UNIT 6 ENZYMES

Structure

6.1 INTRODUCTION

In Unit 5, you learnt about an important class of biomolecules, called proteins. The variety of functions which the proteins perform in the cell are crucial to life and undeniably, the most important of these functions is carried out by a group of proteins called enzymes. An enzyme is a biological catalyst. It speeds up or catalyses a biochemical reaction. A living cell, which we described in Unit 1 of this course, depends upon a large number of such biochemical transformations for its own survival, and that of the organism of which it is a part. Since each biochemical reaction requires a separate enzyme to catalyse it, a cell must contain a large number of different enzymes. Acting in a co-ordinated fashion, enzymes regulate the speed and direction of all biochemical changes. Thus, understanding enzymes and their properties is important in understanding metabolism, which we shall discuss in Block 3 of this course. Therefore, in this unit you will learn about enzymes and understand their functioning in the living cell. Besides this you will also learn that many enzymes need other nonprotein organic molecules or metal ions as cofactors for their proper functioning. Some vitamins and minerals act as coenzymes/ cofactors for these biocatalysts. Most of the vitamins and minerals are basically required to maintain proper cell function, growth and reproduction. We shall discuss vitamins and minerals separately in Unit 7 of this course.

You will recall from Unit 5 that enzymes are globular proteins. However, they differ from other proteins in that they act as biocatalysts.

Objectives

After studying this unit, you should be able to:

- describe the biological significance and general characteristics of enzymes,
- classify enzymes into six major groups on the basis of their biochemical actions,
- explain mechanism of enzyme action in physico-chemical terms,

- describe effect of substrate and enzyme concentration as also the role of pH and temperature on reaction rate,
- distinguish between competitive and noncompetitive inhibition,
- explain the various mechanisms of enzyme regulation,
- define isoenzymes, and
- describe importance of enzymes in health sciences.

6.2 GENERAL CHARACTERISTICS OF ENZYMES

Enzymes are a specialised class of proteins, and, as already mentioned, they act as biocatalysts in the metabolic reactions. They are responsible for most of the activities that take place within the living system and more than two thousand enzymes have been identified. Although enzymes are important in maintaining life, they have many medical and commercial uses also. For example, determining the level of particular enzymes in blood gives a clue to the extent of heart muscle damage after a heart attack. On the commercial side, enzymes have been used for centuries, as in fermentation to make alcoholic beverages.

All the enzyme catalysed reactions in the living system are collectively defined as **metabolic reactions**. Metabolism consists of **catabolic** and **anabolic** reactions. In catabolic reactions larger molecules are broken down to produce smaller products and energy, whereas in anabolic reactions, the cell uses energy to produce molecules which it needs for growth and repair. Both these series of reactions occur continuously in the cell.

We shall now describe some characteristics of enzymes and also explain some terms used in this unit.

6.2.1 Chemical Nature

Enzymes, for a long time were associated with the whole living organism with its cellular structures intact. However, **Buchner** in 1897 demonstrated that enzymes were organic substances produced by living cells and did not require intact cellular organisation for their catalytic activity. The exact chemical nature of enzymes was established thirty years later by **Sumner**. He showed that urease, an enzyme which catalyses the hydrolysis of urea, could be crystallised and was shown to possess properties characteristic of proteins.

$$\underset{\text{urea}}{H_2N-\overset{\overset{O}{\|}}{C}-NH_2} + H_2O \xrightarrow{\text{Enzyme urease}} 2NH_3 + CO_2$$

This result was confirmed by **Northrop** and **Kunitz**, who crystallised several enzymes and established that they were protein in nature. In 1960, the laboratories of **Stein** and **Moore** established the chemistry of the enzyme ribonuclease by deducing its amino acid sequence and a few years later, **Merrifield** (1969) achieved its total synthesis on the basis of its known amino acid sequence. This provided the ultimate proof that enzymes are no different from other chemicals of nonbiological origin. A complete understanding of the chemistry of an enzyme came with the work of **Philips** (1965) who determined the three dimensional structure of lysozyme. Using this information, Philips and his group proposed a chemical mechanism for the catalytic process of lysozyme. It was for the first time that such a feat was accomplished. The primary amino acid sequence and the three dimensional structure of many enzymes is now known. This knowledge has also thrown extensive light on the general features governing enzyme structure, function, regulation and evolution.

Ribonuclease catalyses the breakdown of ribonucleic acid.

Lysozyme is an enzyme which degrades bacterial cell wall.

Considerable diversity of structure is seen in the enzymes. Many enzymes are simple protein molecules. This implies that the protein molecule in itself is the true catalyst. However, many enzymes require the presence of additional nonprotein molecules for the full expression of their catalytic function, which means that these enzymes are conjugated protein molecules. Let us first try to learn the meaning of some terms, which we shall be using in this unit. Fig.6.1 gives you a general idea of these terms, as related to the structure and function of an enzyme.

Proteins can be divided into two major classes, according to one classification method. Simple proteins, according to this classification are proteins which produce only amino acids upon hydrolysis, whereas conjugated proteins produce amino acids and other organic or inorganic substances upon hydrolysis.

Apoenzyme: The protein part of an enzyme molecule is known as apoenzyme.

Cofactor: The nonprotein part of an enzyme is called a cofactor. These cofactors are basically the additional chemical groups, which appear in those enzymes that are conjugated protein molecules. Some cofactors may be metal ions such as Mg^{2+}, Zn^{2+} or complex organic molecules, such as nicotinamide adenine dinucleotide. Whatever be the nature of a cofactor, both are required for enzyme activity.

You will learn more about enzyme cofactors in Unit 7.

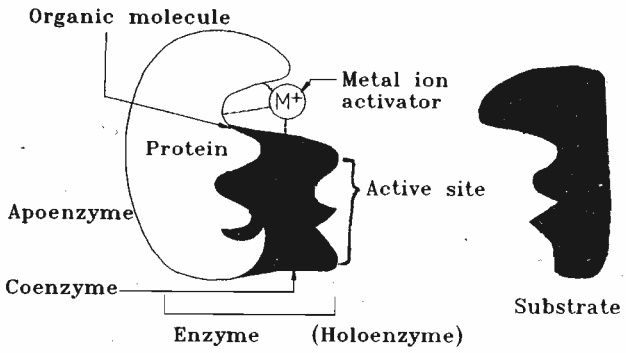


Fig.6.1: Schematic representation of an enzyme

Holoenzyme: The combination of an apoenzyme and the cofactor is known as the holoenzyme:

$$\text{Apoenzyme} + \text{Cofactor} \longrightarrow \text{Holoenzyme}$$

Activator: In case the cofactor of an enzyme molecule is a metal ion such as, Fe^{2+}, Mg^{2+}, Cu^{2+}, K^{+}, Na^{+}, Zn^{2+}, the cofactor is known as an activator.

Coenzyme: When the cofactor of an enzyme is a nonprotein organic molecule, the cofactor is known as a coenzyme. For example, the B group of vitamins are coenzymes that are necessary for proper cellular respiration.

Prosthetic group: A cofactor, which is tightly bound to the apoenzyme, is generally called a prosthetic group. However, it is possible that what is a prosthetic group for one enzyme may be simply a cofactor for another.

Substrate: A chemical substance or substances, the transformation of which is catalysed by an enzyme, is called its substrate. The catalytic transformation of a substrate by an enzyme involves its prior binding to the enzyme, followed by a reaction leading to bond making or bond breaking, resulting in a product.

Active site: The part of an enzyme where all the events of the catalytic process occur is called its active site. Thus, it is the specific area of an enzyme to which the substrate attaches during the reaction. Besides, the reaction is also catalysed and the products subsequently released at this site.

The active site consists of a few amino acid side chains, some known as **binding groups** and the other as **catalytic groups**. The binding side chains can bind to different parts of the substrate by hydrogen bonds and electrostatic or hydrophobic interactions. Thus, binding amino acids can either be polar or nonpolar. However, the amino acid side chains of the catalytic groups are of polar type only, because they bring about changes in the electronic structure of the substrate, which is a prelude to chemical transformations. We may mention here that there is no clear distinction between the amino acid side chains participating in the binding process and those which participate in the chemistry of catalysis. Some amino acid side chains could participate in both these aspects. In Figure 6.2, we have presented a schematic view of an active site.

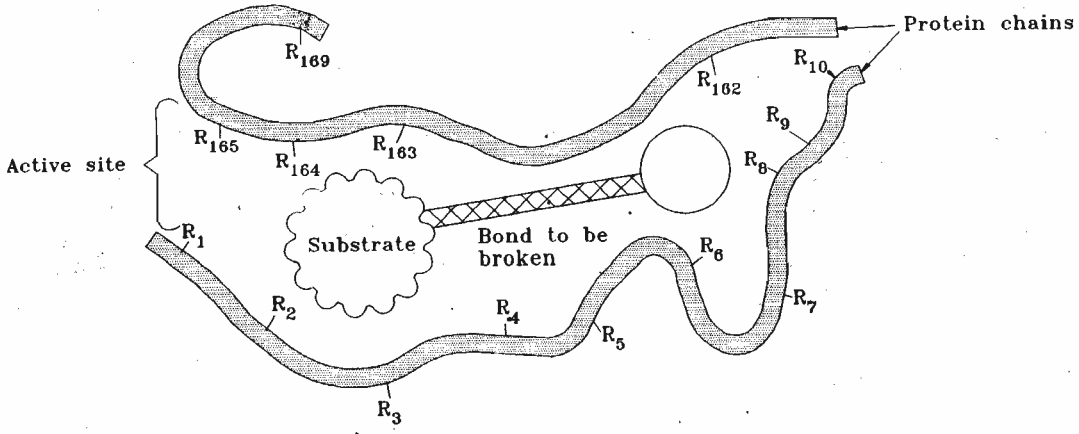


Fig.6.2: A schematic representation of the active site of an enzyme. The letter R with subscript shows the position of the amino acids in the protein chain.

We shall now describe the efficiency of an enzyme as a biocatalyst. You will also learn that enzymes possess enormous catalytic power.

6.2.2 Catalytic Efficiency

Enzymes, in a living cell, perform their functions under very moderate conditions of temperature and pH. Various chemical reactions, which are catalysed by enzymes in the living cell under physiological conditions, would hardly occur outside the cell in the absence of an enzyme. For example, it would take nearly 50 years to digest a single meal without enzymes. Another example, which we can cite, is that of the reversible reaction between carbon dioxide and water to produce carbonic acid. This reaction is catalysed by carbonic anhydrase, an enzyme which is present in most of the tissues, especially in erythrocytes.

RBCs are rich in the enzyme carbonic anhydrase. They can absorb CO_2 as it is produced in the body and transport it back to the lungs where it is released as one of the products of the body.

$$CO_2 + H_2O \underset{}{\overset{\text{Carbonic anhydrase}}{\rightleftharpoons}} \underset{\text{Carbonic acid}}{H_2CO_3}$$

The rate of this reaction is very low, but with the presence of enzymes it is increased to about 10^7 times. Another reaction, which we shall illustrate here is the decomposition of hydrogen peroxide to oxygen and water.

$$\underset{\text{Hydrogen peroxide}}{2H_2O_2} \xrightarrow{\text{Peroxidase}} 2H_2O + O_2$$

This reaction is catalysed by the enzyme peroxidase. Hydrogen peroxide is produced as a byproduct in many biological oxidations. It is highly toxic, and has to be decomposed quickly to prevent any damage to the cell components. This is achieved with the help of peroxidase, which affects an increase in the rate of reaction by about 10^{10} as compared to the uncatalysed reaction.

