
UNIT 4 ENZYMES AND COENZYMES

Structure

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4.1 INTRODUCTION

Enzymes and coenzymes are two of the most important groups of biomolecules, without whose active participation, none of the nutrients discussed in the last three units, can be utilized by the body. As a learner of dietetics, it is therefore essential for you to get the knowledge of different structural and functional aspects of enzymes and coenzymes. This unit focuses on these aspects which will enable you to understand the metabolism of different nutrients which are to be discussed in subsequent units of this course. Remember, this unit along with the next three units forms the basic component of this course.

Objectives

After studying this unit, you will be able to:

- classify enzymes and coenzymes,
- discuss their chemical nature and functions,
- describe the factors on which enzyme activity depends, and
- explain the diagnostic use of enzymes.

4.2 INTRODUCTION TO ENZYMES AND COENZYMES

Let us start by understanding what enzymes are. Enzymes are the *proteins that catalyze biochemical reactions*. Study of these important biochemical reactions was started many years ago, from the time of *Louis Pasteur*, who for the first time demonstrated the fermentation of glucose by yeast. The catalytic agent of yeast cell was subsequently identified and named as *ferment*. At the end of nineteenth century, a cell-free extract of yeast was found to be capable of fermenting glucose by *Buchner brothers*. Considerable advances were made since then in order to properly know the nature of these agents named as *enzymes* and finally their true nature was revealed by *James Sumner* in 1926 after the extraction and crystallization of the enzyme *urease* from jack beans. At present, no less than 150 enzymes have been prepared in crystalline form.

What is the chemical nature of enzymes? Let's find out. We all know that enzymes are proteins. Do you recall the structure of proteins described in Unit 2 earlier? Enzymes are *high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds* as shown in Figure 4.1.

An enzyme can be a large protein made up of several hundred amino acids, or several polypeptides that act together as a unit. Enzymes have molecular weights ranging from 10,000 to 2,000,000.

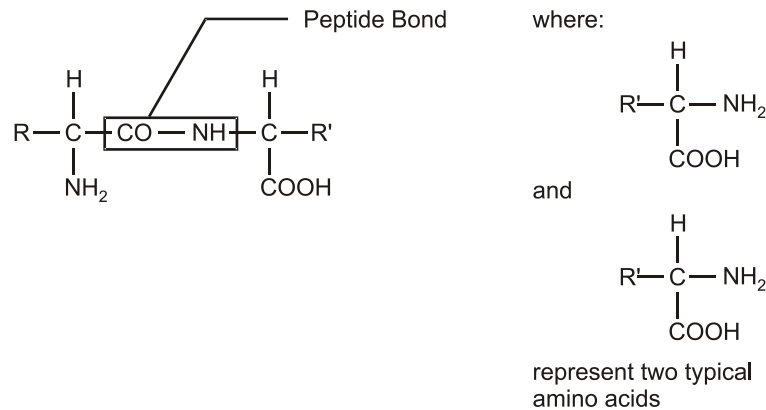


Figure 4.1: Protein structure

Until recently, it was understood that all enzymes are proteins. However, observations made in organelles from plants, yeast, viruses and higher eukaryotic cells, show that RNA can act as an enzyme. Such RNA molecule is called a *ribozyme*. Hence, ribozymes are RNA molecules with catalytic activity. These generally involve transesterification reactions, and most are concerned with RNA metabolism (splicing and endoribonuclease). Recently, a ribosomal RNA component was noted to hydrolyze an aminoacyl ester and thus to play a central role in peptide bond function i.e. having peptidyl transferase activity.

Before we move on to understanding more about enzymes, we should familiarize with the vocabulary and definitions of various terms which you will come across while studying about enzymes.

Definitions of terms related to enzymes

Cofactor	Organic molecules or ions that assist many enzymes in their reactions.
Apoenzyme	The protein portion of an enzyme requiring a cofactor for its reaction.
Holoenzyme	A whole enzyme, as a complete and functional molecule. Generally, a holoenzyme consists of a polypeptide portion (an apoenzyme) and at least one cofactor or another coenzyme.
Enzyme	Proteins that act as catalysts, speeding the rate at which biochemical reactions proceed but not altering the direction or nature of the reactions.
Coenzyme	An organic, nonprotein molecule that binds with an apoenzyme (a protein molecule) to form an active enzyme. Coenzymes are often derived from vitamins.
Endoenzyme	An enzyme which is not secreted or exported out of the cell, but is kept and used by the cell which made it.
Substrate	The specific molecule an enzyme acts upon.
Metabolism	All of the organized chemical reactions in a cell which are under the control of enzymes.
Catabolic reactions	Reactions in which chemical compounds are broken down.
Anabolic reactions	Reactions in which chemical compounds are synthesized.
Enzyme immobilization	The attachment of an enzyme to a solid matrix so that it cannot escape but can still act on its substrate.
Enzyme inactivation	The disappearance of activity of an enzyme (<i>in vivo</i> or <i>in vitro</i>) due to presence of inhibitor molecules or inhibitory conditions (changes in pH, temperature, salt concentration etc.).

Enzyme kinetics	Quantitative characteristics of enzymatic reactions.
Enzyme stabilization	Reducing the chances that an enzyme will inactivate (<i>in vivo</i> or <i>in vitro</i>) by changing the environmental conditions (such as pH, temperature, concentration of salt etc.) or by attaching organic groups to it or changing some of its amino acid subunits.

Having learnt about the various terminologies related to enzymes let us now continue with our study on enzymes.

Enzymes are synthesized within the cells and sometimes can pass through cell membranes under certain conditions. Enzymes which act inside the cell are called as *endoenzymes* and those which are liberated outside the cell are called as *exoenzymes*. While enzymes like those involved in the production of energy belong to the first category, digestive enzymes belong to the second category.

Enzymes though basically are proteins may sometimes have a non-protein component attached to the protein part. This may be an organic compound or a metal ion. While the first is called as *coenzyme*, the second is best known as *cofactor*. The protein part and non-protein part together form the *holoenzyme*, as illustrated in Figure 4.2. Sometimes, the non-protein part remains so tightly bound to the protein part that it cannot be dissociated. Such non-protein part is called as a *prosthetic group*. When the non-protein component dissociates from the protein part, the enzyme loses its catalytic function and is called an *apoenzyme* as can be seen in the Figure 4.2.

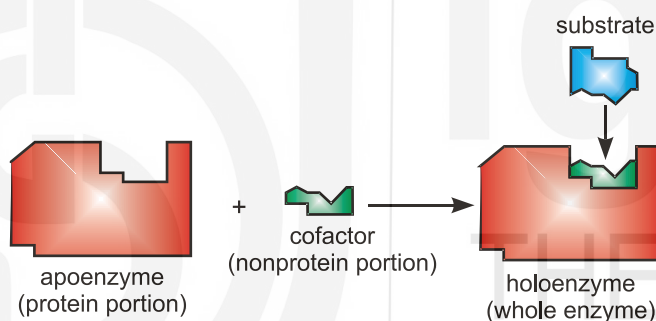


Figure 4.2: Holoenzyme

Thus, coenzymes which are usually vitamins, sometimes form an integral part of the active enzyme and make them indispensable for the enzyme to carry out the catalysis. You have already learnt about the classification of vitamins in the last Unit and know that these are broadly classified as water soluble and fat soluble. Remember that water soluble and not fat soluble vitamins constitute the precursors of coenzymes.

While participating in the mechanism of catalysis, a coenzyme undergoes alteration and its restoration requires the involvement of another enzyme. As coenzymes may participate in a variety of reactions catalyzed by different enzymes, it is always convenient to classify them on the basis of nature of element or group such as hydrogen, carboxyl, amino etc. they transfer in these reactions. We shall learn more about the classification of enzymes in the next section.

4.3 NOMENCLATURE AND CLASSIFICATION OF ENZYMES

Enzymes are generally named by the addition of 'ase' to the root, indicating the substrate on which the enzyme acts. For instance, the enzyme which catalyzes the conversion of maltose into glucose is called as *maltase*. Many common names such as aldolase also persist which do not tell about the substrates, although the type of reaction can be recognized. Besides, trivial names of many enzymes are in use which do not follow this convention at all e.g. pepsin.

In order to follow a systematic nomenclature for all the enzymes and to classify them, the *International Union of Biochemistry (IUB)* has established a system, whereby, the enzymes are placed into one of the six major classes as summarized in Table 4.1. Each class is then subdivided into several classes, which are further subdivided.

The system for classification of enzymes also serves as a basis for assigning code numbers to them. The code numbers are prefixed by *EC (Enzyme Commission)* and contain four numbers separated by points, with the following meaning:

- (a) the first number shows to which of the six main classes an enzyme belongs
- (b) the second figure indicates the sub-class
- (c) the third figure gives the sub-subclass, and
- (d) the fourth figure is the serial number of the enzyme in its sub-class.

So you can see that a number is assigned to each class, sub-class and sub-subclass so that an enzyme gets a four digit number. The fourth digit, in fact, identifies a specific enzyme. For example, *alcohol:NAD oxidoreductase* is assigned the number 1.1.1.1 because it is an oxidoreductase, the electron donor is an alcohol and the acceptor is the coenzyme NAD. Thus, in naming an enzyme, the substrate is stated first followed by the reaction type. The trivial name of the enzyme is alcohol dehydrogenase. Similarly, the EC number of catalase is EC 1.11.1.6. The first digit (1) indicates that the enzyme belongs to oxidoreductase (class 1). EC1.1 indicated subsequent digits representing sub-classes and sub-subclasses.

Major classes of enzymes and the types of reaction catalyzed by them are summarized in Table 4.1.

Table 4.1: Major classes of enzymes and the types of reaction catalyzed by them

Enzyme class	General reaction catalyzed by the class	Specific reactions catalyzed by the member of the class determining the subclass
Oxido-reductases (EC1)	Oxidation and reduction of the substrate.	1. <i>Dehydrogenases</i>: Catalyze removal of two atoms of hydrogen. 2. <i>Oxidases</i>: Catalyze reduction of molecular oxygen. 3. <i>Oxygenases</i>: Catalyze incorporation of molecular oxygen into the substrate. 4. <i>Oxidative deaminases</i>: Catalyze the oxidation of amino compounds with the formation of ammonia. 5. <i>Hydroxylases</i>: Catalyze the introduction of hydroxyl radical into the substrate. 6. <i>Peroxidases</i>: Act on hydrogen peroxide.
Transferases (EC2)	Transfer of groups between two substrates	1. <i>Aminotransferases</i>: Catalyze exchange of amino and keto group between amino and keto acids. 2. <i>Kinases</i>: Catalyze the transfer of phosphate radical. 3. <i>Acyltransferases</i>: Catalyze the transfer of acyl/acetyl groups to an acceptor. 4. <i>Glycosyltransferases</i>: Catalyze the transfer of glycosyl groups.
Hydrolases (EC3)	Hydrolysis of the substrate	1. <i>Peptidases</i>: Catalyze hydrolysis of peptide bonds. 2. <i>Glycosidases</i>: Catalyze hydrolysis of glycosidic bonds. 3. <i>Esterases</i>: Catalyze hydrolysis of carboxylic acid esters.

		4. <i>Phosphatases</i>: Catalyze hydrolysis of phosphoric acid esters. 5. <i>Phosphodiesterases</i>: Catalyze hydrolysis of phosphodiester bonds. 6. <i>Deaminases</i>: Catalyze hydrolysis of amines. 7. <i>Deamidases</i>: Catalyze hydrolysis of amides.
Lyases (EC4)	Removal of groups from substrates non-hydrolytically	1. <i>Decarboxylases</i>: Catalyze removal of carboxyl group from the substrate. 2. <i>Aldolases</i>: Catalyze removal of carbonyl group from the substrate.
Isomerases (EC5)	Isomerization of the substrate	1. <i>Racemases</i>: Catalyze the conversion of D-isomer to L-isomer and vice versa of a compound. 2. <i>Epimerases</i>: Catalyze the formation of an epimer of the substrate. 3. <i>Cis-trans isomerases</i>: Catalyze the inter-conversion of the <i>cis</i> and <i>trans</i> isomer of the substrate. 4. <i>Aldose-ketose isomerases</i>: Catalyze the conversion of aldose to ketose and vice versa. 5. <i>Mutases</i>: Catalyze the intramolecular transfer of a group.
Ligases (EC6)	Joining of two substrates at the expense of the breakdown of one pyrophosphate bond of ATP	1. <i>Synthetases</i>: Catalyze the formation of C–O, C–S, C–N bonds at the expense of ATP. 2. <i>Carboxylases</i>: Catalyze the introduction of carboxyl group with a C–C bond formation at the expense of ATP.

From the classification above, the different classes of enzymes identified include: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Let's get to know more about them and their nomenclature.

Oxidoreductases

To this group (EC 1) belongs all enzymes catalyzing oxidation-reduction reactions. Common names include dehydrogenases, oxidases, reductases and catalases. The substrate (AH_2) that is oxidized is regarded as a hydrogen donor ($AH_2 + B = A + BH_2$). The recommended name is *dehydrogenase* but, as an alternative, the term *reductase* is used. The name oxidase is restricted to enzymes which exclusively use O_2 as the hydrogen acceptor. The second figure in the code number of oxidoreductases indicates the group in the hydrogen donor which undergoes oxidation e.g. CH–OH, CHO, CH=CH, $CHNH_2$, NAD(P)H. Therefore, EC 1.1 indicated acting on the CH–OH group of donors. EC 1.2 acting on the aldehyde or oxo group of donors etc. The third figure indicates the type of acceptor involved: 1 denotes NAD(P), 2 a cytochrome, 3 O_2 , 4 a disulphide, 5 a quinone etc. So, EC 1.1.1 states with NAD^+ or $NADP^+$ as acceptor, EC 1.1.2 with cytochrome as acceptor and so on, the list goes on.

Transferases

These are the enzymes (code EC 2) which catalyze the transfer of a group, e.g. a methyl or glycosyl group, from one compound to another. In many cases, the donor is a cofactor (coenzyme) carrying the group to be transferred. Common names include *acetyltransferase*, *methylase*, *protein kinase* and *polymerase*. The second figure (EC 2.1) in the code number of transferases indicates the group transferred: a carbon group (2.1), a carbonyl group (aldehyde or ketone) (2.2), a glycosyl group (2.3) and so on. The third figure informs of the group transferred: e.g. subclass 2.1 is

subdivided into methyltransferases (2.1.1), hydroxymethyl and formyltransferases (2.1.2) and so on.

Hydrolases

These enzymes (code EC 3) catalyze the hydrolytic cleavage of C–O, C–N, C–C and some other bonds, including phosphoric anhydride bonds. Their trivial names are formed by adding the suffix ... 'ase' to the substrate which they hydrolyze. Examples include *protease*, *nuclease*, *phosphatase*. A number of hydrolases acting on ester, glycosyl, peptide, amide or other bonds are known to catalyze not only the hydrolytic removal of a particular group from their substrates, but also the transfer of this group to a suitable acceptor molecule. Yet, they are not grouped as transferases, because the transfer of a specific group to water as the acceptor molecule is considered to be their main physiological function. The second figure in the code number of hydrolases indicates the nature of the bond hydrolyzed, e.g. esterases (3.1), glycosidases (3.2) and so on. The third figure generally specifies the nature of the substrate, e.g. carboxylic esters (3.1.1), thiol esters (3.1.2), phosphoric monoesters (3.1.3), O-glycosides (3.2.1), N-glycosides (3.2.2) and so on.

Lyases

These enzymes (code EC 4) cleave C–C, C–O, C–N and other bonds by elimination, forming double bonds or conversely adding groups to double bonds. Common names include *decarboxylase*, *aldolase*, *dehydratase* (if water is eliminated) or *hydro-lyase* (if the reverse reaction is more important or the only one which can be demonstrated). *Synthase*, but not *synthetase*, may be used as in tryptophan synthase or cystathionine β -synthase. The second figure in the EC number indicates the bond being cleaved for e.g. C–C-lyases (4.1), C–O-lyases (4.2) and so on. The third figure informs about the group that is eliminated e.g. CO₂ (4.1.1) or H₂O (4.2.1).

Isomerases

These enzymes (code EC 5) catalyze geometric or structural changes within a molecule. According to the type of isomerism, they may be called *racemases*, *epimerases*, *cis-trans isomerases* (5.2), *isomerases*, *tautomerases*, *mutases* or *cyclo-isomerases*. EC 5.1 are racemases or epimerases, EC 5.1.1 indicates acting on amino acids and derivatives and so on.

Ligases (synthetases)

These enzymes (code EC 6) catalyze the linkage of two molecules coupled with the hydrolytic breakdown of a pyrophosphate bond in ATP or an analogous compound. The bonds formed are often high energy bonds. The second figure in the code number indicates the bond formed, e.g. C–O (6.1), C–S (6.2) etc. Examples include *peptide synthase*, *aminoacyl-tRNA synthetase*, *DNA ligase* and *RNA ligase*.

We hope the information given above may have helped you in understanding the classification of enzymes. The system of the nomenclature and the classification of enzymes are based exclusively on the reaction that is catalyzed and does not consider their origin or multiplicity. Enzymes catalyzing the same reaction, but isolated from different species will have varying amino acid sequences so that they may be distinguished by electrophoretic methods. They may have different sizes and net negative charges and may even differ in their catalytic behavior.

With this basic knowledge about enzymes, we shall go on to learn about the specificity of enzymes.

One of the major characteristics of enzymes is that they are highly specific. Enzymes, the organic catalysts, differ from the inorganic catalysts in their extraordinary specificity. Each of the enzymes that have been isolated and studied is found to possess different types of specificities. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four different types of specificity. These are as follows:

- Reaction specificity
- Bond specificity
- Group specificity
- Optical specificity

We shall look at each of these specificities one by one.

- *Reaction specificity*

- Some enzymes catalyze only one reaction acting on a specific substrate. Example: urease and catalase acts only on urea and hydrogen peroxide, respectively. This is also called absolute specificity.
- Many enzymes can catalyze same type of reactions (phosphate transfer, oxidation-reduction, hydrolysis etc.) in several structurally-related compounds. Example: carboxypeptidase acts on protein chains and removes one amino acid at a time from the C-terminal, irrespective of the nature of amino acid.
- A substrate can undergo many reactions but in a specific reaction, an enzyme will catalyze only one of these reactions. Example: citrate synthase converts oxaloacetate to citrate in the presence of acetyl-CoA. But, in absence of acetyl-CoA, oxaloacetate is acted upon by a different enzyme *malate dehydrogenase* with the formation of malate.

- *Bond specificity*

- Some enzymes act on a particular bond (glycosidic, peptide, ester etc.). Examples: pepsin, trypsin, chymotrypsin etc. are all only acting on peptide bonds, though the peptide bonds on which they will attack depend on the amino acids that have formed the bonds.

- *Group specificity*

- Some enzymes prefer a specific functional group to be present on the substrate molecules. Example: alcohol dehydrogenase acts on alcohols having –OH group.
- Some proteases act on the peptide bonds depending on the amino acids involved in the formation of the bonds. Example: chymotrypsin hydrolyzes only the peptide bond which is formed by the carboxyl group of phenylalanine, tyrosine or tryptophan.

- *Optical specificity*

- Some enzymes exhibit absolute optical specificity for at least a portion of the substrate molecule. For example, the enzyme maltase catalyzes the hydrolysis of α -glycosidic bond between two glucose molecules but not the β -glycosidic bond.

- ii) Enzymes are able to discriminate between the optical isomers and act accordingly. Example: L-amino oxidase acts only on L-amino acids and not on D-amino acids.

Having gone through the section above, it is clear that enzymes can be very specific. Next, let us learn about their mechanism of action. But first, let us recapitulate what we have learnt so far.

Check Your Progress Exercise 1

- 1) Define the following terms:

a) Enzymes

.....

b) Holoenzyme

.....

c) Metabolism

.....

- 2) Differentiate between endoenzymes and exoenzymes, giving suitable examples.

.....

- 3) How are enzymes classified? Explain giving an appropriate example.

.....

- 4) How are enzymes different from inorganic catalysts?

.....

- 5) What do you understand by enzyme specificity? List the four different types of enzyme specificities.

.....

- 6) Match the following:

A

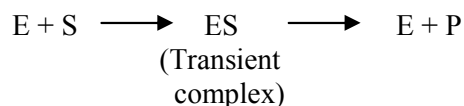
- a) Oxidoreductases
- b) Isomerases
- c) Transferases
- d) Lyases
- e) Hydrolases
- f) Ligases

B

- i) Kinases, Glycosyltransferases
- ii) Synthetases, Carboxylases
- iii) Peptidases, Phosphatases
- iv) Racemases, Mutases, Isomerases
- v) Dehydrogenases, Oxidases, Peroxidases
- vi) Aldolases, Decarboxylases

4.5 MECHANISM OF ENZYME ACTION

L. Michaelis and *M. L. Menten* developed a general theory of enzyme action in 1913. According to *Michaelis* and *Menten*, the enzyme (E) first binds the substrate (S) to form a transient enzyme-substrate complex (ES). This complex then dissociates into the product (P) and the unaltered enzyme (E).



Let us understand how this ES complex forms.

The action of an enzyme is initiated when the reactants i.e. substrates bind at the catalytic sites or active sites on the enzyme molecule. The catalytic site of the enzyme molecule possesses a complex three-dimensional form and provides a cleft, which binds the substrate as shown herewith.



A change in the tertiary or quaternary structure of the enzyme may alter the three dimensional shape of the catalytic site and thus reducing its binding and catalytic activities. The ES complex is formed mainly by non-covalent bonds between specific groups of the substrate molecules and the specific amino acid side chains present at the catalytic site of the enzyme. Different models for enzyme-substrate complex formation exist. Let us look at these models next.

Models for enzyme-substrate (ES) complex formation

There are two popular models to explain the enzyme-substrate interaction. These are:

- Fischer's template or lock and key model, and
- Koshland's induced fit model

The two models are described next.

Fischer's template or lock and key model

According to this model, the catalytic site of the enzyme has a proper conformation compatible to a specific substrate even in the absence of the substrate molecule, as shown in Figure 4.3. The catalytic site binds the substrate and catalyzes the reaction without any change in its own three dimensional conformation. It has become possible to explain the specificity of many enzymes for only one of the stereoisomers of the substrate by this model. This model, however, failed to explain the change in enzyme activity in presence of *allosteric modulators* (low molecular weight regulatory substances that bind at a specific site on the enzyme molecule other than the catalytic site and thereby enhance or inhibit the enzyme activity) or the action of the noncompetitive inhibitors, about which we will learn later in section 4.8 in this Unit.

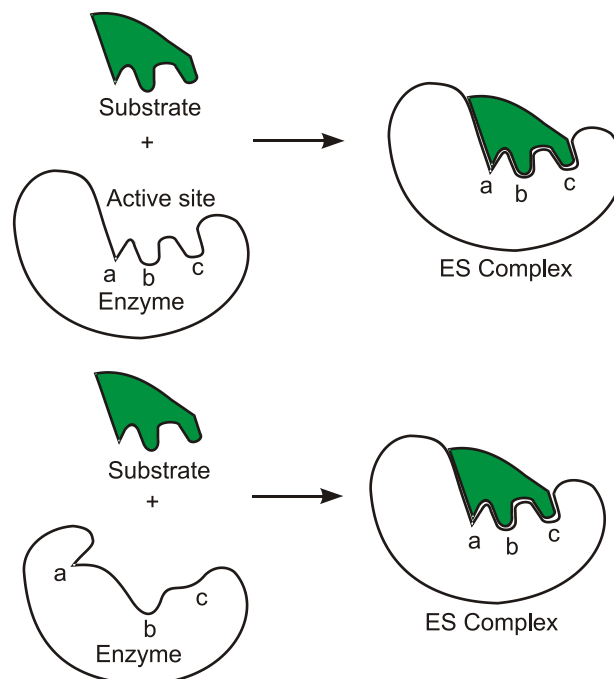


Figure 4.3: Fischer's template or lock and key model and Koshland's induced fit model

Koshland's induced fit model

This model considers a flexibility in the three dimensional conformation of the catalytic site. According to this model, despite having the required amino acids, the catalytic site of the enzyme does not possess the conformation complementary to the substrate in absence of the substrate molecule. Only when the substrate approaches towards the enzyme or during its binding, the conformation of the catalytic site changes so that the enzyme can hold the substrate properly, as shown in Figure 4.4. This model, therefore, can suitably explain the noncompetitive inhibition and allosteric modulation of the enzyme. We will learn about the noncompetitive inhibition later in this Unit in section 4.8.

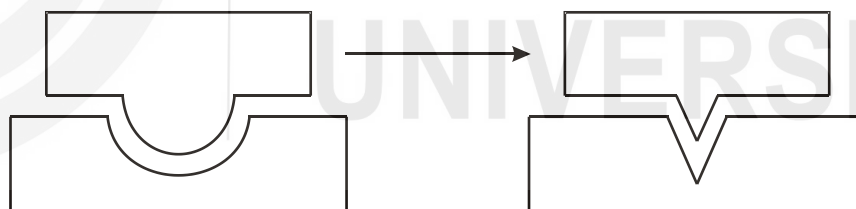


Figure 4.4: Koshland's induced fit model

While on the topic of the mechanism of enzyme action, it is also important to know about the unit of enzyme activity i.e. how to express the activity of an enzyme. Have you heard of the term 'katal'? *Recommended unit of enzyme activity is called as 'katal', which is the amount of an enzyme that transforms 1 mol of substrate into product in one second.*

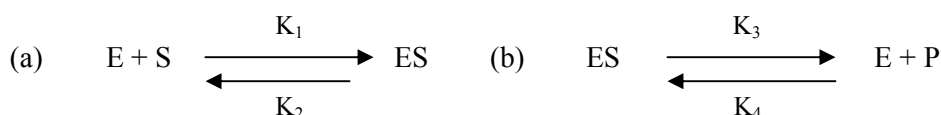
In fact, other than katal, the activity of an enzyme may be expressed in different other ways as highlighted herewith:

- International enzyme unit (IU) is defined as *the amount of enzyme that catalyzes the transformation of 1 μ mol of substrate into product in one minute.*
- Specific activity of an enzyme preparation is expressed as kat/kg or IU/mg of protein.
- Molar activity of an enzyme is kat/mol of the enzyme.
- Turnover number of the enzyme is *the number of molecules of substrate transformed per catalytic site of the enzyme per minute.*

4.6 ENZYME KINETICS

The study of the rate at which an enzyme works is called *enzyme kinetics*.

You have read in the last section that *L. Michaelis* and *M. L. Menten* developed a general theory of enzyme action and kinetics in 1913. According to this theory, we learnt that in an enzyme substrate reaction, the enzyme (E) first combines with the substrate (S) with the formation of an enzyme-substrate (ES) complex, which subsequently breaks down into the product (P) and the enzyme (E) is recovered. Thus, the enzyme catalyzed reaction proceeds in the following two steps:



The reactions are assumed to be reversible and K_1 , K_2 , K_3 and K_4 are the rate constants of each reaction.

Let us examine enzyme kinetics as a function of the concentration of substrate available to the enzyme. The enzyme substrate interaction may be studied with the help of Figure 4.5. In this graph, substrate concentration and velocity of reaction of the enzyme catalysis has been shown on X and Y axis, respectively. When the velocity of a reaction is plotted against different substrate concentrations, as in the present graph, a hyperbolic curve is obtained.

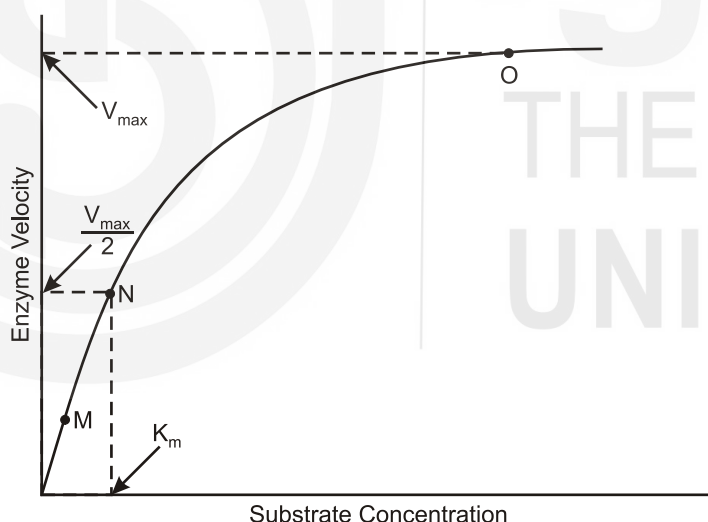


Figure 4.5: Effect of substrate concentration on the reaction velocity of an enzyme catalyzed reaction

M, N and O are three points on the curve representing three stages of enzyme catalyzed reaction. While M represents that stage of the reaction when the substrate concentration is very low and the rate of reaction is directly proportional to the substrate concentration, O represents the stage when the reaction velocity reaches its maximum due to the gradual increase in substrate concentration resulting in a saturation of the active site of the enzyme. In between these two extremities lies the third point N which represents the reaction velocity of the enzyme catalyzed reaction that is half of the maximum velocity.

The mathematical relationship between the initial velocity of an enzyme catalyzed reaction, the concentration of the substrate and certain characteristics of the enzyme are expressed by the Michaelis-Menten equation, which was derived on the basis of *Michaelis-Menten* theory and assumptions of *G. E. Briggs* and *J. B. S. Haldane* and was proposed in 1925.

The *Michaelis-Menten* equation is:

$$v = \frac{V_{\max} \times [S]}{K_m + [S]}$$

where, v is the velocity of the reaction at any stage,

$V_{\max}$ is the maximum velocity,

$[S]$ is the substrate concentration and

K_m is the *Michaelis-Menten* constant, usually expressed in moles per litre.

K_m represents the substrate concentration at which the velocity of the reaction is half of $V_{\max}$. K_m is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the K_m , the greater the affinity (so, lower is the concentration of substrate needed to achieve a given rate).

This equation is widely used to describe the most enzyme catalyzed reactions. Now, we can verify the situations of the three points M, N and O in Figure 4.5 with the help of this equation.

i) *Point M*: At this stage of enzyme-substrate reaction, the substrate concentration is much less than K_m and its value does not change significantly with the increase in substrate concentration. Thus, $[S]$ can be ignored in the denominator of the Michaelis-Menten equation. So it becomes,

$$v = \frac{V_{\max} \times [S]}{K_m}$$

As $V_{\max}$ and K_m are both constants, $V_{\max} / K_m$ ratio may be replaced by a new constant, K . Thus, we have

$$v = K \times [S]$$

So, reaction velocity (v) of the enzyme-substrate reaction at this stage is directly proportional to substrate concentration $[S]$.

ii) *Point N*: At this point, substrate concentration $[S]$ is equal to Michaelis-Menten constant, K_m . Thus the Michaelis-Menten equation becomes:

$$v = \frac{V_{\max} \times [S]}{[S] + [S]}$$

or

$$v = \frac{V_{\max} \times [S]}{2 [S]}$$

or

$$v = \frac{V_{\max}}{2}$$

This clearly established that K_m is the substrate concentration at which the reaction velocity of an enzyme catalyzed reaction is half of maximum velocity.

iii) *Point O*: At this point, the substrate concentration is much higher in comparison to K_m and so K_m may be ignored in the *Michaelis-Menten* equation. Thus it becomes,

$$v = \frac{V_{\max} \times [S]}{[S]}$$

or $v = V_{\max}$

Plotting the reciprocals of the same data points yields a “double-reciprocal” or *Lineweaver-Burk plot*. This provides a more precise way to determine $V_{\max}$ and K_m . Let us get to learn more about this concept.

Lineweaver-Burk equation

Michaelis-Menten equation is often algebraically transformed to other forms for convenience in plotting experimental data. One such form is *Lineweaver-Burk* equation, which is derived by taking the reciprocal of both sides of *Michaelis-Menten* equation. Thus results:

$$\frac{1}{v} = \frac{K_m + [S]}{V_{\max} \times [S]}$$

or,

$$\frac{1}{v} = \frac{K_m}{V_{\max} [S]} + \frac{[S]}{V_{\max} [S]}$$

Now, after rearrangement we get,

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \times \frac{1}{[S]} + \frac{1}{V_{\max}}$$

This is known as *Lineweaver-Burk* equation. Look at Figure 4.6. When $1/v$ is plotted against $1/[S]$, a straight line is obtained having a slope of $K_m / V_{\max}$, an intercept of $1/V_{\max}$ on the $1/v$ axis and an intercept of $-1/K_m$ on the $1/[S]$ axis.

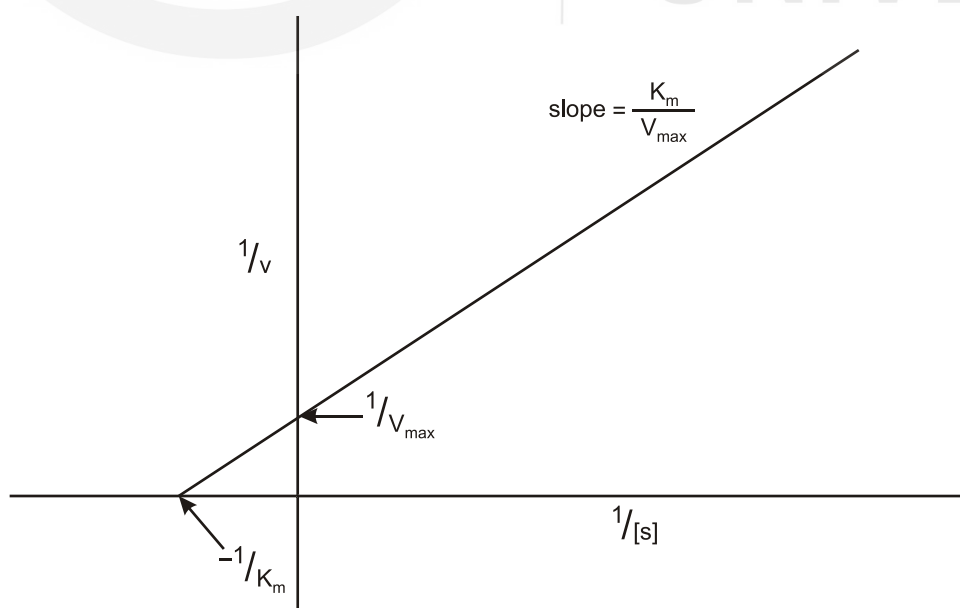


Figure 4.6: Lineweaver-Burk plot

Note that K_m is not a fixed value but may vary with the structure of the substrate, pH and temperature. For enzymes having more than one substrate, each substrate has a characteristic K_m . Affinity of an enzyme for a substrate inversely varies with the K_m . For example, glucose can be acted upon by both hexokinase and glucokinase but hexokinase has lower K_m value for glucose and therefore greater affinity in comparison to glucokinase.

At this stage, we suggest you take a break. Try to recapitulate what you have learnt so far. We agree this section may have been a bit tough. Perhaps give this section another reading. Alternatively, try to attempt the check your progress exercises given next. This will help you clarify your doubts and facilitate your understanding about the topic.

Check Your Progress Exercise 2

- 1) Discuss the general theory of enzyme action.
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- 2) What is meant by lock and key model? What are its drawbacks?
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- 3) Give the four different ways by which the enzyme activity can be expressed.
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- 4) Write down Michaelis - Menten equation.
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- 5) Give the relationship between substrate concentration and reaction velocity.
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- 6) How can affinity of an enzyme for a substrate be judged?
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- 7) Name one model of enzyme-substrate interaction which can explain non-competitive inhibition.
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Earlier we studied about the mechanism of enzyme action. There are various factors which influence the enzyme activity. We shall study about these factors next.

Rate of an enzyme substrate reaction depends on several important factors. These include concentration of enzyme, concentration of substrate, pH, temperature etc. Without the optimum condition of these factors, the enzymes will be unable to exhibit its best activity. These factors are discussed briefly in this sub section.

- *Concentration of enzyme*

Increase in enzyme concentration will increase the rate of an enzyme catalyzed reaction. This is because of the availability of additional catalytic sites to which the substrates can bind. Accordingly a straight line graph is obtained as can be seen in Figure 4.7. Since the concentration of substrate relative to that of enzyme is always very high, increase in enzyme concentration results in increased enzyme activity.

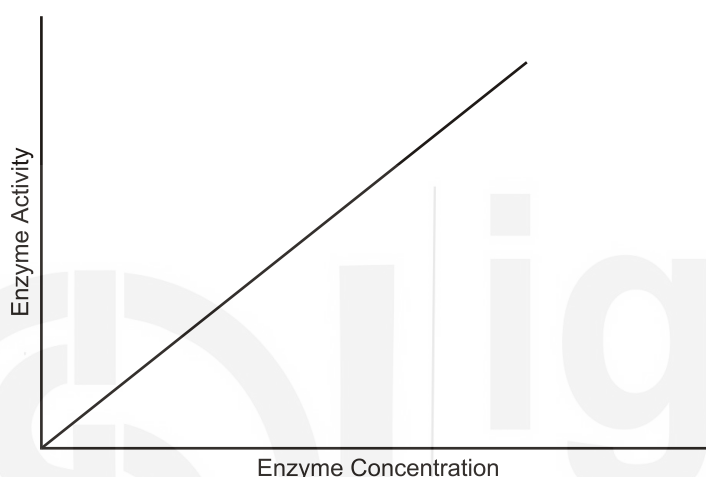


Figure 4.7: Effect of enzyme concentration on enzyme activity

- *Concentration of substrate*

When the concentration of the substrate is low, the rate of enzyme catalyzed reaction also remains low, inspite of concentration of substrate being higher compared to enzyme concentration. This is because at this stage all the catalytic sites of the enzyme are not occupied by the substrate molecules. So, with the increase in substrate concentration, the rate of the reaction also increases until all the catalytic sites of the enzyme are utilized. Rate of the reaction becomes maximum at this point and beyond this, it remains constant as can be seen in Figure 4.8.

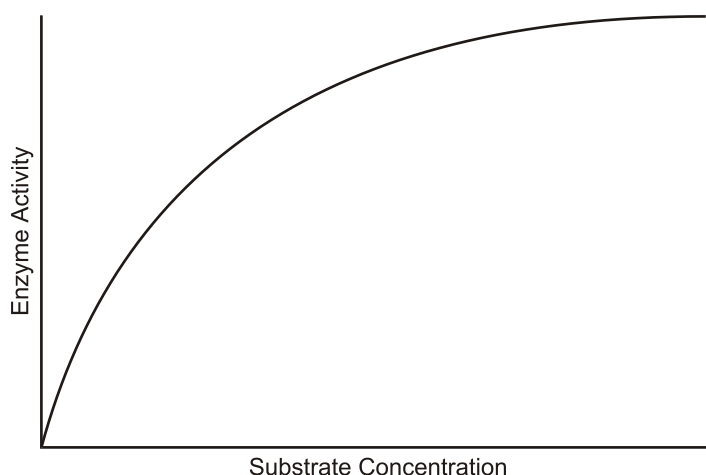


Figure 4.8: Effect of substrate concentration on enzyme activity

- *Temperature*

The rise in temperature accelerates the rate of enzyme-catalyzed reaction up to a certain temperature known as *optimum temperature* for the enzyme. At very high temperature, the enzyme undergoes denaturation and subsequent loss of activity. For most enzymes, the optimum temperatures are close to that of ambient temperature of the cell. For human beings, the temperature is in the region of 37°C while the optimum temperature of plant urease is 60°C. Certain microbial enzymes have a higher optimum temperature that enables them to adjust in a new higher ambient temperature of a new environment.

The effect of temperature on the reaction rate is shown in Figure 4.9. You can see that the rate of the reaction is almost zero at 0°C and this gradually increases with the rise of temperature until the optimum point reaches. Beyond this point, the activity of the enzyme falls due to denaturation and the curve bends reaching ultimately the zero level. It has been found that a rise of 10°C will double the activity of the enzyme.

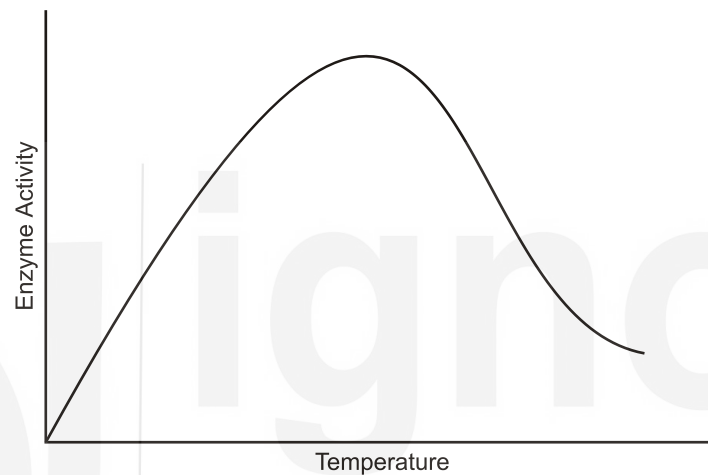


Figure 4.9: Effect of temperature on enzyme activity

- *pH*

Enzymes are influenced by pH changes as they have an ionic character due to the presence of amino and carboxylic groups. All enzymes have an optimum pH for showing highest catalytic activity and a change of this affects their activity. However, within a narrow pH range, the changes in the reaction rate are reversible but if the pH becomes too low or high, denaturation of the enzyme may occur. Most enzymes have an optimum pH range between 5 and 8, although some enzymes like *pepsin* and *trypsin* are most active at high acidic (pH 1.5) and high alkaline (pH 8) condition. A typical curve of enzyme activity against pH changes is presented in Figure 4.10.

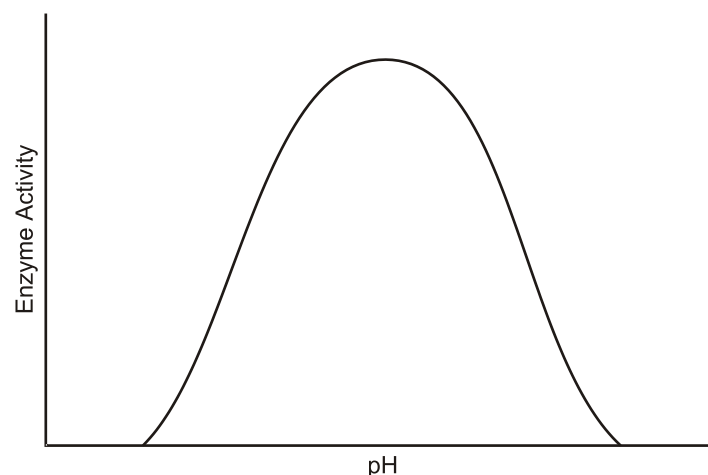


Figure 4.10: Effect of pH on enzyme activity

- *Activators*

Activity of many enzymes is influenced by certain ions called as *activators*. Large number of enzymes such as *hexokinase* that require ATP are also in need of divalent cations like Mg^{2+} or Mn^{2+} . Many enzymes such as *ATPase* require monovalent cations like Na^+ , K^+ or NH_4^+ for maximum catalytic activity. *Amylase* requires Cl^- . Generally, these ions interact with the substrates so that the substrates can bind with the catalytic sites of the enzyme properly. Thus, in absence of the activators, the enzymes become inactive or sluggish.

- *Oxidation*

Some enzymes which have the sulfhydryl ($-SH$) group in the catalytic site are very sensitive to oxidation. Due to oxidation of the $-SH$ group by aerial oxygen or oxidizing agents, a disulfide linkage ($-S-S-$) forms with the subsequent loss of enzyme activity. The enzyme activity can be restored by the reduction of the enzyme by some reducing agent such as cysteine or glutathione.

Having studied about the factors influencing the enzyme activity, we move on to factors which inhibit the enzyme activity in the next section.

4.8 ENZYME INHIBITION

Enzymes are often inhibited by the presence of suitable inhibitors. Much of current drug therapy is based on this. Basically, there are three major classes of enzyme inhibition. These are:

- *Competitive inhibition*, when the substrate and inhibitor compete for binding to the same active site
- *Noncompetitive inhibition*, when the inhibitor binds somewhere else on the enzyme molecule reducing its efficiency, and
- *Uncompetitive inhibition*

Let us learn about each of these classes of inhibition.

- *Competitive inhibition*

This type of inhibition takes place when a compound having a strong structural resemblance to the substrate competes with it for the catalytic site of the enzyme. Once the compound binds, the enzyme cannot convert the inhibitor to products. Increasing substrate concentration, however, is capable of displacing the inhibitor. Thus this type of inhibition is *reversible* in nature. A good example of this is the reaction catalyzed by *succinate dehydrogenase* in the citric acid cycle. You will learn about the citric acid cycle in Unit 6, later in this Course. In this reaction, succinate is converted to fumarate with the aid of this enzyme. Now, the compound malonate is structurally similar to succinate and if present, it will compete with succinate for the catalytic site of succinate dehydrogenase and reduce the product formation. Thus, *malonate* is a competitive inhibitor for this particular reaction. The structure of succinate and malonate is given in Figure 4.11.

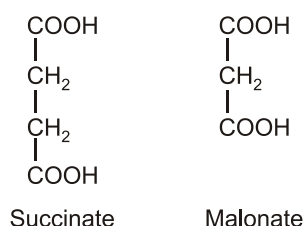


Figure 4.11: Structure of succinate and malonate

In this type of inhibition, the K_m of the enzyme for the substrate shows an apparent increase in the presence of the inhibitor as can be seen in Figure 4.12. This means that by increasing the substrate concentration, enzyme inhibition can be overcome. However, the V_{max} remains unaltered.

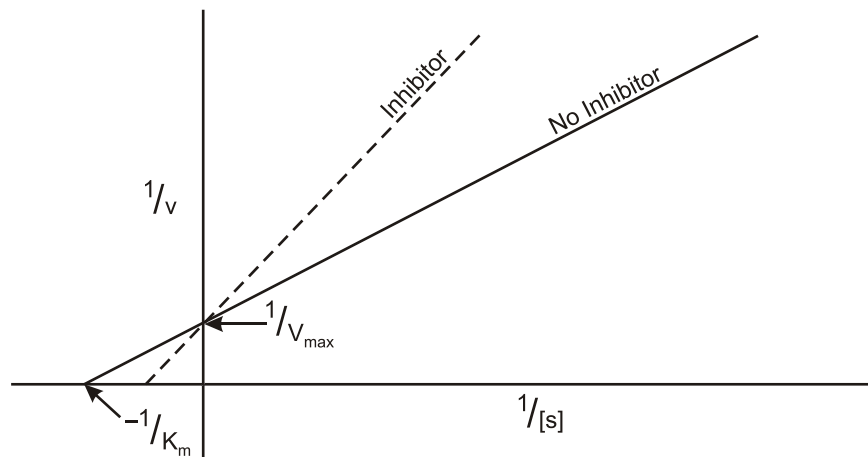


Figure 4.12: Lineweaver-Burk plot for an enzyme-substrate reaction in competitive inhibition

Next, let us learn about the noncompetitive inhibition.

- *Noncompetitive inhibition*

In this type of inhibition, the inhibitor binds at a site on the enzyme other than catalytic site. As there is no competition between the substrate and the inhibitor, the inhibition cannot be reversed in this case by increasing the substrate concentration. It appears that as if inhibitor is removing the enzyme, thus causing a decrease in V_{max} as can be seen in Figure 4.13. No change of K_m , however, occurs.

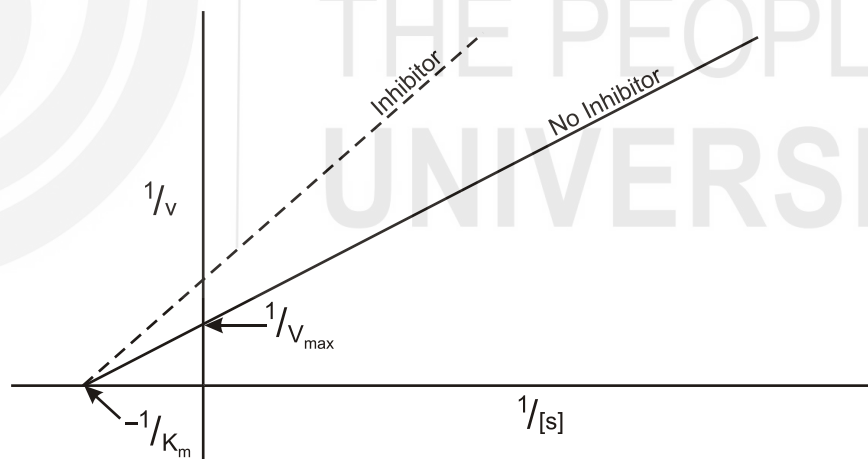


Figure 4.13: Lineweaver-Burk plot for an enzyme-substrate reaction in noncompetitive inhibition

A noncompetitive inhibitor can combine with either the free enzyme or the enzyme-substrate complex, interfering both. The most common type of noncompetitive inhibition is affected by the substances that combine with some functional group of the enzyme (outside the catalytic site) that is essential for maintaining the conformation of the enzyme molecule required for its activity. For example, enzymes possessing the essential $-SH$ group are sometimes inhibited by metals like mercury or copper.

Finally, let us get to know about the third type of enzyme inhibition i.e. uncompetitive inhibition.

- *Uncompetitive inhibition*

In this type of inhibition, the inhibitor only binds with the enzyme-substrate complex making it inactive. As a result, the product formation becomes difficult. In uncompetitive inhibition, both K_m and V_{max} changes as can be seen in Figure 4.14. The former increases while the latter decreases. This kind of inhibition is rare in one substrate reactions but common in two substrate reactions.

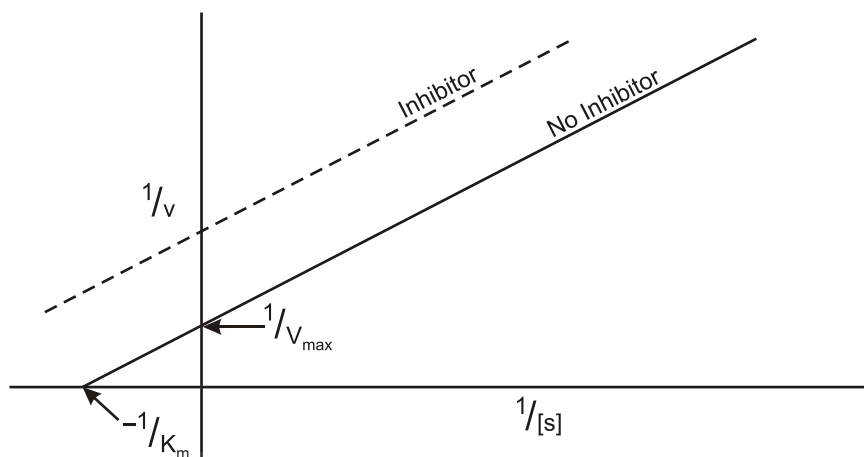


Figure 4.14: Lineweaver-Burk plot for an enzyme-substrate reaction in uncompetitive inhibition

So far we studied about the enzyme mechanism and the factors which influence and inhibit its activity. Our study of enzymes shall be incomplete, without the understanding of the role of enzymes and coenzymes in metabolism. The next section focuses on this aspect. But before moving on to the next section, let us recapitulate what we learnt till now.

Check Your Progress Exercise 3

- 1) Name the environmental factors on which enzyme activity depends. Explain any two of these.

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- 2) What do you mean by 'enzyme inhibition'? What is its significance?

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- 3) Explain the following terms giving suitable examples.

- a) Competitive inhibition

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- b) Uncompetitive inhibition

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.....

- c) Non-competitive inhibition

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.....

- 4) How does the K_m of an enzyme catalyzed reaction change in the presence of a competitive inhibitor?

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4.9 ROLE OF ENZYMES AND COENZYMES IN METABOLISM

You have already learnt that many enzymes require a non-protein part for their optimal activity, which may be a coenzyme or a metal ion. This has also been stated earlier that many water soluble vitamins are acting as coenzymes in their native or derived forms. So, it is important to learn how the coenzymes are assisting different enzymes in catalyzing biochemical reactions. In this sub-section, you will learn about:

- the chemical nature of different coenzymes, and
- the general role of co-enzymes in assisting different enzymes during metabolism.

As coenzymes participate in a variety of functions, they can be classified broadly into two groups:

- Hydrogen transferring coenzymes, and
- Group transferring coenzymes

Let's learn about these two groups of enzymes.

I) *Hydrogen Transferring Coenzymes*

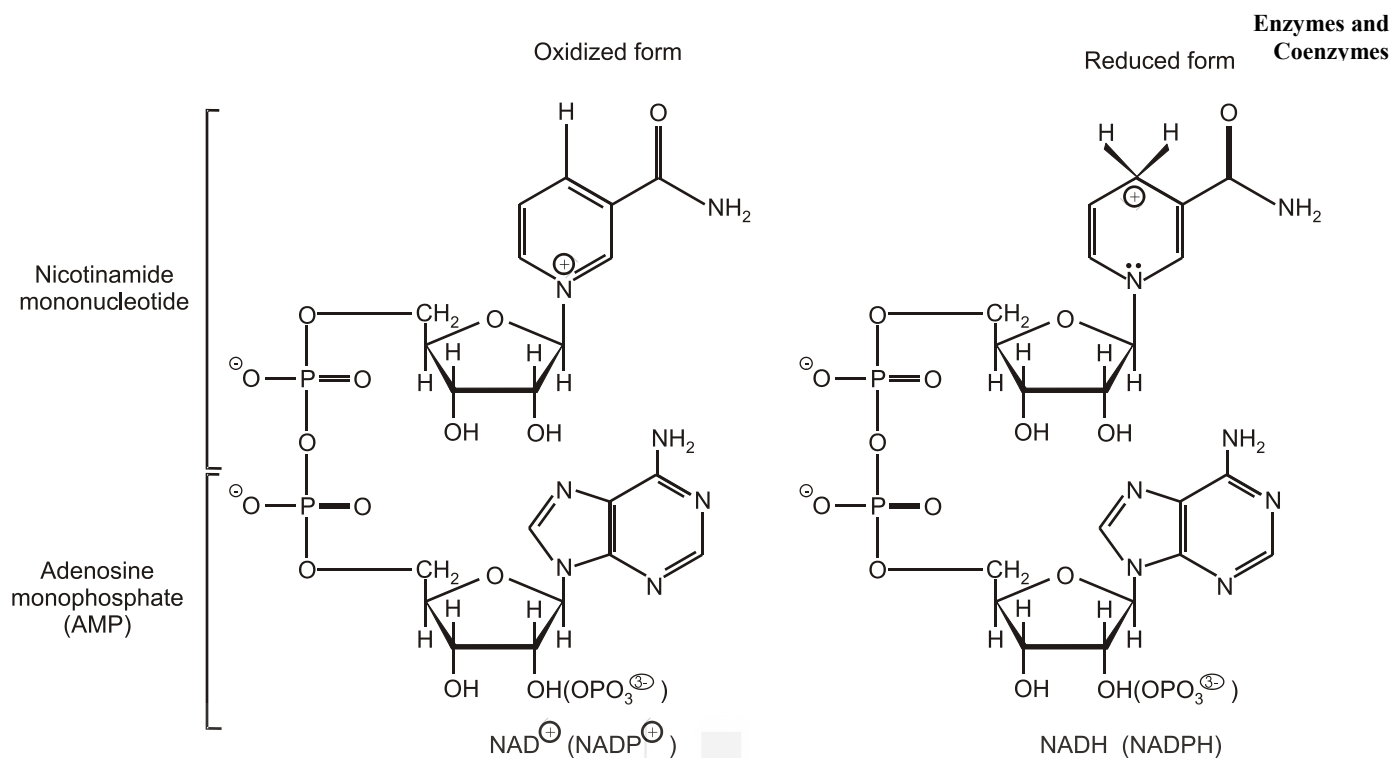
This group consists of three important coenzymes all of which assist different enzymes in oxidation-reduction reactions. These are:

- Nicotinamide nucleotides
- Flavin nucleotides
- Lipoic acid

A brief review of these coenzymes follows.

(a) *Nicotinamide nucleotides*

These coenzymes are derived from the vitamin, niacin. You may recall reading in Unit 3 that they are of two types, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Collectively, they are called as *pyridine nucleotides*. Have a look at Figure 4.15. In NAD^+ , the pyridine ring is attached to a ribose molecule through glycosidic bond and the phosphate provides a link between adenosine and nicotinamide riboside. In $NADP^+$, an additional phosphate group is present in carbon atom 2 of the ribose molecule of adenosine component.



Oxidized and reduced forms of NAD (and NADP). The pyridine ring of NAD⁺ is reduced by addition of a hydride ion to C-4 when NAD⁺ is converted to NADH (and when NADP⁺ is converted to NADPH). In NADP, 2'-hydroxyl group of the sugar ring of adenosine is phosphorylated.

Figure 4.15: Oxidized and reduced forms of NAD and NADP

Both NAD⁺ and NADP⁺ act as hydrogen acceptors during oxidation-reduction reactions in the body. Dehydrogenation is the primary mechanism of biological oxidation in which two hydrogen atoms are removed from the substrate in presence of an acceptor. The hydrogen atoms ionize to yield two H⁺ and two electrons.

The nicotinamide ring of NAD⁺ or NADP⁺ accepts a proton and two electrons which are equivalent to H⁻. The other H⁺ remains as such. All reactions catalyzed by them are reversible. Table 4.3 lists some of the enzymes, all dehydrogenases, which are dependent on these coenzymes.

Table 4.3: Enzymes requiring nicotinamide nucleotide coenzymes

Enzyme	Coenzyme	Substrate	Product
Lactate dehydrogenase	NAD ⁺	Lactate	Pyruvate
Alcohol dehydrogenase	NAD ⁺	Primary and secondary alcohols	Corresponding aldehydes and ketones
Glutamate dehydrogenase	NAD ⁺	Glutamate	α-ketoglutarate and ammonia
Glyceraldehyde-3-phosphate dehydrogenase	NAD ⁺	Glyceraldehyde-3-phosphate	1,3-diphosphoglyceric acid
Glucose-6-phosphate dehydrogenase	NADP ⁺	Glucose-6-phosphate	6-phosphogluconolactone
Malate dehydrogenase	NAD ⁺	Malate	Oxaloacetate
Isocitrate dehydrogenase	NADP ⁺	Isocitrate	α-ketoglutarate

Flavin nucleotides are derived from the vitamin B₂ (riboflavin) and are actively involved in hydrogen transfer reactions. You may recall reading in the last unit about the two coenzymes that are produced from riboflavin, i.e. flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Look at Figure 4.16 for the structure of FAD. FMN is the active component of riboflavin and is formed by the addition of a phosphate group and FAD is formed by the combination of FMN with one molecule of adenosine triphosphate (ATP). Both, FMN and FAD, are involved in many oxidation-reduction reactions. Enzymes that require the presence of FMN for their catalytic activity include glycolic acid oxidase, L-amino oxidase etc. FAD containing enzymes are succinate dehydrogenase, D-amino oxidase etc.

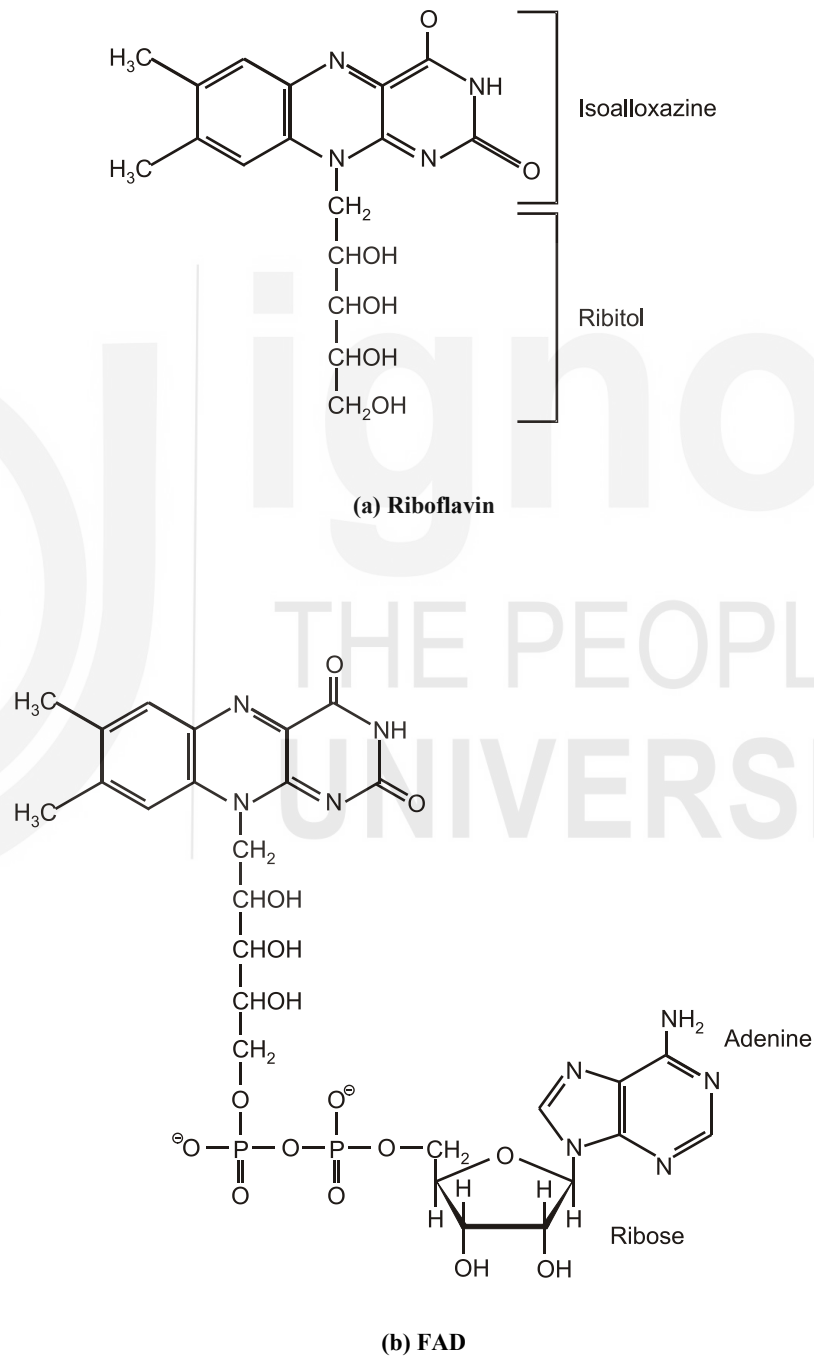


Figure 4.16: Structures of Riboflavin and its coenzyme FAD

(c) *Lipoic acid*

Lipoic acid acts in the transfer of hydrogen during oxidative decarboxylation reactions. It occurs both in oxidized and reduced forms. Refer Figure 4.17 (a) for its structure. Figure 4.17 (b) shows the structure of lipoamide, where lipoic acid is bound in an amide linkage to the ϵ -amino group of a lysine residue (blue) of dehydrolipoamide acyl-transferases. The complex reactions of the carbohydrate metabolism catalyzed by pyruvate dehydrogenase system and α -ketoglutarate dehydrogenase system require the participation of lipoic acid. These reactions will be discussed later in detail in Unit 6 covering carbohydrate metabolism.

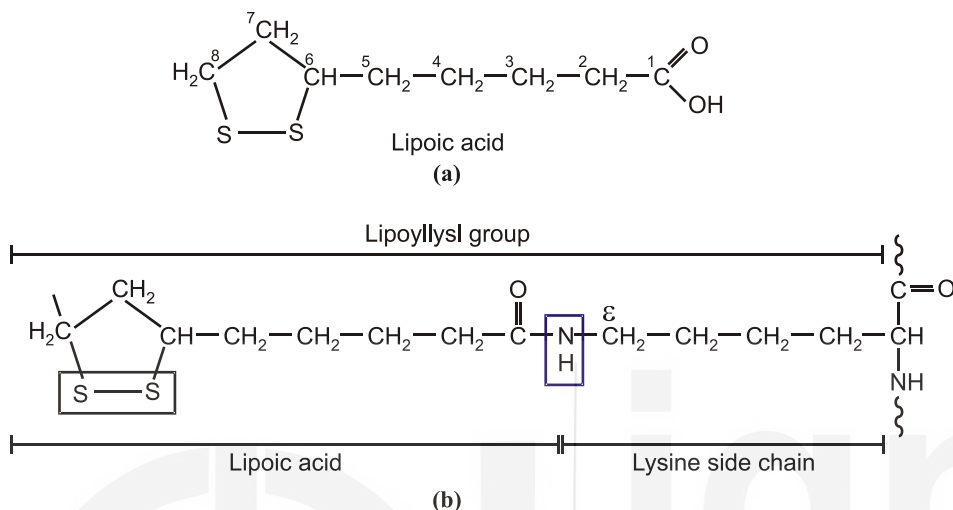


Figure 4.17: Structures of (a) Lipoic acid (b) Lipoamide

II) *Group Transferring Coenzymes*

This group consists of the following coenzymes involved in different metabolic reactions, where the transfer of any functional group is taking place:

- Biotin
- Thiamin diphosphate (TDP)
- Pyridoxal phosphate
- Coenzyme A (CoA)
- Tetrahydrofolic acid (THF)
- Cobamide coenzymes
- Adenosine triphosphate (ATP)

You may recall reading about these enzymes in the last unit on vitamins, particularly under the section on water soluble vitamins. There we talked about them as derivatives of water soluble vitamins. We shall look at these enzymes once again to help you understand them better.

• *Biotin*

This water soluble B group vitamin participates in the transfer of carboxylic groups. Two important enzymes carrying out carboxylation reactions are *pyruvate carboxylase* and *acetyl CoA carboxylase*. Pyruvate carboxylase contains four biotin molecules attached to the enzyme protein. The biotin dependent enzymes require ATP (adenosine triphosphate) which is converted to ADP (adenosine diphosphate) during the reaction.

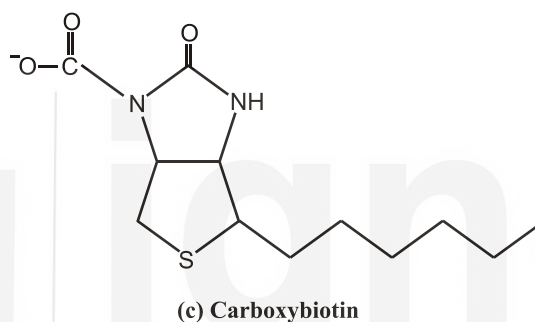
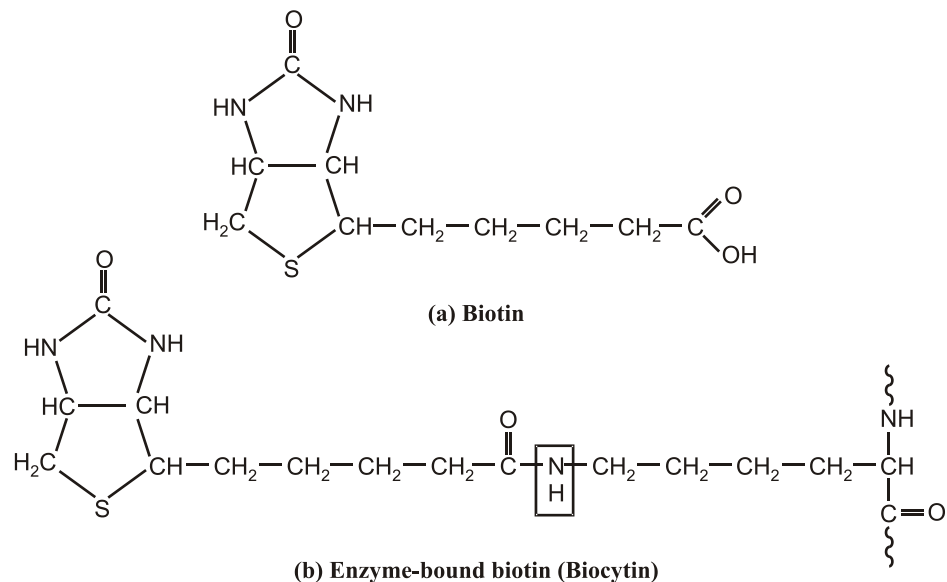


Figure 4.18: Structure of Biotin and enzyme-bound biotin

- *Thiamine diphosphate*

Thiamine diphosphate (TDP) is the coenzyme responsible for the transfer of aldehyde and glyoxal groups. Figure 4.19 presents the structure of TDP.

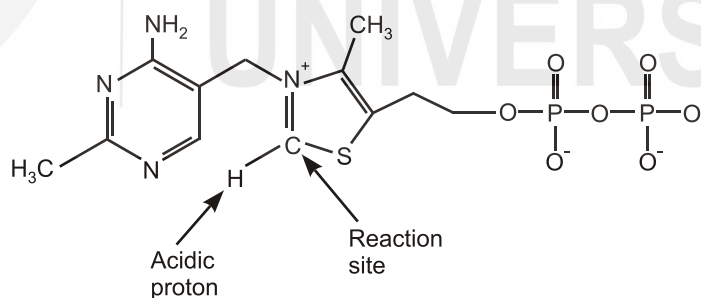


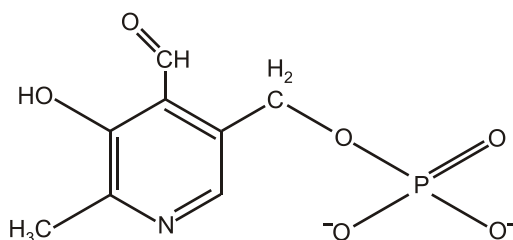
Figure 4.19: Structure of thiamine diphosphate

The most important metabolic reaction catalyzed by TDP is the oxidative decarboxylation of α -keto acids such as pyruvic acid. The involvement of TDP in the decarboxylation of pyruvic acid was so well known that the coenzyme was also popularly called as co-carboxylase. It is also actively taking part in the conversion of pyruvic acid to acetyl-CoA and α -ketoglutarate to succinyl-CoA that are catalyzed by pyruvate dehydrogenase and α -ketoglutarate dehydrogenase complex, respectively during carbohydrate metabolism. You shall learn about this in more details in Unit 6.

Another important metabolic reaction catalyzed by TDP is the conversion of xylulose-5-phosphate to sedoheptulose-7-phosphate catalyzed by the enzyme transketolase in pentose phosphate pathway of carbohydrate metabolism. Look up Unit6, section 6.8 for knowing more about the pentose phosphate pathway.

- *Pyridoxal phosphate*

Pyridoxal phosphate is derived from pyridoxine (vitamin B₆) and is involved in amino acid metabolism. The other two compounds, pyridoxal and pyridoxamine, about which you learnt in the last unit, having the properties of vitamin B₆ also occur as phosphate derivatives. Enzymes that are dependent on B₆ phosphate coenzymes catalyze a variety of reactions such as *transamination* (transfer of amino group from an amino acid to a keto acid), *decarboxylation* (removal of carboxyl group) and *racemization* (transformation of one isomer to another). The structure of pyridoxal phosphate is shown in Figure 4.20.



Pyridoxal Phosphate (PyP; Vitamin B₆)

Figure 4.20: Structure of pyridoxal phosphate (Pyp; Vitamin B₆)

Enzyme glutamate oxaloacetate transaminase catalyzes the reaction between glutamate and oxaloacetate with the formation of α -ketoglutarate and aspartate due to transfer of one amino group from glutamate to oxaloacetate. Pyridoxal phosphate acts as the carrier of the amino group. Whenever it accepts the amino group, it transforms to pyridoxamine phosphate, and after the release of the amino group, it again becomes pyridoxal phosphate. Aspartate is decarboxylated by aspartate decarboxylase taking the help of pyridoxal phosphate.

L-alanine transforms to D-alanine by *alanine racemase* which also requires pyridoxal phosphate as the co-enzyme.

- *Coenzyme A*

Coenzyme A is derived from the vitamin *pantothenic acid*. This is abbreviated as CoA. This can be divided into two components, adenosine 3,5-diphosphate and pantotheine, which is formed by the combination of pantothenic acid and mercaptoethylamine. Refer Figure 4.21 to understand its structures well. It gives rise to acyl-CoA derivatives that are mainly formed in ATP dependent synthetase reactions. These are highly reactive and participate in various types of reactions. For example, oxaloacetate is converted to citrate in presence of citrate synthetase by accepting acyl group of acyl-CoA. During carbohydrate metabolism, the pyruvate is converted by the pyruvate dehydrogenase complex to acetyl-CoA by the active participation of coenzyme A. Also, in the oxidation of fatty acids, CoA has an important role to play as you will find out in Unit 7.

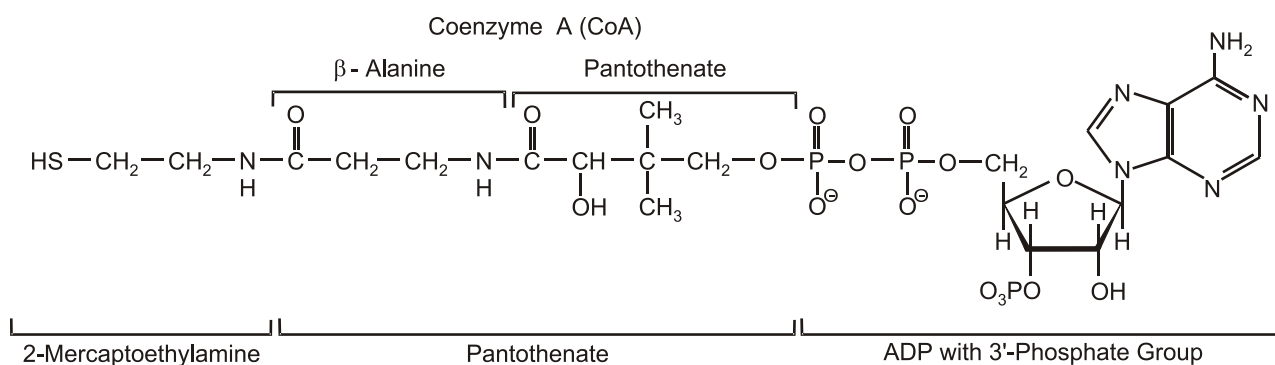
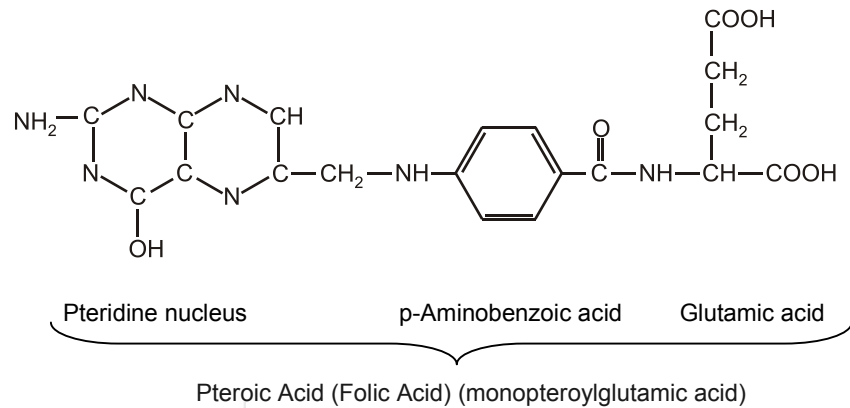


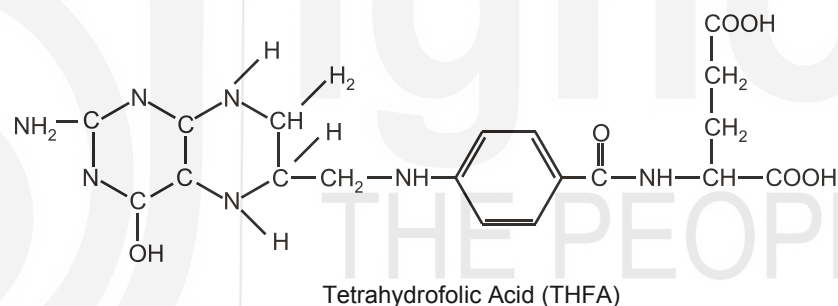
Figure 4.21: Structure of coenzyme A

- *Tetrahydrofolate*

Coenzyme tetrahydrofolate, as you may already know, is derived from the vitamin folic acid. Have a look at the Figure 4.22 for structures of folic acid and tetrahydrofolate – the coenzyme. It is responsible for the transfer of one carbon fragments at the oxidation level of formate, formaldehyde and methanol. The two most important metabolic reactions in which tetrahydrofolate participates are the biosynthesis of purine (refer to Unit 8, sub-section 8.3.1) and methionine.



(a) Vitamin: Folic Acid

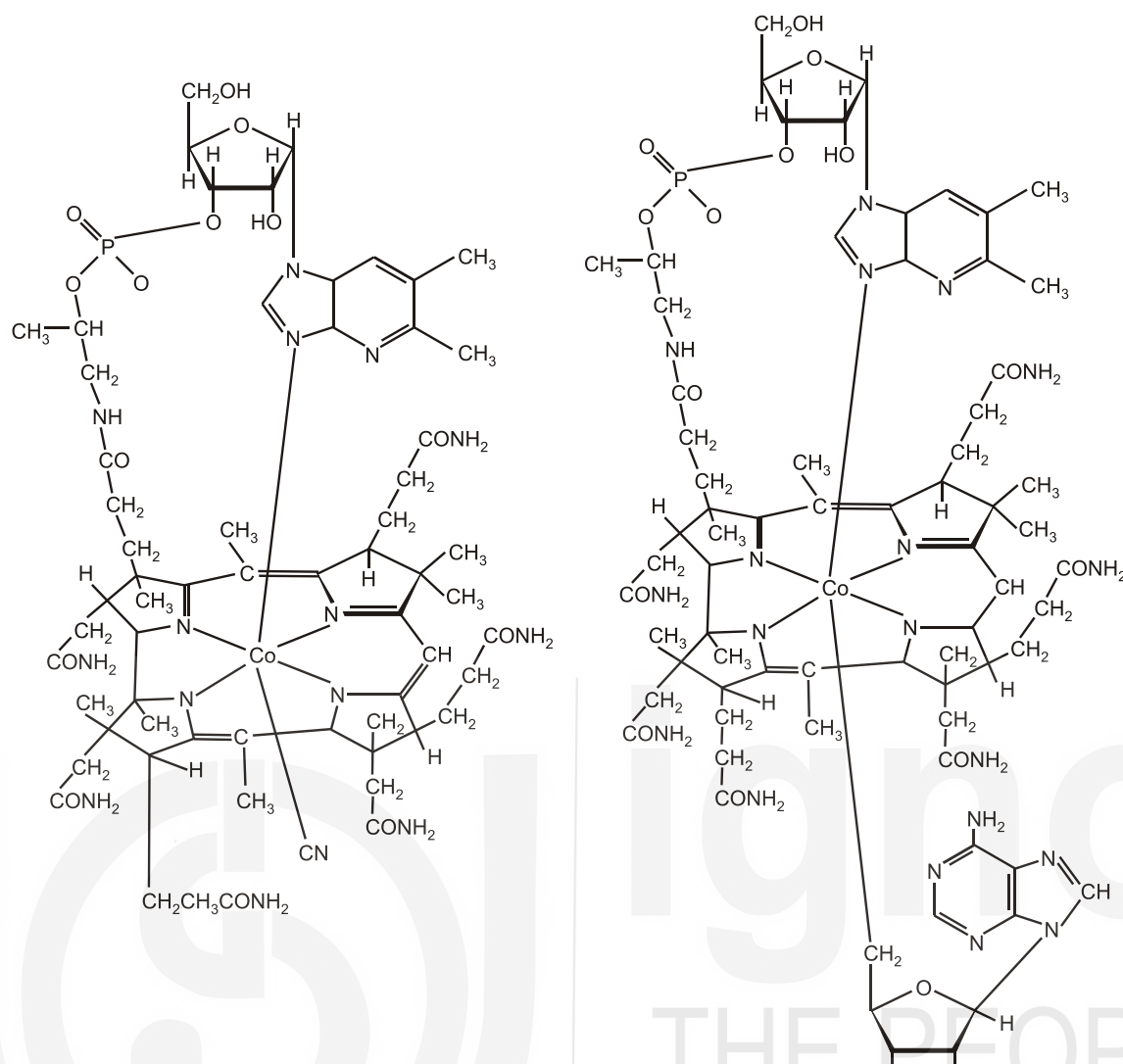


(b) Coenzyme: Tetrahydrofolic Acid (THFA)

Figure 4.22: Structures of folic Acid and tetrahydrofolic acid

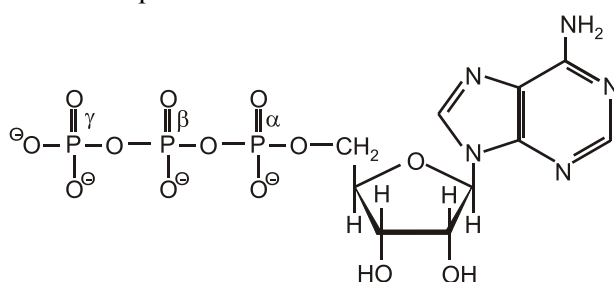
- *Cobamide coenzymes*

Cobamide coenzymes are the derivatives of vitamin B₁₂. The structure of cobamide-coenzyme is very complex and is shown in Figure 4.23. They participate in many biochemical reactions. L-methylmalonyl CoA is converted to succinyl CoA by the action of the enzyme methylmalonyl CoA isomerase. In this reaction, the –CO–SCoA group is transferred by the cobamide-coenzyme. An important reaction catalyzed by this coenzyme as an integral part of the ribonucleotide reductase is the reduction of ribonucleotides to deoxyribonucleotides.

Figure 4.23: Structure of vitamin B₁₂ and its coenzyme

- *Adenosine triphosphate*

Though adenosine triphosphate (ATP) often functions as a second substrate, it can also serve as a coenzyme by modulating the activity of specific enzymes. The compound consists of adenine connected to a ribosyl 5'-triphosphate. See Figure 4.24 for its structure. As a co-substrate, ATP is utilized by various kinases for the transfer of the terminal phosphate group to various acceptors. For example, glucose is converted to glucose 6-phosphate in presence of the enzyme hexokinase by accepting phosphate from ATP. This will be discussed many a times in the Units 6 and 7 covering carbohydrate and lipid metabolism.



ATP donates PO_4^{2-} group

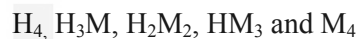
Note the Greek lettering for the different phosphate groups

Figure 4.24: Structure of ATP

So far we have studied about the different enzymes and coenzyme involved in metabolism. Interestingly, it is known that enzymes catalyzing essentially the same reaction may differ in various ways. These are called *isozymes*. The next section focuses on isozymes.

4.10 ISOZYMES

Sometimes an enzyme present in the same organism is found to have different molecular forms but catalyzing the same reaction. These are called *isozymes*. The 1964 Committee recommended that "*multiple enzyme forms*" in a single species should be known as isozymes. Among many enzymes known to have isozymes, the most studied is *lactate dehydrogenase (LDH)*, which is an important enzyme of carbohydrate metabolism. This enzyme exists in five possible forms in most vertebrates. In fact two basically different types of LDH occur predominantly in the heart and muscle. The former consists of four identical monomers (H). The latter (muscle LDH) also forms due to the combination of four identical monomers (M) but having different amino acid composition, in comparison to the former. Different combinations of H and M monomers yield three additional hybrid enzymes possessing four monomers each. The possible combinations of H and M monomers therefore, produce five isozymes of LDH. These are:



Isozymes differ from each other not only in the amino acid composition, they also have different electrophoretic property, thermolability, immunological properties and kinetic properties such as substrate affinities i.e. different K_m values.

Estimation of isozymes of some enzymes can be used for the clinical diagnosis of different diseases. Let us learn about this aspect next.

4.11 ENZYMES IN CLINICAL DIAGNOSIS

The rationale for measuring plasma or serum enzyme levels is based on the premise that these levels reflect changes that have occurred in a specific tissue or organ. Enzymes present in the blood are of two types – one type such as thrombin (associated with blood coagulation) has a functional role and is present in high concentration, the other type has no functional role in the blood and is present in very small amount. The latter types of enzymes mainly originate from different tissues or organs. An insult in the form of any disease may cause changes in cell membrane permeability or increased cell death, resulting in the release of intracellular enzymes into the blood. As a result, the concentration of these enzymes increases in the blood. In the diagnosis of specific organ involvement in a disease process, it would be ideal if enzymes unique to each organ could be identified. But, this seldom occurs as the metabolism of different organs is not unique. *Alcohol dehydrogenase* and *acid phosphatase* are two such enzymes, the levels of which in the blood can be used as specific tools for the diseases of liver and prostate, respectively. However, the ratio of enzymes varies from organ to organ. This fact, combined with a study of the kinetics of appearance and disappearance of particular enzymes from the blood, enables one to identify the involvement of a specific organ in a disease. Both *glutamate-oxaloacetate transaminase (GOT)* and *glutamate-pyruvate transaminase (GPT)* are present in the liver but the concentration of GPT is more than GOT. In liver dysfunction, both these enzymes are leaked out from liver and as a result, their level in the blood increases but the rise is more in case of GPT than GOT making it a reliable marker for liver diseases.

Following a heart attack, many enzymes such as *GOT*, *creatine phosphokinase*, *LDH* etc. are released from the myocardium but their time of release differs, enabling one to establish when the attack occurred and whether the treatment is effective or not.

Enzymes that are often estimated in the plasma or serum for confirmatory or suggestive diagnosis of different diseases are listed in Table 4.4 along with their normal values. This has to be kept in mind that these values will vary according to the procedure used for their estimation and generally all laboratories standardize their own normal values.

This is to note that though increase of enzyme activities in the cerebrospinal fluid sometimes occurs e.g. increase in lactate dehydrogenase activity in meningitis, it is not reflected in the blood.

Isozymes are often estimated to specify a diseased organ/tissue. Two different isozymes of alkaline phosphatase are present in liver and bones. So, a rise of serum alkaline phosphatase may occur either due to liver damage or due to problems related to bones. In this situation, estimation of specific isozymes will give a clear picture for the rise in the enzyme level in serum and this enables one to pin point the affected organ/tissue.

Table 4.4: Common enzymes of diagnostic importance

Enzyme	Normal value in the serum	Concentration increases in conditions
Acid phosphatase	1-5 units/dl	Metastatic carcinoma in the prostate.
Alkaline phosphatase	5-13 units/dl	Rickets, hyperparathyroidism, obstructive jaundice, kidney disease, metastatic carcinoma, osteoblastic sarcoma. Isozymes of alkaline phosphatase can distinguish liver lesions from bone lesions in cases of metastatic carcinoma.
Amylase	0.8-32 IU/L	High intestinal obstruction, acute pancreatitis.
Acetyl cholinesterase	3-5 IU/ml	Nephrotic syndrome.
Creatine phosphokinase	5.5-75 units/L (Male) 6-50 units/L (Female)	Muscular dystrophy, myocardial infarction.
Glutamate-oxaloacetate transaminase (SGOT)	5-40 units/dl	Myocardial infarction, slightly elevated in liver diseases.
Glutamate-pyruvate transaminase (SGPT)	5-35 units/dl	Acute liver diseases, slightly elevated in cardiac necrosis.
Lipase	0.2-1.5 units/dl	Acute pancreatitis, pancreatic carcinoma.
Glucose-6-phosphate dehydrogenase (in RBC)	3.5-7.5 units/ml	Haemolytic anaemia often associated with administration of anti-malarial or sulphonamide drugs and after eating fava bean.
Lactate dehydrogenase	90-200 IU/L	Myocardial infarction, acute hepatitis, renal tubular necrosis.

Check Your Progress Exercise 4

- 1) What are coenzymes? How are the coenzymes grouped?

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2) Name the type of reactions in which the following co-enzymes are involved.

- a) NAD^+ and NADP^+
- b) FAD and FMN
- c) Lipoic acid
- d) Biotin
- e) TDP
- f) Tetrahydrofolate

3) Match the following:

A

- a) Niacin
- b) Riboflavin
- c) Thiamine
- d) Biotin

B

- i) TDP
- ii) NAD^+ and NADP^+
- iii) FAD and FMN
- iv) Pyruvate carboxylase

4) What is the main role of tetrahydrofolate in different biochemical reactions?

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5) What are isozymes? How many isozymes of LDH are known?

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6) Name any two enzymes which are determined during the clinical diagnosis of the following diseases:

- a) Heart attack
- b) Liver diseases
- c) Acute pancreatitis

4.12 LET US SUM UP

In this we unit covered the structural and functional aspects of enzymes and coenzymes – two of the most important group of biomolecules. While enzymes are almost always protein in nature, coenzymes are mainly derived from water-soluble vitamins. You now have a clear idea about the different classes of enzymes and the type of reactions they catalyze. This knowledge will greatly help you to understand the different metabolic reactions that will be described in the subsequent sections.

One of the main differences between an inorganic catalyst and enzyme is the high degree of specificity of the latter which has been discussed in the light of models for enzyme-substrate interaction. Enzyme being a protein is particularly sensitive to different environmental factors and its activity changes accordingly. These along with other important factors governing the activity of an enzyme have been dealt with in this unit.

Enzyme kinetics was further discussed in this Unit and so also the application of Michaelis-Menten equation. You now understand how the affinity of an enzyme for a particular substrate can be judged on the basis of its K_m value. Impact of different inhibitors on the K_m and V_{max} of an enzyme is also taken into consideration.

Further, in this unit we got the idea how different coenzymes are taking part in different enzyme-catalyzed reactions with suitable examples. This will also greatly help us in learning different metabolic reactions in the subsequent units of this course. Importance of serum enzymes and isozymes in the diagnosis of different diseases is well known. This has been discussed comprehensively. Thus this unit has covered all important aspects of enzymology but emphasis has been given only on the topics relevant to our need as nutritional biochemists.

4.13 GLOSSARY

Active site	: the site on the surface of an enzyme to which substrate or substrates bind.
Cleft	: partially divided
Conformation	: specific arrangement of the molecule.
Denaturation	: change from original structures.
Electrophoretic	: ability to move in an electric field.
Haemolytic anaemia	: liberation of haemoglobin from erythrocyte causing anaemia.
International Enzyme	: the amount of enzyme that catalyses the transformation of 1 μ mol of substrate into product in 1 minute.
Isozymes	: an enzyme having different molecular forms but catalyzing the same reaction.
Katal	: the amount of enzyme that transforms 1 mol of substrate into product in one second.
Metabolism	: synthesis or breakdown of bio-molecules.
Metastatic carcinoma	: cancer that can be transferred from one part of the body to other unrelated parts.
Muscular dystrophy	: defects in the muscle due to faulty nutrition.
Myocardial infarction	: necrosis of myocardium due to interruption of blood supply.
Myocardium	: the middle layer of the heart wall.
Necrosis	: cell death.
Nephrotic syndrome	: kidney disease due to degeneration of renal tubule.
Osteoblastic sarcoma	: a kind of tumor.
Pancreatitis	: inflammation of the pancreas.
Prosthetic group	: a non-protein part of the enzyme which remains tightly bound to the protein part.
Rickets	: a condition in children due to vitamin D deficiency.
Thermolability	: temperature sensitivity.
Trivial	: less important.
Turnover number	: the number of molecules of substrate transformed per catalytic site of the enzyme per minute.

4.14 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

- 1) a) Enzymes are the proteins that act as catalysts, speeding the rate at which biochemical reactions proceed but not altering the direction or nature of the reactions.
 b) A holoenzyme is whole enzyme, as a complete and functional molecule. Generally, a holoenzyme consists of a polypeptide portion (an apoenzyme) and at least one cofactor or other coenzyme.
 c) Metabolism is all of the organized chemical reactions in a cell which are under the control of enzymes.
- 2) Enzymes which act inside the cell are called as endoenzymes and those which are liberated outside the cell are called as exoenzymes. While enzymes like those involved in the production of energy belong to the first category, digestive enzymes belong to the second category.
- 3) The enzymes are classified by assigning code numbers to them. The code numbers are prefixed by EC (Enzyme Commission) and contain four numbers separated by points, with the following meaning:
 - a) the first number (EC1) shows to which of the six main classes an enzyme belongs
 - b) the second figure (EC1.1) indicates the sub-class
 - c) the third figure (EC1.1.1) gives the sub-subclass, and
 - d) the fourth figure (EC1.1.1.1) is the serial number of the enzyme in its sub-class.

For example, alcohol : NAD⁺ oxidoreductase is assigned the number 1.1.1.1 because it is an oxidoreductase, the electron donor is an alcohol and the acceptor is the coenzyme NAD⁺. Thus, in naming an enzyme the substrate is stated first followed by the reaction type.
- 4) Enzymes, the organic catalysts, differ from the inorganic catalysts in their extraordinary specificity.
- 5) The enzymes are specific in action that is, they catalyze a particular reaction or a particular type of chemical bond or functional group. This is referred to as enzyme specificity. The 4 different types of specificities are reaction specificity, bond specificity, group specificity and optical specificity.
- 6) a) – v)
 b) – iv)
 c) – i)
 d) – vi)
 e) – iii)
 f) – ii)

Check Your Progress Exercise 2

- 1) The general theory of enzyme action given by Michaelis and Menten, states that the enzyme (E) first binds the substrate (S) to form a transient enzyme-substrate complex (ES). This complex then dissociates into the product (P) and the unaltered enzyme (E).

$$E + S \longrightarrow ES \longrightarrow E + P$$
- 2) According to lock and key model, the catalytic site of the enzyme has a proper conformation compatible to a specific substrate even in the absence of the substrate molecule. The catalytic site binds the substrate and catalyzes the reaction without any change in its own three dimensional conformation. This model, however, failed to explain the change in enzyme activity in presence of allosteric modulators or the action of the noncompetitive inhibitors.

- 3) The enzyme activity can be expressed as ‘katal’, which is the amount of an enzyme that transforms 1 mol of substrate into product in one second.

Other than katal, the activity of an enzyme may be expressed in different other ways as:

- International enzyme unit (IU)
- Specific activity of an enzyme preparation, expressed as kat/kg or IU/mg of protein.
- Molar activity of an enzyme, expressed as kat/mol of the enzyme.
- Turnover number of the enzyme.

- 4) The Michaelis-Menten equation is:

$$v = \frac{V_{\max} \times [S]}{K_m + [S]}$$

where, v is the velocity of the reaction at any stage, $V_{\max}$ is the maximum velocity, $[S]$ is the substrate concentration and K_m is the Michaelis-Menten constant, usually expressed in moles per litre.

- 5) The relationship between substrate concentration and reaction velocity is best explained through the hyperbolic curve. When substrate concentration is low, rate of reaction is directly proportional to the substrate concentration. A gradual increase in the substrate concentration results in saturation of active enzyme site ($V_{\max}$). In between, lies the reaction velocity i.e. half of maximum velocity. In this position,

$$v = \frac{V_{\max} \times [S]}{K_m}$$

Here, $V_{\max}$ and K_m are both constants, $V_{\max} / K_m$ ratio may be replaced by a new constant, K . Thus, we have:

$$v = K \times [S]$$

So, reaction velocity (v) of the enzyme-substrate reaction at this stage is directly proportional to substrate concentration $[S]$.

- 6) Affinity of an enzyme for a substrate can be judged by K_m . The enzyme affinity inversely varies with the K_m .
- 7) Koshland's induced fit model can explain noncompetitive inhibition.

Check Your Progress Exercise 3

- 1) Concentration of enzyme, concentration of substrate temperature, pH, activators, oxidation are the environmental factors on which enzyme activity depends. A brief explanation of two factors follows:
- a) Concentration of enzyme: Increase in enzyme concentration will increase the rate of an enzyme catalyzed reaction. This is because of the availability of additional catalytic sites at the beginning of the reaction to which the substrates can bind.
 - b) Concentration of substrate: At low concentration, the rate of reaction also remains low. This is because at this stage all the catalytic sites of the enzyme are not occupied by the substrate molecules. So, with the increase in substrate concentration, the rate of the reaction also increases until all the catalytic sites of the enzyme are utilized. Rate of the reaction becomes maximum at this point and beyond this, it remains constant.

- 2) Enzymes are often inhibited by the presence of suitable inhibitors. This is referred to as enzyme inhibition. Much of current drug therapy is based on this, hence it is significant.
- 3) a) Competitive inhibition takes place when a compound having a strong structural resemblance with the substrate competes with it for the catalytic site of the enzyme. Once the compound binds, the enzyme cannot convert the inhibitor to products. Increasing substrate concentration, however, is capable of displacing the inhibitor. Thus this type of inhibition is reversible in nature. A good example of this is the reaction, catalyzed by succinate dehydrogenase in the citric acid cycle. In this reaction, succinate is converted to fumarate with the aid of this enzyme. Now, the compound malonate is structurally similar to succinate and if present, it will compete with succinate for the catalytic site of succinate dehydrogenase and reduce the product formation. Thus malonate is a competitive inhibitor for this particular reaction.
- b) In uncompetitive inhibition, the inhibitor only binds with the enzyme-substrate complex making it inactive. As a result, the product formation becomes difficult. In uncompetitive inhibition, both K_m and V_{max} changes. The former increases while the latter decreases.
- c) In noncompetitive inhibition, the inhibitor binds at a site on the enzyme other than catalytic site. As there is no competition between the substrate and the inhibitor, the inhibition cannot be reversed by increasing the substrate concentration. For example, enzymes possessing the essential $-SH$ group are sometimes inhibited by metals like mercury or copper.
- 4) The K_m of the enzyme for the substrate shows an apparent increase in the presence of an inhibitor.

Check Your Progress Exercise 4

- 1) Coenzyme is an organic, non-protein molecule that binds with an apoenzyme to form an active enzyme. Coenzymes can be grouped as hydrogen transferring coenzymes and group transferring coenzymes.
- 2) a) oxidation-reduction reactions
b) oxidation-reduction reactions
c) oxidative decarboxylation reactions
d) transfer of carboxylic groups
e) transfer of aldehyde and glyoxal groups.
f) Transfer of one-carbon atoms.
- 3) (a) – (ii)
(b) – (iii)
(c) – (i)
(d) – (iv)
- 4) Biosynthesis of purine and methionine is the main role of tetrahydrofolate.
- 5) Isozyme is an enzyme present in the same organism having different molecular forms but catalyzing the same reaction. LDH exists in 5 possible forms.
- 6) a) GOT, creatine phosphokinase, LDH
b) SGOT, SGPT, alkaline phosphatase
c) amylase, lipase