (i) High substrate specificity, (ii) Role of metal ions in catalysis, (iii) Modifications to substrates preventing enzymatic catalysis of unwanted side reactions.

(iv) Nucleoside Monophosphate Kinases (NMP Kinases): NMP kinases catalyze the transfer of a phosphate group from a nucleoside triphosphate (NTP) to a nucleoside monophosphate (NMP). The hydrolysis

of the NTP with a water molecule resulting in the transfer of the phosphate group to water is an unwanted competing reaction. These are depicted in the reactions and figures 3.16 and 3.17 below.



**Fig. 3.16** NMP Kinase catalyzed reaction between NMP and ATP leading to the transfer of a phosphoryl group and formation of NDP and ADP



**Fig. 3.17** Structure of adenylate kinase. Image from RCSB PDB ([www.pdb.org](http://www.pdb.org)) of PDB ID 4AKE, C.W. Muller, G.J. Schlauderer, J. Reinstein and G.E. Schulz (1996), Adenylate kinase motions during catalysis: an energetic counterweight balancing substrate binding, *Structure* 4:147-156.



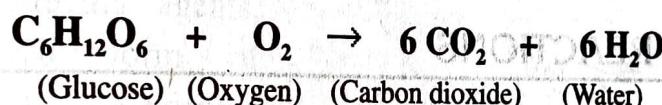
NMP kinases are known to be active only in the presence of divalent metal ions such as  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$ . Unlike earlier examples where metal ions are a part of the enzyme active site, here the metal ion is not involved directly with the enzyme. The metal ion coordinates with the ATP substrate and makes it conform to a three-dimensional structure. This structure enables the formation of an ATP- $\text{Mg}^{2+}$ -enzyme complex that also brings the NMP in close proximity with the right orientation to the active site. This step has been

reported to be an example of "induced fit. This leads to the phosphoryl group transfer from ATP to NMP. It also excludes water from the active site and prevents the undesired ATP hydrolysis reaction. Hence, NMP kinase has two important features: (i) metal ion complexation with substrate instead of the enzyme and (ii) induced fit mechanism facilitating catalysis.

### 3.6 PHOTOSYNTHESIS

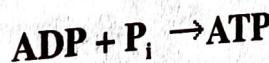
All living organisms need energy to survive. They obtain their energy from the food they consume. They can be either produce their own food or consume other organisms to obtain it. Organisms that make their own food such as plants and algae are called *autotrophs*, whereas animals that source their energy by consuming other organisms are called *heterotrophs*.

The food that heterotrophs consume is digested and absorbed as simple molecules. The carbohydrates in particular are converted to simple sugars such as glucose, absorbed in the cells and oxidized to carbon dioxide and water in an organelle called mitochondria.



This process is called **cellular respiration**. The oxygen required for this process is sourced from the atmosphere through the lungs and transported systemically to all the tissues in the body. The oxidation reaction is an exergonic process, *i.e.* releases energy. Each mole of glucose upon oxidation provides around 2880 KJ of free energy.

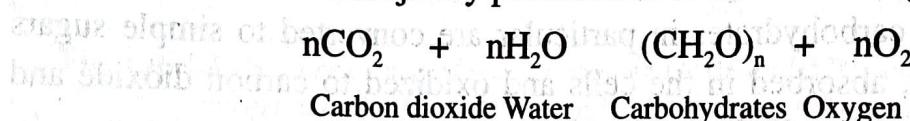
This free energy is transferred and stored in a chemical molecule called ATP (Adenosine-5'-Triphosphate). Each mole of glucose upon metabolism can provide up to 38 moles of ATP and each mole of ATP can store around 30 KJ of free energy.



ATP serves as the common denomination for all energy transactions in all organisms. ATP is transported to different sites where free energy is needed to drive forward **endergonic** (requires free energy to be supplied to the reactants) processes. The hydrolysis of ATP to Adenosine-5'-Diphosphate (ADP) with the loss of the inorganic phosphate in an exergonic step as shown above. When this is coupled to endergonic reactions, the free energy released by the ATP hydrolysis used to drive the endergonic reactions forward. The ADP is recycled to the mitochondria to form ATP. In summary, heterotrophs, use atmospheric oxygen to oxidize sugars, convert it to chemical energy, and

