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**From Ipsit Chakrabarti**
**Dinabandhu Andrews College**
**Associate Professor**
**Department of Zoology**

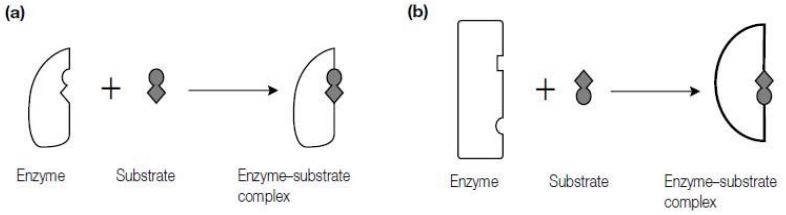
# ENZYMES

**Definition :** Enzymes are biological catalysts that increase the rate of a chemical reaction within or outside the cells without any change in enzyme structure after the reaction. Nearly all enzymes are proteins, and are made up of polypeptide chains. Enzyme reacts to its substrate with its own active or catalytic site. Enzymes are also highly specific with respect to the substrates, temperature and pH. In addition, enzyme activity can be regulated, varying the concentration of substrates or other molecules.

**Active site :** The active site of an enzyme is the region that binds the substrate and converts it into product. It is usually a relatively small part of the whole enzyme molecule and is a three-dimensional structure formed by amino acid residues in the polypeptide chains. The active site is a cleft or crevice on the surface of the enzyme that helps the binding of the substrate. The substrate(s) is bound in the active site by multiple weak forces (electrostatic interactions, hydrogen bonds, van der Waals bonds, hydrophobic interactions ).By binding enzyme forms an enzyme–substrate complex. There is a specific site within active site ,known as catalytic site , which ultimately acts on the substrate to produce product**.** The enzyme is now free to bind another molecule of substrate and begin its catalytic cycle again.

Two models have been proposed to explain how an enzyme binds its substrate:

**(a)Lock-and-key model :** In the lock-and-key model proposed by Emil Fischer in 1894, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock (*Fig. 1a*). The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the right alignment.
**(b)Induced-fit model :**In the induced-fit model proposed in 1958 by Daniel E.Koshland, Jr., the binding of substrate induces a conformational change in the active site of the enzyme (*Fig. 1b*). The binding of substrate to the enzyme induces a conformational change in the structure of the enzyme such that the active site changes to a shape that is complementary to the substrate only after it has bound to the enzyme. Different enzymes show features of both models, with some complementarity and some conformational change.




(a)Lock-and-key model (b) Induced-fit model

**Enzyme classification :** Many enzymes are named by adding the suffix '-ase' to the name of their substrate, like urease, fructose-1,6-bisphosphatase. However, other enzymes, such as trypsin and chymotrypsin have names that do not denote their substrate.

A system of enzyme nomenclature has been internationally agreed. This system places all enzymes into one of six major classes based on the type of reaction catalyzed (*Table 1*). Each enzyme is then uniquely identified with a four-digit classification number. Thus trypsin has the Enzyme Commission (EC) number 3.4.21.4, where the first number (3) denotes that it is a hydrolase, the second number (4) that it is a protease that hydrolyzes peptide bonds, the third number (21) that it is a serine protease with a serine residue at the active site, and the fourth number (4) indicates that it was the fourth enzyme in this class.

## International classification of enzymes

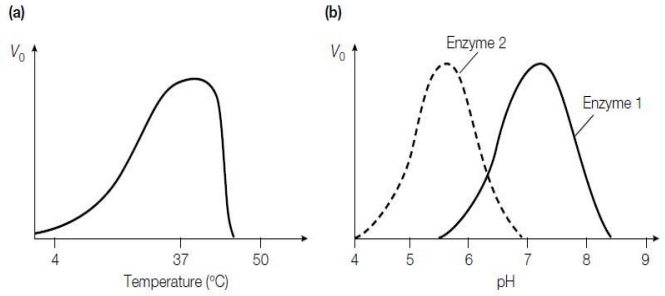
| Class | Name | Type of reaction catalyzed | Example |
|---|---|---|---|
| 1. | Oxidoreductases | Transfer of electrons | Alcohol dehydrogenase |
| 2. | Transferases | Transfer of functional groups | Hexokinase |
| 3. | Hydrolases | Hydrolysis reactions | Trypsin |
| 4. | Lyases | Cleavage of C–C, C–O, C–N and other bonds, often forming a double bond | Pyruvate decarboxylase |
| 5. | Isomerases | Transfer of groups within a molecule | Maleate isomerase |
| 6. | Ligases (or synthases) | Bond formation coupled to ATP hydrolysis | Pyruvate carboxylase |

