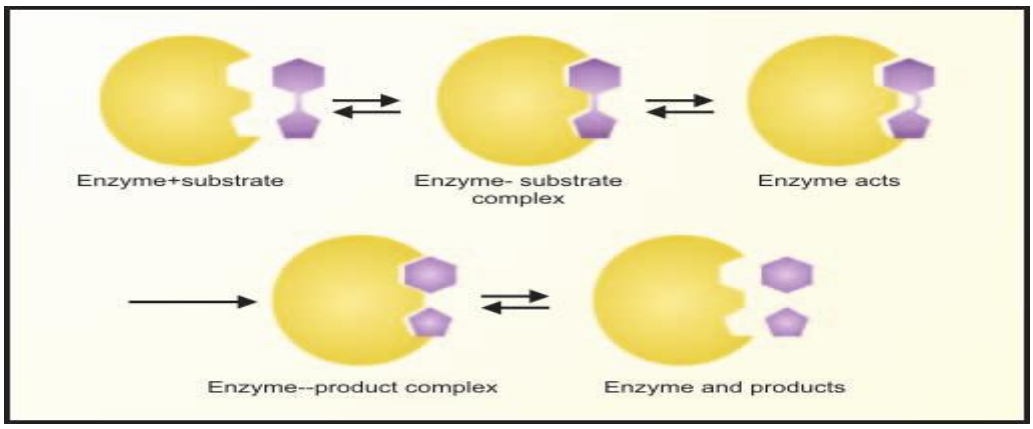
Enzymes and clinical enzymalogy :

Most chemical raction, particulary those for the oxidation or transformation of organic compounds, proceed imperceptily, or not at all at 37 °C becouse they require elevated temperature for their action. living cells cannot be subjected to high temperature becouse of the delicate nature of protoplasm: most protein solutions coagulate at temperature above 56 °C. biological systems, however, have developed numerous catalysts that enable the necessary metabolic reactions to occur at body temperature. The biologic catalysts speed up reaction rate and are called enzymes.

Enzymes are biocatalysts. Life is possible due to the co-ordination of numerous metabolic reactions inside the cells. Lack of enzymes will lead to block in metabolic pathways causing inborn errors of metabolism. The substance upon which an enzyme acts, is called the substrate. The enzyme will convert the substrate into the product or products. All enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins. They are heat labile, soluble in water, precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid) and contain 16% weight as nitrogen. Enzymes are present in all body cells, and each function in a specific reaction. enzymes catalyze all essential reactions (oxidation, reduction, hydrolysis, esterification, synthesis, molecular interconversion) that supply the energy and/or chemical changes nessessary for vital activites (muscle contraction, nerve conduction, respiration, digestion, growth, reproduction, mantanice of the body temerature). As catalysis enzymes are not used up in the process and not change the equilibrium point of the reaction: they merely accelerate the rate for reaching equilibrium.

Enzyme action

The first step in the action of enzyme is the formation of an enzyme substrate complex : that is, the binding of the substrate molecule to the target protein enzyme. Some enzymes require the presence of a coenzyme, anonprotein molecule, before they can function. Most coenzymes are small, heat stable compounds that usually derivative of particular members of the vit. B family, such as thiamine phosphate (B1), pyridoxal (B_6), nucleotides riboflavein (B2), and others.



Enzyme-substrate complex

Enzymes have an active site, the actual place where partcular bond in the substrate are strined and ruptured. The active site has a specific three dimentional configration, depending on the amino acid sequence of the enzyme; the sequence determines the distribution of changes around the site. Part or all of the substrate fits into the active site in the same minner as a key matches a lock, a process that confers specificity upon the enzyme. Certain enzymes requir the presence of a particular ion to induce the nessasery configration for proper binding to the substrate; such inorganic ions are called activaters. The most common activeters are calcium, magnesium, and zinc, but other cations, such as iron, manganese and copper , are required by certain enzymes. Anions may be required by some enzymes for full activity; chloride ion is essential for amylase action.