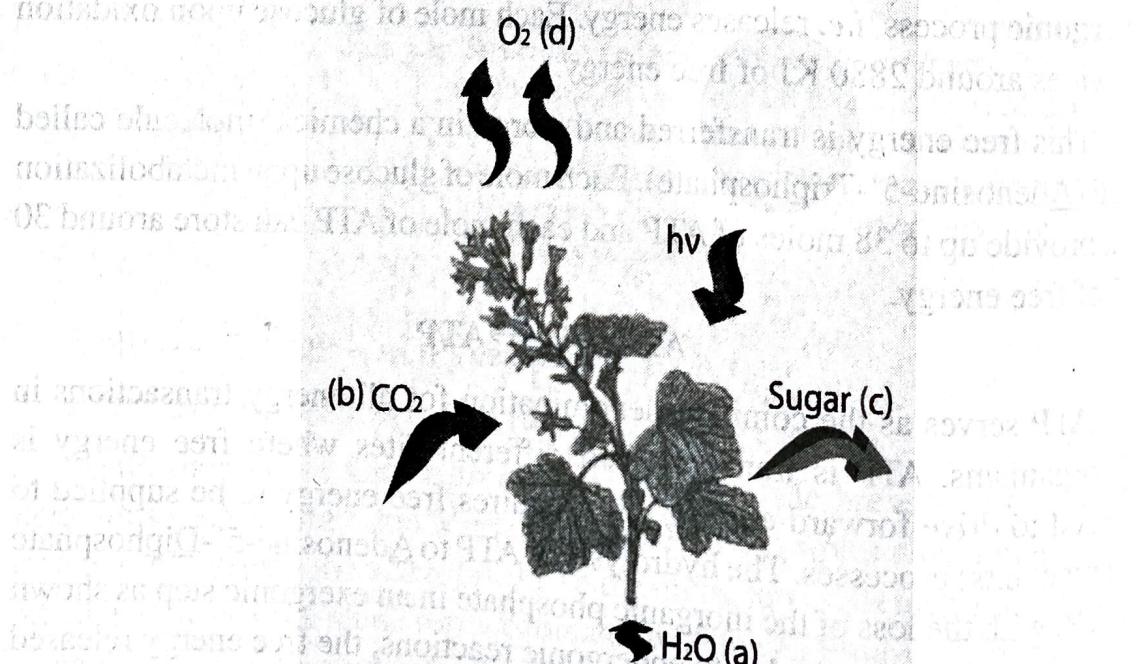
store it in ATP. The ATP is utilized as and when required to drive reactions and processes for conducting life functions.

Photosynthesis as the name suggests in the process of transforming light energy to chemical energy that enables synthesis of molecules. In this process, light energy from the sun is used by plants, algae, and cyanobacteria to reduce carbon dioxide in the presence of water to form carbohydrates (sugars). In plants, this process occurs in leaves and is highly endergonic. The free energy needed to drive this reaction is provided by absorption of photons in sunlight as shown in figure 3.18. Photosynthesis also results in the production of oxygen as a by product as shown in the equation below. Organisms conducting this process produce their own food by fixing sunlight and are called *photoautotrophs*. Photosynthesis in essence is the opposite of what we animals do, i.e., use atmospheric oxygen to oxidize food, derive energy from it, and form carbon dioxide as major by product that is exhaled through the lungs.

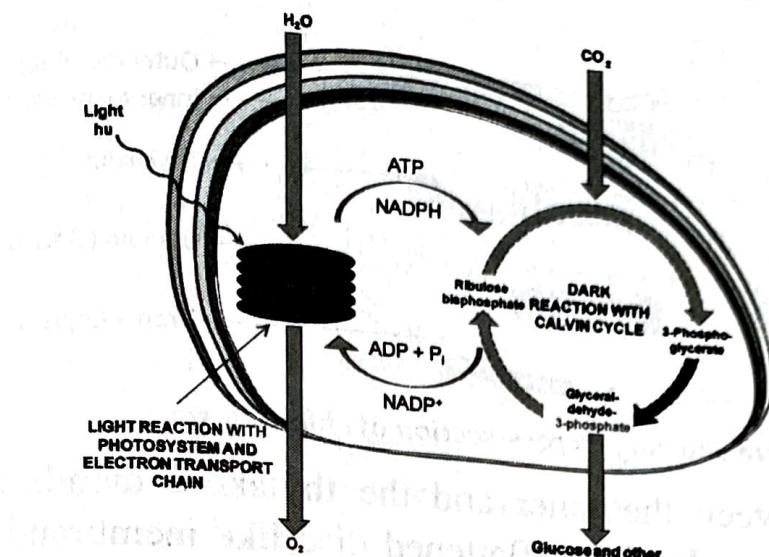


### 3.7 LIGHT REACTIONS

Photosynthesis can be subdivided into two steps: (i) light and (ii) dark reactions (Fig. 3.18). The light reaction occurs in the presence of light and



**Fig. 3.18** Photosynthesis proceeds by the reduction of carbon dioxide in the presence of water and sunlight to produce sugars and oxygen.

