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Enzymes

Introduction

Living cells are unique in their ability to allow complex chemical reactions to proceed rapidly at ambient temperatures. These chemical reactions can also occur outside the living cell but proceed too slowly. The complex metabolic pathways in a cell do not exist under such sluggish conditions. The principal agents which take part in the remarkable transformations in cells are the enzymes. Enzymes synthesized by living cells speed up thermodynamically possible reactions so that the rate of such reactions becomes compatible with the biochemical reactions essential for the living cells. Enzymes function at very small concentrations out of proportion to the change they catalyze. Enzymes are highly specific in that one enzyme does not catalyze all reactions, but thousands of different enzymes are required to catalyze the thousands of chemical reaction occurring in cells. Due to the protein nature of enzymes, they can be easily inactivated by heat, strong acids or bases, organic solvents, heavy metals etc. The role of enzymes was recognized in the process of investigation on the mechanism of digestion and fermentation. Von Helmont in the seventeenth century suggested that digestion involved transformation of food materials chemically through "ferments". In 1812, Kirchhoff noted that starch can be converted into sugar by dilute acid; but the acid did not undergo any change. During the beginning of nineteenth century, in biological materials also, the catalytic power was identified. Kirchhoff later noted that starch is not only hydrolysed by acid, but also by a ferment (diastase). Payen and Persoz (1833) precipitated diastase from malt extract. It was Kuhno (1878) who introduced the term "enzyme", to avoid confusion from the word "ferment". Sumner (1926) obtained for the first time the enzyme urease in a crystalline

form. Northrop (1930) later crystallized pepsin and by 1956 about 75 enzymes have been crystallized.

Exo-and Endoenzymes

In early times the enzymes were called as the ferments which were distinguished into organized ferments (like *yeast*, in which the living cell was considered essential) and unorganized ferments (like pepsin, the protein hydrolysing enzyme in the stomach, which contained no living cells). Later on it was proved by Buchner in 1897 that there is an essential difference between organized and unorganized ferments as ground yeast cells could also cause the fermentation of sugar. At present, enzymes which normally act within cell are called *endoenzymes* and those which normally work after they have been secreted by the cells are called *exoenzymes*. The enzymes of the digestive tract are good examples of exoenzymes.

The substance upon which the enzyme acts is called **substrate**. A common system of naming enzymes is to add the ending 'ase' to the root of the substrate. For example, the enzyme that hydrolyses sucrose is called *sucrase*. Sometimes, an enzyme is named by adding the ending-*ase* to a word descriptive of the reaction which it catalyses. For example, an oxidase enzyme catalyses an oxidation. However, many of the enzymes were known long before their systems of naming were suggested. Hence, many of the common enzymes are usually referred to by their old names. A good example is *pepsin*, the protein hydrolysing enzyme of the stomach. Today it would be perfectly proper to speak of pepsin as the gastric protease.

Chemical nature of enzymes

Concerning the chemical nature of enzymes it may be said that they all contain protein. Some are simple proteins and others consist of a protein combined or associated with a non-protein component. *Urease*, for example, is a protein which converts urea into carbon dioxide and ammonia. It is a globulin. In 1926, Sumner received the Noble prize for being the first to prepare a pure enzyme. Since then many enzymes have been prepared in crystalline form which have been found to be proteins. Many enzymes, notably those associated with oxidations and reductions appear to be made up of two parts : one is protein, and the other is simple organic compound. The two parts appear to be in loose chemical combination, and the complete enzyme is called a *holoenzyme*. When such an enzyme is submitted to dialysis, the protein does not pass through the membrane, but the simpler organic compound does. The protein part of the enzyme has been called an *apoenzyme*, and the part which passes through the membrane has been called the *coenzyme* or the *prosthetic group* of the enzyme. Neither the apoenzyme nor the coenzyme is active by itself, but when they are mixed together, the active enzyme is regenerated. Apoenzymes, being

proteinaceous in nature, are destroyed by heat, probably because of denaturation. Coenzymes are never destroyed by heat.

Activators and kinases

Many enzymes are stimulated by the presence of simple molecules or ions. The salivary amylase, for example, works better in the presence of chloride ions. Similarly, bile salts accelerate the working of pancreatic lipase. Several bivalent ions, such as Mg, Mn, Co and Ni, are known to stimulate the action of certain enzymes. These simple enzyme stimulators are called *activators*.

Some enzymes, as produced by the cell, are inactive and require another substance to activate them. The inactive form of the enzyme is called a *zymogen* or *proenzyme*, and the substance that converts it into an active form is called a *kinase*. In the pancreas, for example, there is a proenzyme *trypsinogen*, which is converted into active *trypsin* by a kinase called *enterokinase*, found in the intestinal juice.

Activation of enzymes

Some enzymes are synthesized by cells in an inactive form, known as *proenzymes* or *zymogens*. Examples are trypsinogen, chymotrypsinogen and pepsinogen. Zymogens can be converted into active enzymes by removing an inhibitory or a blocking peptide. Trypsinogen is converted into active trypsin by the removal of a hexapeptide consisting of valine, aspartic acid and lysine from the amino terminal and bringing about a change in the conformation of the protein.

$$\text{Trypsinogen} \xrightarrow{\text{Trypsin}} \text{Trypsin} + \text{Hexapeptide (valine, aspartic acid, lysine)}$$

A second type of activation involves the maintainance of the integrity of sulfhydryl groups (−SH) in the enzyme molecule. The sulfhydryl groups may either maintain or form the active site of enzyme. Oxidation of these groups into disulfide form (−S−S) leads to inactivation while reduction of the disulfide to −SH groups by a suitable reducing agent activates the enzyme. *Papain*, a proteolytic enzyme is inactivated on exposure to oxygen. When treated with a suitable reducing agent, the −S−S group is converted to −SH group and the enzyme is activated. Other examples are pyruvate carboxylase and triose phosphate isomerase.

Another type of activation involves the participation of a *cofactor* in addition to the enzyme protein molecule. Cofactors may be (i) prosthetic groups, (ii) coenzymes and (iii) metal activators. A *prosthetic group* is a cofactor firmly bound to the enzyme protein. Examples are flavine adenine dinucleotide (FAD) firmly bound to succinate dehydrogenase and porphyrin of peroxidase. A *coenzyme* is a small heat stable, organic molecule which readily dissociates from the enzyme protein. It can be removed by dialysis. Examples are DPN^+, TPN^+, thiamine pyrophosphate and tetrahydrofolic

acid. The *metal activators* are the mono and divalent metal cations such as Mg^{++}, Ca^{++}, Zn^{++}, Ni^{++}, K^{+} which are loosely or firmly bound to enzyme proteins by chelation to phenolic, amino, phosphoryl or carboxyl groups.

A fourth type of activation is related to regulatory enzymes. These enzymes are made up of several subunits and consist of two receptor sites which are non-overlappping and distinct. The first site, the *active site* binds to the substrate molecule while the second site binds to another compound and this site is known as *allosteric site*. This binding leads to a change in the quaternary structure or a conformational change in the enzyme protein molecule. The active site is also modified accordingly leading to a change in enzyme activity. Examples for this type of regulatory enzymes are aspartate transcarbamylase and its allosteric effector cytidylic acid ; phosphofructokinase and ADP ; muscle phosphorylase b activated by 5,—AMP and acetyl CoA carboxylase of mammalian tissues activated by citrate.

Isozymes

An *isozyme* is an enzyme which has multiple molecular forms in the same animal or organism. Lactate dehydrogenase occurs in five forms in vertebrates. These can be separated electrophoretically. Two basically different types of LDH occur. The first is inhibited strongly by low concentrations of pyruvate and occurs in the heart. The second occurs in muscles and is less easily inhibited by pyruvate. The heart enzyme consists of four identical subunits which are called 'H' subunits. The muscle enzyme consists of four identical subunits, the 'M' subunits. The two types of subunits differ in their aminoacid composition and immunological properties, and are produced by two separate genes.

The two types of enzymes play different physiological roles. Pheasants and chicken require sudden energy for brief flights but cannot fly continuously. Therefore, they have only M LDH in their breast muscle. In contrast, the petrel which fly continuously for prolonged periods have H LDH in flight muscles. Intermediate fliers have equal amounts of the two enzymes.

Table 4.1. Some coenzymes and prosthetic groups of enzymes.

Coenzyme or Prosthetic group	Function	Source
1. Flavine Adenine Dinucleotide	Hydrogen acceptor	Riboflavine
2. Flavine Mono Nucleotide	Hydrogen acceptor	Riboflavine
3. Nicotinamide Adenine Dinucleotide	Hydrogen acceptor	Nicotinic acid
4. Nicotinamide Adenine Dinucleotide Phosphate	Hydrogen acceptor	Nicotinic acid
5. Pyridoxal Phosphate	Aminotransferase, amino decarboxylase	Pyridoxine

6. Lipoic acid	Oxidative decarboxylation	Component of vitamin B complex
7. Thiamine pyrophosphate	Oxidative decarboxylation	Thiamine
8. Tetrahydrofolic acid	Carbon transfer	Folic acid
9. Ubiquinone	Hydrogen acceptor	–
10. Biotin	CO_2 transfer	Biotin
11. Cobamide	Group transfer	Cobalamine
12. Ascorbic acid	Hydroxylation	Vitamin C
13. Coenzyme A	Acyl transfer	Pantothenic acid
14. Adenosine Triphosphate	Transphosphorylation	–

Mechanism of enzyme action

Enzymes attach to the substrate either chemically or by absorption, rendering it more unstable. As the substrate breaks down, the enzyme is liberated and attaches itself to more substrate molecules, and the process is repeated Thus, a small amount of the enzyme reacts with a large amount of the substrate. The colloidal nature of the enzymes makes available large surfaces for the adsorption of the substrate.

Nature of catalysis

Enzymes are catalysts peculiar to living matter. A *catalyst is defined as a substance that accelerates a chemical reaction but is not consumed in the overall process*. The hydrolysis of sucrose is catalysed by acid. The important feature of such chemical reactions is that the amount of catalyst has no stoichiometric relationship to the quantity of substance altered. The efficiency of a catalyst is expressed as the moles of the substrate transformed per mole of the catalyst in unit time. Enzymes are highly efficient catalysts. Pure enzymes may transform as many as 10,000 to 10,00,000 moles of substrate per minute per mole of enzyme. Thus, very minute quantities of enzymes are sufficient to bring about a chemical change in a substrate.

Another important point of enzyme catalyzed reactions is the directed nature of the reaction. Enzymes produce a more uniform reaction in which the yield of products is high.

Thermodynamic principles

In order to understand the nature of enzyme catalysed reactions, it is necessary to understand some thermodynamic principles. Thermodynamics deals with the flow of energy in a chemical reaction or molecular transformation. The first and second laws of thermodynamics deal with the major concepts of thermodynamics, and enable us to understand (i) the direction of a chemical reaction i.e. whether a reversible reaction proceeds from left to right or right to left as it is written, (ii) whether the progress of the chemical reaction will result in useful work done or liberation of energy or (iii) in order for the reaction to proceed, energy must be supplied from an external source.

highly folded and that the active site is formed from contiguous parts of different folds. Denaturation of enzyme is unfolding of the tertiary structure and always results in a loss of enzyme activity. Presence of its substrate tends to protect an enzyme from denaturation.

Specificity of enzymes

Enzymes are very specific in their action. Some enzymes such as *urease* attack a specific compound and no other, some attack many different molecules having similar chemical structures. For example, *maltase* hydrolyses α–glycosides and emulsin β–glycosides. Others attack large groups of similar compounds. For example, pepsin hydrolyses all soluble native proteins.

Emil Fischer has explained enzyme specificity by assuming that an enzyme must fit its substrate as a key-fits a lock. Some enzymes are master keys which fit many locks.

Reversibility of enzyme action

Most of the chemical reactions taking place in protoplasm are reversible; that is, the reaction may proceed in either direction, depending upon conditions. Reversible reaction is not completed unless the end products of reaction are removed. Under normal conditions the reaction proceeds until an equilibrium is established. For example, when ethyl acetate is allowed to stand in contact with water, it slowly hydrolyses to form acetic acid and ethyl alcohol.

$$CH_3COOC_2H_5 + H_2O \rightleftharpoons CH_3COOH + C_2H_5OH$$

If ethyl alcohol and acetic acid are mixed, they will slowly combine to form ethyl acetate and water. According to the law of mass action, the speed of a reaction is proportional to the concentration of the reacting substances. In the foregoing equation, if one starts with ethyl acetate and water, the initial concentration of these substances will, of course, be high and the reaction will proceed from left to right. As soon as acetic acid and ethyl alcohol are formed, they will react with each other to reverse the original reaction. At first, this reaction will be very slow because the concentration of acetic acid and ethyl alcohol are low. However, as the concentrations of these two substances increases, the speed of the reaction from right to left also increases, until finally it equals the speed of the reaction in the opposite direction. At this point, equilibrium will be established. If an end product of either reaction are continuously removed, the reaction proceeds to completion in the direction of the removed end product.

Enzymes added to a reacting mixture do not cause the reaction to go to completion but simply hasten the establishment of an equilibrium. The same enzyme will hasten the equilibrium in either direction. Thus, if to an ethyl acetate-water mixture an ester-hydrolysing enzyme is added, the ester is hydrolysed very rapidly to form the equilibrium mixture. If the enzyme is

added to an alcohol acetic acid mixture, the same equilibrium mixture is rapidly formed. What has just been said is an example of the *reversibility of enzyme action*.

When protein is eaten, enzymes in the digestive tract hydrolyse it to form amino acids. These amino acids, which are the end products of the reaction, are removed by being absorbed into the blood stream. In this way, it is possible for the reaction to go to completion. In the body tissues, possibly the same enzymes are responsible for the synthesis of these amino acids into tissue proteins.

It should be pointed out that not all of the reactions occurring in the protoplasm are reversible and that in certain cases reversible reactions require different enzymes for catalysing the reaction in the opposite directions.

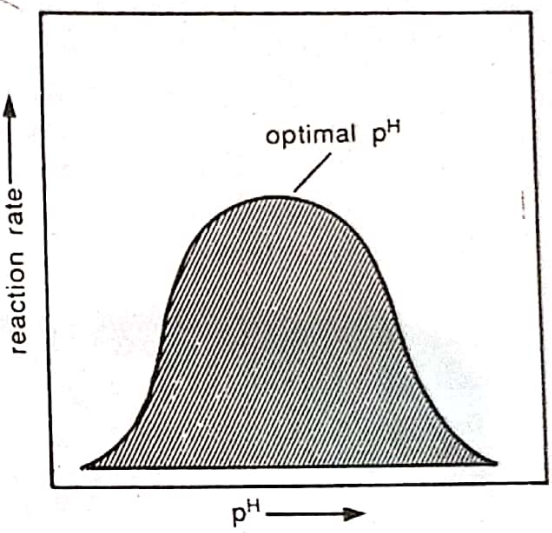

Fig. 4.1. Effect of pH on the enzyme activity.

Factors influencing enzyme action

Enzymes are usually present in very small quantities in biological systems and it is not possible to measure their quantity. They are usually measured in terms of activity. A number of factors influence their activity. To study the effect of these factors, the velocity of the reaction in different conditions is measured, by quantitatively estimating the formation of products or disappearance of the substrate.

Effect of hydrogen ion concentration. Enzyme activity is influenced by varying the hydrogen ion concentration. (Fig. 4.1) *The opimum pH is that pH at which a certain enzyme causes a reaction to progress most rapidly.* On either side of the optimum, the velocity of reaction is lower and at certain pH values an enzyme may become totally inactivated. In enzyme reactions, therefore, buffers are used to maintain the hydrogen ion concentration at optimum or most favourable. The following table shows the optimum pH for some enzymes.

Enzyme	Source	Optimum pH
Invertase	Intestine	6.2
Amylase	Saliva, pancreas	5.6–7.2
Lipase	Pancreas	7.0
Pepsin	Stomach	1.5–2.5
Trypsin	Pancreas	8–11
Urease	Soybean	7.2–7.9

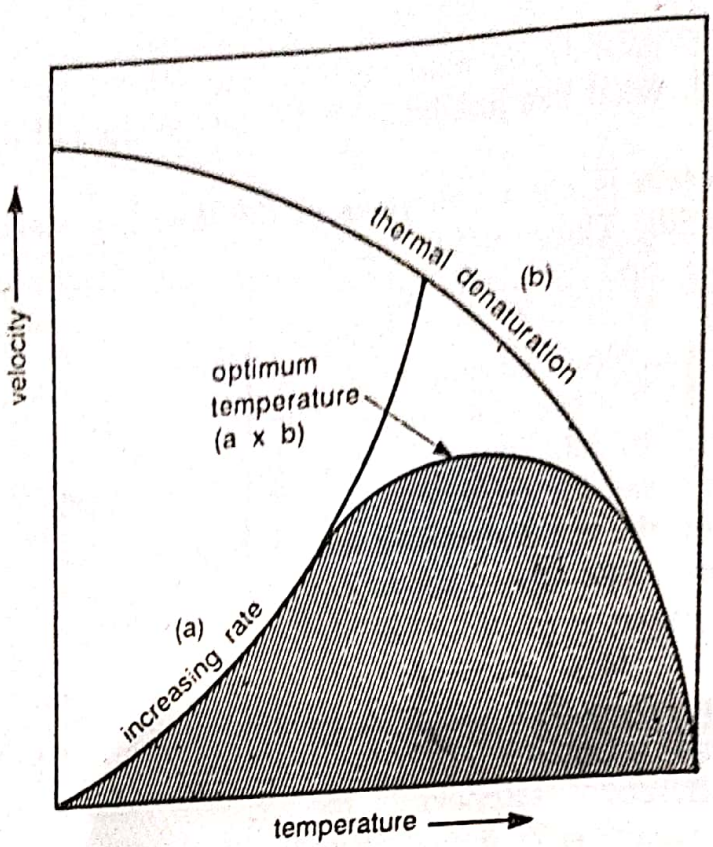

Fig. 4.2. Effect of temperature on reaction rate of an enzyme-catalyzed reaction. (*a*) represents the increasing rate of a reaction as a function of temperature. (*b*) represents the decreasing rate as a function of thermal denaturation of the enzyme protein. The shaded area represents the combination of $a \times b$.

Effect of temperature. The rate of an enzyme catalyzed reaction is increased from 1.1 to 3 times for every 10°C rise in temperature. An optimum temperature is usually reached at 40–50°C for animal enzymes. For plant enzymes it is higher, usually between 50–60°C. High temperature

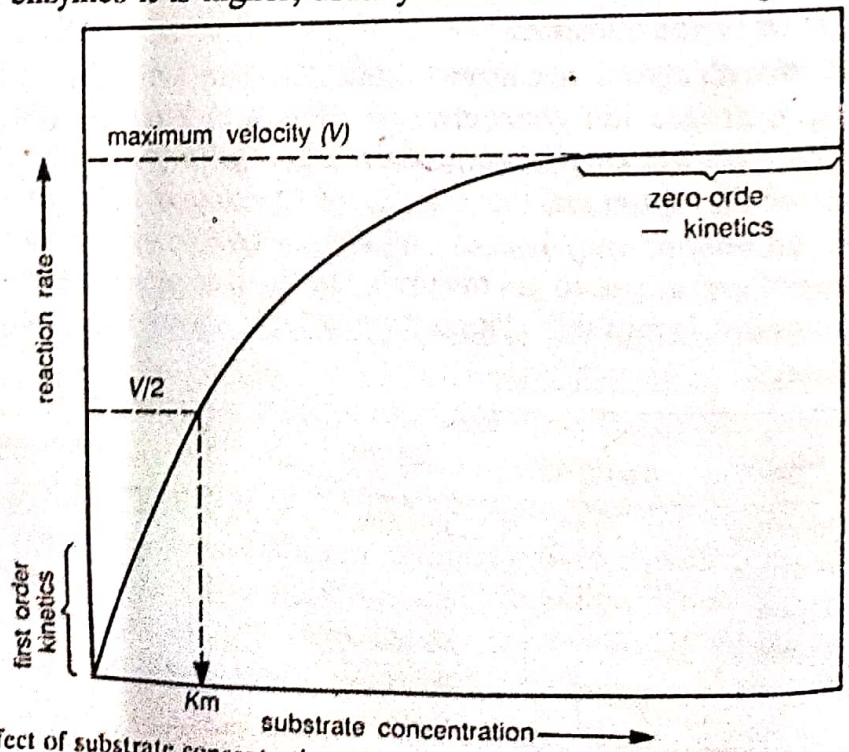

Fig. 4.3. Effect of substrate concentration of reaction rate, assuming that enzyme concentration is constant.

Enzymes

above this range decreases the rate of enzyme activity as the enzyme becomes denatured (Fig. 4.2). Majority of enzymes are denatured above 60°C. For short periods of time, enzymes can withstand high temperatures.

Concentration of enzyme. The velocity of an enzyme catalyzed reaction is directly proportional to the concentration of the enzyme provided the substrate is present in excess.

Influence of time. The velocity of the reaction is the amount of product formed per unit of time. If a curve is plotted with the amount of product formed against the incubation time, usually a plateau is obtained. The slowing may be due to a number of causes; the substrate available may decrease; accumulation of products increases the reverse reaction; the product may have a harmful effect on the reaction by denaturing the enzyme or changing the pH away from the optimum.

Concentration of substrate. The rate of reaction increases as the quantity of substrate is increased for a given quantity of enzyme. In the beginning the rate of reaction is linear but as shown in Fig. 4-3. the reaction rate becomes a hyperbolic type. This shows that the reaction is biphasic, The linear part of the curve in the initial stages follows first order kinetics.

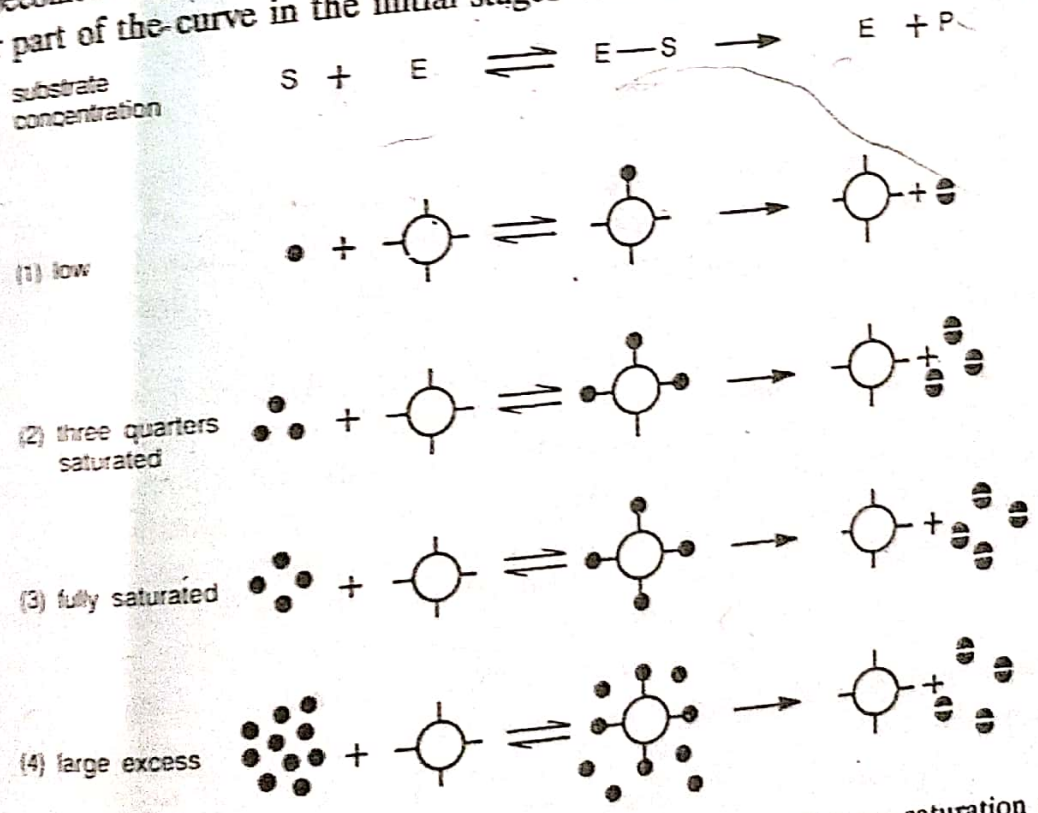

Fig. 4.4. Diagrammatic demonstration of effect of substrate concentration on saturation of active sites on enzyme surface. Note that for a unit time interval, cases 3 and 4 give the same amount of P (product) despite the large excess of substrate in case 4.

In the second phase a plateau is reached where the velocity is constant and the reaction is zero order type. The zero order reaction starts from the

point at which further increase in the substrate concentration does not have any affect on the enzyme activity. The substrate completely saturates the enzyme. The zero order portion approaches a limiting or maximal velocity, usually called V_{max} or V_m. The fact that a saturation point of the substrate concentration is reached, suggests not only the formation of a enzyme-substrate complex but also that there is a definite number of active sites at which this interaction can occur (*Fig.* 4.3).

Determination of the activity of enzymes has clinical importance in the diagnosis of various diseases. It is preferable to use substrate concentrations that will give zero-order kinetics so that variations in substrate concentrations do not influence the measurements of the enzyme activity. It is only under the conditions of zero order kinetics that the velocity will vary with the enzyme concentration.

Mechanism of enzyme action. Detailed studies show that enzyme reacts with the substrate forming an enzyme-substrate intermediary complex. The overall reaction can be given as follows.

$$\underset{\text{Enzyme}}{E} + \underset{\text{substrate}}{S} \rightleftharpoons \underset{\text{Enzyme substrate complex}}{ES} \rightleftharpoons \underset{\text{Enzyme}}{E} + \underset{\text{products}}{P}$$

However, the reaction is not so simple and is more likely.

$$E + S \rightleftharpoons ES \rightleftharpoons EP_2 + P_1$$

$$EP_2 \rightleftharpoons E + P_2$$

The ES may consist of several stages. Evidence so far available is in favour of the formation of such enzyme-substrate complexes. It is difficult to explain the high specificity of enzymes unless they in fact physically combine with their substrates.

Since the enzyme can act on the substrate only after combining with it, the rate of the enzyme-catalysed reaction, other conditions being constant, must depend on the frequency with which enzyme molecules encounter substrate molecules (Fig. 4.4). When the substrate concentration is very low, enzyme molecules encounter substrate molecules only rarely. Consequently the rate of the reaction is low. At any given instant only a small proportion of enzyme molecules are acting on substrate molecules. The remainder are unoccupied and waiting for substrate molecules to come along.

At high substrate concentrations, enzyme molecules meet the substrate more frequently, and the rate of the reaction is higher. At any given instant, a larger proportion of enzyme molecules are acting on the substrate molecules and the number of free or unoccupied enzyme molecules is comparatively less. Thus, avaliable enzyme molecules are used for greater effects.

If the concentration of substrate is further increased, enzyme molecules encounter substrate molecules so frequently that as an enzyme molecule reacts with one substrate molecule, it immediately finds another molecule to