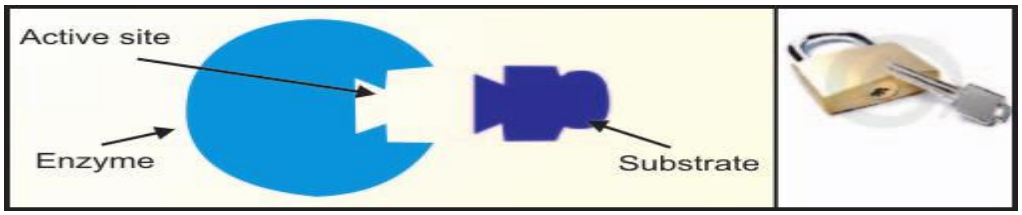


Figure : Enzyme and substrate are specific to each other. This is similar to key and lock

After the enzymatic action (ruptere of a particular bond in the sustrate) has occurred, the products of the reaction are no longer bound and the enzyme is recycled as the prodiucts diffuse away. The free enzyme can bind another substrate molecule repeat its action.

Activation energy is defined as the energy required to convert all molecules in one mole of a reacting substance from the ground state to the transition state. Enzymes are said to reduce the magnitude of this activation energy. This can be compared to making a tunnel in a mountain, so that the barrier could be lowered (Fig. below). For example, activation energy for hydrolysis of sucrose by H + is 26,000 cal/mol, while the activation energy is only 9,000 cal/mol when hydrolysed by sucrase.

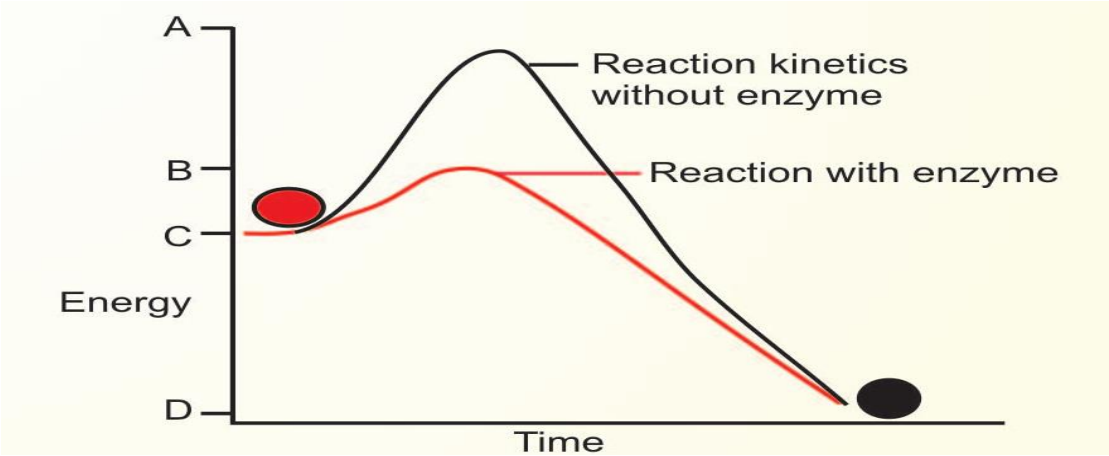


Figure : effect of enzymes on lowering activation energy

Enzymes inhibition

Becouse the catalytic action of an enzyme depends on its specific configration as a protein, many substrate can inhibit this activity by changing the configration of the enzyme (binding metal cation, drugs, or other charged molecules to the protein).

Competitive Inhibition

i) The inhibitor molecules are competing with the normal substrate molecules for attaching with the active site of the enzyme.