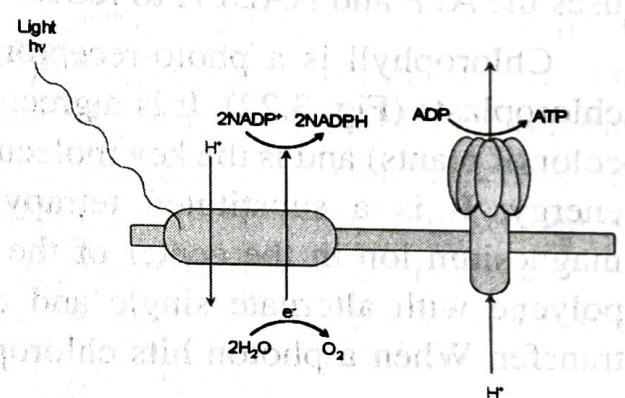


**Fig. 3.19** Photosynthesis involving light and dark reactions in the chloroplast

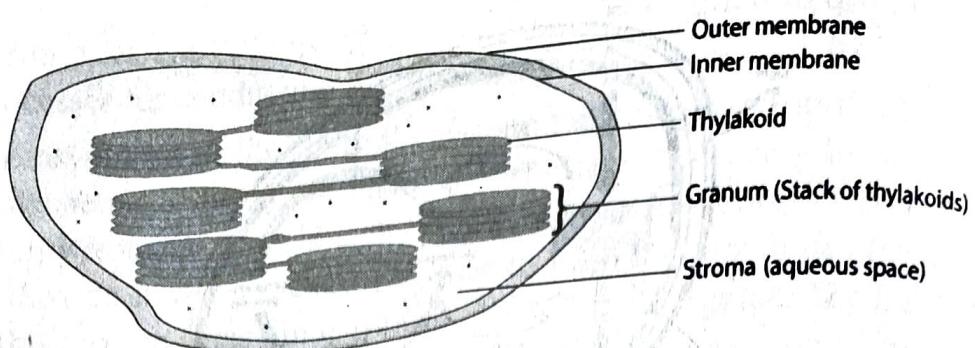
photons from sunrays are used to excite electrons in pigments called chlorophylls present in the chloroplast of leaves (Figures 3.19 and 3.20). The electrons are transported through an electron-transport chain that results in a proton-motive force and production of reducing agents, NADPH (reduced form of Nicotinamide Adenine Dinucleotide Phosphate) and ATP. The **dark reaction** steps occur even in the absence of light is also called the Calvin cycle. In this process, reducing power generated in light reaction step is utilized to reduce carbon dioxide to 3-phosphoglycerate, a 3-carbon molecule which forms the building block for other carbohydrates. Discussion under photosynthesis is divided into two parts. In the first part, photosynthesis in chloroplasts leading to formation of ATP and NADPH is discussed and in the next part, Calvin cycle, utilization of the NADPH to produce carbohydrates is discussed.

#### Photosynthesis in chloroplasts with chlorophylls as the key energy-trapping molecules

The chloroplast is the organelle in plant leaf cells responsible for photosynthesis. It is around  $5\mu\text{m}$  in length with an outer, inner and a thylakoid membrane (Fig. 3.21).



