Enzymes

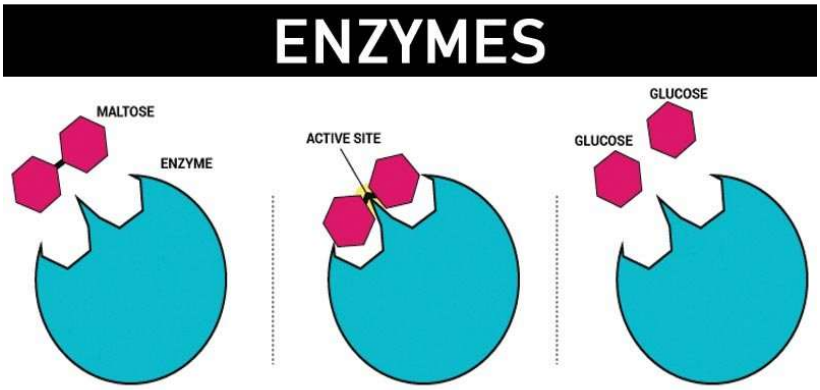
What are Enzymes?

- The chemical reactions in the cell are catalyzed by the biological catalysts called enzymes. Almost all enzymes are highly specialized proteins. (Exception: Ribozymes –Ribozymes are RNA with catalytic activity).
- Enzymes are the biological macromolecules which speed up the rate of biochemical reactions without undergoing any change. They are also called as biological catalysts.
- An enzyme is a highly selective catalyst that greatly accelerates both the rate and specificity of metabolic reactions.

Brief History about Enzymes

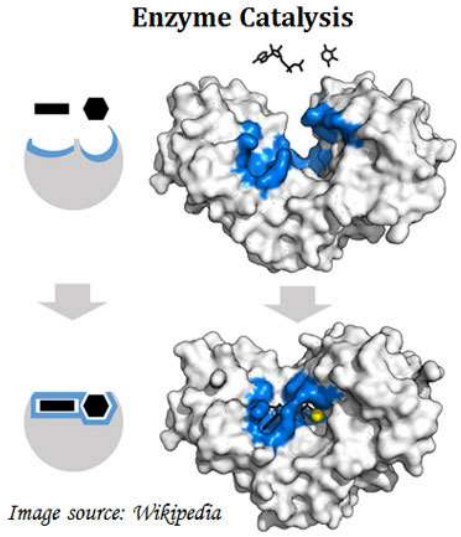
Louis Pasteur

- Ø The history of biochemistry is the history of enzyme research.
- Ø Louis Pasteur reported fermentation of sugar into alcohol by yeast is catalyzed by "**ferments**".
- Ø Frederick W. Kuhne coined the term **ENZYME** for the 'ferments'.
- Ø The first enzyme discovered was **Diastase** from malt by Anselme Payen in 1833.
- Ø The first crystallized enzyme is **Urease** by James Sumner.



Characteristics of Enzymes

- Ø The enzymes have extraordinary catalytic power.
- Ø Enzymes accelerate reactions up to 1014 to 1020 times.
- Ø Enzymes have a high degree of specificity for their substrates and reactions.
- Ø They function in an aqueous solution.
- Ø Enzymes work under a mild condition of temperature and **pH**.



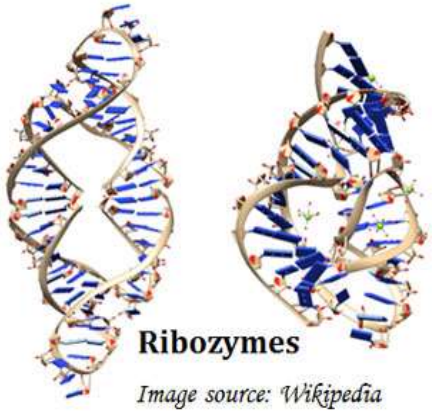
- Ø Enzymes make macromolecules from simple precursors.
- Ø The enzymes act in an organized sequence.

Ø Enzymes make macromolecules from simple precursors.
Ø The enzymes act in an organized sequence.
Ø They catalyze the hundreds of step-wise reaction.
Ø Enzymes can regulate metabolic pathways and these enzymes are **regulatory enzymes**.
Ø In some genetic disorders, there may be a deficiency one or several enzymes (Eg. albinism).
Ø Enzyme reduces the activation energy of the reaction.

