Indira Gandhi National Open University
School of Sciences

BCHET-149
MOLECULES OF LIFE

Block

3

ENZYMES

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BLOCK 3: ENZYMES

In continuation to the study of biomolecules in this course, another class of biomolecules are discussed. Thus, in Block 3 you will study about another very important biomolecules viz., enzymes. These molecules are responsible for almost all the physiological processes in the cell, hence the living beings.

Block 3 comprises three units. In Unit-7, **'Enzymes: Nature and Classification'** an introduction to the nature of enzymes is discussed. The unit deals with the nomenclature, classification and characteristics of enzymes. Unit 8 is on **'Enzyme: Action and Regulation'** which discusses the mechanism of enzyme action, enzyme kinetics, factors affecting enzyme reaction rate and the regulation of enzyme activity. Unit 9 on **'Enzymes and Drug Action'** deals with the significant role of enzymes in medicine. The unit differentiates medicine from drug, pharmacokinetics from pharmacodynamics and explains the important drug-receptor theory of drugs in a living system. It also explains the process of enzymes as drug targets and the concept of structure activity relationship.

Expected Learning Outcomes

After studying this block, you should be able to:

- describe the nomenclature, classification and characteristics of enzymes,
- explain the mechanism of enzyme action, enzyme kinetics, factors affecting enzyme reaction rate and the regulation of enzyme activity, and
- explain the drug-receptor theory and the mechanism involved in enzymes as drug targets.

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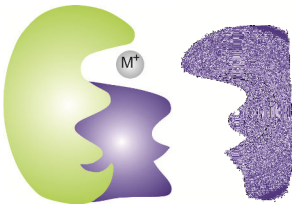


UNIT 7

ENZYMES: NATURE AND CLASSIFICATION

Structure

7.1 INTRODUCTION

In Unit 6, 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. You studied in Unit 1 about the living cell. The cell depends upon a large numbers of biochemical transformations for its own survival and hence that of the organism to which it belongs. Since each biochemical reaction requires a separate enzyme to catalyse it, a cell must contain a large number of different enzymes. The enzymes regulate the speed and direction of all biochemical changes. Thus, understanding enzymes and their properties is important in understanding metabolism, the latter would be discussed later in this course.

In the beginning of this unit of you will study about the nomenclature of enzymes. As the number of enzymes is very large, it is very essential to understand their various types. It is dealt under the classification of enzymes. In order to understand the functioning in the living cell it is very important to understand the characteristics of enzymes that have been explained next in the unit. You will study more about the mechanism of functioning of enzymes in the next unit.

Expected Learning Outcomes

After studying this unit, you should be able to:

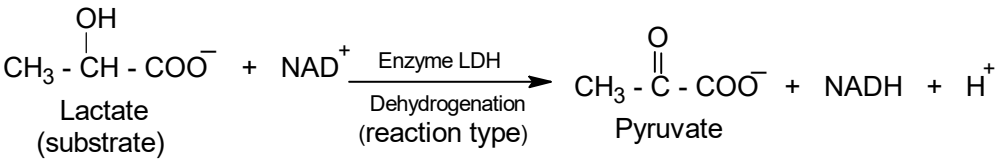
- ❖ define enzymes and explain the basic nature of these molecules;
- ❖ explain the different ways of naming enzymes;
- ❖ describe the chemical nature and catalytic efficiency of enzymes;
- ❖ explain the specificity of action and regulation of enzyme activity; and
- ❖ classify enzymes into six major groups on the basis of their biochemical actions.

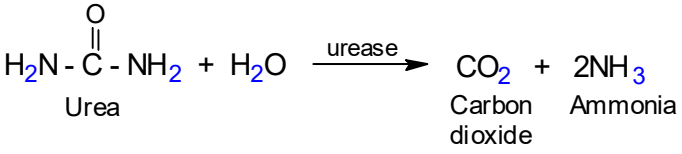
7.2 NOMENCLATURE OF ENZYMES

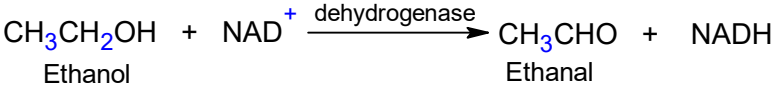
The term enzyme was introduced by W. Kühne in 1878 for certain substances in yeast (in *zymin*) that were responsible for fermentation. These are a specialised class of proteins, and, as already mentioned, these act as biocatalysts in metabolic reactions. These are responsible for most of the activities that take place within the system and till now more than two thousand enzymes have been identified. In nature, enzymes help millions of chemical reactions to occur at extraordinary speeds and under moderate conditions. In the absence of these biocatalysts the reactions would proceed at a very low rate and under rigorous conditions. As enzymes are important in maintaining life, they have many medicinal 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 in industrial production, as in fermentation to make alcoholic beverages.

Metabolic reactions consists of **catabolic** and **anabolic** reactions. In catabolic reactions larger molecules are broken down to produce energy, whereas in anabolic reactions, the cell uses energy to produce molecules which it needs for growth and repair.

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 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.







This nomenclature worked fine initially when the number of enzymes was not that large. However, with large number of enzymes this system of nomenclature did not work well due to overlap and other problems. Therefore the International Commission on enzymes was set up by the International Union of Biochemistry (IUB) in 1957 to impart the nomenclature of enzymes. The Commission on Enzymes (EC) considered the question of a systematic and logical nomenclature for enzymes and recommended two types of nomenclature; one **systematic** and the other **working** or **trivial**. The nomenclature has been revised and updated from time to time.

In the systematic way all the known enzymes are given code numbers depending on their classes and sub-classes etc. These are collected in a list called as enzyme list, in which the common names follow immediately after the code number and are described as **recommended** names. Thus, enzymes have been assigned code numbers with four parts:

- 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 sub-sub-class, denoting the exact specification of the reaction removed.
- The fourth number refers to the serial number of the enzyme, in its sub-sub-class.

As an illustrative example, the number of lactate dehydrogenase is 1.1.1.27 and for hexokinase it is 2.7.1.10.

You would note that although systematic names describe the enzyme system accurately, they are otherwise not very convenient for day to day use. Hence, old trivial names/common names continue to be used in the biochemical literature. The classification of enzymes is based on the coding system which you would study in the following subsection.

7.3 CLASSIFICATION OF ENZYMES

The enzyme commission has classified enzymes by assigning an EC (enzyme) commission number to these which consist of four parts as mentioned in the previous subsection. The following three principles are to be followed in classifying enzymes.

1. An enzyme is to be named by adding the suffix ‘–ase’ to the name of the substrate on which it acts.
2. The chemical reaction catalysed is specific for an enzyme so it forms the basis of classifying or naming the enzyme.
3. The enzymes are classified on the basis of the type of reaction catalysed.

Thus the classification is based on the reactions enzymes 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:

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

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.

$$\text{ATP} + \text{D - Hexose} \xrightleftharpoons{\text{Hexokinase}} \text{ADP} + \text{D - Hexose 6-phosphate}$$

Hydrolases

A very large number of enzymes catalyse hydrolytic reactions. Many of the digestive enzymes, such as amylase, sucrose, 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.**

$$\text{Triacylglycerol} + H_2O \xrightleftharpoons{\text{Pancreatic lipase}} \text{2 - Monoacyglycerol} + \text{2 Fatty acids}$$

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, fructose bisphosphate aldoase, is an example of this type. The full systematic name of this enzyme is **D-fructose-1, 6-bisphosphate-D-glyceraldehyde 3-phosphate lyase,** signifying that the substrate, D-fructose-1, 6-bisphophate is degraded in such a way as to give D-glyceraldehyde-3-phosphate as a product.

$$\text{D -Fructose -1, 6-bisphosphate} \xrightleftharpoons{\text{Fructose - bisphosphate aldoase}} \text{D-Glyceraldehyde 3-phosphate} + \text{Dihyroxyacetone phosphate}$$

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.

D - Glyceraldehyde 3-phosphate $\xrightleftharpoons{\text{Triosephosphate isomerase}}$ Dihydroxyacetone phosphate

Ligases (synthetases)

The function of this class of enzymes is to join two molecules at the cost of energy generated by the hydrolysis of a high energy bond. An enzyme known by its trivial name, isoleucyl-t RNA synthetase, carries the systematic name, **L-isoleucine: t RNA^{ile} ligase (AMP-forming).** The systematic name indicates that L- isoleucine is joined to isoleucine-specific t RNA acceptor, the process being accompanied by splitting of ATP to give AMP and pyrophosphate.

ATP + L-Isoleucine + tRNA^{ile} $\rightleftharpoons$ AMP + Pyrophosphate + L- Isoleucyl-tRNA^{ile}

SAQ 1

Write T for true and F for false in the following statements:

a) Enzyme urease which catalyses the hydrolysis of urea is nonprotein in nature. [ ]

b) In alcohol: NAD oxidoreductase NAD acts as an oxidant. [ ]

c) The biochemical reaction catalysed is specific for an enzyme. [ ]

d) The second number in the recommended name of the enzyme is related to the type of the reaction catalysed. [ ]

SAQ 2

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

a) Urease [ ]

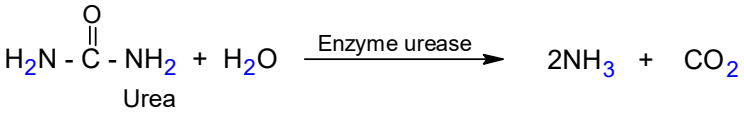
b) LDH [ ]

c) Alcohol dehydrogenase [ ]

d) Amylase [ ]

7.4 GENERAL CHARACTERISTICS OF ENZYMES

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.



This result was confirmed by **Northrop** and **Kuntiz,** who crystallised several enzymes and established that these were proteins 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. Let us study the chemical nature and some other characteristics that are very specific to enzymes with more clarity in the following subsections.

Ribonuclease catalyses the breakdown of ribonucleic acid.

7.4.1 Chemical Nature

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 success was accomplished. The primary amino acid sequence and the three-dimensional structure of many could be achieved. 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.

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 look into the types of groups associated with the enzymes and the terms used for these. Fig. 7.1 gives you a general idea of these terms, as related to the structure and function of an enzyme.

You would recall from previous unit that proteins which produce only amino acids upon hydrolysis are called simple proteins, 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 study more about these in Unit 8.