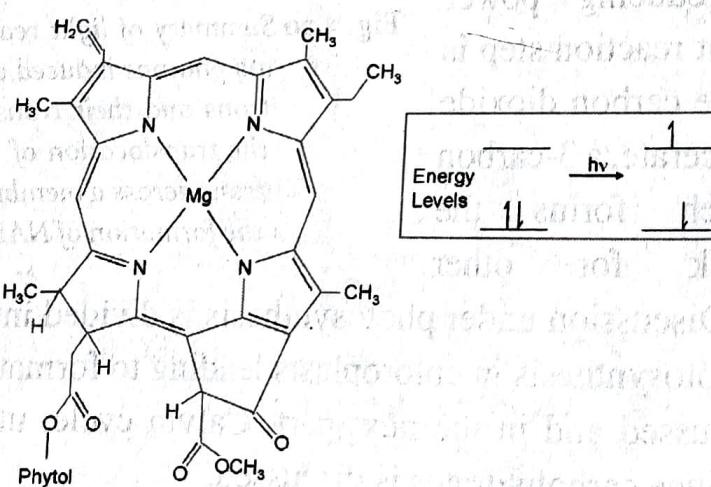
**Fig. 3.20** Summary of light reaction step showing photons induced excitation of electrons and their transport resulting in the translocation of protons and electrons across a membrane. This leads to the formation of NADPH and ATP.



**Fig. 3.21** Diagram showing a cross-section of chloroplasts.

The space between the inner and the thylakoid membrane is called "stroma". The stroma also hosts flattened disc like membranous structures called thylakoids. The thylakoids in turn are stacked to form a granum. The grana are interconnected with a lumen like stroma lamellae. The thylakoid contains "light harvesting machinery" composed of light harvesting proteins, reaction centers, electron-transport chains and ATP synthase that forms ATP and NADPH in the stroma. The stroma hosts the "enzymatic machinery" that uses the ATP and NADPH to reduce carbon dioxide to sugars.

Chlorophyll is a photo-receptor molecule present in the thylakoids of chloroplasts (Fig. 3.22). It is a green pigment (which accounts for the green color of plants) and is the key molecule involved in the "trapping" of sunlight energy. It is a substituted tetrapyrrole with nitrogens coordinated to a magnesium ion in the center of the molecule. Chlorophyll is a conjugated polyene with alternate single and double bonds which facilitate electron transfer. When a photon hits chlorophyll molecules, it excites ground state

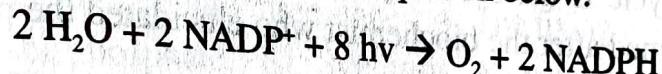


**Fig. 3.22** Chlorophyll, the light receptor green pigment found in chloroplasts of plant leaf cells. Phytol is a highly hydrophobic 20 carbon alcohol bound to an ester in chlorophyll. In the inset is the energy diagram showing the photon induces excitation of electrons from lower to higher energy level.

electrons to a higher energy state (Fig. 3.22, inset). The excited electron may return to ground state and dissipate the energy as heat. However, if suitable electron acceptor chlorophyll is nearby, the electron may be transferred. The electron can get transferred through a series of donor-acceptor pairs transferring the reducing power to NADPH. The proton gradient formed during the process aids the formation of ATP as explained in further in detail below.

## Photosystems in photosynthesis

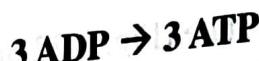
As explained earlier, in plants, photosynthesis occurs in chloroplasts of leaf cells. Nature has endowed leaves with large specific surface area necessary for trapping of incident light energy. The photosynthesis in plants occurs due to the interaction of two thylakoid transmembrane protein complexes called photosystems. The two photosystems are termed photosystem I (PS I) and photosystem II (PS II). The stoichiometry of the reactions occurring in PS I and PSII is shown in a simplified form in equation below.



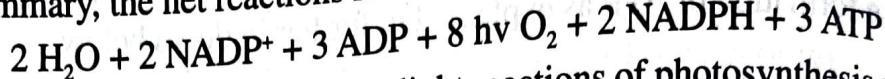
PS I and PS II systems follow a co-operative strategy to harvest light energy from 8 photons, produce light-induced charge separation and an electron-transport chain that reverses an thermodynamically unfavorable endergonic reaction in the reverse direction and temporarily store the chemical energy in 2 molecules of NADPH in the stroma. This NADPH is subsequently used in the dark reaction to reduce  $\text{CO}_2$  to carbohydrates in the dark reaction. Also during this process, 2 molecules of water are oxidized to produce a molecule of oxygen in the lumen.

## ATP synthesis in chloroplasts

During photosynthesis, protons are sourced from the stroma and transferred to the lumen. As the thylakoid membrane is impermeable to protons, the lumen is markedly more acidic (around pH 4) than the stroma and this provides a proton gradient across the membrane. This potential difference also generates a proton-motive force across the membrane. This force is harvested by ATP synthase enzyme present across the thylakoid membrane (Fig. 3.20). The ATP synthase complex consists of two units,  $\text{CF}_1$  and  $\text{CF}_0$ . The  $\text{CF}_0$  is closer to the lumen side of membrane and it routes the protons from the lumen to the stroma (Refer to Chapter 4). This helps the  $\text{CF}_1$  unit present on the stromal side of the membrane to catalyze the formation of ATP from ADP. In this reaction,  $12 \text{H}^+$  are translocated across the membrane for the catalytic phosphorylation of three ADP molecules. A simplified stoichiometric equation of this process is shown below.



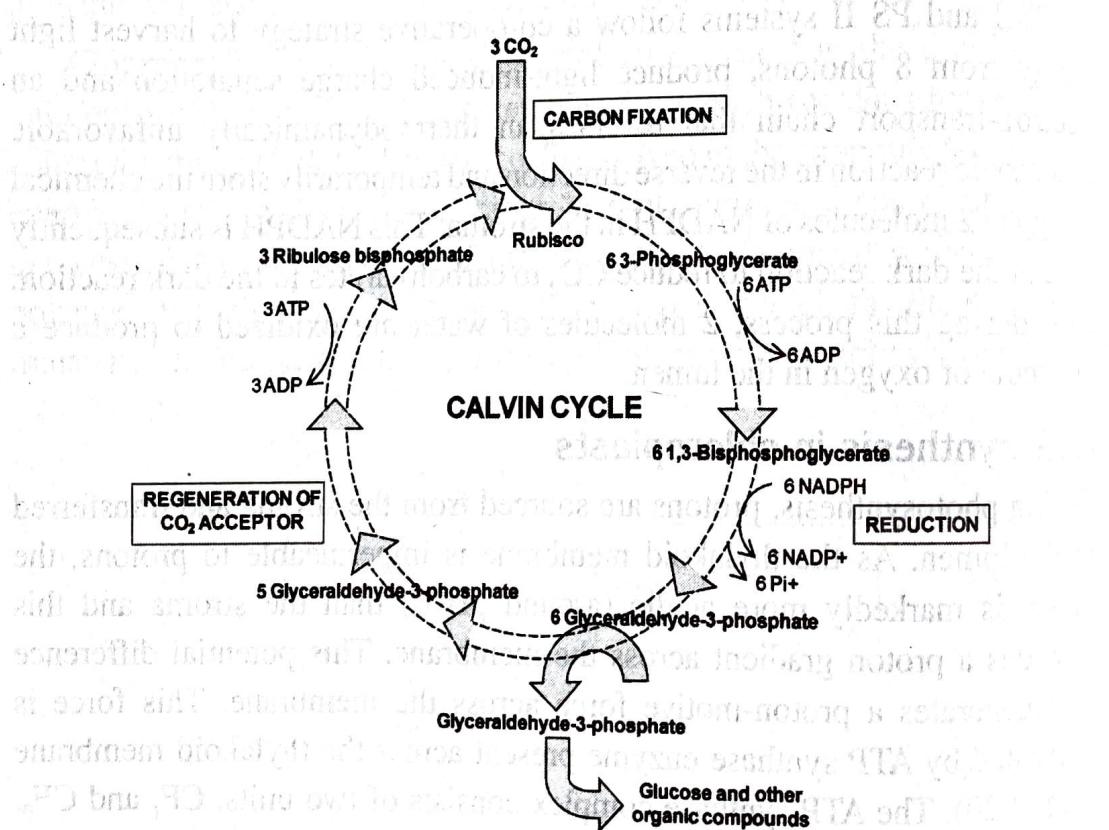
The reaction releases the ATP to the stroma and is used for the dark reactions for conversion of  $\text{CO}_2$  to carbohydrates. This process of using light energy to effect the phosphorylation of ADP is called photophosphorylation. In summary, the net reactions in the light reactions in photosynthesis:



This equation shows that overall, light reactions of photosynthesis utilize 8 photons to oxidize two water molecules and produce two NADPH, three ATP molecules in the stroma, and an oxygen molecule in the lumen. While the NADPH and ATP find use in the subsequent dark reactions, the oxygen finds its way to the atmosphere.