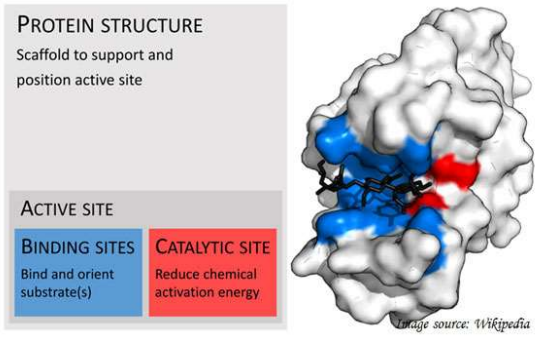
Properties of Enzymes

- Nearly all enzymes are proteins, although a few catalytically active RNA molecules have been identified.
- Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below 100oC, atmospheric pressure and neutral pH) as compared with the corresponding chemical reactions.
- Enzymes are catalysts that increase the rate of a chemical reaction without being changed themselves in the process.
- Enzymes are highly specific with respect to the substrates on which they act and the products that they form.
- Enzyme activity can be regulated, varying in response to the concentration of substrates or other molecules.
- They function under strict conditions of temperature and pH in the body.

Enzymes Structure
Ø All enzymes are proteins except Ribozymes. Ribozymes are specialized RNA molecules with catalytic activity.

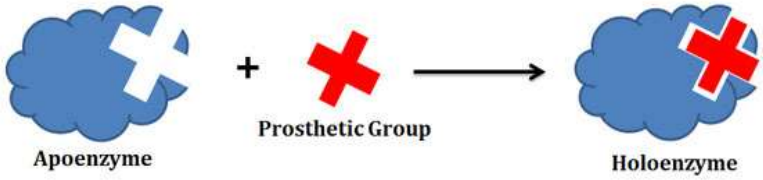


Ø The catalytic activity of an enzyme depends on the integrity of the enzyme's native conformation.
Ø The primary, secondary, tertiary & quaternary structures of protein are essential for its catalytic properties.
Ø The denatured enzyme will not have catalytic activity.
Ø Most of the enzymes consist of multi-subunits (more than one polypeptide chains).
Ø Some enzymes require no chemical groups for activity other than their amino acid residues.
Ø Others enzymes require additional chemical components (one or more) for their activity.
Ø Enzymes are much larger than their substrates.
Ø The smallest enzyme 4-oxalocrotonate tautomerase consists of 62 amino acid residues.
Ø The largest enzyme **Fatty acid synthase** consists of ~ 2000 amino acid residues.
Ø Even though most of the enzymes contain thousands of amino acids only 2–4 amino acids are directly involved in the catalysis.
Ø Binding Sites in the enzyme:
Substrate binding site: the areas of an enzyme where the substrate binding occurs.
Catalytic site: one or many sites, located near to the binding site. They perform the catalysis.
Active site: Binding site and catalytic site together called active site.



Cofactor site: Additional sites for the binding of cofactors.
Allosteric site: Additional sites for the binding of allosteric modulators. Allosteric modulators are involved in the regulation of enzymatic activity.

Apoenzyme and Holoenzyme
Ø **Apoenzyme** (apoprotein): The protein part of an enzyme is called apoenzyme.
Ø **Prosthetic group**: The non-protein part of an enzyme is called the prosthetic group.
Ø **Holoenzyme**: The fully functional apoenzyme and the required prosthetic group together are called holoenzyme.
Ø Holoenzyme = Apoenzyme + Prosthetic Group



Cofactors and Coenzymes
Ø The prosthetic groups of an enzyme are of different types and they are broadly categorized into two groups.
(1). Cofactors
(2). Coenzymes
Cofactors
Ø **Cofactors**: *A non-protein chemical compound in an enzyme that is bound to an enzyme is called the cofactor.*
Ø They are tightly bound to the enzyme.

- Ø Cofactors may be organic groups or inorganic groups.
- Ø Inorganic cofactors include metal ions such as Fe2+, Mg2+, Mn2+, Zn2+ and iron-sulfur clusters.
- Ø Organic cofactor includes **Flavin** and **Haem**.
- Ø Cofactors are required for the proper functioning of enzymes.
- Ø Some enzymes require several cofactors.
- Ø Example: The pyruvate dehydrogenase of the link reaction of respiration requires **five** cofactors. They are:

(1). Metal ion
(2). Loosely bound thiamine pyrophosphate (TPP)
(3). Covalently bound lipoamide
(4). Flavin adenine dinucleotide (FAD)
(5). Co-substrates (NAD, Coenzyme-A and Mg2+)

Coenzymes

- Ø **Co-enzyme:** *Additional chemical component in the enzyme (prosthetic group) which is complex organic or metallo-organic molecules.*
- Ø The main difference from cofactor is that coenzymes are **NOT** tightly bound to the enzyme.

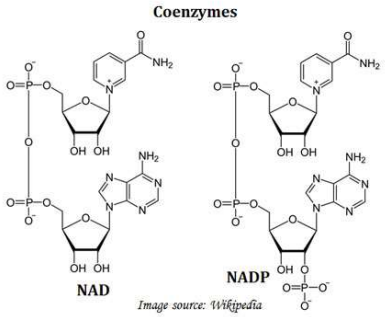


Image source: Wikipedia

- Ø Coenzymes act as the carriers of specific functional groups.
- Ø They transport chemical groups from one enzyme to another
- Ø Most of the coenzymes are derived from vitamins.
- Ø Co-enzyme is released from the enzyme's active site during the reaction.
- Ø Usually, coenzymes are chemically modified after the catalytic reaction.
- Ø Thus, the coenzymes are considered as the second substrate.
- Ø Examples for co-enzymes: NADHH+, NADPHH+, ATP
- Ø One coenzyme is common to many different enzymes.
- Ø Example: The NADHH+ is a coenzyme for about 700 different enzymes in human.
- Ø Coenzymes are continuously generated in the cell.
- Ø Their concentration is maintained at a steady level in the cell.

Activation Energy

Ø The term activation energy was introduced by Svante Arrhenius (1889).

Ø Definition: "The minimum energy that must be input to a chemical system, containing potential reactants, in order for a chemical reaction to occur".

Ø In simple term, the minimum energy required to start a chemical reaction.

Ø For a chemical reaction to proceed at a reasonable rate, there should exist an appreciable number of molecules with energy equal to or greater than the activation energy.

Ø The activation energy of a reaction is denoted as Ea.

Ø The Ea is given in units of kilo-joules per mole.

Coenzymes and prosthetic groups

- Many enzymes require the presence of small, non-protein units or cofactors to carry out their particular reaction.
- Cofactors may be either one or more inorganic ions, such as Zn2+ or Fe2+ or a complex organic molecule called a coenzyme.
- A metal or coenzyme that is covalently attached to the enzyme is called a prostheticgroup (heme in hemoglobin).
- Some coenzymes, such as NAD+, are bound and released by the enzyme during its catalytic cycle and in effect function as co-substrates. Many coenzymes are derived from vitamin precursors.

Holo enzymes and Apo enzymes

A complete catalytically-active enzyme together with its coenzyme or metal ion is called a holoenzyme.
The protein part of the enzyme on its own without its cofactor is termed an apoenzyme.

Isoenzymes

- Isoenzymes are different forms of an enzyme which catalyze the same reaction, but which exhibit different physical or kinetic properties, such as isoelectric point, pH optimum, substrate affinity or effect of inhibitors.
- Different isoenzyme forms of a given enzyme are usually derived from different genes and often 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. The five isoenzymes can be resolved electrophoretically.

Active site of Enzymes

- 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 entity formed by amino acid residues that can lie far apart in the linear polypeptide chain.
- The active site is often a cleft or crevice on the surface of the enzyme that forms a predominantly nonpolar environment which enhances 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; and in some cases by reversible covalent bonds.

Substrate Specificity of Enzymes

- The properties and spatial arrangement of the amino acid residues forming the active site of an enzyme will determine which molecules can bind and be substrates for that enzyme.
- Substrate specificity is often determined by changes in relatively few amino acids in the active site.
- This is clearly seen in the three digestive enzymes trypsin, chymotrypsin and elastase**.**

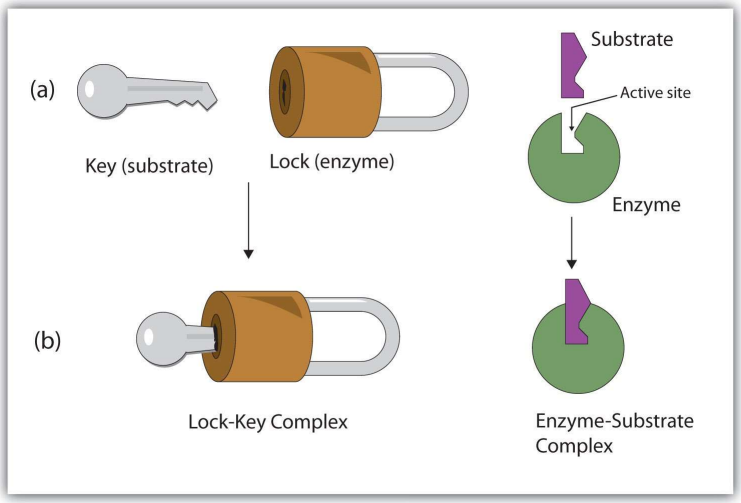
Mechanism of Action of Enzymes

- The substrate(s) is bound in the active site by multiple weak forces which result into the enzyme-substrate complex.
- Once bound active residues within the active site of the enzyme act on the substrate molecule to transform it first into the transition state complex and then into product, which is released.

- The enzyme is now free to bind another molecule of substrate and begin its catalytic cycle again.

The Substrate-Enzyme Binding

Originally two models were proposed to explain how an enzyme binds its substrate.



The Lock and Key Model

- In the lock-and-key model proposed was proposed by Emil Fischer in 1894.
- According to the model, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock.
- The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the right alignment.

The Induced Fit Model

- In the induced-fit model was proposed by Daniel E. Koshland, Jr., in 1958.
- It states that the binding of substrate induces a conformational change in the active site of the enzyme.
- In addition, the enzyme may distort the substrate, forcing it into a conformation similar to that of the transition state.
- For example, the binding of glucose to hexokinase induces a conformational change in the structure of the enzyme such that the active site assumes a shape that is complementary to the substrate (glucose) only after it has bound to the enzyme.
  The reality is that different enzymes show features of both models, with some complementarity and some conformational change.

Nomenclature of Enzymes

- Many enzymes are named by adding the suffix '-ase' to the name of their substrate.
  Example. Urease is the enzyme that catalyzes the hydrolysis of urea, and fructose-1,6-bisphosphatase hydrolyzes fructose-1,6-bisphosphate.
- However, other enzymes, such as trypsin and chymotrypsin, have names that do not denote their substrate.
- Some enzymes have several alternative names.
- To rationalize enzyme names, 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. Each enzyme is then uniquely identified with a four-digit classification number.
  Example: Trypsin has the Enzyme Commission (EC) number 3.4.21.4, where

1. the first number (3) denotes that it is a hydrolase
2. the second number (4) that it is a protease that hydrolyzes peptide bonds
3. the third number (21) that it is a serine protease with a critical serine
4. residue at the active site, and
5. the fourth number (4) indicates that it was the fourth enzyme to be assigned to this class.

- For comparison, chymotrypsin has the EC number 3.4.21.1, and elastase 3.4.21.36.

Classification of Enzymes

1. Oxidoreductases

- Catalyze oxidation-reduction reactions where electrons are transferred.
- These electrons are usually in the form of hydride ions or hydrogen atoms.
- The most common name used is a dehydrogenase and sometimes reductase is used.
- An oxidase is referred to when the oxygen atom is the acceptor.

2. Transferases

- Catalyze group transfer reactions.
- The transfer occurs from one molecule that will be the donor to another molecule that will be the acceptor.
- Most of the time, the donor is a cofactor that is charged with the group about to be transferred.
- Example: Hexokinase used in glycolysis.

3. Hydrolases

- Catalyze reactions that involve hydrolysis.
- It usually involves the transfer of functional groups to water.
- When the hydrolase acts on amide, glycosyl, peptide, ester, or other bonds, they not only catalyze the hydrolytic removal of a group from the substrate but also a transfer of the group to an acceptor compound
- For example: Chymotrypsin.

4. Lyases

- Catalyze reactions where functional groups are added to break double bonds in molecules or the reverse where double bonds are formed by the removal of functional groups.
- For example: Fructose bisphosphate aldolase used in converting fructose 1,6-bisphospate to G3P and DHAP by cutting C-C bond.

5. Isomerases

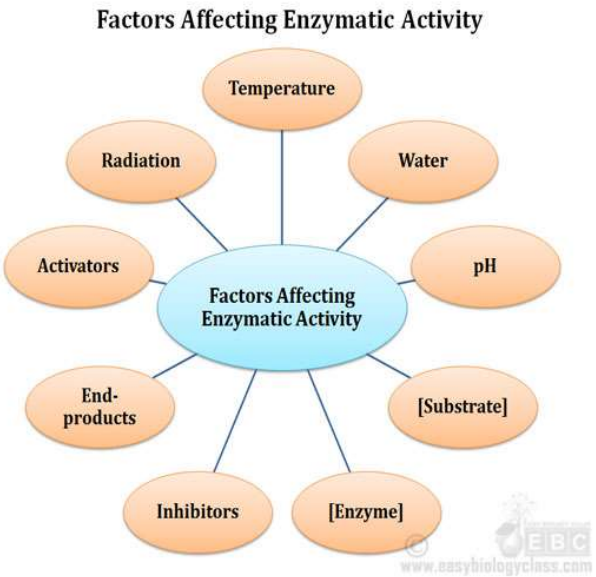
- Catalyze reactions that transfer functional groups within a molecule so that isomeric forms are produced.
- These enzymes allow for structural or geometric changes within a compound.
- For example: phosphoglucose isomerase for converting glucose 6-phosphate to fructose 6-phosphate. Moving chemical group inside same substrate.

6. Ligases

- They are involved in catalysis where two substrates are ligated and the formation of carbon-carbon, carbon-sulfide, carbon-nitrogen, and carbon-oxygen bonds due to condensation reactions.
- These reactions are coupled to the cleavage of ATP.

Factors Affecting Enzymatic Activity

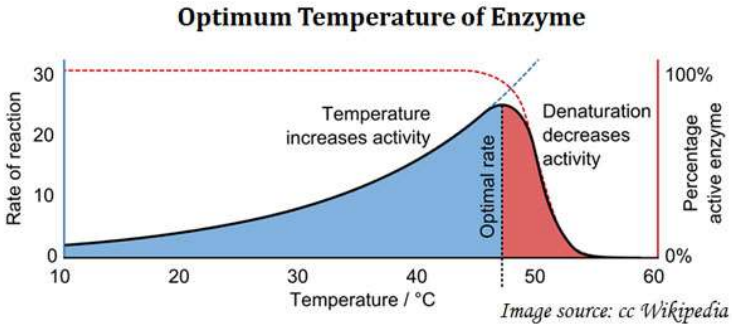
Ø The catalytic activities of enzymes are affected by multiple factors. They can be summarized as:



(1). Temperature
(2). Water
(3). Hydrogen ion Concentration (pH)
(4). Concentration of Substrate
(5). Concentration of Enzyme
(6). Inhibitors

(1). Temperature

- The enzymatic activity will be optimum at normal temperature.
- At very low temperature the activity of enzyme will be minimal or zero.
- The increase in temperature (up to a certain limit) can increase the enzyme's catalytic activity. (Study the graph below).



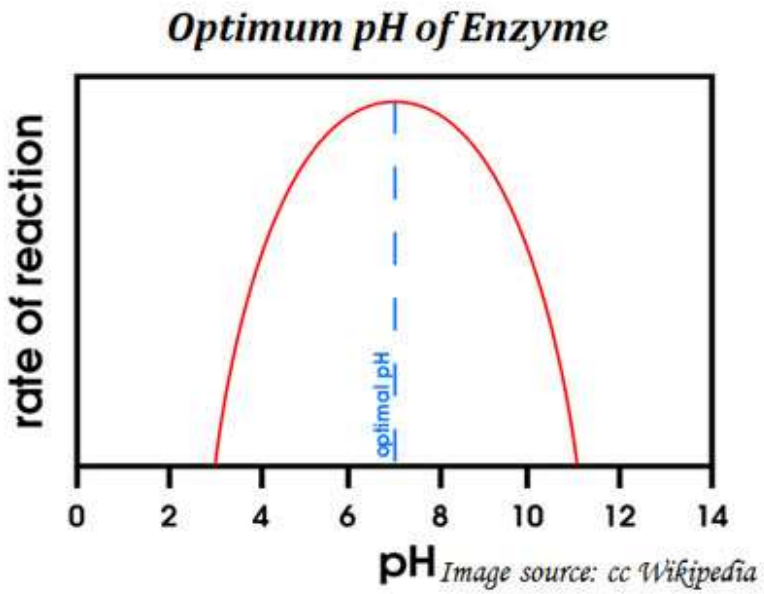
- The maximum activity is observed between 30 to 45ºC for most of the enzymes.
- Beyond 45ºC the enzyme activity is reduced drastically.
- Beyond 60-70ºC the enzymes were **denatured**.
- **Temperature coefficient or Q10**: *Increase in the velocity of enzymatic reactions when the temperature is increased by 10°C.*
- The Q10 of majority of enzymes will be 2 at a temperature between 0 and 40°C.

(2). Water

- Enzyme activity is suppressed in the absence of water.
- In dry seeds, the enzymes are almost inactive.
- Hydration of the cells is necessary for the enzyme activity.
- Water provides a medium for the enzymatic reaction to take place.
- In many cases, water is one of the reactants.

(3). Hydrogen Ion Concentration (pH)

- Enzymes are active only over a limited range of **pH**.
- Most of the enzymes are specific to a particular pH.



Ø Examples:
$. **Trypsin** is active in alkaline medium.
$. **Diastase** is active in the neutral medium.
$. **Pepsin** show optimum activity acidic pH.
Ø Each enzyme will have an optimum pH where the velocity of the reaction is maximum.
Ø A bell-shaped curve is obtained for the effect of pH and enzymatic activity.
Ø Hydrogen ions in the medium influence the ionic charges on the amino acids in the enzymes particularly on the active site.

(4). Concentration of Substrate

Ø Increase in the substrate concentration increases the activity of the enzyme until all the active sites of the enzyme molecule are saturated with the substrate.
Ø After this saturation the rate of enzymatic reaction becomes steady.
Ø Then, the addition of the substrate will not have a positive effect on the velocity of reaction.
Ø Higher concentration of substrate can nullify the effects of competitive inhibitors in the medium.

(5). Concentration of Enzyme

Ø Enzymes have extraordinary catalytic power.
Ø A small amount of enzyme is enough for a large amount of substrate.
Ø Increase in the concentration of enzyme will increase the rate of reaction (if there is enough substrate is available in the medium).
Ø Increased number of enzyme molecules will have more active sites.
Ø Besides, at high concentration of the enzyme, the effect of inhibitors will be less.

(6). Accumulation of end products

Ø The end product accumulation retards the enzymatic activity.
Ø The active sites of the enzymes become crowded with the products.
Ø Thus, the substrate molecules will have comparatively lesser chances of combining with the active sites.
Ø Inhibition by end products is also a regulation mechanism of the enzyme such as Feed Back Inhibition or Allosteric Modulation.

(7). Inhibitors

Ø Inhibitors in the reaction can inhibit enzymatic activity.
Ø Type of inhibition depends on the nature of the inhibitor.
Ø Inhibitors are less effective when the concentration of enzyme and substrate is higher.
Ø Inhibitors are of different types:
(1). Competitive inhibitor
(2). Noncompetitive inhibitors
(3). Uncompetitive inhibitors

Significance of Enzymes

1. In the absence of an enzyme, biochemical reactions hardly proceed at all, whereas in its presence the rate can be increased up to 107-fold. Thus, they are crucial for normal metabolism of living systems.
2. Besides in the body, extracted and purified enzymes have many applications.

• Medical applications of enzymes include:

1. To treat enzyme related disorders.
2. To assist in metabolism
3. To assist in drug delivery.
4. To diagnose & detect diseases.
5. In manufacture of medicines.

• Industrial applications of enzymes include:

1. Amylase, lactases, cellulases are enzymes used to break complex sugars into simple sugars.
2. Pectinase like enzymes which act on hard pectin is used in fruit juice manufacture.
3. Lipase enzymes act on lipids to break them in fatty acids and glycerol. Lipases are used to remove stains of grease, oils, butter.
4. Enzymes are used in detergents and washing soaps.
5. Protease enzymes are used to remove stains of protein nature like blood, sweat etc.