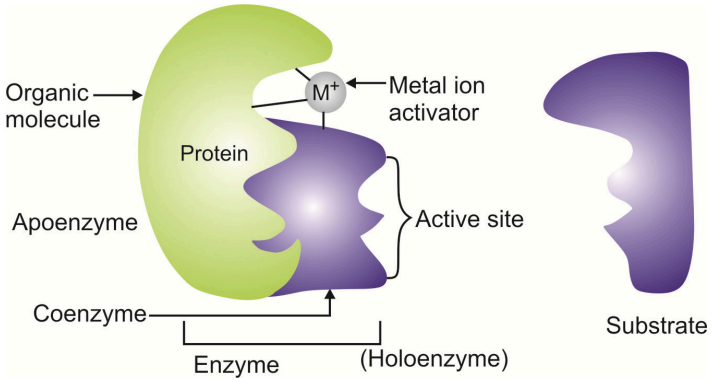


Fig. 7.1: Schematic representation of an enzyme.

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. You will get some more information about coenzymes in the next unit.

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 pre-stage to chemical transformations. In Fig. 7.2, a schematic view of an active site is represented.

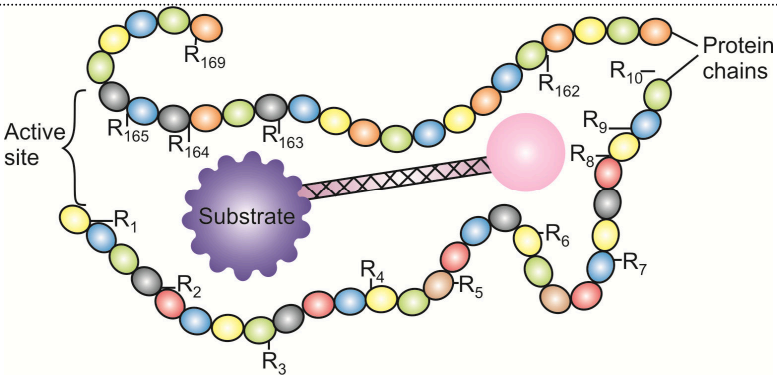


Fig. 7.2: A schematic representation of the active site of an enzyme. The letter R with subscript shows the position of the amino acids in the protein chain.

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

7.4.2 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 (Red Blood Corpuscles, RBCs).

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.

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

Carbonic acid

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.

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

Hydrogen peroxide

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.

This reaction is catalysed by the enzyme peroxidise. Hydrogen peroxide is produced as a by-product 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 7.1 gives you some examples of rate enhancements brought about by enzymes. We shall consider various factors that lead to this rate acceleration in the next unit.

Table 7.1: 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}

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 study about the optimal conditions of temperature and pH in Unit 8. The turnover numbers of some enzymes are given in Table 7.2. As it is evident from the table the rate at which an enzyme catalyses a reaction, will very from enzyme to enzyme.

Table 7.2: 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 study about enzymatic. specificity of action.

7.4.3 Specificity of Action

An aspect of catalysis that distinguishes an enzymic from its nonenzymic counterpart, is the specificity displayed by the former in the 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 on 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 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 process 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 channelized in a particular direction. The specificity of enzymes can be explained by two theories described below.

Lock and Key Theory of Specificity

About a century ago, Email Fischer had proposed the **"lock and key"** hypothesis to explain the specificity of enzyme-substrate interaction. According to this, 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. 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. The 'lock and key' theory is depicted schematically in Fig. 7.3.

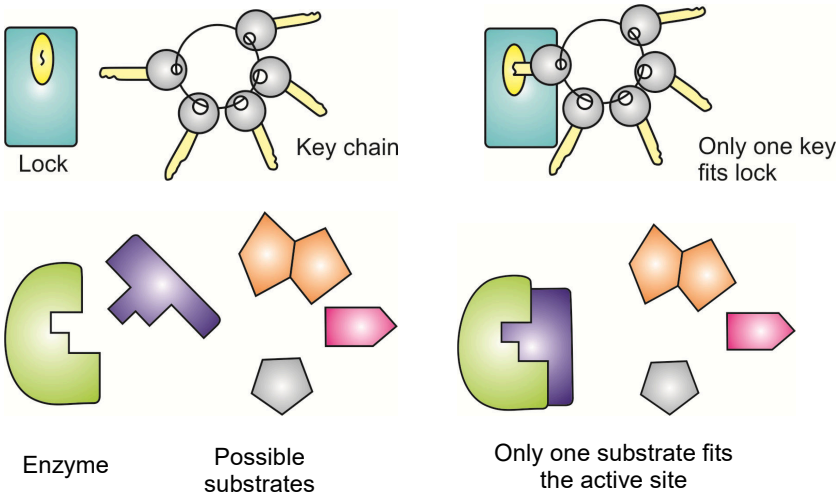


Fig. 7.3: In "lock and key" hypothesis, the active site conforms precisely to the substrate molecule.

Induced Fit Theory of Specificity

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 and vice versa. This theory is also diagrammatically illustrated in Fig. 7.4.

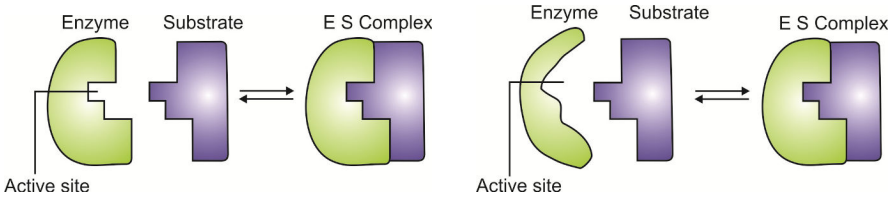


Fig. 7.4: In "induced fit" theory, the active site is induced to take a complementary shape by the substrate molecule.

7.4.4 Regulation of Enzyme Activity

An interesting feature of enzymic catalysis which is not shown by its non-enzymic counterpart, is its ability to be regulated by small ions or small molecules which may be substrate, 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 turning of enzyme activity in response to changing conditions in the cell. You will learn more about this aspect of enzyme function in Unit 8.

SAQ 3

Choose the correct option to complete the following statement.

The binding of an enzyme to the substrate involves:

a) only hydrogen bonds.

b) only electrostatic interactions.

c) hydrogen bonds and electrostatic interactions.

d) only van der Waals interactions.

SAQ 4

How is a cofactor different from an activator?

7.5 SUMMARY

- Enzymes are biocatalysts which increase reaction rate enormously. These 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.

- 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. In the systematic way all the known enzymes are given code numbers depending on their classes and sub-classes etc.
- Enzymes have been grouped into six major classes on the basis of the nature of the reactions 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.
- Many enzymes (conjugated proteins) require nonprotein part without which these 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 “lock and key” and “induced fit” hypothesis are proposed to explain the specificity of enzyme-substrate interaction.
- The regulatory properties of enzymes enable fine tuning of enzyme activity in response to the needs of the cell, under changing physiological conditions.

7.6 TERMINAL QUESTIONS

1. How does an oxidoreductase differ from a transferase? Illustrate your answer with an example.
2. Describe the active site of an enzyme.
3. List the characteristics of enzymes and explain how are the enzyme catalysed reactions different from the chemical catalysed reactions.
4. Name and differentiate between the two models proposed for explaining the specificity of action of enzymes.
5. Differentiate between coenzymes and prosthetic group.

7.7 ANSWERS

Self-Assessment Questions

1. a) False b) True c) True d) False

2. a) and d)

3. c)

4. Cofactor: the nonprotein part of an enzyme is called a cofactor. These are additional chemical groups for example, Mg^{2+}, Zn^{2+}. Activator: when the cofactor of an enzyme molecule is a metal like Fe^{2+}, Cu^{2+}, it is called an activator.

Terminal Questions

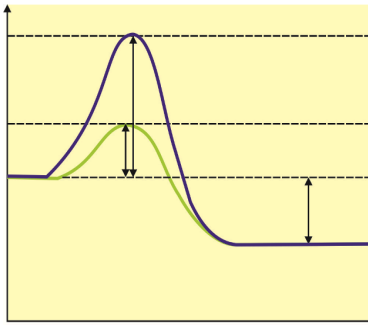
1. An oxidoreductase takes part in physiological oxidation-reduction process. On the other hand, a transferase catalyses the transfer of a chemical group, from one substrate to another. Lactate dehydrogenase is an example of an oxidoreductase and hexokinase is an example of a transferase.

2. 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 breaking processes.

3. Most of the enzymes are chemically proteinaceous in nature however, some of these have a nonprotein part too. These are characterised by high catalytic efficiency, remarkable specificity and regulatory properties. The enzymes increase the reaction rate enormously as compared to the chemical catalysed reaction.

4. The “lock and key” and “induced fit” hypothesis are proposed to explain the specificity of enzyme-substrate interaction. According to “lock and key” model, most of the enzymes are specific to only one particular substrate. This is comparable to a lock which opens up with only one particular key.

5. When the cofactor of an enzyme is a nonprotein organic molecule, the cofactor is known as a coenzyme. A cofactor, which is tightly bound to the apoenzyme, is generally called a prosthetic group.



UNIT 8

ENZYMES: ACTION AND REGULATION

Structure

8.1 INTRODUCTION

In the previous unit you studied about enzymes as the important biological catalysts. Now you are familiar with the nature, classification and some general characteristics of these molecules. As is clear that enzymes are very specific for a reaction and act on a specific substrate, it becomes important to learn how do these act on substartesi.e. the mechanism of enzyme action. In this unit this is what you are precisely going to study.The transition state theory and the concept of activation energy explain the action of enzymes on substrates. The unit begins with the mechanism of action and the role of coenzymes and cofactors in the biological reactions. You have studied in the previous unit that cofactors are some of the metal ions attached to the main

enzymic moiety where as coenzymes are the nonprotein parts. Some vitamins and minerals

act as coenzymes or cofactors for these biocatalysts. Most of the vitamins and minerals are basically required to maintain proper cell function, growth and reproduction.

You are very well familiar with the kinetics of chemical reactions. Enzyme kinetics is similar to that of other chemical reactions in concept and related to the reaction rates. You will study the various factors that affect the rate of biological reactions. The enzymes being biological catalysts need to be regulated so that the reactions are taken to an appropriate stage. Acting in a co-ordinated fashion, enzymes regulate the speed and the direction of all biochemical changes. You will study how do enzyme regulate biotransformations in the last section of this unit.

The next unit deals with the important role of enzymes in pharmaceuticals.