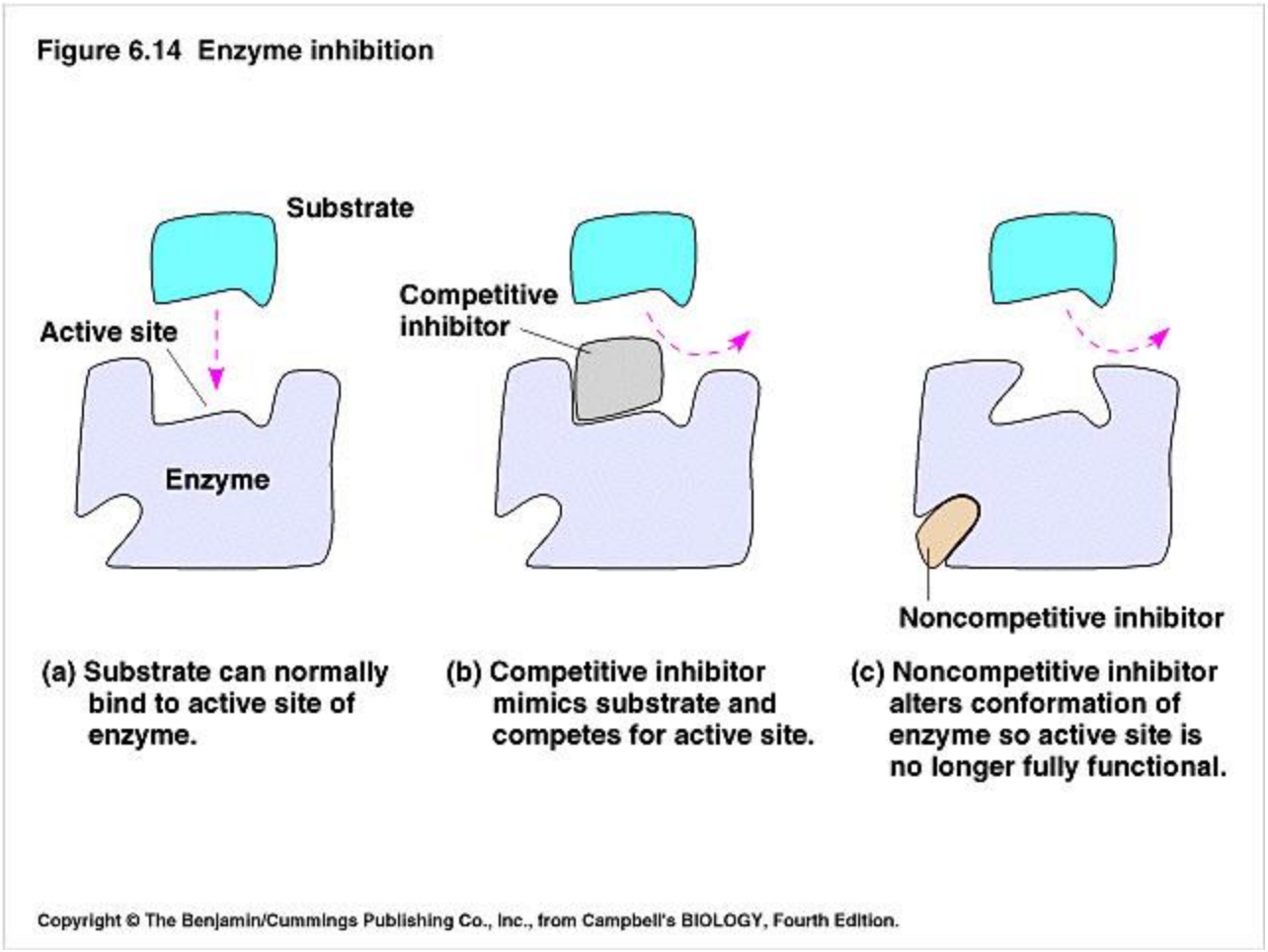
E + S------→ E-S --------→E + P

E + I -------→E-I

Since E-I (enzyme–inhibitor complex) can react only to reform the enzyme and inhibitor, the number of enzyme molecules availabl for E-S formation is reduced.

Non-competitive Inhibition

A variety of poisons, such as iodoacetate, heavy metal ions (silver, mercury) and oxidising agents act as irreversible non- competitive inhibitors. The inhibitor usually binds to a different domain on the enzyme, other than the substrate binding site.