### 3.8 CALVIN CYCLE OF THE DARK REACTION

The Calvin cycle is the second part of the photosynthesis process (figure 3.23). It is named after the biochemist who elucidated the pathway. It is also



**Fig. 3.23** Steps involved in Calvin Cycle. In the first stage, carbon dioxide from the atmosphere is fixed by condensing it with ribulose 1,5-bisphosphate. This is an exergonic reaction facilitated by ribulose 1,5-bisphosphate carboxylase also known as RuBisCO. In the second stage, the 3-phosphoglycerate produced in the first stage is reduced to glucose through a series of reactions. In the final stage, 5-carbon ribulose 1,5-bisphosphate is regenerated from the 6-carbon and 3-carbon phosphate intermediates observed in the first two stages.

named as the dark reaction because in contrast to the light cycle, these reactions do not depend of the presence of light for their activity. The dark reactions take place in the stroma of chloroplasts and utilize the ATP and NADPH produced in the light reactions to reduce carbon dioxide to carbohydrates such as hexoses. The carbon dioxide is sourced from the atmosphere through pores called stomata present in plant leaves and stems. The hexoses, mainly glucose synthesized by the fixing of atmospheric carbon dioxide are used as fuel, for synthesis of metabolic intermediates and stored in cells as starch and sucrose. The stored energy is also utilized by organism higher in the food chain. Calvin cycle is the single most important reaction on earth responsible for incorporating carbon atoms from the atmosphere into living systems.

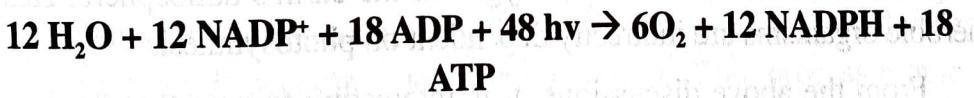
Thus, the light and dark reactions work in tandem to harness light energy (photons) and convert carbon dioxide to chemical energy (hexoses). The Calvin cycle can be divided into three stages: A) The fixing of carbon dioxide by ribulose 1,5-bisphosphate, B) Synthesis of hexose sugars, and C) Regeneration of ribulose 1,5 bisphosphate.

The net stoichiometry for dark reaction in simplified form is:

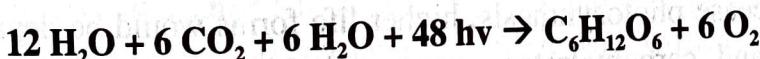


Thus the net stoichiometry for dark reaction shows that 18 ATP and 12 NADPH are required to reduce carbon in its completely oxidized form ( $\text{CO}_2$ ) to its reduced glucose form.

The net stoichiometry for photosynthesis can be obtained by first multiplying the equation for light reactions by 6 to obtain:



This equation for light reaction when summed up with the reaction for dark reaction shown earlier provides the net stoichiometry for the photosynthesis process.



Thus, the light and dark reactions work in tandem in chloroplasts, harness 48 photons from sunlight and reduce 6 molecules of carbon dioxide to one molecule of glucose. It also produces 6 molecules of oxygen in the process. Photosynthesis, utilizes light energy to overcome the endergonic energy barrier to synthesize chemical energy which is mainly converted to starch and

stored in plants. It also issues oxygen that populates the earth's atmosphere and helps maintain the oxygen levels in the earth's atmosphere.

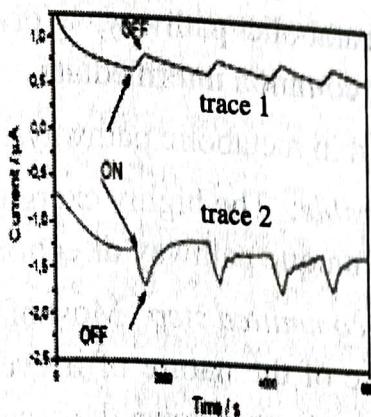
## **Significance of photosynthesis**

From the point-of-view of chemical thermodynamics, the formation and maintenance of ordered and complex structure of living things on earth is not possible without the input of energy. If the earth were to be an isolated system, then the energy has to be sourced from within the system. This would mean the slow depletion of energy sources on earth and the system will tend to a position, wherein sustenance of life forms would be impossible. Fortunately, the earth is not an isolated system, and this energy is obtained from an external source, the sun. Sun is a hydrogen fusion reactor that generates electromagnetic waves that includes the visible range as well. A fraction of the light energy that impinges on earth is used by photosynthesis in plants to synthesize and store organic chemical energy reserves in the form of starch, sucrose, micronutrients etc. The herbivores consume these plants and use the carbohydrates stored in plant cells as an energy source. The herbivores in turn are consumed by carnivores as an energy source. Man is an omnivore who consumes plants as well as animals. Finally the decomposers in the food chain degrade dead and decaying organisms. The sunlight driven chemical energy synthesized by plants as carbohydrates emerges as the almost sole source of energy that traverses through the food chain.

The photosynthesis reaction uses water to reduce carbon dioxide in the light reaction. This is accomplished by using water as an electron and hydrogen donor. This is a highly endergonic reaction, but is accomplished by the absorption of photons. The oxygen produced by light reaction of photosynthesis in plants is the main source of oxygen in the earth's atmosphere. Hence, all aerobic organisms are indirectly dependent on photosynthesis.

From the above discussions, it is interesting to note that the sun is the ultimate source of all energy on earth and that almost all organisms are dependent on the carbohydrates and oxygen produced during photosynthesis either directly or indirectly as the source of energy in their food. If plants were to cease photosynthesis, higher life forms would be deprived of their oxygen and carbohydrates source and this would slowly lead to their decimation and even extinction.

As the sun is the source of all our energy, it would be interesting to use photosynthesis as tool for harnessing solar energy and converting it directly to electrical energy. This interesting application has been recently reported in literature and explained in figure 3.24.



The figure on the left shows a lamp illuminating a cactus plant with implanted electrodes for measuring oxygen and glucose levels. The light acted as an external stimulus to trigger photosynthesis. The adjacent plot shows levels of glucose (trace 1) and oxygen (trace 2) corresponding to durations when the lamp was either switched on or off. The plot shows that the electrodes responded in real time to visible light from the lamp. This suggests that photosynthesis induced

generation of glucose and oxygen can be harvested by the electrodes to produce electrical energy. This is an example of biofuel cell which transforms visible light to electricity. Thus visible sunlight can be converted to electricity in a green, renewable, and sustainable manner.

Reprinted with permission from "Dynamic Measurements of Photosynthesis in a Living Plant to Sunlight Transformation into Electricity, Victoria Flexer et al., Anal. Chem., 2010, 82 (4), pp 1444–1449", Copyright 2010, American Chemical Society.

Fig. 3.24 Biofuel cells utilizing photosynthesis as a source of electrical energy.

### 3.9 METABOLISM AND BIOENERGETICS

Metabolism is a key process in living organisms in which they obtain and use free energy to carry out day-to-day functions for survival and reproduction. In order to perform these functions, metabolic processes *acquire energy from photosynthesis, utilize it to convert nutrients to macromolecules, assemble these macromolecules into cellular structures, and breakdown macromolecules into cellular fuel for biological functions*. Metabolic processes are divided into two processes, **anabolism** and **catabolism**.

**Anabolism** is the *biosynthesis of biomolecules* such as nucleotides, proteins, lipids, and polysaccharides from simple precursor molecules. Energy is used in this process (*endergonic process*). This process requires the free energy of ATP hydrolysis and NADH oxidation. As stated in Chapters 1 and 2, biomolecules are made up of mostly carbon, hydrogen, oxygen, and nitrogen. On the other hand, **catabolism** is the *breakdown of biomolecules* to simple metabolites. In catabolism, energy is generated (*exergonic process*) for use by living organisms. The free energy is conserved by the reduction of  $\text{NADP}^+$  to  $\text{NADPH}$  or by coupling exergonic reactions to ATP synthesis.

Several metabolic pathways are functioning in a living organism. As stated earlier in this chapter, enzymes are the basic units of metabolism. Each metabolic pathway is a series of interconnected enzymatic reactions that result in production of a particular product. In the anabolic pathway, few

common metabolites are used as starting materials and diverge into a wide range of biomolecules. In the catabolic pathway, a divergent range of biomolecules converge by forming common intermediates.

Following features are observed in metabolic pathways:

- Metabolic pathways are irreversible:* The highly exergonic nature of the metabolic pathways gives them unique pathway direction.
- Every metabolic pathway has a committed step.* Most of the reactions are close to equilibrium but because of the nature of irreversible exergonic reactions, the intermediates can go further down the reactions.
- All metabolic pathways are regulated.* The regulation is achieved through the first committed step which is also the rate-limiting step of the biosynthetic pathway.
- Metabolic pathways in eukaryotic cells occur in unique cellular compartments listed below.

**Mitochondrion** – Citric acid cycle, oxidative phosphorylation, amino acid catabolism.

**Cytosol** – Glycolysis, pentose phosphate pathway, fatty acid biosynthesis, gluconeogenesis.

**Nucleus** – DNA replication, RNA transcription, RNA processing.

**Lysosomes** – Enzymatic digestion of cellular components.

**Golgi Apparatus** – Post translational modification of membrane and secretory proteins, formation of plasma membranes and secretory vesicles.

**Rough Endoplasmic Reticulum** – Synthesis of membrane-bound and secretory proteins.

**Smooth Endoplasmic Reticulum** – Lipid and steroid biosynthesis.

**Peroxisomes** - Oxidative reactions involving amino acid oxidases and catalase, glyoxylate cycle reactions in plants.

ATP is energy currency of the cell that connects catabolism to anabolism. ATP is generated by the oxidation of metabolic fuels; glucose, amino acids, and fatty acids. The cleavage of  $\gamma$ -phosphate anhydride bond of ATP by hydrolysis releases free energy. Besides ATP, there are other high-energy phosphorylated compounds such as phosphoenolpyruvate, 1,3-bisphosphoglycerate, phosphocreatine, and inorganic pyrophosphate.

**Glycolysis** is a metabolic pathway in which glucose is catabolized to two molecules of pyruvate. D-glucose is a major fuel for most organisms and occupies the central position in the metabolic pathways of a living organism.

Breakdown of glucose yields important metabolic intermediates for other biosynthetic reactions. Glycolysis occurs in the cytosol of the cells and does not require oxygen (anaerobic process) for the reactions. It consists of 10 enzyme catalyzed reactions producing 2 molecules of ATP and 2 molecules of NADH per molecule of glucose.

Pyruvate and NADH produced by glycolysis undergo further reactions in the citric acid cycle and electron transport chain, respectively, in the mitochondria. The electron transport chain generates a proton gradient that drives the synthesis of 5 ATP molecules from ADP and Pi. In addition, pyruvate formed through the process of glycolysis is converted to acetyl-CoA by pyruvate dehydrogenase (generating additional 2 moles of NADH/glucose and another 5 ATPs by oxidative phosphorylation). The acetyl-CoA enters the citric acid cycle where it is completely oxidized into CO<sub>2</sub>. The electrons liberated by oxidation are captured by NAD<sup>+</sup> or FAD which are then transferred via the electron transport chain to form O<sub>2</sub>. Altogether, the whole process results in the net production of 32 molecules of ATP per molecule of glucose. The citric acid cycle generates several metabolic intermediates that are precursors for fatty acids, amino acids, nucleotide bases, cholesterol, and porphyrin synthesis.

The metabolic processes are integrated and regulated by several factors including the level of substrates, substrate transporters, enzymes, hormones, neurotransmitters, etc. Epinephrine is one of the major neurotransmitters that can regulate the concentrations of glucose in blood. Likewise, glucagon, insulin, cortisol, and gut- and brain-derived hormones regulate the levels of glucose in the blood to effectively regulate the metabolic process.

## Chapter highlights

- Enzymes are proteinaceous in nature and act as ‘catalysts’ in living organisms facilitating biochemical reactions to occur faster
- Enzymes lower the requirement of activation energy, specific in their actions, and enhance the reaction kinetics.
- Several factors such as enzyme concentration, substrate concentration, temperature, pH, cofactors, and inhibitors can modify the functional capabilities of the enzymes.
- Proteases are enzymes that catalyze the hydrolytic cleavage of peptide bonds in proteins and peptides
- Carbonic anhydrase is a family of enzymes that catalyze the interconversion of carbon dioxide and water to bicarbonate and protons.

The enzyme plays a key role in the elimination of carbon dioxide through the lungs which is formed as a result of aerobic metabolism.

- Restriction enzymes are hydrolytic enzymes that cleave viral DNA at specific sequences called recognition sequences.
- Nucleoside monophosphate kinases catalyze the transfer of a phosphate group from a nucleoside triphosphate (NTP) to a nucleoside monophosphate (NMP).
- Photosynthesis is a process in which light energy is used to reduce carbon dioxide and oxidize water to form carbohydrates and oxygen. Light and dark reactions constitute photosynthesis mostly occurring in plants, algae, and cyanobacteria.
- Living organisms require energy which is obtained through synthesis (anabolism) and breakdown (catabolism) of biomolecules. Glycolysis, citric acid cycle, and electron transport chain are some of the key metabolic pathways through which ATP, the energy currency of the cell, is generated.