**Coenzymes and prosthetic groups :** Many enzymes require the presence of small, nonprotein units or cofactors to carry out their particular reaction. Cofactors may be either one or more inorganic ions, such as Zn or Fe, or a complex organic molecule called a coenzyme.
A metal or coenzyme that is covalently or strongly attached to the enzyme is called a prosthetic group (heme in hemoglobin). A complete active enzyme together with its coenzyme or metal ion is called a holoenzyme. The protein part of the enzyme without its cofactor is termed an apoenzyme. Some coenzymes, such as NAD, are bound and released by the enzyme during its catalytic cycle and in effect function as cosubstrates. Many coenzymes are derived from vitamin precursors. **Examples :** Coenzyme A, FAD, FMN ,NAD, NADP.

**Isoenzymes (isozymes)** : Isozymes are different forms or structure of an enzyme which catalyze the same reaction, but which exhibit different physical or kinetic properties, such as optimum pH, substrate affinity or effect of inhibitors. Different isoenzyme forms of an enzyme are usually derived from different genes and occur in different tissues of the body.
An example of an enzyme which has different isoenzyme forms is lactate dehydrogenase (LDH) which catalyzes the reversible conversion of pyruvate into lactate in the presence of the coenzyme NADH. LDH is a tetramer of two different types of subunits, called H and M, which have small differences in amino acid sequence. The two subunits can combine randomly with each other, forming five isoenzymes that have the compositions H4, H3M, H2M2, HM3 and M4.
**Effect of different factors on enzyme activity :**
**Temperature :** Temperature affects the rate of enzyme-catalyzed reactions in two ways. First, a rise in temperature increases the thermal energy of the substrate molecules, and hence increases the rate of the reaction. Further increase in the thermal energy of the molecules breaks the multiple weak, noncovalent interactions (hydrogen bonds, van der Waals forces, etc.) which hold the three-dimensional structure of the enzyme together . Ultimately this will lead to the denaturation (unfolding) of the enzyme, but even small changes in the three-dimensional shape of the enzyme can alter the structure of the active site and lead to a decrease in catalytic activity. For many mammalian enzymes this is around 37 degree Centigrade, but there are also organisms which have enzymes adapted to working at considerably

higher or lower temperatures. For example, *Taq* polymerase that is used in the polymerase chain reaction is found in a bacterium that lives at high temperatures in hot springs.

**pH :** Each enzyme has an optimum pH at which the rate of the reaction that it catalyzes is at its maximum. Small deviations in pH from the optimum value lead to decreased activity due to changes in the ionization of groups at the active site of the enzyme. Larger deviations in pH lead to the denaturation of the enzyme protein itself, due to interference with the many weak noncovalent bonds maintaining its three-dimensional structure. A graph of *V*0 plotted against pH will usually give a bell shaped curve (*Fig. 3b*). Many enzymes have a pH optimum of around 6.8, but there is great diversity in the pH optima of enzymes, due to the different environments in which they are adapted to work. For example, the digestive enzyme pepsin is adapted to work at the acidic pH of the stomach (around pH 2.0).




The effect of (a) temperature and (b) pH on enzyme activity.

The **Michaelis–Menten model** uses the following concept of enzyme catalysis:

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P}.$$

The enzyme (E), combines with its substrate (S) to form an **enzyme–substrate complex** (ES). The ES complex can dissociate again to form E+S, or can proceed chemically to form E and the product P. The **rate constants** *k*1, *k*2 and *k*3 describe the rates associated with each step of the catalytic process. It is assumed that there is no significant rate for the backward reaction of enzyme and product (E+P) being converted to ES complex. [ES] remains approximately constant until nearly all the substrate is used, hence the rate of synthesis of ES equals its rate of consumption over most of the course of the reaction; that is, [ES] maintains a **steady state**. From the observation of the properties of many enzymes it was known that the initial velocity (*V*0) at low substrate concentrations is directly proportional to [S], while at high substrate concentrations the velocity tends towards a maximum value, that is the rate becomes independent of [S] . This maximum velocity is called *V*max . The initial velocity (*V*0) is the velocity measured experimentally before more than approximately 10% of the substrate has been converted to product in order to minimize such complicating factors as the effects of reversible reactions, inhibition of the enzyme by product, and progressive inactivation of the enzyme.
Michaelis and Menten derived an equation to describe these observations, the Michaelis–Menten equation:

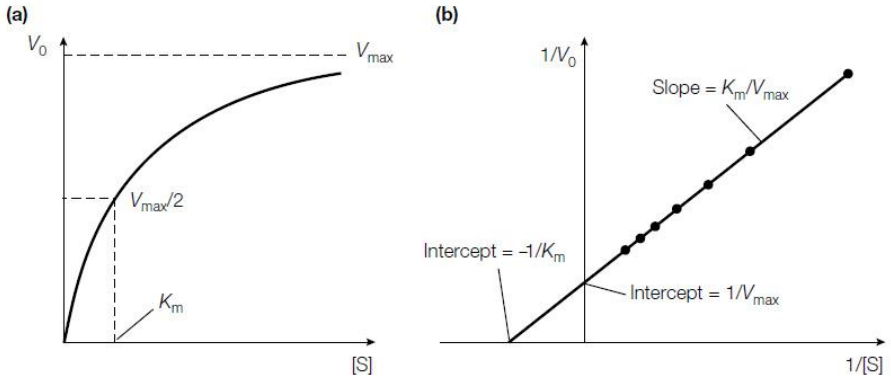
$$V_0 = \frac{V_{\max} \cdot [\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

The equation describes a hyperbolic curve of the type . In deriving the equation, Michaelis and Menten defined a new constant, *K*m, the Michaelis constant :

$$K_\mathrm{m} = \frac{k_2 + k_3}{k_1}$$

Km is a measure of the stability of the ES complex, being equal to the sum of the rates of breakdown of ES over its rate of formation. For many enzymes *k*2 is much greater than *k*3. Under these circumstances *K*m becomes a measure of the affinity of an enzyme for its substrate since its value depends on the relative values of *k*1 and *k*2 for ES formation and dissociation,
respectively. A high *K*m indicates weak substrate binding (*k*2 predominant over *k*1), a low *K*m indicates strong substrate binding (*k*1 predominant over *k*2). *K*m can be determined experimentally by the fact that its value is equal to the substrate concentration at which the velocity is equal to half of *V*max.

**Lineweaver–Burk** Plot :Because *V*max is achieved at infinite substrate concentration, it is impossible to estimate *V*max (and hence *K*m) from a hyperbolic plot. However, *V*max and *K*m can be determined experimentally by measuring *V*0 at different substrate concentrations . Then a double reciprocal or Lineweaver–Burk plot of 1/*V*0 against 1/[S] is made . This plot is a derivation of the Michaelis–Menten equation, which gives a straight line, with the intercept on the *y*-axis equal to 1/Vmax, and the intercept on the *x*-axis equal to -1/*K*m. The slope of the line is equal to Km/Vmax.

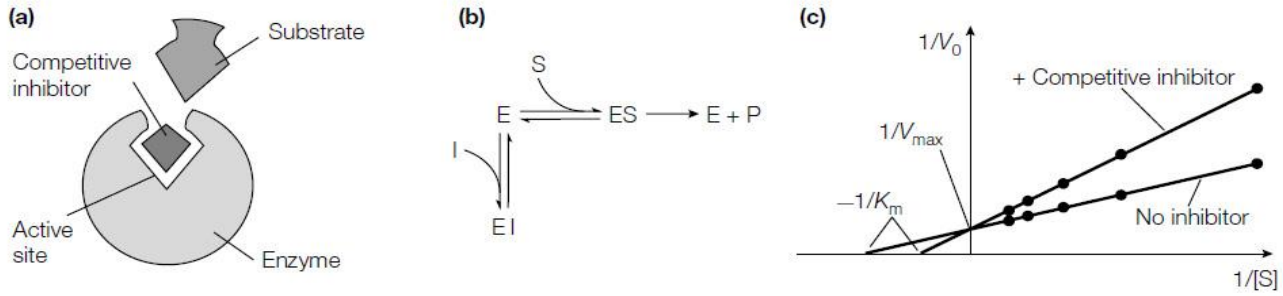



A few enzymes do not follow the Michaelis–Menten kinetics. These enzymes, such as aspartate transcarbamoylase (ATCase), are called allosteric enzymes.

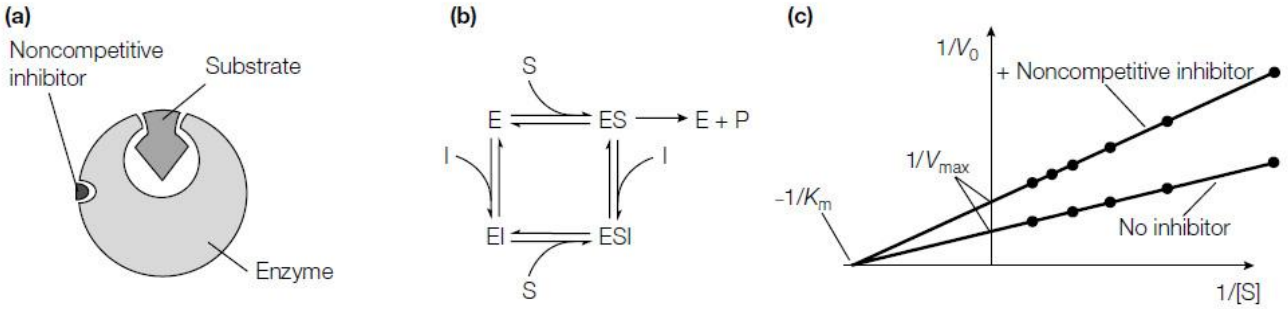
**Enzyme inhibition :**Any molecule which acts directly on an enzyme to lower its catalytic rate is called an inhibitor. The catalytic rate of an enzyme can be lowered by inhibitor molecules. Many inhibitors exist, including normal body metabolites, foreign drugs and toxins. Enzyme inhibition can be of two main types: irreversible or reversible. Reversible inhibition can be subdivided into competitive and noncompetitive.

**Irreversible inhibition :** Inhibitors which bind irreversibly to an enzyme often form a covalent bond to an amino acid residue at or near the active site, and permanently inactivate the enzyme. Susceptible amino acid residues include Ser and Cys residues which have reactive –OH and –SH groups, respectively.

**Competitive inhibition :**A competitive inhibitor typically has close structural similarities to the normal substrate for the enzyme. Thus it competes with substrate molecules to bind to the active site.The enzyme may bind either a substrate molecule or an inhibitor molecule, but not both at the same time.The competitive inhibitor binds reversibly to the active site. At high substrate concentrations the action of a competitive inhibitor is overcome because a high substrate concentration will successfully compete out the inhibitor molecule in binding to the active site. Thus there is no change in the *V*max of the enzyme but the apparent affinity of the enzyme for its substrate decreases in the presence of the competitive inhibitor, and hence *K*m increases.




**Noncompetitive inhibition :** A noncompetitive inhibitor binds reversibly at a site other than the active site noncompetitive and causes a change in the overall three-dimensional shape of the inhibition enzyme that leads to a decrease in catalytic activity. Since the inhibitor binds at a different site to the substrate, the enzyme may bind the inhibitor, the substrate or both the inhibitor and substrate together .The effects of a noncompetitive inhibitor cannot be overcome by increasing the substrate concentration, so there is a decrease in *V*max. In noncompetitive inhibition the affinity of the enzyme for the substrate is unchanged and so *K*m remains the same.




**Allosteric enzymes:** These enzymes are multi-subunit proteins, with one or more active sites on each subunit. The binding of substrate at one active site induces a conformational change in the enzyme that influences the other active sites, to react with substrate molecules. In addition, allosteric enzymes may be controlled by effector molecules (activators and inhibitors) that bind to the enzyme at a site other than the active site (either on the same subunit or on a different subunit), thereby causing a change in the conformation of the active site which alters the rate of enzyme activity.