Enzymes, definition, terminology & classifications:

OVERVIEW: Virtually all reactions in the body are mediated by enzymes, which are protein catalysts that increase the rate of reactions without being changed in the overall process. Among the many biologic reactions that are energetically possible, enzymes selectively channel reactants (called substrates) into useful pathways. Enzymes thus direct all metabolic events.

Nomenclature:

Each enzyme is assigned two names. The first is its short, recommended name, convenient for everyday use. The second is the more complete systematic name, which is used when an enzyme must be identified without ambiguity. A. Recommended name Most commonly used enzyme names have the suffix "-ase" attached to the substrate of the reaction (for example, glucosidase and urease), or to a description of the action performed (for example, lactate dehydrogenase and adenylyl cyclase). [Note: Some enzymes retain their original trivial names, which give no hint of the associated enzymic reaction, for example, trypsin and pepsin.].

Systematic name:

In the systematic naming system, enzymes are divided into six major classes (Figure 1), each with numerous subgroups. For a given enzyme, the suffix -ase is attached to a fairly complete description of the chemical reaction catalyzed, including the names of all the substrates; for example, lactate: NAD+ oxidoreductase. [Note: Each enzyme is also assigned a classification number.] The systematic names are unambiguous and informative, but are frequently too cumbersome to be of general use.

Classes of enzymes

1. Oxidoreductase enzymes that catalyze oxidations and reductions.

2. Transferases—enzymes that catalyze transfer of moieties such as glycosyl, methyl, or phosphoryl groups.

3. Hydrolases—enzymes that catalyze hydrolytic cleavage of C-C, C-O, C-N, and other covalent bonds.

4. Lyases—enzymes that catalyze cleavage of C-C, C-O, C-N, and other covalent bonds by atom elimination, generating double bonds.

5. Isomerases—enzymes that catalyze geometric or structural changes within a molecule.

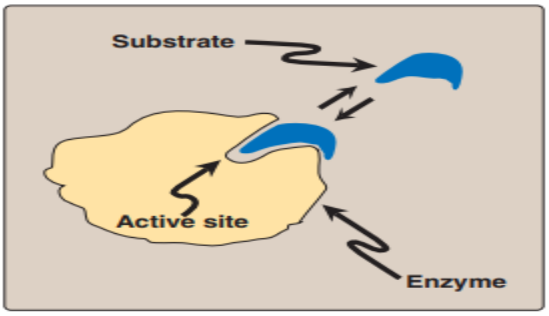
6. Ligases—enzymes that catalyze the joining together (ligation) of two molecules in reactions coupled to the hydrolysis of ATP.

Properties of enzymes:

Enzymes are protein catalysts that increase the velocity of a chemical reaction and are not consumed during the reaction. [Note: Some RNAs can act like enzymes, usually catalyzing the cleavage and synthesis of phosphodiester bonds. RNAs with catalytic activity are called ribozymes, and are much less commonly encountered than protein catalysts.]

Active sites:

Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid side chains that participate in substrate binding and catalysis (Figure 2). The substrate binds the enzyme, forming an enzyme–substrate (ES) complex. Binding is thought to cause a conformational change in the enzyme (induced fit) that allows catalysis. ES is converted to an enzyme–product (EP) complex that subsequently dissociates to enzyme and product.



Figue-1: Schematic representation of an enzyme with one active site binding a substrate molecule

Catalytic efficiency: Enzyme-catalyzed reactions are highly efficient, proceeding from 10^3–10^8 times faster than uncatalyzed reactions. The number of molecules of substrate converted to product per enzyme molecule per second is called the turnover number, or k cat, and typically is 10^2 –10^4 s^{-1}. C.

Specificity:

Enzymes are highly specific, interacting with one or a few substrates and catalyzing only one type of chemical reaction. [Note: The set of enzymes made in a cell determines which metabolic pathways occur in that cell.]

Holoenzymes:

Some enzymes require molecules other than proteins for enzymic activity. The term holoenzyme refers to the active enzyme with its nonprotein component, whereas the enzyme without its nonprotein moiety is termed an apoenzyme and is inactive. If the nonprotein moiety is a metal ion such as Zn^{2+} or Fe^{2+}, it is called a cofactor. If it is a small organic molecule, it is termed a coenzyme. Coenzymes that only transiently associate with the enzyme are called cosubstrates. Cosubstrates dissociate from the enzyme in an altered state (NAD^+ is an example). If the coenzyme is permanently associated with the enzyme and returned to its original form, it is called a prosthetic group (FAD is an example). Coenzymes frequently are derived from vitamins. For example, NAD^+ contains niacin and FAD contains riboflavin.

Mechanism of enzyme action

How enzymes work:

The mechanism of enzyme action can be viewed from two different perspectives. The **first treats** catalysis in terms of energy changes that occur during the reaction, that is, enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction. **The second perspective** describes how the active site chemically facilitates catalysis.

Energy changes occurring during the reaction virtually all chemical reactions have an energy barrier separating the reactants and the products. This barrier**, called the free energy of activation**, *is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product*. For example, Figure 1: shows the changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state (high-energy intermediate),

$$A \longleftrightarrow T^* \longleftrightarrow B$$

Free energy of activation: The peak of energy in Figure 1- is the difference in free energy between the reactant and T*, where the high-energy intermediate is formed during the conversion of reactant to product. Because of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.

Rate of reaction: For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of an enzyme, only a small proportion of a population of molecules may possess enough energy to

achieve the transition state between reactant and product. The rate of reaction is determined by the number of such energized molecules. **In general, the lower the free energy of activation, the more molecules have sufficient energy to pass through the transition state, and, thus, the faster the rate of the reaction.**

Alternate reaction pathway: An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an alternate reaction pathway with a lower free energy of activation (Figure 1). The enzyme does not change the free energies of the reactants or products and, **therefore, does not change the equilibrium of the reaction. It does, however, accelerate the rate with which equilibrium is reached.**

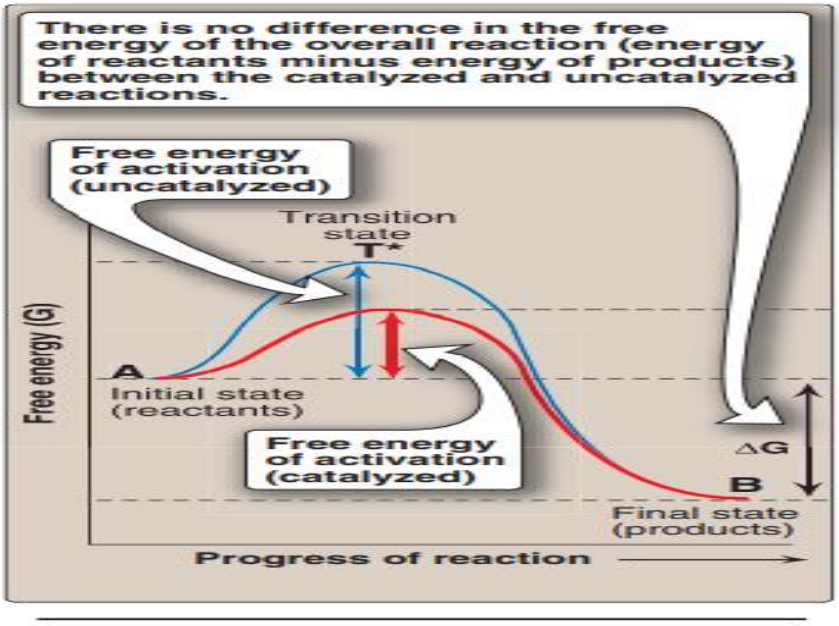


Figure 1: Effect of an enzyme on the activation energy of a reaction.

Michaelis-Menten Theory:

Lenor Michaelis and Maud Menten put forward the **Enzyme-Substrate complex theory.** The enzyme (E) combines with the substrate (S), to form an enzyme-substrate (ES) complex, which immediately breaks down to the enzyme and the product (P) (Fig. 2).

$$\mathbf{E + S \rightarrow E\text{–}S\ Complex \rightarrow E + P}$$

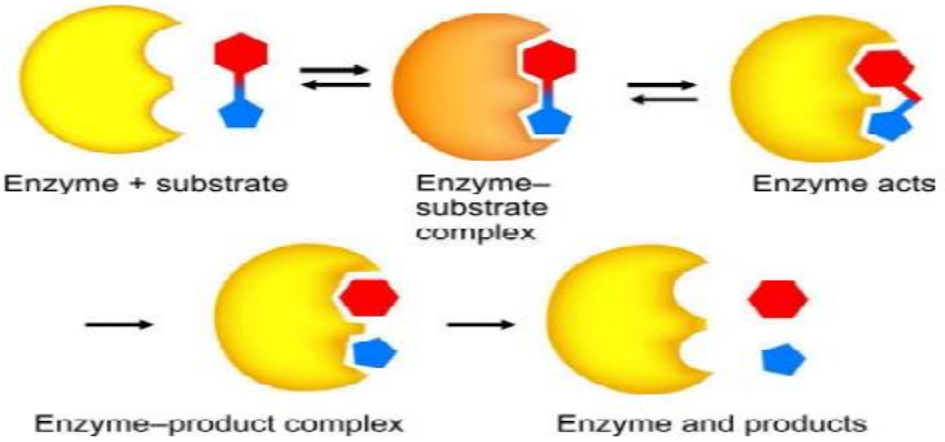


Figure. 2: Enzyme substrate complex

Fischer's Template Theory :

i. It states that the three dimensional structure of the active site of the enzyme is complementary to the substrate. Thus, enzyme and substrate fit each other (Fig. 3).
ii. The explanation is that substrate fits on the enzyme, similar to lock and key. The key will fit only to its own lock.

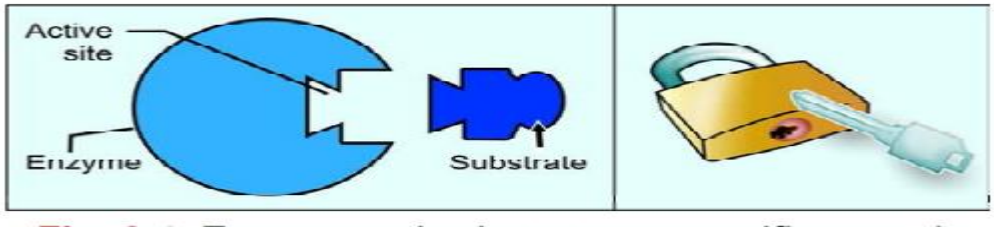


Figure-3: Enzyme and substrate are specific to each other. This is similar to key and lock (Fischer's theory)

Koshland's Induced Fit Theory

i. Conformational changes are occurring at the active site of enzymes concomitant with the combination of enzyme with the substrate.
ii. At first, the substrate binds to a specific part of the enzyme, and this leads to more secondary binding and conformational changes.
iii. The substrate induces conformational changes in the enzyme, such that precise orientation of catalytic groups is effected.

FACTORS INFLUENCING ENZYME ACTIVITY :

1. Enzyme Concentration

Velocity of reaction is increased proportionately with the concentration of enzyme, when substrate concentration is unlimited (Fig. 4).

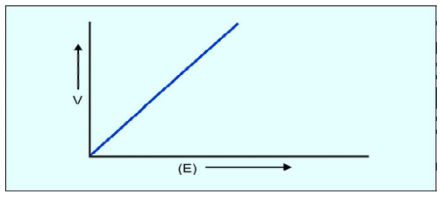


Figure-4: Effect of enzyme concentration

2. Substrate Concentration:

As substrate concentration is increased, the velocity is also correspondingly increased in the initial phases; but the curve flattens afterwards. The maximum velocity thus obtained is called Vmax.

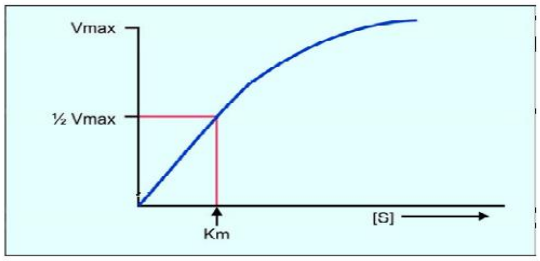


Figure-5 : Effect of substrate concentration (substrate saturation curve)

3. Effect of pH:

i. Each enzyme has an optimum pH, on both sides of which the velocity will be drastically reduced. The graph will show a bell shaped curve (Fig. 6).

ii. The pH decides the charge on the amino acid residues at the active site.

iii. Usually enzymes have the optimum pH between 6 and 8. Some important exceptions are Pepsin (with optimum pH 1-2); alkaline phosphatase (optimum pH 9-10) and acid phosphatase (4-5).

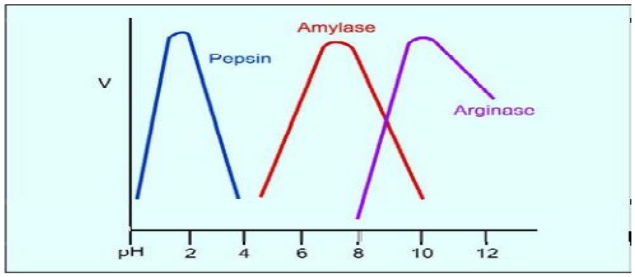


Fig. 6: Effect of pH on enzyme velocity

4. Effect of Temperature

i. The velocity of enzyme reaction increases when temperature of the medium is increased; reaches a maximum and then falls (Bell shaped curve). The temperature at which maximum amount of the substrate is converted to the product per unit time is called the optimum temperature (Fig. 7).

ii. As temperature is increased, more molecules get activation energy, or molecules are at increased rate of motion. So their collision probabilities are increased and so the reaction velocity is enhanced.

iii. But when temperature is more than 50°C, heat denaturation and consequent loss of tertiary structure of protein occurs. So activity of the enzyme is decreased.

iv. Most human enzymes have the optimum temperature around 37°C.

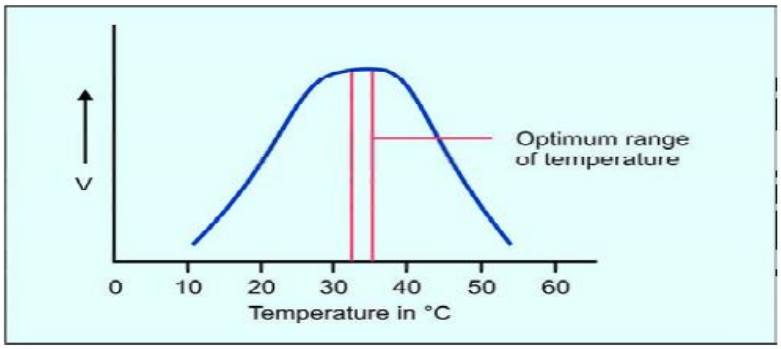


Figure-7: effect of temperature on enzyme reaction velocity

Clinical significant of enzyme assays

enzymes in clinical diagnosis :

Plasma enzymes can be classified into two major groups.

First, a relatively small group of enzymes are actively secreted into the blood by certain cell types. For example, the liver secretes zymogens (inactive precursors) of the enzymes involved in blood coagulation.

Second, a large number of enzyme species are released from cells during normal cell turnover. These enzymes almost always function intracellularly, and have no physiologic use in the plasma. In healthy individuals, the levels of these enzymes are fairly constant and represent a steady state in which the rate of release from damaged cells into the plasma is balanced by an equal rate of removal of the enzyme protein from the plasma. Increased plasma levels of these enzyme may indicate tissue damage (Figure 1).

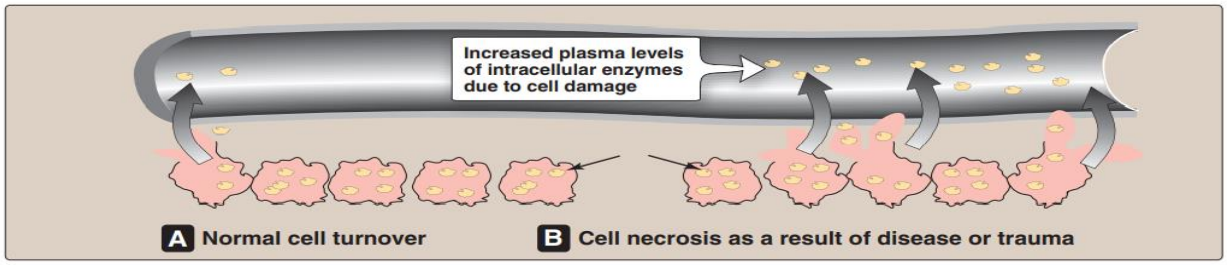


Figure-1: Release of enzymes from normal and diseased or traumatized cells.

A. Alteration of plasma enzyme levels in disease states:

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues. The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Thus, determining the degree of elevation of a particular enzyme activity in the plasma is often useful in evaluating the prognosis for the patient.

B. Plasma enzymes as diagnostic tools:

Some enzymes show relatively high activity in only one or a few tissues. The presence of increased levels of these enzymes in plasma thus reflects damage to the corresponding tissue. For example, the enzyme alanine aminotransferase is abundant in the liver. The appearance of elevated levels of ALT in plasma signals possible damage to hepatic tissue. [Note: Measurement of ALT is part of the liver function test panel.] Increases in plasma levels of enzymes with a wide tissue

distribution provide a less specific indication of the site of cellular injury and limits their diagnostic value.

C. Isoenzymes and diseases of the heart: Most isoenzymes (also called isozymes) are enzymes that catalyze the same reaction. However, they do not necessarily have the same physical properties because of genetically determined differences in amino acid sequence. For this reason, isoenzymes may contain different numbers of charged amino. Different organs frequently contain characteristic proportions of different isoenzymes. The pattern of isoenzymes found in the plasma may, therefore, serve as a means of identifying the site of tissue damage. For example, the plasma levels of creatine kinase (CK) are commonly determined in the diagnosis of myocardial infarction. They are particularly useful when the electrocardiogram is difficult to interpret, such as when there have been previous episodes of heart disease.

1. Quaternary structure of isoenzymes: Many isoenzymes contain different subunits in various combinations. For example, creatine kinase (CK) occurs as three isoenzymes. Each isoenzyme is a dimer composed of two polypeptides (called B and M subunits) associated in one of three combinations: CK1 = BB, CK2 = MB, and CK3 = MM. Each CK isoenzyme shows a characteristic electrophoretic mobility. [Note: Virtually all CK in the brain is the BB isoform, whereas in skeletal muscle it is MM. In cardiac muscle, about one-third is MB with the rest as MM.]

2. Diagnosis of myocardial infarction: Measurement of blood levels of proteins with cardiac specificity is used in the diagnosis of myocardial infarction (MI) because myocardial muscle is the only tissue that contains more than 5% of the total CK activity as the CK2 (MB) isoenzyme. Appearance of this hybrid isoenzyme in plasma is virtually specific for infarction of the myocardium. Following an acute MI, this isoenzyme appears approximately 4–8 hours following onset of chest pain, reaches a peak of activity at approximately 24 hours, and returns to baseline after 48–72 hours. Troponin T and troponin I are regulatory proteins involved in myocardial contractility. They are released into the plasma in response to cardiac damage. Cardiac troponin I (cTnI) is highly sensitive and specific for damage to cardiac tissue. cTnI appears in plasma within 4–6 hours after an MI, peaks in 8–28 hours, and remains elevated for 3–10 days. Elevated serum troponins then, are more predictive of adverse outcomes in unstable angina or myocardial infarction than the conventional assay of CK2.