Expected Learning Outcomes

After studying this unit, you should be able to:

- explain the transition state theory of mechanism of enzyme action;
- explain the concept of activation energy for enzyme catalysed reactions;
- describe the biological role of cofactors and coenzymes;
- describe the effect of substrate and enzyme concentration and alsothe role of pH and temperature on biological reaction rates;
- distinguish between competitive and non-competitive inhibition; and
- explain the various mechanisms of enzyme regulation.

8.2 MECHANISM OF ENZYME ACTION

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.

All the enzyme catalysed reactions in the living system are collectively defined as **metabolic reactions.**

In general, the enzyme catalysed reactions proceed through various steps.

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

$$E + S \rightleftharpoons ES$$

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

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

Activated complex

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

$$E\,S^{*} \rightleftharpoons E\,P$$

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

$$E\,P \rightleftharpoons E + P$$

These steps are pictorially depicted in Fig. 8.1.

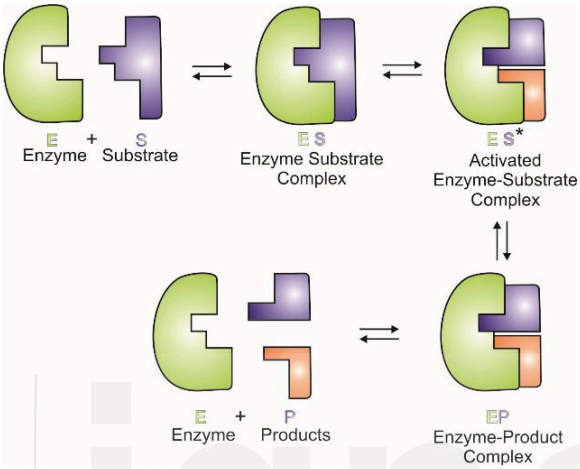


Fig. 8.1: Diagrammatic representation of various steps in enzyme catalysis.

All the above events 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 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 enhancement 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 rearrangement 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.

8.2.1 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. This can be shown as given in Fig. 8.2.

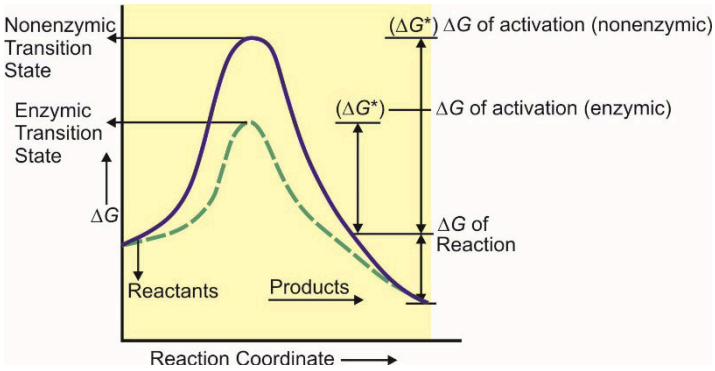
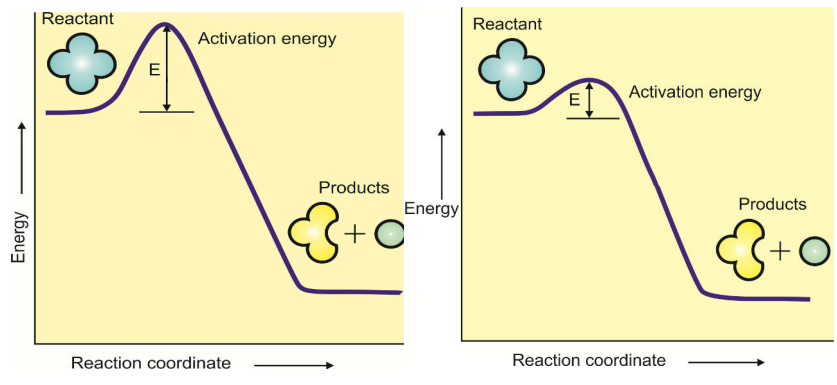


Fig. 8.2: Change in free energy level during the progress of a reaction.

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 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 the transition state. For this concentration to increase, with a corresponding increase in reaction rate, the free energy of activation has to decrease (Fig. 8.3). For this to happen, the transition state may be reached with a smaller expenditure of heat or enthalpy of activation ($\Delta H^{\#}$)or a smaller decrease in the entropy of activation ($\Delta S^{\#}$). This is precisely what enzymes achieve and thus bring about a remarkable increase in the reaction rate. It should be clear from Fig. 8.2 and Fig. 8.3, 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 do not change the equilibrium point of the reaction which is related to the free energy of the reaction (ΔG; Fig. 8.3) but merely speed up the rate at which the equilibrium point is reached.



(a) **(b)**

Fig. 8.3: Effect of enzymes on the activation energy: (a) Nonenzymatic reaction; (b) Enzymatic reaction.

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

8.2.2 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 nonezymatic 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 as 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 nonezymatic 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** and shown in Fig. 8.4.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 the proximity effect of an enzyme has been mimicked in which two interacting groups were attached to a single molecule. It has been demonstrated that a rate increase of about 10^4fold can be realised by this factor alone.

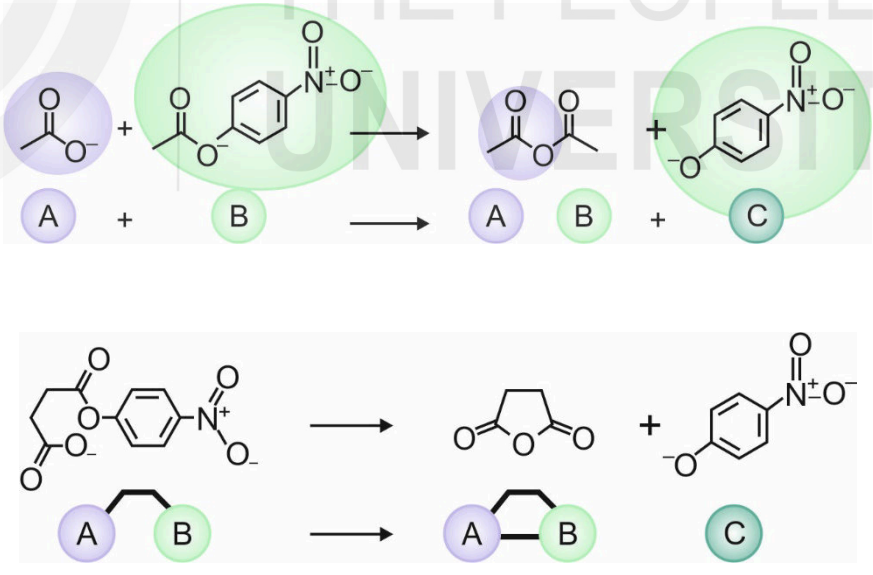


Fig. 8.4: Proximity effect on reaction rates.

The other condition of fruitful collisions, 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). The orientation effect is

shown in Fig. 8.5. 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.

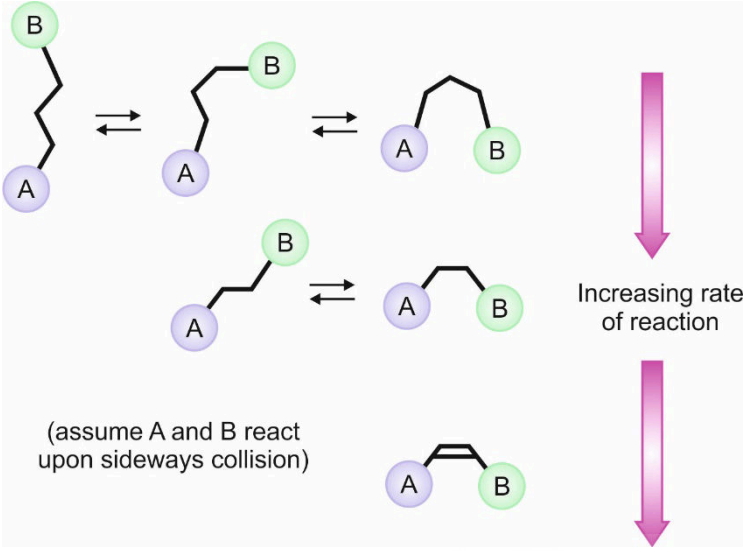


Fig. 8.5: Orientation effect on reaction rates.

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. It is the binding energy of enzyme-substrate interaction at the active side, 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"**. This suggests that when an enzyme-substrate complex is formed, the conformation 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 catalyst because they bring together interacting molecules in a proper orientation. Also the functional groups at the active site provide donors and acceptors in high local concentration. Their groups are also properly positioned to bring about a reaction.

SAQ 1

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.

8.3 ENZYME KINETICS

Enzyme kinetics deals with rates of enzymatic reactions and the various parameters that govern these rates, such as concentration of substrates, 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.

8.3.1 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 is observed 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 reaction, 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 light 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 the factors affecting the rate of biochemical reactions.

8.4 FACTORS AFFECTING ENZYME REACTION RATE

Several factors affect the rate at which enzymatic reactions proceed. These factors are enzyme concentration, substrate concentration, temperature, pH, and the presence of any inhibitors or activators. These are discussed in the following subsections.

8.4.1 Concentration of Substrate

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 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 the 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 unaffected by further increase in substrate concentrations. This is illustrated in Fig. 8.4.

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

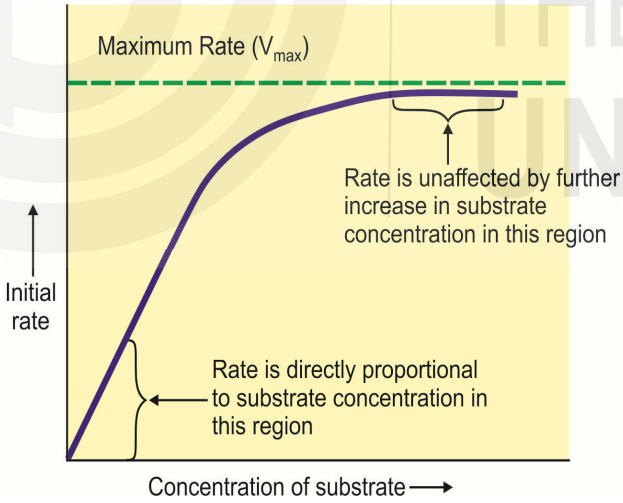


Fig. 8.4: Variation of reaction rate with substrate concentration keeping enzyme concentration constant.

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 nonenzymatic reaction, the reaction rate is dependent on the number of effective collisions between the reactants. The number of such effective collisions 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 enzymatic reaction, if the

concentration of one reactant is kept constant, 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 release 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 for reaction. Thereafter, there will be no further increase in the rate of reaction.