The classes of enzymes:

1. Oxidoreductases catalyze oxidations and reductions.
2. Transferases catalyze transfer of groups such as methyl or glycosyl groups from a donor molecule to an acceptor molecule.
3. Hydrolases catalyze the hydrolytic cleavage of C–––C, C––O, C–––N, P––O, and certain other bonds, including acid anhydride bonds.
4. Lyases catalyze cleavage of C––C, C––O, C––N, and other bonds by elimination, leaving double bonds, and also add groups to double bonds.
5. Isomerases catalyze geometric or structural changes within a single molecule.
6. Ligases catalyze the joining together of two molecules, coupled to the hydrolysis of a pyrophosphoryl group in ATP or a similar nucleoside triphosphate.

CO-ENZYMES

i) Enzymes may be simple proteins, or complex enzymes, containing a non-protein part, called the prosthetic group.
ii) The protein part of the enzyme is then named the apo-enzyme, the prosthetic group the co- enzyme; and these two portions combined together is called the holo-enzyme.
iii) The co-enzyme is essential for the biological activity of the enzyme. A co-enzyme is a low molecular weight organic substance, without which the enzyme cannot exhibit any reaction.
iv) One molecule of the co-enzyme is able to convert a large number of substrate molecules with the help of enzyme.

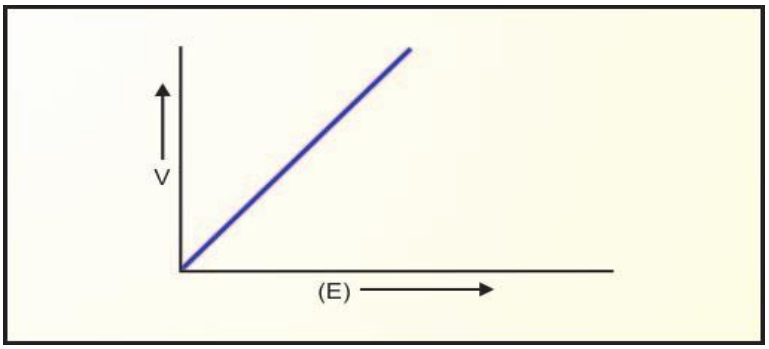
Metallo-enzymes

i) These are enzymes which require certain metal ions for their activity. Some examples In certain cases, e.g. copper in Tyrosinase, the metal is tightly bound with the enzyme.
ii) In other cases, when metal ion is removed from the enzyme, the activity of the enzyme will be minimal; but when the metal ion is added, the activity is enhanced. They are called ion-activated enzymes, e.g. chloride ions will activate salivary amylase.

Factors affecting the velocity of enzyme reactions

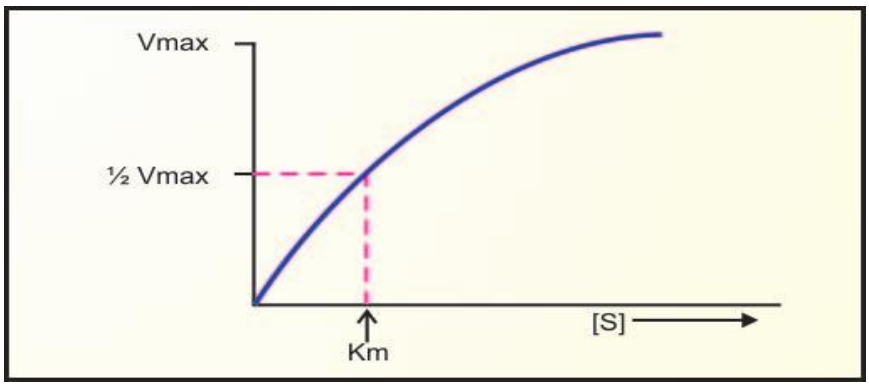
The velocity of enzyme reaction is affected by various factros such as:

1. enzyme concentration
   when the concentration of substrate is high and essentially constant during enzyme reaction, the velocity of the reaction is directly proportional to the enzyme concentration. The reaction proceeds more rapidly when more enzyme molecules are present to bind the abundant substrate, a mass action effect.