It is thus obvious that enzymes increase the reaction rate enormously, which is of utmost significance in the biological system. Wherever it has been possible to make comparisons, a rate enhancement of about 10^4 to 10^{14} has been observed with the presence of enzymes in biochemical reactions. Table 6.1 gives you some examples of rate enhancements brought about by enzymes.

Table 6.1: Comparison of nonenzymic and enzymic catalysis

Substrate	Catalyst	Temperature (K)	Rate constant (k) $(mol\ dm^{-3})^{-1}\ s^{-1}$
Amide (hydrolysis)			
benzamide	H^+	325	2.4×10^{-6}
benzamide	OH^-	326	8.5×10^{-6}
benzoyl-L-tyrosinamide	α-chymotrypsin	298	14.9
Urea (hydrolysis)	H^+	335	7.4×10^{-7}
	urease	294	5.0×10^{6}
Hydrogen peroxide (decomposition)	Fe^{2+}	295	56
	peroxidase	295	3.5×10^{7}

We shall consider various factors that lead to this rate acceleration in Section 6.4.

The catalytic efficiency of an enzyme is expressed in terms of its **turnover number**. This number indicates the number of substrate molecules which are transformed in one unit of time by one molecule of an enzyme, under optimal conditions of temperature and pH. You will learn about optimal conditions of temperature and pH in Section 6.5. We have given turnover numbers of some enzymes in Table 6.2. As will be evident from this table, the rate at which an enzyme catalyses a reaction, will vary from enzyme to enzyme.

Table 6.2: Turnover numbers of some enzymes

Enzyme	Turnover number (sec^{-1})
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetyl cholinesterase	25,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase	15

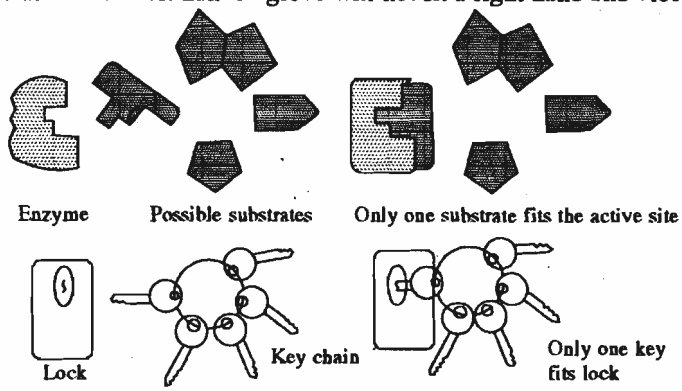
An important aspect of enzyme catalysis is their specificity in acting on a particular substrate(s), corresponding to a particular reaction or a class of reactions. In the next subsection, you will learn about enzymatic specificity of action.

6.2.3 Specificity of Action

An aspect of catalysis that distinguishes an enzymic from its nonenzymic counterpart, is the specificity displayed by the former in its action on the substrate. Enzymes are highly specific in their action, both with respect to the substrate they act upon and the kind of reaction they catalyse. Thus, the same substrate undergoing two different types of chemical transformations will be acted upon by two different enzymes. Physiologically the term specificity refers to the ability of an enzyme to recognise and transform one particular substrate in a mixture of several substrates. Therefore, it follows that **the specificity of an enzyme for a substrate not only involves the strength of binding of the substrate to the enzyme but also the velocity of the catalysed reaction.**

The degree of specificity varies from one enzyme to another. Some enzymes have a very low specificity. This enables them to act on a variety of substrates, provided they contain a particular susceptible bond such as, a peptide bond, a phosphate ester bond or a carboxylic ester bond. These enzymes are of the degradative type, such as peptidases, phosphatases or esterases. They generally participate in digestive processes where a narrow specificity would be expensive for the economy of the organism. There are other enzymes of intermediate specificity such as those which display group specificities. Thus, carboxypeptidase A is specific for the removal of C-terminal amino acids containing a free carboxyl group. Another enzyme of intermediate specificity is hexokinase which catalyses the phosphorylation of a variety of D-hexoses. Besides the above mentioned enzymes of variable specificity, some enzymes show absolute specificity for a particular substrate only, and the best example of this type is the enzyme urease, which splits only urea. Many enzymatic reactions also display stereochemical specificity. For example, D-amino acid oxidase is specific for D-amino acids only and will not affect L-amino acids. As can be expected biosynthetic enzymes tend to be highly specific so that anabolic activities are channelised in a particular direction.

About a century ago, Emil Fischer had proposed the **"lock and key"** hypothesis to explain the specificity of enzyme-substrate interaction. Although, this hypothesis still holds good in part, the specificity of an enzyme with respect to its substrate or a bond to be broken, is determined by the shape of the active site, its topographical features and the proper disposition in space of the amino acid residues participating in the process of substrate binding and catalysis. In the light of modern knowledge, one may say that not only have the lock and key to fit each other but they have to be flexible enough for the best fit. This constitutes what is known as the **"induced fit"** theory and can be compared to a hand slipping into a glove, which then causes or more appropriately 'induces' a fit. However, the specificity is still retained as a left handed glove will not fit a right hand and vice versa. We



Most of the enzymes are specific for only one particular substrate. This is comparable to a lock, which opens up with only one particular key.

Enzymes have been classified on the basis of the type of reactions they catalyse and we shall describe the six major classes of enzymes which have been identified. This classification is

have diagrammatically illustrated the "lock and key" hypothesis and the "induced fit" theory in Fig.6.3.

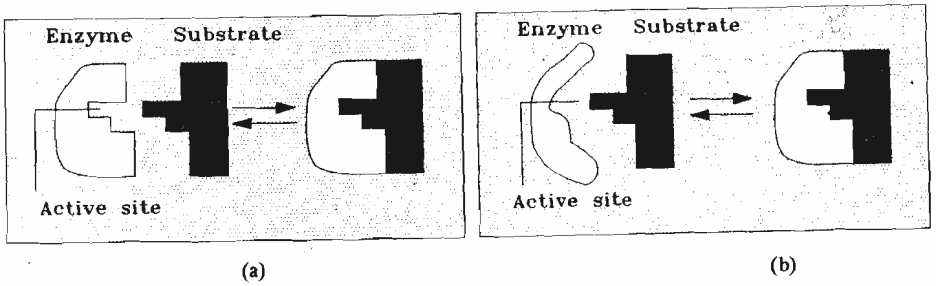


Fig. 6.3 : a) In "lock and key" hypothesis, the active site conforms precisely to the substrate molecule
b) In "induced fit" theory, the active site is induced to take a complementary shape by the substrate molecule

6.2.4 Regulation of Enzyme Activity

An interesting feature of enzymic catalysis which is not shown by its nonenzymic counterpart, is its ability to be regulated by small ions or small molecules which may be substrates, substrate analogues or substances structurally unrelated to the substrate. Quite often such regulatory molecules are the end products of a biosynthetic pathway which inhibit the early enzymes in the pathway, thus regulating their own formation. This is one of the most fascinating properties of enzymes, which enables fine tuning of enzyme activity in response to changing conditions in the cell. You will learn more about this aspect of enzyme function in Section 6.6.

SAQ 1

Tick [√] mark the correct statements:

a) Enzyme urease which catalyses the hydrolysis of urea was shown to be nonprotein in nature. [ ]

b) The combination of an apoenzyme and a cofactor is known as the holoenzyme. [ ]

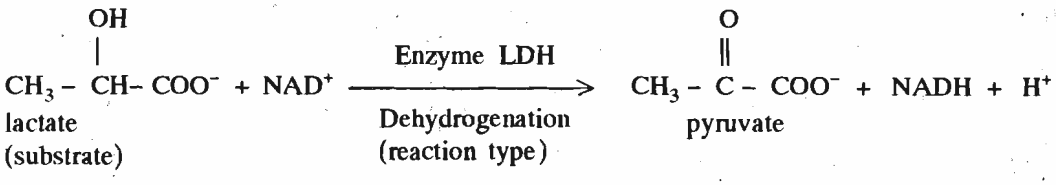
c) Specificity of an enzyme for its substrate involves strength of binding as well as rate of catalysis. [ ]

d) The rates at which different enzymes catalyse their reactions are the same. [ ]

In the foregoing sections you studied the biological importance of a class of proteins, called enzymes. We then described the general characteristics of these biocatalysts and also defined various terms which we have used in this unit. We shall now briefly discuss the classification and nomenclature of the enzymes in the next section.

6.3 CLASSIFICATION OF ENZYMES

Although systematic names are used to designate the enzymes, more often common names are employed to identify them. The common name of an enzyme is generally derived by naming the substrate and the type of reaction it catalyses and adding the suffix *-ase* to it. For example, the oxidation of lactate to pyruvate involves the removal of hydrogen atoms from adjacent atoms, i.e., dehydrogenation. Nicotinamide adenine dinucleotide (NAD) is the oxidising agent used in this reaction. The enzyme which catalyses this reaction is named as lactate dehydrogenase or LDH in short.



isoleucyl- tRNA synthetase, carries the systematic name, **L-isoleucine: $tRNA^{ile}$ ligase (AMP-forming)**. The systematic name indicates that L-isoleucine is joined to

Enzymes have been classified on the basis of the type of reactions they catalyse and we shall describe the six major classes of enzymes which have been identified. This classification is based on the reactions they catalyse and the substrates they act on. We shall illustrate each class of enzyme with an example.

Oxidoreductases

This class of enzymes participates in physiological oxidation- reduction processes i.e., they catalyse electron transfer. An example of this class is the enzyme, **alcohol:NAD oxidoreductase**, which indicates that alcohol acts as the electron donor and NAD^+ as the electron acceptor:

$$\text{Ethyl alcohol} + NAD^+ \xrightarrow{\text{Enzyme}} \text{Acetaldehyde} + NADH$$

This enzyme is also known by its trivial or common name, alcohol dehydrogenase. You would, however, observe that the common name does not completely describe the substrates undergoing the reaction.

Transferases

This class of enzymes catalyses the transfer of a chemical group from one substrate to another. The transferred groups could be amino, methyl, alkyl, acyl, sulphate or phosphate, etc. A typical example is an enzyme commonly designated by its trivial name, hexokinase. The systematic name of this enzyme is **ATP: D-hexose 6-phosphotransferase**, indicating that ATP is the phosphate donor, D-hexose is the phosphate acceptor and the transfer occurs to the hydroxyl on 6-carbon position of hexose.

$$\text{ATP} + \text{D- hexose} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{D- hexose 6- phosphate}$$

Hydrolases

A very large number of enzymes catalyse hydrolytic reactions. Many of the digestive enzymes, such as amylase, sucrase, lipase and all the proteases, which cause the breakdown of food materials, belong to this group. An enzyme of this group, commonly known by its trivial name, pancreatic lipase, degrades lipids and is known by its systematic name **triacylglycerol acylhydrolase**.

Pancreatic lipase is virtually specific for the hydrolysis of primary acyl groups i.e., at C-1 and C-3 positions of triacylglycerol. As a result of this 2-monoacylglycerols are the main end products of triacylglycerol digestion.

$$\text{Triacylglycerol} + H_2O \xrightarrow{\text{Pancreatic lipase}} \text{2- monoacylglycerol} + 2 \text{ fatty acids}$$

Lyases

These are enzymes which catalyse the elimination of chemical groups without hydrolysis, resulting in the formation of a double bond. An enzyme known by its common or trivial name, fructosebisphosphate aldoase, is an example of this type. The full systematic name of this enzyme is **D-fructose- 1, 6-bisphosphate D-glyceraldehyde 3-phosphat- -lyase**, signifying that the substrate D-fructose- 1,6-bisphosphate is degraded in such a way as to give D-glyceraldehyde- 3-phosphate as a product.

$$\text{D-fructose-1,6-bisphosphate} \xrightarrow{\text{Fructose-bisphosphate aldoase}} \text{D-glyceraldehyde 3-phosphate} + \text{dihydroxyacetone phosphate}$$

Isomerases

Enzymes of this class catalyse isomerisations, and include racemases, epimerases and mutases. An enzyme known by its trivial name, triosephosphate isomerase, has the systematic name of **D-glyceraldehyde 3-phosphate ketol-isomerase**, signifying that the aldose, D-glyceraldehyde 3-phosphate is converted to its isomer ketose i.e., dihydroxyacetone phosphate.

$$\text{D- glyceraldehyde 3- phosphate} \xrightarrow{\text{Triosephosphate isomerase}} \text{Dihydroxyacetone phosphate}$$

Ligases (synthetases)

The function of this class of enzymes is to join together two molecules at the cost of energy generated by the hydrolysis of a high energy bond. An enzyme known by its trivial name,

isoleucyl- tRNA synthetase, carries the systematic name, **L-isoleucine: $tRNA^{ile}$ ligase (AMP-forming)**. The systematic name indicates that L-isoleucine is joined to isoleucine-specific tRNA acceptor, the process being accompanied by splitting of ATP to give AMP and pyrophosphate.

$$ATP + L\text{– isoleucine} + tRNA^{ile} \longrightleftharpoons AMP + pyrophosphate + L\text{–isoleucyl– } tRNA^{ile}$$

Enzymes have also been assigned four part code numbers. The first number indicates the type of reaction catalysed, as per the classification scheme described above. The second number shows the subclass which refers to the substrates which participate in the reaction or the bond attacked. The third number is indicative of subsubclass, denoting the exact specification of the reaction catalysed, such as the nature of the electron acceptor or the type of chemical grouping removed. The fourth number refers to the serial number of the enzyme, in its subsubclass. As an illustrative example, the number for lactate dehydrogenase is 1.1.1.27 and for hexokinase it is 2.7.1.1.

You would note that although systematic names describe the enzyme system accurately, they are otherwise, unwieldy for day to day use. Hence, old trivial names/common names continue to be used in the biochemical literature.

SAQ 2

Identify hydrolases from the following enzymes. Tick [√] mark the appropriate box.

a) Urease [ ]

b) LDH [ ]

c) Alcohol dehydrogenase [ ]

d) Amylase [ ]

Let us now discuss how enzymes increase the rate of a reaction. We shall briefly describe the various physico-chemical factors that contribute to the catalytic power of enzymes.

6.4 MECHANISM OF ENZYME ACTION

In Section 6.2.2, you learnt about the enormous catalytic power of enzymes, i.e., they are responsible for a very large increase in the rate of a chemical reaction. In this section let us attempt to understand the mechanism of enzyme activity. In the next section, you will learn about the factors that influence the rates of enzymatic reactions.

In general, the enzyme catalysed reactions proceed through various steps, as illustrated in Fig.6.4.

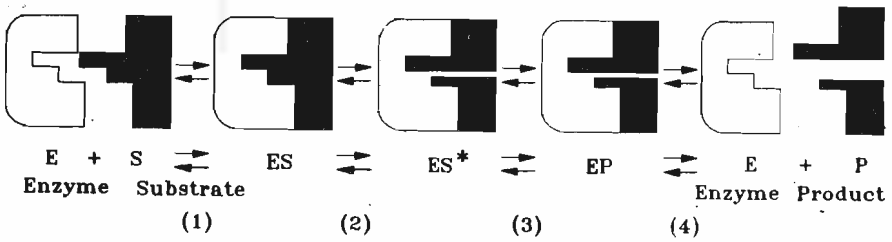


Fig. 6.4: Diagrammatic representation of various steps in enzyme catalysis

In the first step the substrate (S) gets attached to the surface of an enzyme (E), forming an enzyme-substrate complex (ES):

$$E + S \longrightleftharpoons ES$$

After this complex formation, the substrate becomes activated and the bonds in the substrate become polarised:

$$E + S \longrightleftharpoons ES^{*}$$

activated complex

In the next stage of the reaction, the activated complex undergoes a chemical change to form an enzyme-product complex:

$$ES^* \longrightarrow EP$$

The last step involves the release of the products and the enzyme is made available for further catalysis.

$$EP \longrightarrow E + P$$

You will recall from Section 6.2.1, that all the above events will take place at the surface of the enzyme, i.e., at the active site, where the binding and the catalytic groups will be involved in the transformation of the substrate. The most critical step in enzyme catalysis is the formation of the activated complex and the interactions between the enzyme and substrate are generally of noncovalent type, i.e., electrostatic, hydrophobic, hydrogen bonding, etc. Though, in some cases, actual covalent bonds are also formed, the interaction between an enzyme and a substrate has to be weak enough, so that enzyme-product complex can break apart to release the product and thus regenerate the enzyme.

We shall emphasise here that the astronomical rate enhancements associated with enzymes are not a magical effect, but a phenomenon, which can be understood qualitatively in accordance with the principles of physical and organic chemistry. It is also generally accepted that usual reactions such as nucleophilic, electrophilic, homolytic reactions and rearrangements etc. are involved in the transformation of the substrate in enzymatic reactions. Also the efficiency of enzymatic reactions has been accounted for by suggesting factors like proximity effect and orientation effect. However, the precise quantitative contribution of each of the physico chemical factors to final rate enhancement in any particular enzyme is still a matter of guess only.

Let us now discuss, in simple terms, the principles underlying catalytic power of enzymes.

6.4.1 Transition State Theory of Chemical Reactions

A chemical reaction can occur if it is thermodynamically feasible. This means that the conversion of reactants to products must result in a negative free energy change, i.e., ΔG must be negative under the existing conditions, such as the concentrations of reactants and products (Fig. 6.5).

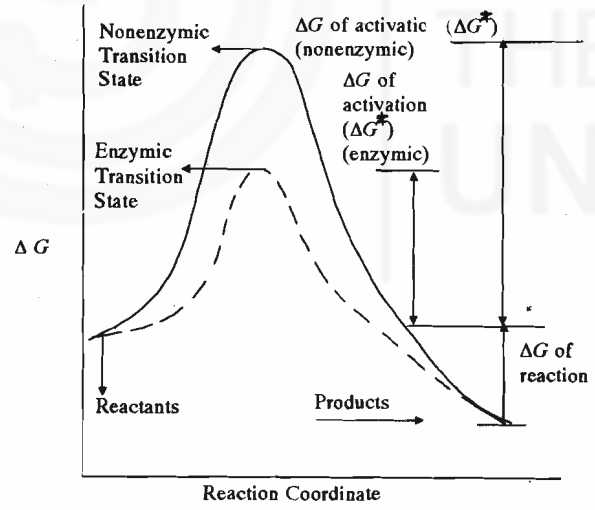


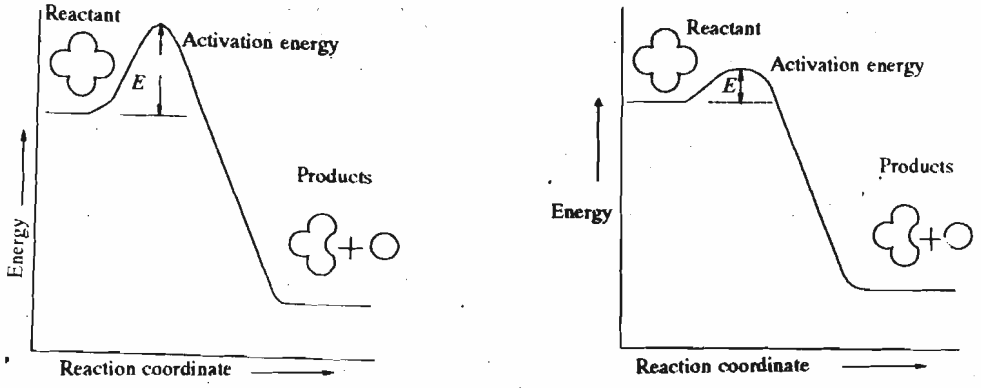
Fig.6.5: Change in free energy level during the progress of a reaction from reactants to products

As is evident from the reaction coordinate, the reactants have to overcome a free energy barrier before the products can be formed. This energy barrier is known as the **activation energy** (E) of the reaction. The molecular structure and conformation corresponding to the peak position in this profile is called the **transition state** of the reaction. In the transition state the chemical bonds are in the process of being formed or broken. It is, therefore, the most activated and hence highly unstable entity in the reaction pathway.

The concept of the transition state helps to express the rate of reaction, in terms of the free energy of activation. Since the molecules in the transition state are the ones which lead to the formation of products, the rate of reaction will depend on the concentration of molecules in

$\Delta G = \Delta H - T\Delta S$

the transition state. For this concentration to increase, with a corresponding increase in reaction rate, the free energy of activation has to decrease (Fig. 6.6). For this to happen, the transition state may be reached with a smaller expenditure of heat or enthalpy of activation ($\Delta H^{\ddagger}$) or a smaller decrease in the entropy of activation ($\Delta S^{\ddagger}$). This is precisely what



(a) No enzyme present (b) Enzyme present

Fig. 6.6: Effect of enzymes on the activation energy. The reactant molecules have to get over the energy barrier to form the products.

enzymes achieve and thus bring about a remarkable increase in the reaction rate. It should be clear from Fig.6.5 and Fig. 6.6, that enzymes only decrease the free energy of activation, so that more and more of reactant molecules pass the energy barrier to form the products. Thus, the enzymes donot change the equilibrium point of the reaction which is related to the free energy of the reaction (ΔG ; Fig. 6.5) but merely speed up the rate at which the equilibrium point is reached.

We shall now discuss in qualitative terms how enzymes cause a decrease in energy of activation.

6.4.2 How Enzymes Lower the Activation Energy

A chemical reaction results from random collisions between reacting molecules. This means the reactants have to come very close to each other. In a nonenzymatic reaction, the probability of such an event is low. Even if the reactants collide, most of the collisions are not effective, i.e., they do not result in a chemical reaction. Only those collisions will be effective and yield products where the reactant molecules have adequate energy and their participating groups in the reaction are properly oriented with respect to each other. However, such a effective collision is a rare event and occurs with a low probability. Further, the proper orientation of groups causes a decrease in the entropy of the system. This decrease in entropy contributes to high values for free energy of activation, which explains the low reaction rate of a nonenzymatic reaction.

An enzyme on the other hand possesses an active site which binds the interacting molecules and thus brings them close enough to react (in effect collide). This is known as the **proximity effect**. As a result of this effect extremely high concentrations of reacting molecules are attained, resulting in a large increase in reaction rates (in effect, increase in collision rates). Thus, enzymes "collect" substrates from the reaction medium and "make them to collide". By constructing model organic compounds in which two interacting groups were attached to a single molecule, the proximity effect of an enzyme has been mimicked. It has been demonstrated that a rate increase of about 10^4 fold can be realised by this factor alone.

The other condition of a fruitful collision, namely the proper orientation of the reacting groups, is also realised by the enzymes. The active site of an enzyme with the help of its binding groups, holds the interacting molecules in a correct rigid orientation with respect to each other. This is known as the **orientation effect**. Thus, substrates are precisely oriented at the active site and hence properly positioned for reaction (in effect collision). This effect has also been imitated and a rate increase of 10^4 observed. Thus, a total rate enhancement of 10^8 results from proximity and orientation effects alone in enzyme catalysis.

With these effects an enzyme overcomes the unfavourable entropic barrier, which is characteristic of nonenzymic reactions. However, the enzyme pays a price to overcome the loss of entropy and it is the binding energy of enzyme- substrate interaction at the active site, which is used to pay for this price. It is thus easy to understand how the enzyme decreases the free energy of activation of a reaction.

Another contributing factor to enzyme catalysis is the **"induced fit"**, which we mentioned in subsection 6.2.3. This suggests that when an enzyme-substrate complex is formed, the conformations of both the enzyme as well as the substrate change. This conformation change in the substrate produces a **strain** in the form of distortion of bond angles and bond lengths which brings it closer to the transition state. This, in turn, reduces the energy of activation required for converting a substrate into its products. We may mention here that using model systems, it has been shown that subjecting a substrate to strain can increase its rate of reaction by a factor of 10^8.

Lastly, the catalytic functional groups at the active site also contribute to rate enhancement. You will recall that many organic reactions are catalysed by H^+ or OH^- ions. However, in a biological medium, with the exception of gastric secretions, the concentration of these ions is very low. At the active site of an enzyme, various acidic or basic groups acting as catalytic groups can act as proton donors or acceptors. They thus effectively catalyse a biochemical reaction, as their simultaneous action on the substrate can be much more significant than the chance encounter of a reactant with an acidic or a basic group in the nonenzymic reaction.

We can summarise here that enzymes are highly effective catalysts because they bring together interacting molecules in a proper orientation. Also the functional groups at the active site provide proton donors and acceptors in high local concentration. These groups are also properly positioned to bring about a reaction.

You can check your understanding of enzymes by attempting the following SAQ.

SAQ 3

Fill in the blanks with appropriate words.

a) Enzymes ..... the rate at which equilibrium is attained in a reaction.

b) Enzymes ..... the activation energy of a reaction.

c) Enzymes overcome the entropic barrier to chemical reaction by ..... and ..... effects.

d) In the ..... state, the chemical bonds are in a process of being formed or being broken.

In the foregoing section we explained how the enzyme molecule is able to bring about a rate enhancement. You will now learn about the kinetics of enzymatic reactions. We shall describe the factors that have a direct effect on the rate of enzymatic reactions.

6.5 ENZYME KINETICS

Enzyme kinetics deals with the rates of enzymatic reactions and the various parameters that govern these rates, such as concentration of substrate, concentration of enzyme, pH, temperature and the presence of various substances that may be inhibitors or activators. Study of enzyme kinetics is important because it throws some light on the mechanism of action of an enzyme. Such a study also provides information on the behaviour of an enzyme in the cellular environment and gives us a clue regarding the regulatory mechanisms available to the organism for fine control of enzyme activity.

Before we describe the factors which influence reaction rates, let us briefly discuss how reaction rates are measured.

Measurement of Reaction Rates of Enzymes

The activity of an enzyme can be measured by monitoring the time- dependence of the chemical change that occurs during an enzymatic reaction. The enzyme is incubated with the substrate under optimum conditions. The reaction proceeds with the disappearance of the substrate and the formation of a new product. These changes are monitored by withdrawing small aliquots and analysing them for the formation of the product or disappearance of the substrate. In some cases such measurements can be made directly on the incubation mixture without the necessity of withdrawing aliquots. For example, in several oxidation-reduction reactions, where NAD or NADP is the electron acceptor, the progress of the reaction can be continuously monitored by following the reduction of NAD (or NADP) to the reduced product NADH (or NADPH) which absorbs at 340 nm. Same principle is applied to monitor many other enzyme catalysed reactions, where either the substrate or the product has a unique and easily measurable absorption spectrum.

Let us now learn how concentration of substrate can alter rate of a biochemical reaction.

6.5.1 Concentration of Substrate

The condition of initial reaction rate is important because it ensures that very little product is present to initiate the reverse reaction, which may complicate results.

In order to outline the effect of substrate concentration on reaction rate, we shall discuss the reaction between a single substrate and an enzyme for the sake of simplicity. If we consider an enzyme as a reactant, then this is equivalent to a chemical reaction between two chemical substances i.e., enzyme and its substrate. However, there is an important difference between enzymic and nonenzymic systems with respect to the dependence of rate on the reactant concentrations. For example, if we carry out a nonenzymic reaction between two reactants A and B, keeping the concentration of A constant and changing the concentration of B, then the initial rate of product formation will be directly proportional to the concentration of the reactant B. If on the other hand the conditions of an enzymic reaction are so arranged that the enzyme concentration is kept constant and the concentration of substrate is changed, then the initial reaction rate varies hyperbolically with increasing substrate concentrations. The rate reaches a maximum velocity at high substrate concentration which remains uneffected by further increase in substrate concentrations. This is illustrated in Fig. 6.7.

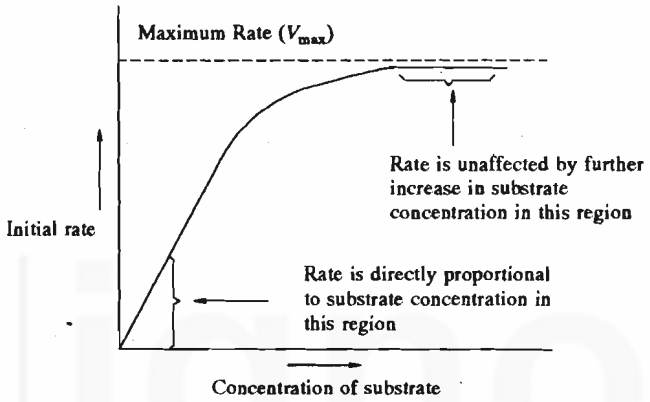


Fig. 6.7 : Variation of reaction rate with substrate concentration keeping enzyme concentration constant

The fact that in an enzymic system, as opposed to a nonenzymic system, the reaction velocity reaches a saturating value at high substrate concentrations can be explained as follows. In a nonenzymic reaction, the reaction rate is dependent on the number of effective collisions between the reactants. The number of such effective collisons would increase in direct proportion to the concentration of one of the reactants, if the concentration of the other reactant is kept constant. On the other hand, in an enzymic reaction the effective collision between the enzyme and the substrate leads to the formation of an enzyme-substrate complex, in which the substrate is firmly bound to the enzyme at its active site. This complex then breaks down to give the product (P) and releases the original enzyme. Since the number of active sites is limited by the enzyme concentration (held constant), the reaction rate will increase with substrate concentration only till all these sites are filled. Under these conditions the system will give maximum possible rate of reaction. Thereafter, there will be no further increase in the rate of reaction.

The shape of the curve as shown in Fig. 6.7, depicting the dependence of reaction rate on the substrate concentration, is a hyperbola. Michaelis and Menton were the first to recognise this relation for enzyme catalysed reactions and put the same in a mathematical form in 1913. This equation for enzyme kinetics is known as the **Michaelis-Menton equation** :

$$\text{Rate of reaction} = v = \frac{V_{max} \times [S]}{[S] + K_m}$$

Where [S] is the molar concentration of the substrate and V_{max} is the maximum possible rate for a given enzyme concentration. K_m, also called Michaelis constant, is numerically equal to the concentration of the substrate at that stage when the observed rate of the enzyme catalysed reaction is one half of the maximum rate i.e., $v = \frac{1}{2} V_{max}$. This will be clear to you from Fig. 6.8. From the above equation, it is clear that rate will tend to acquire a maximum value when [S] is very high.

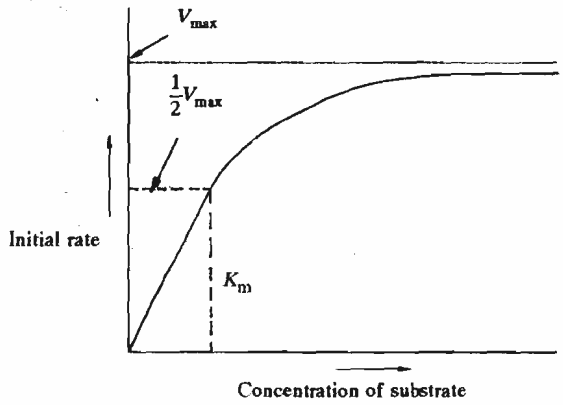


Fig. 6.8 : K_m is equal to the concentration of the substrate required to give an initial reaction rate corresponding to half of V_{max}

We shall now briefly explain what V_{max} and K_m convey in enzyme kinetics.

Significance of V_{max}

At V_{max}, all the enzyme molecules have formed enzyme-substrate complex (ES) and are continuously catalysing the conversion of substrate into the product. Thus at V_{max} value the enzyme is fully saturated. V_{max} values can be used to compare the activity of various enzymes, if they happen to catalyse the same reaction.

Significance of K_m

As we have mentioned already, K_m is equal to the concentration of the substrate at $\frac{1}{2}V_{max}$.

Since at V_{max} all the enzyme molecules have formed enzyme-substrate complex, it, therefore, follows that the concentration of substrate (K_m) required to convert half of enzyme molecules to ES complex, is a measure of the affinity of the enzyme for substrate. A small value of K_m signifies the high affinity of the enzyme for the substrate, since a low concentration of substrate would be needed to saturate the enzyme. Similarly, a large value for K_m would indicate a relatively high concentration of substrate for saturating the enzyme, thus signifying a low affinity of the enzyme for its substrate.

You have thus learnt that increase in substrate concentration has a corresponding affect on the rate up to a certain point, beyond which the rate remains unaffected to any further increase in concentration of substrate. We shall now discuss the role of enzyme concentration in the kinetics of enzymic reactions.

6.5.2 Concentration of Enzyme

In enzymic reactions, the enzyme concentration is generally very low, as compared to the substrate concentration. The rate, therefore, is proportional to the concentration of the enzyme, provided a purified enzyme is used and substrate concentration is very high. Under these conditions, for example, if enzyme concentration is doubled, the rate of conversion of substrate to product shows a corresponding increase (Fig. 6.9).

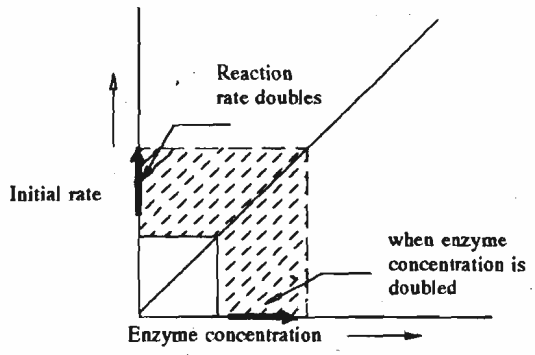


Fig. 6.9 : Variation of rate of catalysed reaction with concentration of the enzyme

Let us now discuss how catalytic activity of an enzyme is affected by the pH of the solution in which the enzymic reaction occurs.

6.5.3 Effect of pH

An enzyme catalysed reaction is strongly influenced by the hydrogen ion concentration, i.e. pH of the reaction mixture. Usually a small change in pH causes an appreciable change in the ability of an enzyme to function as a biological catalyst. The curve showing rate of enzyme catalysed reaction against change in pH of the reaction mixture is generally bell-shaped (Fig. 6.10). You would observe that enzyme activity is maximum only in a narrow pH range and decreases at both higher and lower pH.

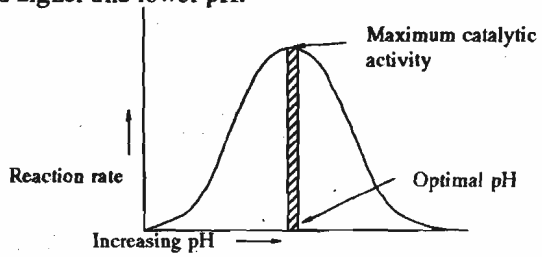


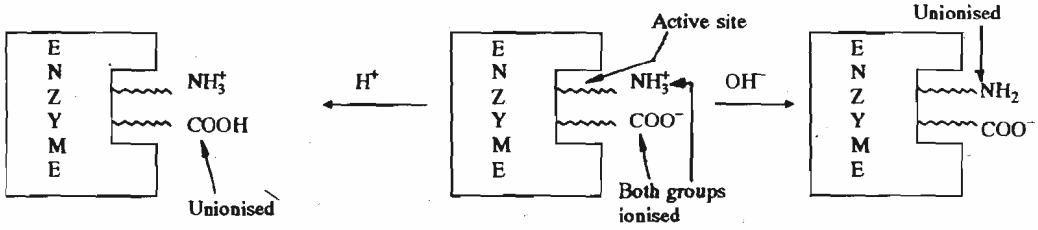
Fig. 6.10 : Effect of pH on the activity of an enzyme

The pH where an enzyme shows maximum reaction rate is known as its **optimal pH**. Most enzymes have a maximum catalytic activity around pH 7, which happens to be the pH of most of the biological fluids. However, many enzymes do have maximum activity at higher or lower pH than this. An important example is that of pepsin, which is a digestive enzyme of the stomach. It has maximum activity around pH 1.5, which is the pH of gastric juice. We have listed optimal pH values of some enzymes in Table 6.3.

Table 6.3

Enzyme	Optimal pH values of some enzymes
Pepsin	1.5
α-Glucosidase	5.4
Urease	6.7
α-Amylase	7.0
Carboxypeptidase	7.5
Trypsin	7.8
Alkaline phosphatase	9.5

In qualitative terms, this effect of pH on enzyme activity can be attributed to several factors, some or all of which may operate at the same time. The change in pH can cause denaturation of the enzymic protein, rendering it inactive. Further, you may recall from subsection 6.2.1 that active site of an enzyme consists of amino acid side chains which form binding and catalytic groups. The catalytic activity may be possible only when these side chains are in a correct state of ionisation. The state of ionisation of these side chains would in turn depend on the pH of the reaction mixture, thus influencing enzyme activity. We will illustrate this with an example. Let us suppose an enzyme needs two charged/ionised side chains ($\sim\!\!\sim NH_3^+$ and $\sim\!\!\sim COO^-$) at its active site for catalysis. This ionisation state would be possible only in a particular pH range, corresponding to the optimal pH:



Below the optimal pH (decreased or no catalytic activity)

Optimal pH (maximum catalytic activity)

Above the optimal pH (decreased or no catalytic activity)

As you would observe from the above representation, at acidic pH (lower pH value than optimal pH) the $-COO^-$ group gets protonated to $-COOH$ and at basic pH (higher pH than optimal pH), the $-NH_3^+$ group gets deprotonated to $-NH_2$. Consequently in both these situations the catalytic activity decreases for lack of proper combination of the ionisable side chains in the active site. Lastly, the effect of pH on enzyme activity could also result from a change in ionisation state of a charged substrate with change in pH, affecting its binding to the enzyme.

You will now learn about the fourth factor which influences the rate in an enzyme catalysed reaction.

6.5.4 Effect of Temperature

You will recall that increase in temperature leads to increased frequency of collisons between reactants. Thus rates of chemical reactions increase with increase in temperature. However, in the case of enzymatic reactions this increase occurs only within a narrow temperature range beyond which the rate decreases sharply, suggesting that the enzyme may have an **optimal temperature**, where the rate is maximum as shown in Fig. 6.11.

For most of the enzymes optimal temperatures are at or above those of the cells in which they occur.

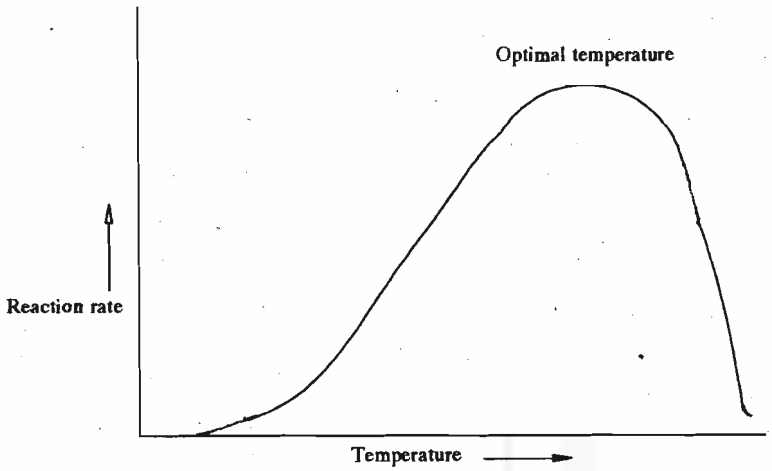


Fig. 6.11 : Effect of temperature on the activity of an enzyme

As you would have observed, enzyme activity increases with temperature. This, evidently, is due to increase in the number of collisions between the enzyme and substrate molecules, and also due to an increase in the energy of these collisions. With further increase in temperature, enzyme activity decreases sharply. The latter is due to the denaturation of the enzyme proteins with heat. We can thus say that though reaction rate increases with temperature, the rate of inactivation of the enzyme also increases simultaneously. The concept of optimal temperature for enzyme activity does not seem to be a satisfactory one, since it is the result of an increase in enzyme activity due to increase in temperature, and loss of activity due to inactivation of the enzyme. Since the latter factor is time dependent, the optimal temperature will itself depend upon the time taken for rate determination at a particular temperature. The change in rate of reaction for any 10° rise in temperature is known as its Q_{10}, or temperature coefficient value. This value is close to 2 for most chemical reactions. For, enzymatic reactions on the other hand the Q_{10} values are lower.

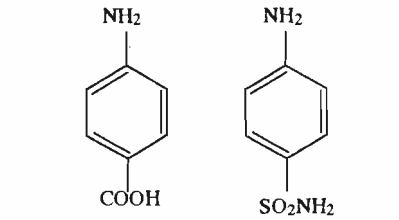
The activity of most of the enzymes is usually destroyed by heat. However, some exceptions are the enzymes of bacteria, living in hot springs at temperatures of 60-80°C.

In the above subsections you learnt about the factors which effect enzyme activity. We shall now describe the enzyme **inhibitors**, which can decrease the rate of enzymatic reactions.

6.5.5 Enzyme Inhibition

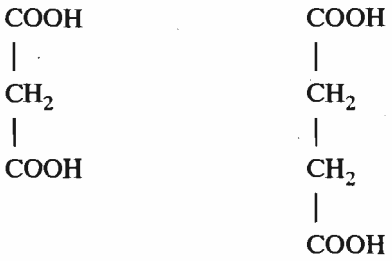
Enzyme activity is inhibited by several factors, such as unfavourable pH conditions, rise (or sometimes fall) in temperature or presence of protein precipitants, such as alcohol, acetone and trichloroacetic acid. Inhibitory action, caused by these agents is of a general nature and nonspecific. Of greater interest in terms of structure and function of enzymes, are inhibitors known as competitive and noncompetitive inhibitors.

Competitive inhibitors have a structure resembling that of the substrate. The structural similarity between the substrate and the inhibitory compound enables the inhibitor to compete with the substrate for binding to the active site of the enzyme. This prevents the access of the substrate to the active site and the formation of ES complex. Since the substrates of enzymes are generally metabolites, participating in metabolic transformations in the cell, competitive inhibitors are known as **antimetabolites**. One of the best known examples of a competitive inhibitor is sulphanilamide. This is a competitive inhibitor of *p*-amino benzoate, a compound utilised in the synthesis of folic acid coenzymes essential for the transfer of one carbon fragments. It is, therefore, easy to understand why sulphanilamide is successfully used as a drug, inhibiting the growth of bacteria. In fact many other drugs also work by acting as competitive inhibitors in enzymatic reactions necessary for the growth and survival of a large number of bacteria.



p-Amino benzoic acid Sulphanilamide

Another well known competitive inhibitor is malonic acid, which inhibits the oxidation of succinic acid to fumaric acid by the enzyme succinic dehydrogenase. The competitive action of malonic acid against succinic acid can be appreciated by a comparison of their structures, which are closely similar.



Malonic acid Succinic acid

It is possible that the active site of the enzyme mistakenly accepts malonic acid instead of succinic acid as its substrate, causing competitive inhibition. You will find a diagrammatic representation of competitive inhibition in Fig. 6.12.

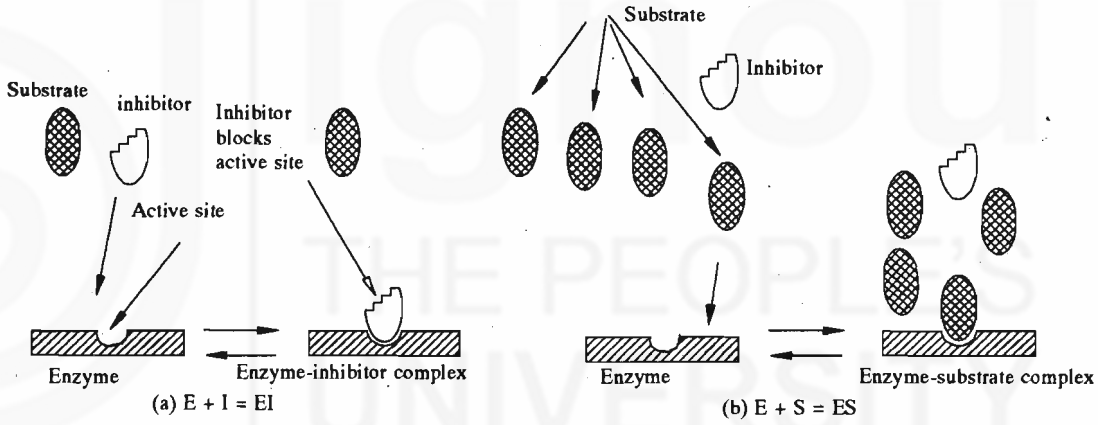


Fig. 6.12 : a) Formation of an enzyme-inhibitor complex
b) By increasing substrate concentration, ES concentration gets increased and EI concentration decreases which reverses the effect of the inhibitor

A characteristic feature of competitive inhibition is that it can be reversed by increasing the substrate concentration. We can detect competitive inhibition by a study of the rate of enzymatic reaction in the presence, and in the absence of a competitive inhibitor. You can

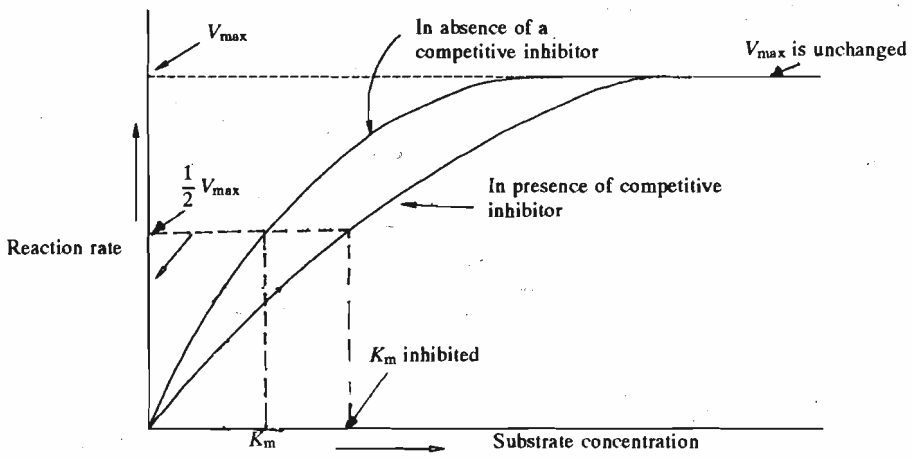


Fig. 6.13 : When a competitive inhibitor is present, V_{max} remains unchanged but K_m is increased

observe from Fig. 6.13 that V_{max} remains unchanged in the presence of a competitive inhibitor, whereas K_m is increased, since a higher substrate concentration would be needed to out-compete with the inhibitor and to achieve the saturation of half of the enzyme molecules.

Noncompetitive inhibitors inhibit enzyme action by combining with a group essential for the activity of the enzyme or by removing a metal ion involved in the activity of the enzyme. These compounds act by converting the enzyme into an inactive or less active form. Such inhibitors cannot be displaced by excess substrate and hence are called non competitive inhibitors. Since their action does not involve displacement of the substrate, they have no effect on the K_m of the substrate. However, since the inhibitor makes the enzyme less active, V_{max} is affected.

SAQ 4

Tick [√] mark the correct statement.

Increasing the temperature of an enzymatic reaction is accompanied by

a) activity remaining constant [ ]

b) activity increasing continuously [ ]

c) activity decreasing continuously [ ]

d) activity first increasing and then decreasing [ ]

SAQ 5

Tick [√] mark the appropriate statement.

A competitive inhibitor of an enzyme acts by

a) modifying one of the amino acid residues of the enzyme [ ]

b) modifying structure of the substrate [ ]

c) competing with the substrate to bind to the active site of the enzyme [ ]

d) increasing temperature of the reaction mixture [ ]

We have described the various factors that alter the rate of an enzymatic reaction, and you studied about enzyme inhibitors also. You will recall that enzymes bring about enormous changes in the rates of biochemical reactions. This makes life possible for an organism. However, their importance for a living system is not only limited to increasing the rates of biochemical reactions. Their activity can also be controlled or **regulated**. This is highly significant in a living system, for rates of individual biocatalytic reactions can be controlled and combined into different metabolic pathways. These individual metabolic pathways are in turn integrated into an overall metabolic system in the living organism. Let us now discuss more about the regulation of enzyme activity in the following section.

6.6 REGULATION OF ENZYME ACTIVITY

Lehninger defined the living cell as a self assembling, self adjusting, self perpetuating isothermal system of molecules that exchanges matter and energy with its environment. The cell accomplishes this by a process called metabolism, in which many consecutive chemical reactions are organised into metabolic pathways. You will recall that metabolism consists of anabolism and catabolism. The term anabolism refers to those pathways of metabolism in which low molecular weight precursors are transformed into more complex substances which include carbohydrates, lipids, nucleic acids and proteins, leading to the synthesis of new cellular components and overall growth. Catabolism on the other hand is the process by which complex and simple substances are broken down in other metabolic pathways to produce energy, either in the form of heat or in the form of high energy compounds. Both the anabolic and catabolic pathways are interconnected.

It is self evident that such a complex system of biochemical transformations has to be so regulated that concentrations of certain key metabolites are controlled, both in terms of time and space, to direct metabolism in a desired direction, and not allow it to drift into the ultimate products of aerobic metabolism i.e., carbon dioxide and water. Since metabolism is driven by enzymes, regulation of metabolism necessitates regulation of enzyme action. We shall now look at some of the principal mechanisms by which enzyme activity is regulated.

6.6.1 Regulation by Substrate or Product

The intracellular concentration of a substrate can sometimes regulate the action of the corresponding enzyme. Such regulation is possible when the K_m value of the enzyme is much higher than the intracellular concentration of the substrate, so that enzyme activity is first order with respect to substrate concentration.

The product of an enzymatic reaction is expected to have a structure resembling that of the substrate and may act as an inhibitor of the reaction, when it accumulates in sufficient concentration. However, the significance of such product inhibition in enzyme regulation cannot be very high since one would expect the product to accumulate to a high concentration before significant inhibition is achieved. Also it is difficult to conceptualise the control of a metabolic pathway, if the product of one particular enzyme inhibits that enzyme independently of the needs of the whole pathway.

An important biological mechanism which controls enzyme activity is **allosteric regulation**. This interaction may bring about inhibition or stimulation of activity by altering the activity of key enzymes. This is possible when enzymes interact with the molecules produced in the cell. Let us explain more about this type of regulation.

6.6.2 Allosteric Regulation

Regulation of some of the biosynthetic pathways occurs by this mode. In such cases, the **first enzyme** of a biosynthetic pathway is strongly inhibited by the **end product** of that pathway (also called feedback inhibition). For example, aspartate carbamoyl transferase, the enzyme which catalyses the first step in pyrimidine biosynthesis in *E. coli*, is inhibited by the final product of this pathway, which is CTP, a pyrimidine nucleotide (Fig. 6.14).

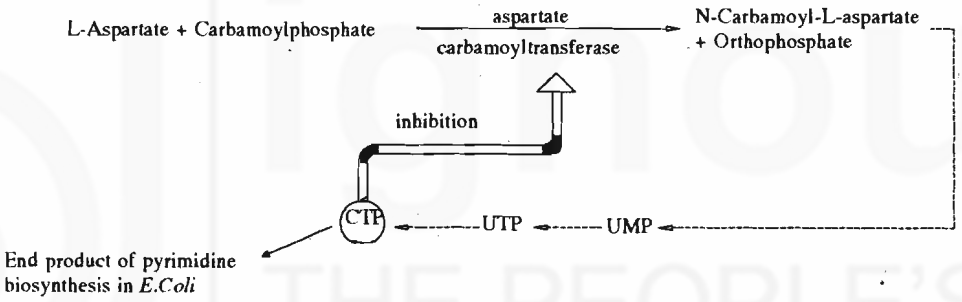


Fig. 6.14 : Inhibition of the first enzyme by the end product of the pathway

Interestingly ATP which is a purine nucleotide, activates this enzyme (Fig. 6.15). Thus, as a result of the opposing action of these two types of nucleotides, production of purine and pyrimidine nucleotides is properly balanced for the synthesis of nucleic acids. On the basis of

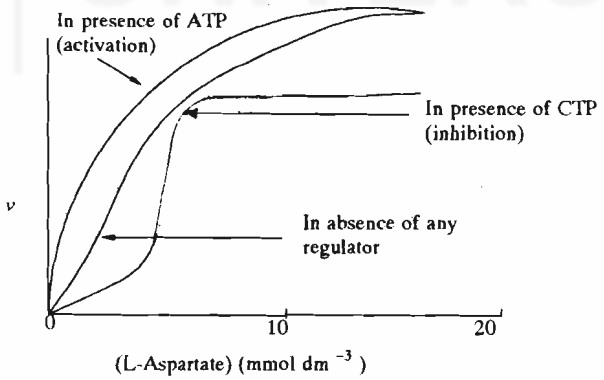


Fig. 6.15 : Effect of CTP and ATP molecules (regulators) on the kinetics of aspartate carbamoyltransferase from *E. Coli*

work carried out on several other pathways, the following general conclusions regarding this type of regulation have emerged.

- **Only the first enzyme** of the biosynthetic pathway is affected, due to the feedback inhibition **by the final product** of the pathway.
- This final product of the metabolic pathway bears no structural resemblance to the substrate or product of the first enzyme, thus, providing a mode of enzyme regulation independent of the substrate or product (also refer to subsection 6.6.1).

- The regulatory molecules, also called **effectors**, being structurally different from the substrate or product of the first reaction, do not bind to the active site of the first enzyme, but would perform their regulatory function by binding at another site on the enzyme, called the **allosteric site**. This binding is reversible. Enzymes so regulated are called **allosteric enzymes**.
- Such allosteric enzymes do not exhibit normal Michaelis-Menton or hyperbolic kinetics. In such cases, the plot of velocity against substrate concentration shows sigmoidal behaviour (Figs. 6.15 & 6.16). This behaviour indicates that at certain concentrations of the substrate, the enzyme activity is much more influenced by changes in concentration of the substrate, than would be the case for an enzyme characterised by normal kinetic behaviour. Sigmoidal kinetics of a regulatory enzyme can be changed to hyperbolic kinetics by subjecting the enzyme to physical or chemical treatment (Fig. 6.16).

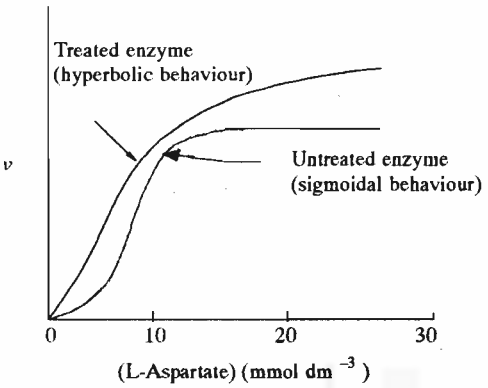


Fig. 6.16 : Comparison of kinetics of the treated and untreated regulatory enzymes, aspartate carbamoyltransferase from *E.Coli*

- Regulatory enzymes generally posses an oligomeric structure like haemoglobin (subsection 5.4.6) which is an oxygen transport protein of blood. The multiple subunits which constitute the oligomeric structure of these enzymes, are held together by weak noncovalent forces. The binding of the regulator molecule to such an enzyme at the allosteric site brings about a reversible conformational change i.e., a change of shape in the enzyme subunit, causing a change in the structure of the active site (Fig. 6.17), leading to alteration in enzyme activity.

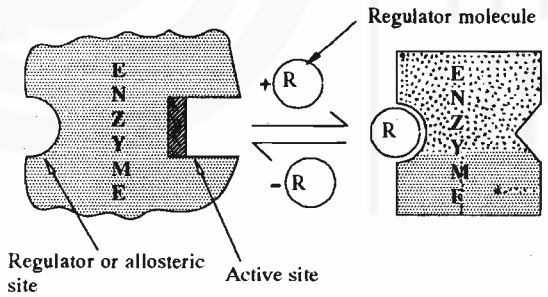


Fig. 6.17 : Diagrammatic representation of the reversible structural change in a regulatory enzyme, brought about by a regulator

You will recall that in the case of haemoglobin, a plot of percent oxygen saturation against oxygen pressure is sigmoidal in nature (Fig. 5.9). The sigmoidal kinetics of regulatory enzymes denotes interaction between subunits, so that changes in the active site structure of the first subunit would affect active site structures of other subunits through rearrangement of noncovalent interactions. Such interactions between subunits, in response to the binding of a regulator molecule to the allosteric enzyme, are called **cooperative interactions.**

Regulation of enzyme activity by conformational changes in the enzyme, induced by the binding of regulator to its allosteric site, is possibly the most important means of metabolic regulation or control. Another mechanism by which enzyme activity is regulated, involves reversible covalent modification of the enzyme. Let us understand this mechanism in the next subsection.

6.6.3 Regulation by Reversible Covalent Modification of the Enzyme

Several enzymes, crucial to biosynthetic and degradative pathways, are regulated by reversible covalent modification of their side chains. These enzymes are present in two different forms, possessing different catalytic efficiency. These forms are interconvertible by the action of other enzymes, some of which catalyse the modification of the side chains, whereas others catalyse reversal of this modification.

We shall illustrate this with two enzymes, phosphorylase and glycogen synthase. These enzymes are involved in the degradation and biosynthesis of glycogen, respectively. The enzyme phosphorylase catalyses the following reaction:

$(\text{Glycogen})_n$ + orthophosphate $\rightleftharpoons$ $(\text{Glycogen})_{n-1}$ + α – D – glucose 1 – phosphate

The enzyme phosphorylase has two forms. The active form (**a**) and the inactive form (**b**). The only structural difference between the two forms is that side chain of serine, at position 14 in the sequence of the enzyme, is phosphorylated in the form **a** but not in the form **b**. This phosphorylation of the enzyme is catalysed by the enzyme phosphorylase kinase, in presence of ATP and Mg^{2+}. The reversal of this process i.e., the conversion of phosphorylase **a** to phosphorylase **b** is accomplished in the presence of another enzyme, phosphorylase phosphatase, which catalyses the removal of the phosphoryl group. These reactions are schematically represented in Fig. 6.18.

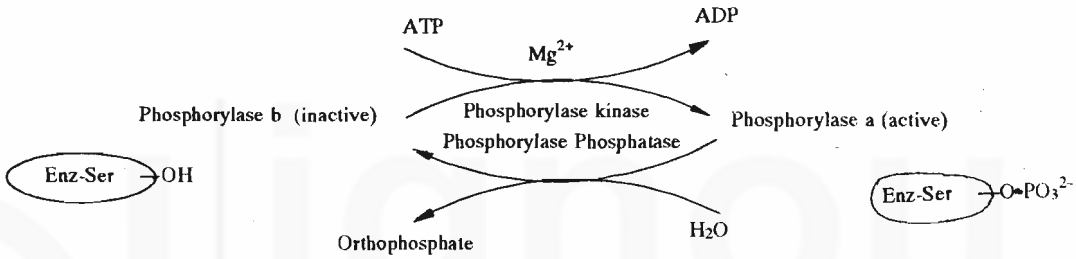


Fig. 6.18 : Schematic representation of the interconversion of phosphorylase a and phosphorylase b

Similarly the other enzyme, glycogen synthase, which catalyses the synthesis of glycogen (as opposed to phosphorylase, which catalyses its breakdown), is converted to an inactive form by phosphorylation, a process which also leads to the activation of phosphorylase. It is thus clear that the simultaneous activation of phosphorylase and inactivation of glycogen synthase in a reversible manner, are complementary processes that allow a well integrated control of glycogen metabolism.

Reversible conversion of active to inactive enzyme forms is a potent mechanism of enzyme regulation. The amounts of the active and inactive forms of the enzyme increase or decrease rapidly, because their interconversion is enzyme catalysed. This also results in a large amplification of the initial signal, where a series of modifying enzymes act in succession to promote a final metabolic event. The continuous activation and inactivation of the enzyme also enables the enzyme system to be more responsive to metabolic requirements.

SAQ 6

Tick [√] mark the following statements as true or false.

a) Allosteric effectors function by binding not to the active, but to an alternative site on the enzyme.

[True/False]

b) Enzyme phosphorylation can result either in a more active or a less active enzyme.

[True/False]

c) In the feed back mechanism the end product of the metabolic pathway does not inhibit the first enzyme of the pathway.

[True/False]

d) Reversible interconversion of an active enzyme to the inactive form does not regulate enzyme activity.

[True/False]

You will now briefly learn that some enzymes can exist in more than one form, which differ in their kinetics, as well as regulatory properties. Genetic factors are responsible for these different forms, and each form is tuned to a function in a specific tissue.

6.7 ISOENZYMES

Sometimes different forms of an enzyme, catalysing the same reaction, occur in a species. They are called **isozymes** or **isoenzymes**. They may arise from minor modification of the same amino acid sequence or they may have different amino acid sequences. These changes are genetically determined. **The term isoenzyme is reserved for those forms of an enzyme which catalyse the same reaction, are isolated from the same species and differ in amino acid sequences because of genetic reasons.** The isoenzymes generally differ in their kinetic and regulatory properties and their distribution is tissue specific. This suggests that the kinetic and regulatory properties of a particular isoenzyme are attuned to the metabolic requirements of a particular tissue, where it is found in abundance. The nomenclature of the isoenzymes is based on their electrophoretic mobilities towards the anode. Thus hexokinase isoenzymes are numbered I to IV, with isoenzyme I having the lowest mobility. Isoenzymes of lactate dehydrogenase represent an interesting case of the origin of isoenzymes. All isoenzymic forms of this enzyme consist of four subunits. These subunits are of two different types, α and β. These combine in five different ways viz. α_4, $\alpha_3\beta$, $\alpha_2\beta_2$, $\alpha\beta_3$ and β_4, to respectively produce five different isoenzymes, LDH-1 to LDH-5 (Fig. 6.19). LDH-1 has the highest electrophoretic mobility towards anode. LDH-1 is predominently present in heart and LDH-5 is mostly present in skeletal muscle and liver. These isoenzymes differ both in their heat lability and sensitivity to inhibition by excess substrate.

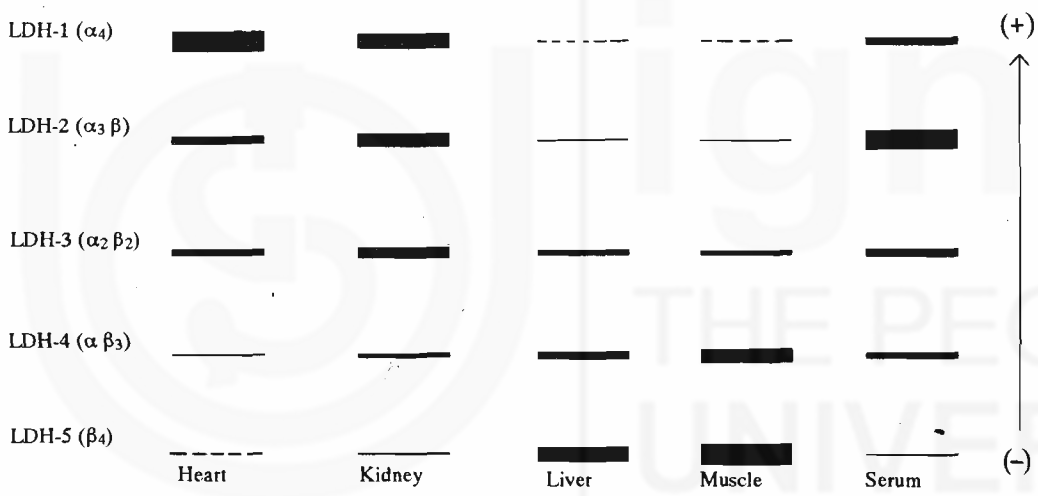


Fig. 6.19 : Electrophoretic mobility of isoenzymes of lactate dehydrogenase

Several enzymes such as alkaline phosphatase, hexokinase, amylase, and glucose 6-phosphate dehydrogenase, exist in isoenzymic forms, which are, however, not as exhaustively studied as those of lactate dehydrogenase.

SAQ 7

Tick [√] mark the most appropriate statement.

Isoenzymes are:

a) electrophoretic variants of the same enzyme. [ ]

b) electrophoretic variants produced by modification of side chains. [ ]

c) electrophoretic variants of the same enzyme which are genetically determined. [ ]

d) different enzymes acting on the same substrate. [ ]

In this unit we mainly discussed the role of enzymes as biocatalysts, regulating metabolic pathways in the organism. However, you may recall from Section 6.2 that enzymes have many commercial, as well as medical uses also. We will attempt to give you some idea of the significance of enzymes as important "tools" in medical science. Let us, therefore, give you a brief account of their role in health sciences.

6.8 ENZYMES IN HEALTH SCIENCES

In the present times enzymes are used to diagnose and to treat diseases. Enzymology is an essential part of the day to day life of modern clinicians. The diagnostic value of certain enzymes arises from their differential distribution between the blood plasma and cells of other tissues. For example, the enzymes involved in blood coagulation are found exclusively in the plasma. On the other hand, many other enzymes are present in much higher concentrations in the tissue cells, than in blood. These are released into the blood and various biological fluids only when there is routine destruction of the cells. Their normal levels in plasma are insignificant, being more than one million times lower than their concentration in the cells. In case of cell destruction or injury by such cases as a damaged heart or uncontrolled growth of cancer cells, the plasma levels of these cellular enzymes are elevated significantly. These changes in plasma concentration of particular enzymes are estimated by the clinicians and used not only to detect cell damage but also to suggest the site of cell damage. The degree of elevation of plasma concentrations of these enzymes also gives a clue to the extent of cellular damage. These enzyme assays have become a critical diagnostic tool in the detection of heart, liver, pancreas, skeletal muscles, bone and malignant diseases. In Table 6.4 you will find a list of some enzymes, clinical assay of which are used for detecting particular diseased states.

Table 6.4 : Enzymes assayed in medical diagnostics

Enzyme	Used in determination of
Lactate dehydrogenase (LDH)	heart or skeletal muscle damage
Alkaline phosphatase	liver and bone disease
Serum glutamate oxaloacetate transaminase (SGOT)	heart and liver disease
Creatine phosphokinase (CK)	myocardial infarction and muscle diseases
Acid phosphatase	cancer of the prostate
α-Amylase	pancreatitis

We shall illustrate the use of enzyme assay in myocardial infarction, which in simple language means a heart attack. In this clinical condition the blood supply to the heart muscle is blocked and some of the cells of the heart muscle die, thereby, releasing an abnormal amount of their enzymes into the blood. Monitoring the blood concentration of three enzymes — CK, SGOT and LDH, helps a physician in identifying a heart attack and its severity. You will find a graph of the concentration of these enzymes after a heart attack in Fig. 6.20.

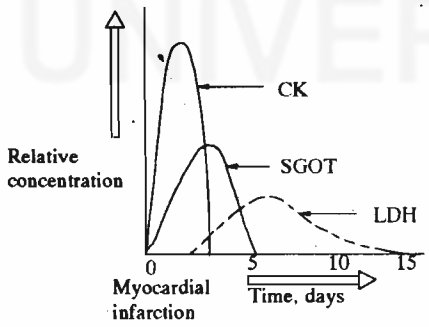


Fig. 6.20 : After a heart attack, concentration of CK increases rapidly and then falls. SGOT level also increases rapidly and then falls. One to two days after an attack, LDH concentration begins to increase, gradually rises for three to four days, and then decreases gradually over the next seven to ten days.

Enzymes also find extensive use as laboratory reagents. A common test, involving use of enzymes, is to measure glucose concentration in urine, as in case of diabetes. Two enzymes, namely glucose oxidase and peroxidase, are used in this test. They are contained on a test strip which is momentarily dipped in the urine sample. A colour change, on the strip after sometime, is compared to a colour scale to measure the concentration of glucose. In this test glucose oxidase catalyses conversion of glucose to gluconic acid and hydrogen peroxide. Peroxidase then catalyses the reaction of hydrogen peroxide and *o*-toluidine to give a coloured product, the colour intensity of which indicates the glucose concentration.

$$\text{D-Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$o\text{-toluidine} + H_2O_2 \xrightarrow{\text{peroxidase}} \underset{\text{(schiff base)}}{\text{coloured product}} + H_2O$$

Enzymes are also used in treatment of many medical problems. For example, trypsin and chymotrypsin are used in severe burns, for they decompose the proteins of the clotted blood, pus and dead skin on the burned areas. Similarly many nasal sprays contain enzymes to clear up congestion. And probably you might have used some digestive enzymes when you had a heavy spicy meal, just to aid your digestion.

6.9 SUMMARY

- Enzymes are biocatalysts which increase reaction rates enormously. They are responsible for most of the chemical reactions which take place in the living organisms.
- Enzymes are protein in nature, with considerable diversity of structure. Some enzymes are simple proteins, whereas many are conjugated proteins.
- Many enzymes (conjugated proteins) require a nonprotein part without which they cannot function. These nonprotein parts, called cofactors, are either metal ions or low molecular weight organic compounds. These organic cofactors are mostly derived from B group of vitamins. The organic cofactors are generally known as coenzymes.
- Enzymes are characterised by an active site, where the substrate (reactant) binds, undergoes transformation and then the products are subsequently released. The active site consists of amino acid side chains, some of which act as binding groups while others act as catalytic groups. The amino acid side chains of catalytic groups, are of polar type only.
- Enzymes are characterised by high catalytic efficiency, remarkable specificity and regulatory properties. Enzymes can increase reaction rates a billion fold, which enables biochemical reactions to occur under physiological conditions. They are highly specific, both with regard to the substrate and the reaction they catalyse. The degree of specificity varies from one enzyme to another. The regulatory properties of enzymes enables fine tuning of enzyme activity in response to the needs of the cell, under changing physiological conditions.
- Enzymes have been grouped into six major classes on the basis of the nature of the reaction they catalyse. These classes are: Oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Each enzyme has been assigned a four number identification code, indicating the major class, type of substrate or the bond cleaved, the actual reaction catalysed and the serial number of the enzyme in its subsubclass.
- Enzymes increase reaction rate enormously by lowering the energy of activation of the reaction. This is achieved by bringing the reactants in close proximity and in a correct orientation for the reaction to occur. Also at the active site, substrate is subjected to stress and strain, which facilitates bond making or breaking. The energy for this is derived from favourable enzyme - substrate interactions at the active site.
- Various factors govern the rate of enzymic reactions. The plot of enzyme velocity versus substrate concentration shows a plateau at high substrate concentration, indicating that the reaction rate after reaching a maximum value is independent of further increase in substrate concentration.
- The substrate concentration required for half maximal velocity (V_{max}) is called the Michaelis Constant K_m. This is a useful parameter for determining catalytic potential of an enzyme under physiological conditions and also gives an idea of the affinity of the enzyme for its substrate.
- Enzyme activity is proportional to the concentration of the enzyme and is dependent on pH and temperature of the medium.
- Enzymes are inhibited by reagents which compete with the substrate for the active site. These substances are known as competitive inhibitors. The other type of substances which inhibit the enzyme by inactivating it are called noncompetitive inhibitors.

- Enzymes are regulated by the end product of a biosynthetic pathway. The final end product inhibits the first enzyme of that pathway by binding to an allosteric site which is distinct from the active site. The inhibition results from a conformational change in the oligomeric structure of the first enzyme.
- In another type of regulation, the amino acid side chains of enzymes are reversibly modified, mainly by phosphorylation or dephosphorylation. This produces an active or an inactive enzyme.
- Sometimes enzymes catalysing the same reaction exist in different electrophoretic forms in different tissues of the same species. These different forms are known as isoenzymes and result due to genetic factors. Lactate dehydrogenase is an interesting case of isoenzymes.
- Enzymes have a diagnostic value in medical sciences. Various enzyme assays are employed to confirm, locate and also indicate the severity of a disease in a human being. Enzymes are used as laboratory reagents and are also employed in treating various diseased conditions.

6.10 TERMINAL QUESTIONS

1. How does an oxidoreductase differ from a transferase? Illustrate your answer with an example.
2. How does the principle of competitive inhibition of enzyme action help in designing drugs against bacterial diseases?
3. Describe active site of an enzyme.
4. Discuss the need for regulation of enzyme action.
5. Briefly describe important features of allosteric regulation.
6. Explain what is 'transition state' of a reaction?
7. How does an enzyme contribute to the lowering of energy of activation of a chemical reaction?
8. Describe the origin of isoenzymes of lactage dehydrogenase.
9. What is K_m and what does a low value of K_m signify?

6.11 ANSWERS

Self Assessment Questions

1. b and c
2. a and d
3. a - increase; b - lower; c - proximity and orientation; d - transition
4. d
5. c
6. a - True; b - True; c - False; d - False
7. c

Terminal Questions

1. An oxidoreductase takes part in physiological oxidation-reduction process. On the other hand, a transferase catalyses the transfer of a chemical group, from one substrate to another. Lactate dehydrogenase is an example of an oxidoreductase, and hexokinase is an example of a transferase.
2. Disease causing bacteria sometimes depend upon a particular enzyme reaction for their survival and growth. Such bacteria can be killed by inhibiting the crucial enzyme reaction by a competitive inhibitor, which can be so designed as to partially resemble the substrate. This competitive inhibitor or drug, displaces the real substrate from the active site and inhibits enzyme activity, killing the bacteria.

3. The portion of an enzyme which binds to the substrate and catalyses its chemical transformation is called the active site of an enzyme. The active site has hydrophobic and hydrophilic amino acids which bind to the substrate by hydrophobic, electrostatic and hydrogen bonded interactions. Some of the hydrophilic amino acids, which are charged or uncharged also participate in bond making and bond breaking processes.

4. Metabolic activities of the cell lead to the biosynthesis of cellular components and to the generation of energy needed to sustain life. Such a complex system of biochemical transformations has to be regulated to channelise crucial metabolities into the required pathway and not allow them to drift into the ultimate products of aerobic metabolism i.e., carbon dioxide and water. Since metabolism is driven by enzymes, it stands to reason that regulation of metabolism is essentially regulation of enzyme action.

5. An important feature of allosteric regulation is that the end product of a pathway inhibits the first enzyme of that pathway, by binding to an allosteric site or a site distinct from the enzymic active site. The inhibition results from a conformational change in the oligomeric structure of the first enzyme. Such allosteric enzymes do not show normal kinetic behaviour, for in their case, the plot of rate versus concentration is not a hyperbola but sigmoidal. The sigmoidal kinetics shows that interaction of the substrate at one active site affects other active sites of the oligomeric structure through inter-subunit contacts.

6. The energy profile of a chemical reaction shows that the reactants have to overcome an energy barrier before they are converted into products. The structure and conformation of reactant molecules corresponding to the peak position of this energy barrier is called the 'transition state' of the reaction. This state represents molecules in their most energised form where bonds are in the process of being made or broken.

7. Enzymes lower the energy of activation by bringing the reactants in close proximity, and correctly orienting them towards each other, thus, in effect increasing their concentration by several orders of magnitude. The entropic disadvantage which chemical reactions suffer from, is thus overcome by the enzyme. The energy for this process is derived from favourable enzyme- substrate interactions at the active site. The energy from these interactions is also used to subject the substrate to stress and strain to facilitate the bond making and bond breaking processes.

8. Lactate dehydrogenase isoenzymes consist of four subunits of two types, α and ß, which are coded for by two different genes. These two types of subunits combine in different proportions viz., α_4, $\alpha_3\beta$, $\alpha_2\beta_2$, $\alpha\beta_3$ and β_4, to give the five isoenzymes.

9. Concentration of the substrate expressed in moles per litre that gives half maximal velocity of the enzyme, is known as K_m. A small value of K_m indicates high affinity of the enzyme for the substrate.

UNIT 7 VITAMINS, COENZYMES AND MINERALS

Structure

7.1 INTRODUCTION

You have already learnt from the preceding units that proteins, carbohydrates, fats and nucleic acids are the major constituents of living cells. In Unit 6 we described a specialised class of protein, the enzymes. You are aware by now that enzymes function as biological catalysts and through enzyme regulation, metabolism in the living organism is also regulated. Life, as we mentioned earlier, would not be possible without the invaluable role of these molecules. However, you will recall that many enzyme molecules require cofactors for their functioning. These cofactors can be small organic molecules (coenzymes) or simple metal ions. In this unit you will learn about vitamins and minerals, as many of these molecules perform the role of cofactors. However, the role of vitamins and minerals, in general, is not only limited to being cofactors of enzymes. It is only a part of their job. The cell, for its proper functioning also requires vitamins and some other elements, in trace amounts. These molecules are essential for normal growth, well being and proper functioning of human beings. In this unit we shall briefly describe the vitamins, their deficiency symptoms, and dietary sources, their relationship with coenzymes and their biochemical functions. We shall also discuss the role of minerals and trace elements in the physiology and biochemistry of humans. With the knowledge gained from the study of various biomolecules, especially carbohydrates, lipids and proteins, it will be easier for you to learn about bioenergetics and metabolism. This we shall describe in Block 3, where you will learn how the body converts food into energy. You will be able to relate, more clearly, the role of enzymes, coenzymes and other trace substances in metabolism, after studying Block 3.

Objectives

After studying this unit, you should be able to:

- define vitamins and explain their biological significance,
- classify vitamins into water soluble and fat soluble types,
- describe the biochemical functions of water soluble vitamins and also their role as coenzymes in intermediary metabolism,
- describe the role of fat soluble vitamins, and
- explain the significance of minerals and trace elements in human beings.