The shape of the curve as shown in Fig. 8.4, 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., $v = 1/2 V_{max}$. This will be clear to you from Fig. 8.5. From the above equation, it is clear the rate will tend to acquire a maximum value when [S) is very high.

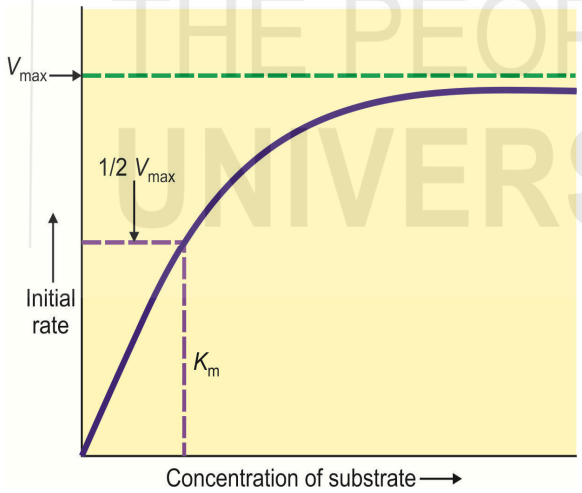


Fig. 8.5 : K_m is equal to the concentration of the substrate required to give an initial reaction rate corresponding to half of V_{max}

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 $v = 1/2 V_{max}$. 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 effect 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.

8.4.2 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 of product shows a corresponding increase (Fig. 8.6).

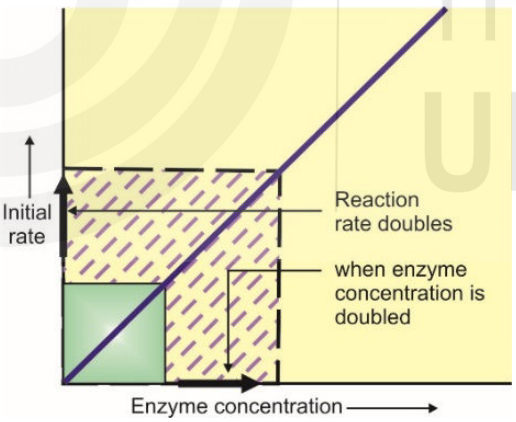


Fig. 8.6: Variation of rate of catalysed reaction with concentration of the enzyme.

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

8.4.3 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. 8.7). You would observe that enzyme activity is maximum only in a narrow pH range and decrease at both higher and lower pH.

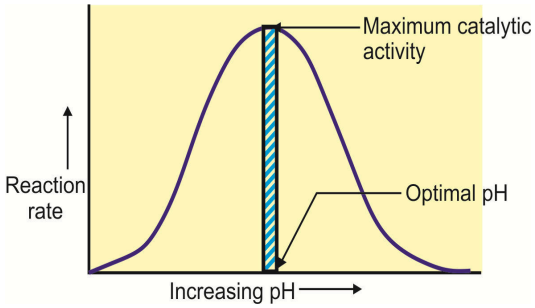


Fig. 8.7: Effect of pH on the activity of an enzyme.

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 in 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 8.1.

Table 8.1: Optimal pH values of some enzymes

Enzyme	**Optimal pH values some enzymes**
Pepsin	1.5
α-Glucosidase	5.4
Urease	6.7
α-Amylase	7.0
Carboxypeptidase	7.5
Trypsin	7.8
Alkaline phosphate	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 7.1 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 (~NH^{+}_{3}) and ~COO^{-}) at its active site for catalytic. This ionisation state would be possible only in a particular pH range, corresponding to the optimal pH. This is depicted in Fig. 8.8.

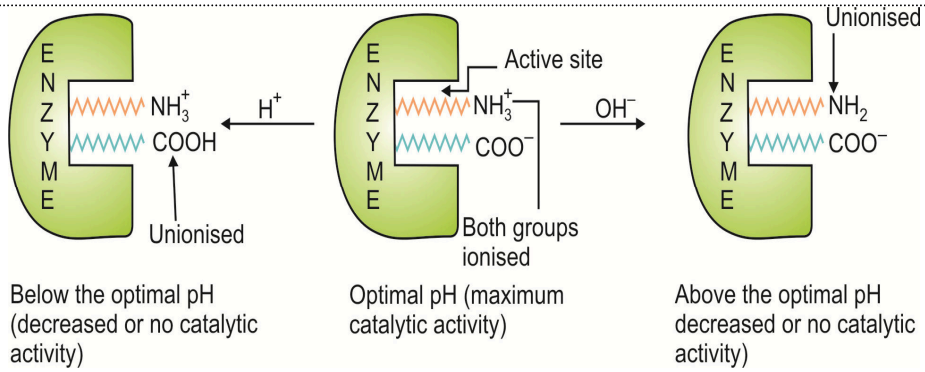


Fig. 8.8: Effect of pH affecting enzyme activity.

As you would observe from the above representation, at acidic pH (lower pH value than optimal pH) the $–COO^-$ groups 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.

8.4.4 Effect of Temperature

You will recall that increase in temperature leads to increased frequency of collisions 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 decrease sharply, suggesting that the enzyme may have an **optimal temperature,** where the rate is maximum as shown in Fig. 8.9.

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

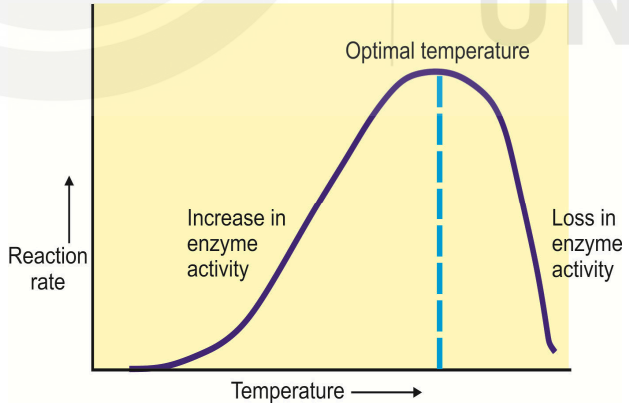


Fig. 8.9: Effect of temperature on the activity of an enzyme.

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.

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 the energy of these collisions. With further increase in temperature, enzyme activity decrease sharply. The latter is due to 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^{o} 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.

8.4.5 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. These will be described in detail in Unit 9.

SAQ 2

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 [ ]

We have described the various factors that alter the rate of an enzymatic reactions. 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.

8.5 REGULATION OF ENZYME ACTIVITY

Lehninger defined the living cell 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 higher 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.

8.5.1 Regulation by Substrate or Product

The intracellular concentrations 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.

When the product of an enzymatic reaction has a structure resembling that of the substrate, it 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 enzyme. This is possible when enzymes interact with the molecules produced in the cell. Let us explain more about this type of regulation.

8.5.2 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. This regulation is represented in Fig. 8.10.

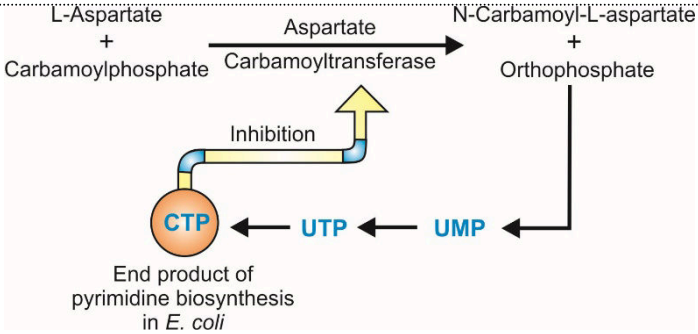


Fig. 8.10: Inhibition of the first enzyme by the end product of the pathway.

Interestingly ATP which is a purine nucleotide, activates this enzyme. This is shown graphically in Fig. 8.11. 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.

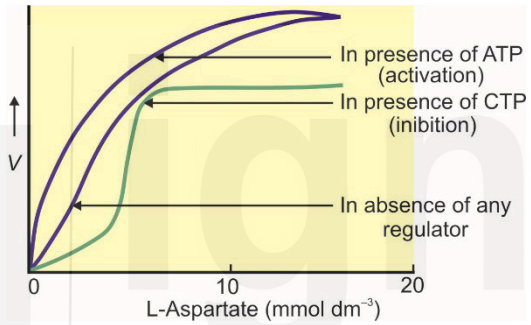


Fig.8.11: Effect of CTP and ATP molecules (regulators) on the kinetics of aspartate carbamoyl transferase from *E.Coli.*

On the basis of the 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 8.5.1).
- 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 enzyme 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. 8.12). The 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 of 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. This kinetic behaviour is compared in case of aspartate carbamoyl transferase as given in Fig. 8.12.

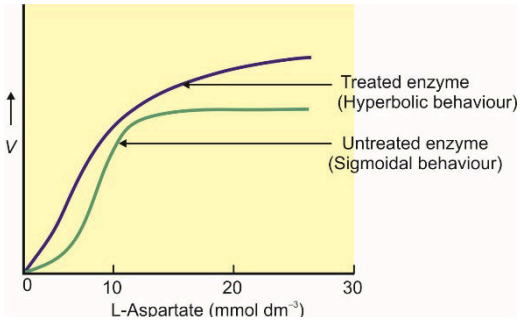


Fig. 8.12: Comparison of kinetics of the treated and untreated regulatory enzymes, aspartate carbamoyl transferase from *E.Coli.*

- Regulatory enzymes generally posses an oligomeric structure like haemoglobin (Unit 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 subunits, causing a change in the structure in the structure of the active site, leading to alternation in enzyme activity. This is depicted in Fig. 8.13 schematically.

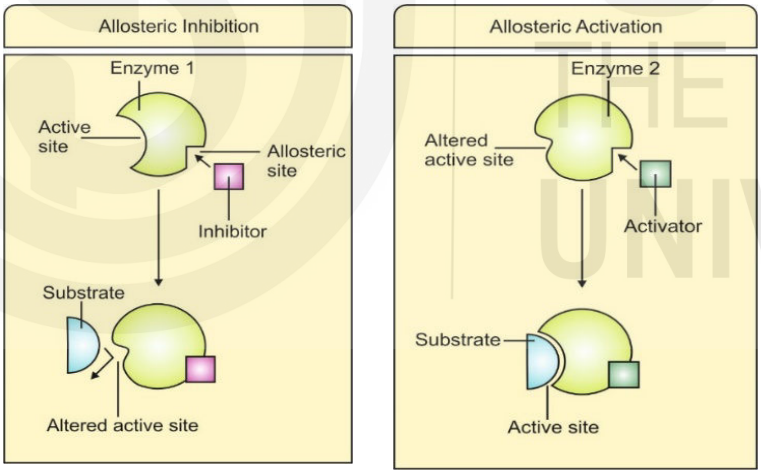


Fig. 8.13: Diagrammatic representation of the reversible structural change in a regulatory enzyme, brought about by a regulator.

You will recall that in the case of haemoglobin, a plot of percent oxygen saturation against oxygen pressure is sigmoidal in nature (Fig. 6.16). 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 change 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.

8.5.3 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:

$$(\text{Glycogen})n \xrightarrow{+\ \text{phosphorylase}} (\text{Glycogen})_{n-1} + \alpha\text{-D-glucose 1-phosphate}$$

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^{3+}. The reversal of the 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 actions are schematically represented in Fig. 8.14.

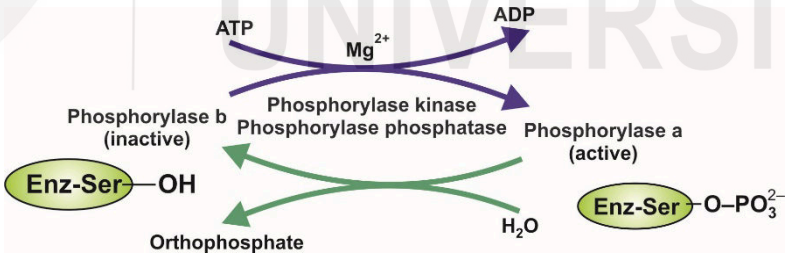


Fig. 8.14 : Schematic representation of the interconversion of phosphorylase a and phosphorylase b.

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 process 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 larger 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 3

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

a) Allosteric effectors function by binding not to the active site, 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 feedback 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.

8.6 ISOENZYMES

Sometimes different forms of an enzyme, catalysing the same reaction, occur in a species. They are called **isozymes** or **isoenzymes.** These 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 isoenzyme 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 consists of four subunits. These subunits are of two different types, α and β. These combine in five different ways viz. A_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 as shown in Fig. 8.16. LDH-1 has the highest electrophoretic mobility towards anode. LDH-1 is predominantly 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.

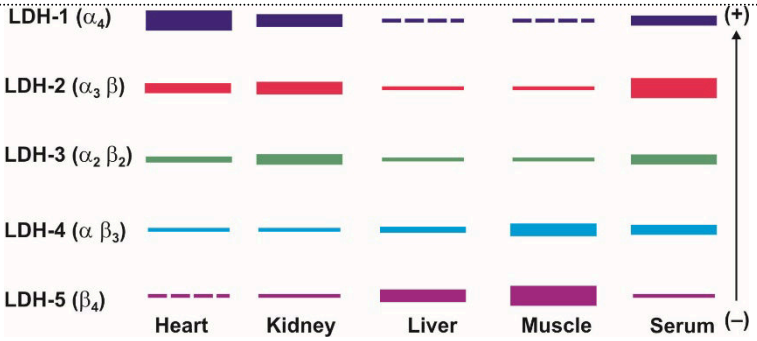


Fig. 8.15: Electrophoretic mobility of isoenzymes of isoenzymes of lactate dehydrogenase.

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 4

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 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 sciences. Let us, therefore, give you a brief account of their role in health sciences.

8.7 ENZYMES IN HEALTH SCIENCE

In the present times enzymes are used to diagnose and to treat diseases. Enzymology is an essential 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 usual 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 so suggest the site of cell damage. The degree of elevation of plasma concentrations of these enzymes also give 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 muscle, bond and malignant diseases. In Table 8.4 you will find a list of some enzymes, clinical assay of which are used for detecting particular diseased states.

Table 8.4: Enzymes assayed in medical diagnostics

Enzyme	Used in the determination of
Lactate dehydrogenase (LDH)	Heart or skeletal muscle damage
Alkaline phosphatase	Liver and bone diseases
Serum Glutamate Oxaloacetate Transaminase (SGOT)	Heart and liver diseases
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 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. 8.16.

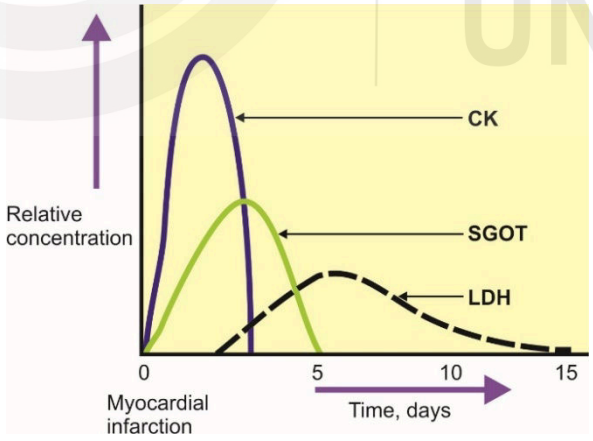


Fig. 8.16 : After a heart attack, concentration of CK increases rapidly and then falls. SGOT level also increases rapidly then falls. One to two days afteran attack, LDH concentration begins to increase, gradually rises for three to four days, and then decreases gradually over the next seven to ten days.

Enzymes also find extensive uses 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 peroxidise, 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 sometimes, 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 this indicates the glucose concentration.

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

$$\textit{o}\text{-Toluidine} + H_2O_2 \xrightarrow{\text{peroxidase}} \underset{\text{(schiff base)}}{\text{Coloured product}} + H_2O$$

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.

SAQ 5

Tick [√] mark the most appropriate statement.

Alkaline phosphatase is used in the determination of:

(a) heart or skeletal muscle damage

(b) liver and bone disease

(c) myocardial infarction and muscle diseases

(d) cancer of the prostate

8.8 SUMMARY

- Enzymes are biocatalysts which increase reaction rate 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 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.

- 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 reactions 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 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 and 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 example 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.

8.9 TERMINAL QUESTIONS

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

8.10 ANSWERS

Self-Assessment Questions

1. a) increase b) lower c) proximity and orientation d) transition
2. d)
3. a) True b)True c) False d) False
4. c)
5. b)

Terminal Questions

1. An oxidoreductase takes part in physiological oxidation-reducing 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. 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 breaking processes.

3. 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 channelize crucial metabolites 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.

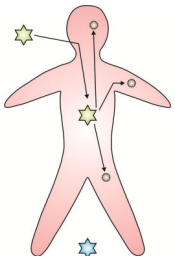
4. An important feature of allosteric regulation is that the end product of a pathway inhibits the first enzyme of that pathway, by binding to an allosteric site or a site distinct from the enzymic active site. The inhibition results from a conformational change in the oligomeric structure of the first enzyme. Such allosteric enzymes do not show normal kinetic behaviour, for in their case, plot of rate versus concentration is not a hyperbole 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.

5. The energy profile a chemical reaction shows that the reactants have to overcome an energy barrier before they 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.

6. 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 and bond making and bond breaking processes.

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

8. Concentrations of the substrate expressed in mole per litre that gives half maximal velocity of the enzyme, is known as K_m. Small value of K_m indicates high affinity of the enzyme for the substrate.



UNIT 9

ENZYMES AND DRUG ACTION

Structure

9.1 INTRODUCTION

In the previous two units you have learnt about enzymes, the naturally occurring biocatalysts, as to how they bring about changes in the biological functions or physiological processes. You know that enzymes are constituted of the biomolecules, proteins specifically. **Drugs** are another class of chemical substances or molecules when administered inside the body, bring about changes in the biological functions or the physiological processes. The drugs are of two types; those with beneficial effects and the others with toxic effects**.** The beneficial and toxic effects of drugs result from their interactions with molecules in the human body. You can easily relate the former to your day-to-day life by the medicines prescribed to you by a doctor when you fall ill. In fact, the prehistoric man also recognised the beneficial or toxic effects of many plants and animal materials. Early written records list remedies of many types, including a few that are still recognised as useful drugs*. The question which you may ask here is: what is the relationship between the enzymes and the drugs especially the beneficial category?*

The intake of chemical substances or drugs affects the action of enzymes directly or indirectly. *How?* You know that enzymes can catalyze multistep chemical reactions and achieve phenomenal rate accelerations by matching protein and substrate chemical groups in the transition state. Some of the most potent and effective drugs take advantage of these chemical interactions and act as inhibitors. The catalytic chemistry of enzymes is the key to

designing potent inhibitors and makes them a special class of drug targets. In this unit you will study how enzymes are good drug targets. We would start with defining some of the terms that you might not have studied earlier in the course and are very important to the study of drugs. We would explain the two very closely related terms often used interchangeably. These are drugs and medicine. This is followed by the action of drugs. You will be introduced to the concept of receptor and how drugs bind with these receptors to produce changes in the biological functions and physiological processes. The structure activity relationship of drugs would also be explained to understand how these help in drug discovery.

Expected Learning Outcomes

After studying this unit, you should be able to:

- define and differentiate between drug and medicine;
- describe the drug receptor theory;
- Explain the structure activity relationships of drugs;
- Discuss enzymes as drug targets.

9.2 COMMON TERMS USED

Let us first get familiar with some of the terms that are essential to understand the concepts involved in the study of drug interaction in human beings. These are explained in brief below.

Dosage form: It is defined as the physical form of a dose of a compound used as a drug intended for administration or consumption.

Dose: The amount of a medicine given at any one time.

Drug formulation: the process of designing by mixing other required substances to the drug and producing the drugs that are eventually sold.

Excipient: inert ingredient added in the drug formulation to protect, support or enhance stability of drug.

Active Pharmaceutical Ingredient: active ingredient in a drug responsible for its effect is called the API.

Pharmacodynamics: The action of drugs on the body with reference to the site and action is called pharmacodynamics

Pharmacokinetics: What the body does with the drug is called pharmacokinetics. It involves absorption, distribution, metabolism and excretion.

Receptor is part of cell or organism which interacts with a drug and produces therapeutic or toxic effects.

Effector is a small molecule like protein that selectively binds to a protein and regulates its biological activity.

Now let us study another two important terms that are different from each other but are used commonly interchangeably.

9.3 MEDICINES AND DRUGS

Most of us may use the terms medicine and drugs interchangeably but scientifically they have different meanings. Medicine is originated from the Latin "*Medicus*" meaning "healing, or physician" and drug is originated from the French word '*drogue*' means a dry herb and Greek "*Pharmacon*" meaning "Drug". Medicine is the formulated form of the drug having definite dose and dosage form. While a drug can be defined as a medicine or other substance which has a physiological effect when ingested or otherwise introduced into the body. We can say that every medicine is a drug but not all drugs are medicines. It can be said that drug is the active pharmaceutical ingredient (API) and does not have a dosage form and dose. On the other hand when the drug is formulated it becomes medicine and with dosage form and dose. As the drug does not have a dosage form it cannot be directly used for treatment. Drugs can be obtained from plant, animal, microorganisms, minerals, synthetic source, semisynthetic source and recombinant DNA technology. While the source of medicine is drug with or without excipient.

Let us suppose there are three drugs with three different therapeutic properties - analgesic (relieves from pain), anti-inflammatory (reduces inflammation or swelling) and antipyretic (decreases body temperature in fever). Let us name these A, B and C. As these have therapeutic properties, these can be classified as drugs. Drug A, though has therapeutic effect, also has potential side effects due to which A cannot be used for treatment, cure and management of any disease, so can't be classified as medicine. Drug B is good but is addictive so it can't be used as medicine. Drug C has beneficial effect and has no harmful side effects so can be used for treatment of diseases and is classified as medicine. The difference between drugs and medicines is summarized in Table 9.1.

Table 9.1: Differences between drugs and medicines

Drug	Medicine
The word 'Drug"is derived from Greek "*Pharmacon"* meaning "Drug".	The word "Medicine" is derived from Latin "*Medicus*" meaning "healing, or physician".
A Drug is any chemical substance which acts on the living body alter the physiological process and is used for prevention, diagnosis, control, and treatment of diseases.	A medicine is the formulated form of drug having definite dose and dosages form which isused for prevention, diagnosis, control, and treatment of disease.
A drug is only active pharmaceutical ingredients (API).	A medicine is the formulation of API with excipients or without excipients.

Drug has no appropriate dosage form and dose.	Medicine has an appropriate dosage form and dose.
Generally, a drug is not using directly for treatment because it needs to be designed in suitable dosage from and dose.	A medicine is used for treatment directly.
Sources of drugs are plants, animals, microorganisms, minerals, synthetic sources, semisynthetic sources, recombinant DNA technology.	Source of medicine is drug and excipient.
All medicines are drugs.	All drugs are not medicines.
Examples: paracetamol, also known as Acetaminophen (analgesic and antipyretic); Morphine (analgesic); Ampicillin (antibiotic).	Examples: Paracetamol tablet, Paracetamol syrup, Paracetamol elixir.

You can proceed to learning the process of drug action after answering the following SAQ.

SAQ 1

Write (T) for true and (F) for false statement(s) in the following.

(a) All drugs are medicines. .

(b) Drugs alter the physiological processes on entering the body.

(c) Drugs have appropriate dosage form.

(d) Medicine is only active pharmaceutical ingredient.

9.4 Processes in Drug Action

As mentioned earlier drug is a chemical substance that when introduced inside the body alters the physiological processes. Once the drug enters the body two processes take place. Firstly, the drug acts on the body and secondly the body metabolises the drug. These two processes are explained by two terms viz. **pharmacodynamics** and **pharmacokinetics,** respectively. These terms have been defined also in Section 9.2. let us understand the two terms in detail in the following subsection.

9.4.1 Pharmacodynamics and Pharmacokinetics

Pharmacodynamics is the study of the biochemical and physiological effects of drugs in the body. It shows the interaction of the drug with tissue receptors which are located on the cell membranes or in the intracellular fluid.

Pharmacokinetics is the study that determines the rapidity and concentration of the drug in the body and its duration of appearance at the target organ, i.e. onset, time of peak action and duration of action. It also determines the route(s) and frequency of administration of drug. The two processes can be depicted pictorially as given in Fig. 9.1.

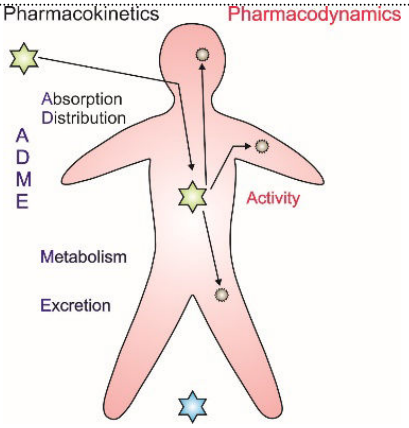
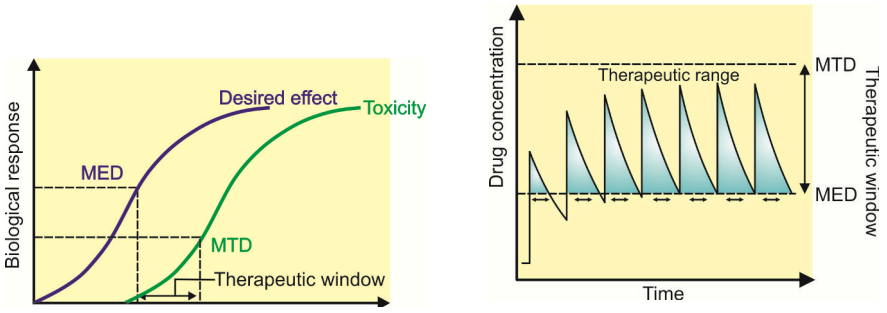


Fig 9.1: Pharmacokinetics and pharmacodynamics of a drug.

When you take a medicine, your body releases the drug to produce a final medicinal product.Then the drug or substance is absorbed and enters the blood. The circulatory system distributes or disperses the drug or substances throughout the fluids and tissues of the body. The drug is then metabolized by the organism and the irreversible transformation of initial compounds to metabolites takes place. The metabolites are then excreted or removed from the body.

Pharmacodynamics plays an important role in drug-response relationship i.e., the relationship between the drug concentration and its effect. It helps in determining the **minimum effective dose** (MED) and the **maximal tolerable dose** (MTD). MED is defined as the dose above which sufficient desired effect is being achieved. The maximal tolerable dose (MTD) is defined as the dose above which intolerable side effects can be observed. The dosage gap between the MED and MTD is termed the **therapeutic window.** The drug-response curve is illustrated in Fig. 9.2 (a) indicating the desired and the side effects. As can be seen the drug concentration is on the x-axis and the y-axis shows the biological effect. **The desired biological effect is shown by the purple line and detrimental side effects by the green line.** The pharmacokinetic profile of the curve is illustrated in Fig. 9.2 (b). As you would note here time is on the x-axis and drug concentration on the y-axis. Also, you can see the unbroken line that specifies the drug concentration and multiple applications of the drug are demonstrated by the multiple peaks which decay with time.



(a) **(b)**

Fig. 9.2: (a) Illustration of typical dose response curves for desired and side effects. (b) Illustration of a dose scheduling pharmacokinetic profile

Let us now study the important aspects of drug action in the next section.

9.5 MECHANISM OF DRUG ACTION

Drugs (except those gene based) like enzymes, just alter the rate of the ongoing activities. They do not cause or produce any new functions to any system, organ or cell. Very few drugs act because of their simple physical or chemical property. Some drugs act due to **physical mass** e.g., bulk laxatives (isabgal), due to **physical form** (petroleum jelly), due to **adsorptive property** (activated charcoal), etc. The drug action can be broadly described as:

(a) **Stimulation**,is an increase in the selective activity of specialized cells, which increases the secretion from a gland.

(b) **Depression**is a reduction/decrease in the activity of specialized cells. For example barbiturates depress central nervous system.

(c) **Irritation**: noxious effect, is the effect of drugs on the growth, nutrition and morphology of living tissues which induce a gross change in cellular function.

(d) **Replacement**,includes certain drugs, which are used to replace some missing endogenous component of the body or when the production of endogenous components is reduced. These are given in deficiency diseases, e.g. insulin in diabetes mellitus.

(e) **Bactericidal and Cytotoxic action** is for killing parasites or cancer cells. Bactericidal activity, which is bacterial death, is induced by certain types of antibiotics. Cytotoxic action is selective for invading cancer cells and altering them without affecting the host cells.

The drugs produce their effects mainly by interacting with a discrete **target** biomolecules, which usually are proteins. These proteins are the targets of drug action and grouped into four major categories, viz. enzymes, ion channels, transporters and receptors. We will discuss in detail about the receptors in the following subsection.

9.5.1 Drug Receptors

You by now know that the beneficial (therapeutic) and toxic effects of drugs result from their interactions with molecules in the patient. Most drugs act by associating with specific macromolecules **in the membrane or inside the cell** and alter the biological function. Such macromolecules are called **receptors.** Thus, receptor is defined as a macromolecule or binding site located on the surface or inside the effector cell that serves to recognize the signal molecule/drug and initiate the response to it, but itself has no other function. The function of receptor is to propagate regulatory signals from outside to the effector cell, amplify the signal, integrate various extracellular and intracellular regulatory signals and adapt to short term and long term changes. The overall scheme of drug action through receptors is depicted in Fig. 9.3.

Ligand (*Latin*: *ligare*—to bind) Any molecule which attaches selectively to a particular receptor or site.

Signal molecules are also called ligands, the molecules that bind specifically to other molecules like receptors.

The binding of a drug to receptor depends on types of chemical bonds that can be established between drug and receptor. The drug receptor interaction is similar to a ligand binding that forms a complex with metal ions. The interactions are of the following types:

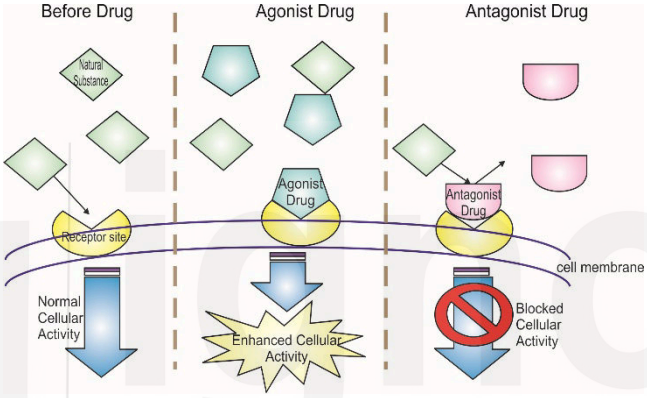
Agonist: When a drug binds with a receptor and produces an effect similar to that of the physiological signal molecule as given in Figure 9.3 (b).

Inverse agonist : When a drug activates the receptor but produces an effect in the opposite to that of a physiological signal molecule.

Antagonist: when a drug prevents the action of an agonist on a receptor or the subsequent response but does not have any effect of its own as shown in Fig. 9.3 (c).

Partial agonist: A drug which activates a receptor to produce submaximal effect but antagonises the action of a full agonist.

The term only indicates affinity or binding without regard to functional change; agonists and competitive antagonists are both ligands of the same receptor.



(a) (b) (c)

Fig. 9.3: Drug action through receptors: a) Natural action b) Agonist drug C) Antagonist drug.

Receptors have dual functions, i.e., they recognise a specific ligand molecule and then cause transduction/transmission/conversion of the signal into a response. As only a handful of transducer pathways are shared by a large number of receptors, the cell is able to generate an integrated response reflecting the sum total of diverse signal input. The transducer mechanisms can be grouped into 4 major categories:

Ligand-gated ion channels (LGICs) are integral membrane proteins that contain a pore which allow the regulated flow of selected ions like Na^+, K^+, Ca^{2+} or Cl^- across the plasma membrane.

(i) **Cell surface receptors**: These are also called ligand-gated ion channels (LGICs). These are integral membrane proteins that have ion selective channels (for Na^+, K^+, Ca^{2+} or Cl^-) across the plasma membranes. When an agonist binds, it opens the channel and causes changes in cytosolic ionic composition, depending on the ion that flows through.

(ii) **G-protein coupled receptors** (GPCR): These are a large family of cell membrane receptors which are linked to the effector (enzyme)channel-carrier protein) through one or more GTP-activated proteins(G-proteins) for response.

(iii) **Enzyme-linked receptors:** These have a subunit with enzymatic property or bind a Janus-Kinase (JAK) on activation. JAK are intracellular, non-receptor tyrosine enzyme kinases.

(iv) **Receptors regulating gene expression** (Transcription factors) are intracellular cytoplasmic or nuclear) soluble proteins.

All the four types of receptors have been described along with their schematic representation is given in Table 9.2.

Table 9.2: Four types of receptors

	Type-1 Ligand-gated ion channels	Type-2 G-protein coupled receptor	Type-3 Enzyme-linked receptor	Type-4 Nuclear receptor
Location	Membrane	Membrane	Membrane	Intracellular
Effector	Channel	Channel or enzyme	Enzyme	Gene transcription
Coupling	Direct	G-protein	Direct	Via DNA
Examples	Nicotinic Receptor, $GABA_A$ R	Muscarinic receptor, Adrenoceptors	Insulin receptor, Growth factors, Cytokine R	Steroid/Thyroid receptors
Time scale for cellular effects	Milliseconds	Seconds	Hours	Hours/Days

The mechanism of drug action can be well understood by drug-receptor theory detailed in subsection 9.5.2. Before you study the receptor theory attempt the following SAQ.

SAQ 2

Match the contents of column (A) with those of column (B) in the following:

(A)	(B)
a) Pharmcokinetics	1) gene transcription
b) Pharmacodynamics	2) produces similar effect
c) Agonist	3) what the body does to the drug
d) Nuclear receptor	4) what the drug does to the body

9.5.2 Drug-Receptor Theory

The biological activity of a drug is related to the stability of drug-receptor complex i.e., affinity of a drug for its receptor. Stability of drug receptor complex is measured by the difficulty in dissociation of the drug-receptor complex. The more the difficulty the more strong is the interaction and more is the drug effect. These interactions are due to **Van der waals, hydrophobic** and **ionic interactions** along with **hydrogen** and **covalent bonding**.There have been several major theories that have been proposed to provide a theoretical basis for understanding, modeling, and thereby predicting the drug response. Three of the most widely known of these are described as follows.

The strength of drug receptor complex is because of interaction between drug and receptor.

- **Occupancy theory**: Clark (1937) propounded a theory of drug action based on the occupation of receptors. The idea being that a response emnates from a receptor only when it is occupied by an appropriate ligand i.e. the drug. When the drug binds with the receptor it stimulates it and produces a biological effect. The biological effect remains till the drug occupies or binds the receptor. Once the drug dissociates from the receptor

the drug action is lost. So we can say that the proportion of occupied receptors is related to the effect of the drug. For example, for full agonists and a linear relationship, 100% occupancy = 100% effect. The interaction between the two molecular species, viz. the drug (D) and the receptor (R) and the effect (E) to be a direct function of the drug-receptor complex (DR) formed can be given by **Clark's equation** as given below.

$$D + R \underset{K_2}{\overset{K_1}{\rightleftharpoons}} DR \longrightarrow E \qquad \text{... (9.1)}$$

Later on it was observed that occupation of the receptor is essential but not itself sufficient to elicit a response; the agonist must also be able to activate i.e., induce a conformational change in the receptor. The ability to bind with the receptor designated as **affinity** and the capacity to induce a functional change in the receptor designated as **intrinsic activity** (IA) or efficacy are independent properties. For example antagonists occupy the receptor but do not activate it. So, a theoretical quantity denoting **strength of stimulus** (S) imparted to the cell was interposed in the Clark's equation given below.

$$D + R \underset{K_2}{\overset{K_1}{\rightleftharpoons}} DR \rightsquigarrow S \longrightarrow E \qquad \text{... (9.2)}$$

Depending on the agonist, DR can generate a stronger or weaker S, probably as a function of the conformational change brought about by the agonist in the receptor.

- **Rate Theory**: It is based on the idea that a response emanates from a receptor in proportion to the kinetic rate of onset and offset of drug binding to the receptor.
- **Operational Model**: It is a modified, semi-empirical approach that is based on occupancy theory modified to incorporate a factor relating agonist-receptor complex and response.

Now that you are familiar with the interactions of a drug with the biological system, it is also important to understand the effect of structure of a drug on its effect in that system. Let us study in brief in the next section.

9.6 STRUCTURE ACTIVITY RELATIONSHIPS (SAR)

Structure activity relationships can be used to predict biological activity from the structure of the lead compound and is a means by which the effect of a drug or toxic chemical on an animal, plant or the environment can be related to its molecular structure.

"Structure of the drug determines its biological function" was first stated by Crum-Brown and Fraser in 1868. Determination of structure and function relationship helps the medicinal chemists to synthesise new drugs. The biological effects of a new chemical compound can often be predicted from its molecular structure using data of other similar compounds. This is because similar compounds may have similar physical and biological properties. There is a relationship between molecular structures and their biological activity, and this principle is referred to as **Structure Activity Relationship** (SAR). This concept is used in drug discovery to characterise existing molecules and guide the synthesis of compounds with increased activity, a different activity from the existing drug and fewer side effects. It is used to determine the

pharmacophore and unwanted side effects. It also helps to know how the minor changes in the molecule would affect the pharmacological activities.

Pharmacophore is part of a molecular structure that is responsible for a particular biological or pharmacological interaction that it undergoes.

SAR is located at the intersection of biology, chemistry, and statistics asdepicted in Fig. 9.4. The focused interlinking of these disciplines has broughtabout the development of a research activity resembling the “science of SAR”.

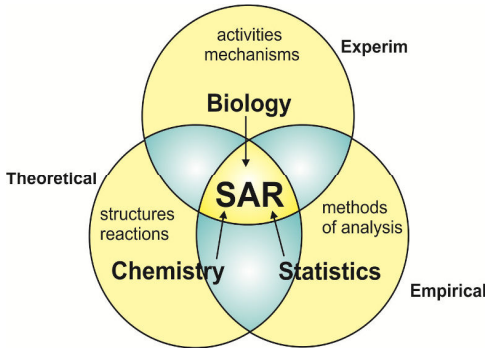


Fig.9.4: SAR located at the intersection of biology, chemistry, and statistics.

The SAR is useful to study the effects of modifications of drug analogues i.e., how modifications will affect transport across membrane, water solubility, receptor binding and pharmacokinetic properties. These modifications can be: change in size and structure of parent compound, stereochemistry of lead molecule (L or D) and nature and degree of substitution. The effect of change in size and structure of parent compound is explained below.

Change in size and structure of parent compound: It is well known that a drug when taken has to be absorbed i.e., cross a biological membrane, transported to the target site and then attach to a receptor to produce its pharmacological effects. For this whole process the structure of the compound/drug is very important as the drug will bind to the receptor only if the structure fits the receptor. If a group is added or removed it not only changes the structure of compound but changes the function also by either enhancing the effect of drug or decreasing the effect of drug. The effect of addition of a few groups is explained in the following paragraphs.

- **Addition of methyl group:** causes an increase in lipophilicity which causes and increases in transport across biological membrane. You have learnt in Unit 1 that biological membranes are a lipid bilayer therefore, increased lipophilicity will increase transport. Increase in transport across biological membrane will increase its biological function. However, increase in lipophilicity will cause a decrease in its property of water solubility which might decrease its activity.
- **Addition of hydroxyl group:** causes an increase in hydrophilicity i.e., increase its ability of solubility. It also causes increase in hydrogen bonding at the target site thus increases the drug activity.
- **Addition of basic groups e.g., amines, amides:** The basic groups when in free form help drug to transport and in salt form helps the bonding of drug with receptor by hydrogen bonds.

SAQ 3

Match the following given in group (A) with that in group (B).

(A)	(B)
a) Clark	1) Lipophilic
b) OH group	2) Hydrophilicity
c) Crum-Brown	3) SAR
d) Methyl group	4) Occupancy theory

9.7 ENZYMES AS DRUG TARGETS

You have read in previous units that enzymes catalyze multistep chemical reactions and achieve phenomenal rate accelerations by matching protein and substrate chemical groups in the transition state. You have also learnt that enzymes act optimally and their activity cannot be either increased or decreased. However, drugs can either increase or decrease the rate of enzymatically mediated reactions. In fact, drugs can act by increasing or decreasing the affinity of enzymes for its substrates. The activity of enzymes can be altered by stimulation of an enzyme which increases its affinity for the substrate and the rate constant (K_m) of the reaction is lowered. Enzyme activity can also be altered by enzyme induction i.e., increasing the synthesis of more enzyme protein. Inhibition of enzymes is another common mode of drug action. The catalytic chemistry of enzymes is the key to designing potent inhibitors and makes them a special class of drug target. The effect of enzyme stimulation, induction and inhibition on the kinetics of enzyme reaction is depicted graphically in Fig. 9.5. In the graph V_{max} is the maximum velocity of reaction, V_{max}(s) of stimulated enzyme, V_{max}(i)in presence of noncompetitive inhibitor, $K_m$rate constant of the reaction, K_m (s)of stimulated enzyme, K_m (i)in presence of competitive inhibitor. **Enzyme induction and noncompetitive inhibition donot change the affinity of the enzyme i.e., K_m is unaltered, whereas enzyme stimulation and competitive inhibition respectively decrease and increase the K_m respectively.**

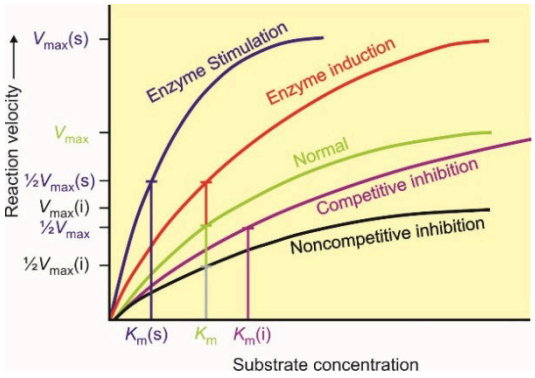


Fig.9.5: Effect of enzyme stimulation,inductionand inhibition on kinetics of enzyme reaction.

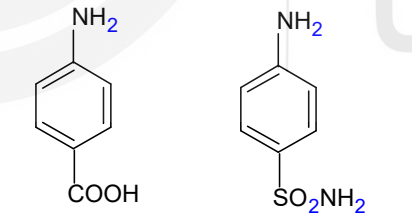
Inhibition of enzymes can occur in different ways. These are explained in the following paragraphs.

- **Nonspecific inhibition:** As enzymes are proteins many drugs alter the tertiary structure of a enzyme with which they come in contact and

denature them and thus enzymes get inhibited. Enzyme activity is also 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. Examples of chemical which inhibit enzymes non specifically are heavy metal salts, strong acids and alkalies, alcohol, formaldehyde, phenol. As these inhibitors cause damage rampantly they cannot be used systemically.

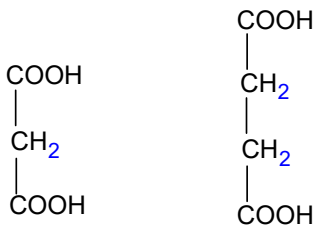
❖ **Specific inhibition:** Many drugs inhibit a particular enzyme without affecting others. Such inhibitionscan be either competitive or noncompetitive in nature. These are explained below.

- **Competitive inhibition**: Competitive inhibitors have a structure resembling that of the substrate. The structural similarity between the substrate and the inhibitory compound enables the inhibitors to compete with the substrate for binding to the active side 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 metabolities, 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 is its substrate, causing competitive inhibition. You will find a diagrammatic representation of competitive inhibition in Fig. 9.6 (b).

As the product is not formed or nonfunctional product is formed as shown in Fig. 9.5 a new equilibrium is achieved in the presence of the drug that is why it is also called **equilibrium type** inhibition. A characteristic feature of competitive inhibitions 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 observe from Fig. 9.5 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.

A **nonequilibrium type** of enzyme inhibition can also occur with drugs which react with the same catalytic site of the enzyme but either form strong covalent bonds or have such high affinity for the enzyme that the normal substrate is not able to displace the inhibitor. In these situations, K_m is increased and V_{max} is reduced.

- **Noncompetitive inhibition**: Noncompetitive inhibitorsinhibit 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 from. Such inhibitors cannot be displaced by excess substrate and hence are called noncompetitive inhibitors. Since their action does not involve displacement of the substrate, they have no effect on the K_m of the substrate. The inhibitor does not attach at the catalytic site but reacts with an adjacent site and alters the enzyme in such a way that it loses its catalytic property as shown in Fig. 9.6 (c). Thus, K_m is unchanged but V_{max} is reduced as shown in Fig. 9.5.

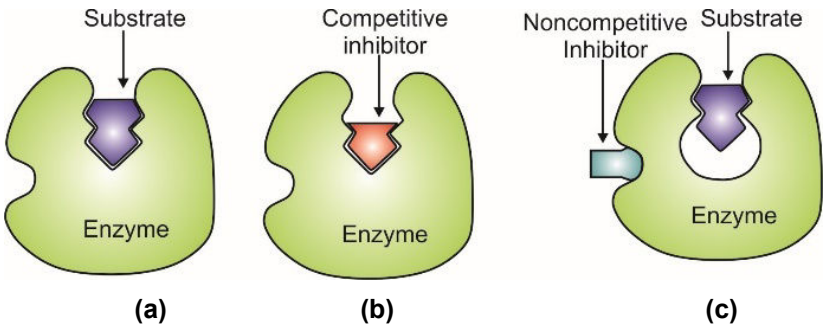


Fig.9.6: Distinction between a Competitive and a Noncompetitive Inhibitor(a) enzyme-substrate complex; (b) a competitive inhibitor binds at the active site and thus prevents the substrate from binding; (c) a noncompetitive inhibitor does not prevent the substrate from binding.

SAQ 4

Fill in the blanks in the following sentences:

(a) Drugs alter the tertiary structure of any enzyme with which they come in contact and denature them such inhibition is called _____

(b) _____ enzyme inhibitors increase the K_m but the V_{max} remains same.

(c) In noncompetitive inhibition, K_m is unchanged but V_{max} is _____.

9.8 SUMMARY

- Medicine is the formulated form of the drug having definite dose and disease. While a drug can be defined as a medicine or other substance which has a physiological effect when ingested or otherwise introduced into the body.
- Pharmacodynamics is the study of the biochemical and physiological effects of drugs in the body. It shows the interaction of the drug with tissue receptors which are located at the cell membranes or in the intracellular fluid.
- Pharmacokinetics is the study that determines the rapidity and concentration of the drug in the body and its duration of appearance at the target organ, i.e. onset, time of peak action and duration of action. It also determines the route(s) and frequency of administration of drug.
- Drugs (except those gene based) like enzymes, just alter the rate of the ongoing activities.The drug action can be broadly described as Stimulation, Depression, Irritation, Replacement, and Bactericidal and Cytotoxic action.
- Most drugs act by associating with specific macromolecules in the membrane or inside the cell and alter the biological function. Such macromolecules are called receptors.
- The drug receptor interactions are agonist, inverse agonist, antagonist, and partial agonist. Agonist: When a drug binds with a receptor and produces an effect similar to that of the physiological signal molecule. Inverse agonist : When a drug activates the receptor but produces an effect in the opposite to that of a physiological signal molecule. Antagonist: when a drug prevents the action of an agonist on a receptor or the subsequent response but does not have any effect of its own. Partial agonist A drug which activates a receptor to produce submaximal effect but antagonises the action of a full agonist.
- Occupancy theory suggests thatwhen the drug binds with the receptor it stimulates it and produces a biological effect. The biological effect remains till the drug occupies or binds the receptor. Once the drug dissociates from the receptor the drug action is lost.

- There is a relationship between molecular structures and their biological activity, and this principle is referred to as Structure Activity Relationship (SAR). This concept is used in drug discovery to characterise existing molecules and guide the synthesis of compounds with increased activity, a different activity from the existing drug and fewer side effects.
- Enzyme activity can be also be altered by enzyme induction i.e., increasing the synthesis of more enzyme protein. Inhibition of enzymes is another common mode of drug action.
- Inhibition of enzymes can occur in non-specific and specific inhibition. Specific inhibition is either competitive or noncompetitive.

9.9 TERMINAL QUESTIONS

1. Differentiate between drug and medicine.
2. What do you understand by pharmcodynamics?
3. Describe in brief the drug action.
4. Describe in brief the drug-receptor interaction.
5. How does change in structure affect the SAR?
6. Why are enzymes good drug targets?

9.10 ANSWERS

Self-Assessment Questions

1. a) F b) T c) F d) F
2. a) -3 b) -1 c) -2 d) -4
3. a) - 4 b) -2 c) - 3 d) - 1
4. a) Nonspecific inhibition b) Competitive c) reduced

Terminal Questions

1. Refer Table 4.2.
2. Pharmacodynamics is the study of the biochemical and physiological effects of drugs in the body. It shows the interaction of the drug with tissue receptors which are located at the cell membranes or in the intracellular fluid.
3. The drug action can be broadly described as:

   (a) Stimulation, is an increase in the selective activity of specialized cells, which increases the secretion from a gland.

   (b) Depression is a reduction/decrease in the activity of specialized cells. For example barbiturates depress central nervous system.

(c) Irritation: noxious effect, is the effect of drugs on the growth, nutrition and morphology of living tissues which induce a gross change in cellular function.

(d) Replacement, includes certain drugs, which are used to replace some missing endogenous component of the body or when the production of endogenous components is reduced. These are given in deficiency diseases, e.g. insulin in diabetes mellitus.

(e) Bactericidal and Cytotoxic action is for killing parasites or cancer cells. Bactericidal activity, which is bacterial death,

4. The drug-receptor interactions are of the following types:

   Agonist: When a drug binds with a receptor and produces an effect similar to that of the physiological signal molecule.

   Inverse agonist : When a drug activates the receptor but produces an effect in the opposite to that of a physiological signal molecule.

   Antagonist: when a drug prevents the action of an agonist on a receptor or the subsequent response but does not have any effect of its own.

   Partial agonist: A drug which activates a receptor to produce submaximal effect but antagonises the action of a full agonist.

5. The structure of the compound/drug is very important as the drug will bind to the receptor only if the structure fits the receptor. If a group is added or removed it not only changes the structure of compound but changes the function also by either enhancing the effect of drug or decreasing the effect of drug. For example, methyl group is added it causes an increase in lipophilicity which causes and increase in transport across biological membrane. Addition of hydroxyl group causes an increase in hydrophilicity. It also causes increase in hydrogen bonding at the target site thus increases the drug activity. Addition of basic groups when in free form help drug to transport and in salt form helps the bonding of drug with receptor by hydrogen bonds.

6. Enzymes catalyse multistep chemical reactions and achieve phenomenal rate accelerations by matching protein and substrate chemical groups in the transition state. Inhibitors that take advantage of these chemical interactions are among the most potent and effective drugs known. The catalytic chemistry of enzymes is the key to designing potent inhibitors and makes them a special class of drug target. The majority of drugs which act on enzymes act as inhibitors and most of these are competitive. Some inhibitors are non-competitive, binding away from the substrate binding domain, competing for cofactor/coenzyme binding, or causing an allosteric conformational change in the 3-dimensional protein structure that prevents substrate interaction. Yet other inhibitors are irreversible and these covalently bind to the enzyme, permanently inactivating catalytic function (these are also known as suicide inhibitors).