Enzyme concetration efffect on reaction velocity

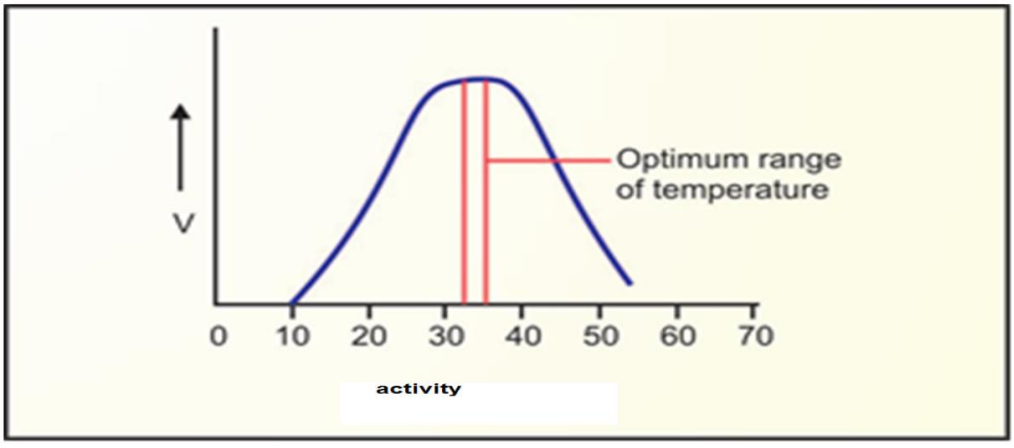
2. substrate concentration
   when the amount of enzyme in a reaction mixture is fixed and the reaction rate is plottted against increasing amount of substrate, the type of curve illustrated in bellow figure. The reaction rate varies directly with substrate concentration when its concentration is low, as illustrated by the linear segment ab curve. The direct proportionality between reaction rate and substrate concentration is known as a first order reaction. At higher substrate concentration, fewer binding site are available for attachment, so the increase in the reaction velocity for each added increase of substrate is progressively diminished. The reaction velocity becomes becomes independent of substrate concentration when the. Binding sites atr satturated



Effect of substrate on reaction velocity

3.pH

Enzymes , being proteins, carry a net charge on the molecule that is sensetive to alerations in pH; in general , the optmium pH range for an enzyme is relatrively small, and the curve produced by plotting enzyme activity versus pH is usually steep, with rapid falling off after the optimum point. Also, the range of permissible pH has a distinct limit before protein denaturation of the enzyme affects activity.



Effect of pH on reaction velocity

4.Temperature

The reaction velocities of most chemical reactions with temperature and approximately double for each 10 °C rise. Again, the preotein nature of enzyme places strict limits on the permissible temperature range becouse most protein become denatured and insoluble within minutes of being subjected to 60 °C and above.

5.Electrolyte environment

Many enzymes are sensetive to certain buffer ions in the incubation mixture; hence, different buffer must be teast before deciding on optmium conditions for measuring activites. Certain cations at a partcular concentration range may be necessary for complete actvation of enzyme, and the total ionic strength of the incubation mixture may also affect activity.

6. inhibitors:

For some enzymes, a product of the reaction may inhibit activity of enzymes. Inorganic phosphate, a reaction product formed during the hydrolysis of organic phosphate ester by alkaline phosphate, is a competitive inhibitors of the enzyme. Also, varoius drugs administration to patients may be competive or noncompetitive inhibitors of certain serum enzymes.

ISO-ENZYMES

They are physically distinct forms of the same enzyme activity. Multiple molecular forms of an enzyme are described as iso-enzymes or isozymes. For example, lactate dehydrogenase has 5 forms. The study of iso-enzymes is useful to understand diseases of different organs.

Clinical enzymology:

Table 7–2. Principal serum enzymes used in clinical diagnosis. Many of the enzymes are not specific for the disease listed.

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's disease)
Creatine kinase	Muscle disorders and myocardial infarction
γ-Glutamyl transpeptidase	Various liver diseases
Lactate dehydrogenase (isozymes)	Myocardial infarction
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases