Enzymes
Fourth Semester (B.Sc Medical)

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About Enzymes

Enzymes are protein molecules in cells: Enzymes are macromolecular biological catalyst. Enzymes accelerate or catalyze chemical reactions. The molecules at the beginning of the process upon which enzymes may act are called Substrates and the enzyme converts these into different molecules, called Products. Almost all metabolic processes in the cell need enzymes in order to occur at rates fast enough to sustain life. The set of enzymes made in cell determines which metabolic Pathways occurs in that cell.

The study of enzyme is called Enzymology.

Enzymes are known to catalyze more than 5000 biochemical reaction types. Most enzymes are proteins although a few are catalytic RNA molecules. Enzymes specificity comes from their unique three-dimensional structures.

Like all Catalysts, enzymes increase the rate of a reaction, by lowering it's activation energy. Some enzymes can make their conversion of substrate to product occur many millions of times faster. An extreme example is Orotidine-5-Phosphate decarboxylase which allows a reaction that would otherwise take millions of years to occur in milliseconds. Chemically, enzymes are like any catalyst and are not consumed in chemical reactions, nor do they after the equilibrium of a reaction. Enzymes differ from most other catalyst by being much more specific.

Enzyme activity can be affected by other molecules:

Inhibitors are molecules that decrease enzyme activity and Activators are molecules that increase activity.

Many drugs and poisons are enzyme inhibitors. An enzyme's activity decreases markedly outside its optimal temperature and PH.

Some Enzymes are used commercially, for example in the synthesis of antibiotics. Some household products use enzymes to speed up the chemical reactions: enzymes in biological washing powders breakdown protein, starch or fat stains on clothes and, enzymes in meat tenderizer break down proteins into smaller molecules, making the meat easier to chew.

Discovery

Man has used many enzymatic processes, such as fermentation of grape juice and sourcing of a milk, for thousands of years, knowing little about their chemistry. In 1950s, Louis Pasteur, a French chemist found that these processes are brought about by the specific microorganisms that provide enzymes (called ferments by him) for them. In 1897, Eduard Buchner, a german chemist and Nobel laureate 1907, found that an extract from yeast fermented glucose like the yeast itself without living cells. So, the term 'enzyme' was coined (Gr.en = in; zyme = leaven). It literally means in 'yeast' but is now used for all biocatalysts. Since then, many enzymes have been isolated from living cells. No enzymes has been synthesized in the laboratory.

Characteristics of Enzymes

The common characteristics of enzymes are listed below:

- Physical Nature: The enzymes are generally colourless but some are coloured also-yellow, brown, red or green. Most of the enzymes are soluble in water, but some such as those located in the mitochondria, are insoluble.
- Chemical Nature: The enzymes are generally complex macromolecules (proteins) with high molecular weights. Some enzymes are conjugated proteins.
- Chemical Activity: The enzymes may break a large molecules into two smaller molecules, or bring two small molecules together to form a larger molecule.

- Changeless Form: The enzymes combine temporarily with the substrate but do not undergo any permanent change in the reaction they catalyze. Neither their presence alters the nature and quantity of the products of the reaction.
- Temperature Sensitivity: The enzymes function best at an optimum temperature (25°C-40°C). Their activity degrees with the decrease as well as increase in temperature and stops at 0°C and above 80°C.
- PH Sensitivity: The enzymes show maximum activity at an optimum PH (6-7.5). Their activity slows with the decrease and increase in PH till it stops.
- Teamwork: The enzymes generally work in teams in the cell, the product of one enzyme controlled reaction serving as a substrate for the next. In germinating seeds, starch is changed into glucose by two enzymes- Amylase and Maltase. Amylase splits the starch into the double sugar maltose, which is then broken by maltase into the single sugar glucose. Eleven different enzymes work sequentially to convert glucose to lactic acid in animal as well as a plant cells.
- Destruction by Poisons: The enzymes are destroyed by poison such as cyanide and iodoacetic acid. Cyanide poisoning is due to the destruction of the respiratory cytochrome enzyme by the cyanide.

Sources of Enzymes

Biologically active enzymes may be extracted from any living organism. A very wide range of sources are used for commercial enzyme production from Actinoplanes to Zymomonas, from spinach to snake venom. Of the hundred or so enzymes being used industrially, over a half are from fungi and yeast and over a one-third are from bacteria with the remainder divided between animal (8%) and plant (4%) sources. A very much larger number of enzymes of find use in chemical analysis and clinical diagnosis. Non-microbial sources provide a large proportion of these, at the present time. Microbes are preferred to plants and animals as source of enzymes because:

- They are generally cheaper to produce.
- Their enzyme contents are more predictable and controllable.
- Reliable supplies of raw material of constant composition are more easily arranged.

- Plant and animal tissues contain more potentially harmful materials than microbes, including phenolic compounds (from plants) and endogenous enzyme inhibitors and proteases.

Some Important industrial enzymes and their sources

Enzyme	Source	Industrial Use
Animal Enzymes		
Catalase	Liver	Food
Chymotrypsin	Pancreas	Leather
Lipase	Pancreas	Food
Rennet	Abomasum	Cheese
Trypsin	Pancreas	Leather

Enzyme	Source	Industrial Use
Plant Enzymes		
Actinidin	Kiwi Fruit	Food
α-Amylase	Malted Barley	Brewing
β-Amylase	Malted Barley	Brewing
Bromelain	Pineapple Latex	Brewing
β-Glucanase	Malted Barley	Brewing
Ficin	Fig Latex	Food
Lipoxygenase	Soybeans	Food
Papain	Papaya	Meat

Enzyme	Source	Industrial Use
Bacterial Enzymes		
α-Amylase	Bacillus	Starch
β-Amylase	Bacillus	Starch
Asparaginase	Escherichia coli	Health
Glucose Isomerase	Bacillus	Fructose syrup
Penicillin amidase	Bacillus	Pharmaceutical
Protease	Bacillus	Detergent
Pullulanase	Klebsiella	Starch

Enzyme	Source	Industrial Use
Fungal Enzymes		
α-Amylase	Aspergillus	Baking
Aminoacylase	Aspergillus	Pharmaceutical
Glucoamylase	Aspergillus	Starch
Catalase	Aspergillus	Food
Cellulase	Trichoderma	Waste
Dextranase	Penicillium	Food
Glucose oxidase	Aspergillus	Food
Lactase	Aspergillus	Dairy
Lipase	Rhizopus	Food
Rennet	Mucor miehei	Cheese
Pectinase	Aspergillus	Drinks
Pectin Lyase	Aspergillus	Drinks
Protease	Aspergillus	Baking
Raffinase	Mortierella	Food

Enzyme	Source	Industrial Use
Yeast Enzymes		
Invertase	Saccharomyces	Confectionery
Lactase	Kluyveromyces	Dairy
Lipase	Candida	Food
Raffinase	Saccharomyces	Food

Nomenclature

Enzyme names end in the suffix- 'ase'.

Exceptions are some old names. Example: Pepsin, Trypsin.

Some old names were also given after the source- 'Papain' (from papaya) 'Bromelain' (from family bromeliaceae).

Most of the name are given on the two basis:

- <u>After substrate</u>: Enzymes are named after the substrate when chemical reaction involved its breakdown. Example:
    a. Protease [Protein to Amino acid]
    b. Lipase [Fats to Fatty acids+Glycerol]
    c. Sucrase [Sucrose to Simple sugar]
    d. Maltase [Maltose to Simple sugar]

- <u>After Chemical Reaction</u>: Majority of the enzymes are given name on the basis of a chemical action. Example:
    a. Dehydrogenase [Dehydrogenation]
    b. Hydrase [Hydration]

c. Invertase [Change in optical rotation]

The Second category of names are:

Collective Names: They are qualified by the addition of name of substrate.

Example:

- Isocitric dehydrogenase
- Succinic dehydrogenase
- Phosphoglyceromutase [3-Phosphoglycerate ⇌ 2-Phosphoglycerate]

Classification of Enzymes

There are two systems of classification of enzymes:

- Older System
- Modern System

1. ***Older System***: It divides enzymes into two groups:
    a. Hydrolysing
    b. Desmolysing

        - Hydrolysing Enzymes: They cause breakage of a substrate by addition of water molecules. They are of several types depending upon substrate hydrolyse.

            1. Carbohydrases: Hydrolyse polysaccharide to disaccharide. Example: Amylase, Cellulase, Maltase, Sucrase.
            2. Esterases: Break ester linkages. Example: Lipase, Phosphatase.

3. Phosphorylase: Break Complex carbohydrates into simple phosphate containing compound by means of phosphoric acid.
4. Protease: Break proteins and proteid into amino acids. Example: Pepsin, Peptidase.
5. Amidases: Release Ammonia from amides. Example: Asparaginase, Urease.

- Desmolysing Enzymes: They are those enzymes which take part in the reaction other than hydrolysis. They cause transfer or addition of carbon chains and addition or removal of other groups. Example: aldolase, dehydrogenase, oxidase, peroxidase, catalase, hydrase, transamylase, trans phosphorylase, carboxylase.

2. ***Modern System***: According to this system, six main divisions of enzymes have been recognised. These six main divisions are:

a. Oxidoreductases: These enzymes take part in transfer of electron or oxidation and reduction reactions. These enzymes include oxidases, dehydrogenases, reductases, peroxidases and catalases. Distinction is also made on the basis of group acted upon like CH—OH, CH—CH, CH≡NH, CH—NH_2, etc.
   - Oxidases: Cause direct oxidation of substrate with the help of a molecular oxygen.

$$AH_2 + ½\ O_2 \rightarrow A + H_2O$$

$$AH_2 + O_2 \rightarrow A + H_2O_2$$

   - Dehydrogenases: Cause oxidation by the removal of hydrogen from substrate.

$$AH_2 \rightarrow A + 2H$$

- Reductases: Cause addition of hydrogen or an electron and removal of oxygen.

$$NO_3^- + NADH_2 \rightarrow NO_2 + NAD^+ + H_2O$$

$$R^{+3} + e^- \rightarrow R^{+2}$$

b. Transferases: They bring about transfer reactions. Transferase are known after the name of the groups transferred by them. Example: Transaminase, Transcarboxylase, Transaldolase, Transketolase, Transphosphorylase.

$$AB + C \rightleftharpoons AC + B$$

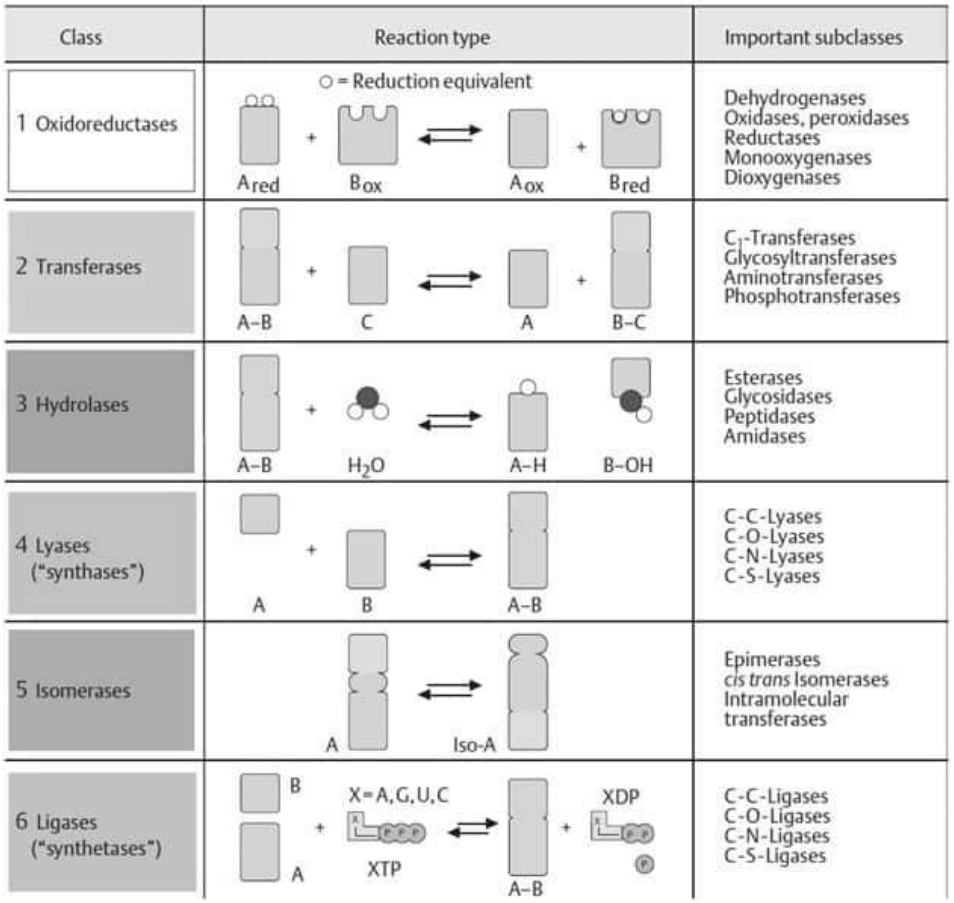
c. Hydrolases: They cause addition of water to variety of bonds. Ex: Ester, Thioether, peptide, non-peptide, C—N, C—C, etc. The group includes digestive enzymes like carbohydrases, lipases, phosphatases, proteases.

$$AB + HOH \rightarrow AH + BOH$$

d. Lyases: The enzymes of this group result in:
   - Direct breakage of different kinds of bones like C—C, C—O, C—N, C—S, etc. without involving hydrolytic change.
   - Removal of groups without hydrolysis.
   - Addition of groups to double bonds.

e. Isomerases: They catalyse rearrangement of atoms or groups inside a molecule to form optical, geometrical or positional isomers. Example: cis-trans isomers, DL-isomers. Their common types are isomerases, epimerases and mutases. Epimerases cause structural change involving position of 1—Carbon group while mutases bring about a shift in position of side group within a molecule.

f. Synthetases or Ligases: They catalyse the synthesis of different types of bonds like C—C, C—N, C—S, C—O, etc. with the help of energy obtained from ATP.



Types of Enzymes

The enzymes are of two types with regard to the site where they act-

- Intracellular (Endoenzymes)
- Extracellular (Exoenzymes)

- Intracellular Enzymes: Most of the enzymes remain and function inside the cells. They are called the intracellular enzymes or endoenzymes. Some occur dissolved in the cytoplasmic matrix. A water extract of ground up liver cells contain all the

11 enzymes necessary to change glucose to lactic acid. Certain enzymes are bound to particles such as ribosomes, mitochondria and chloroplasts in the cell. The respiratory enzymes needed to convert lactic acid to carbon dioxide and water are found in the mitochondria.

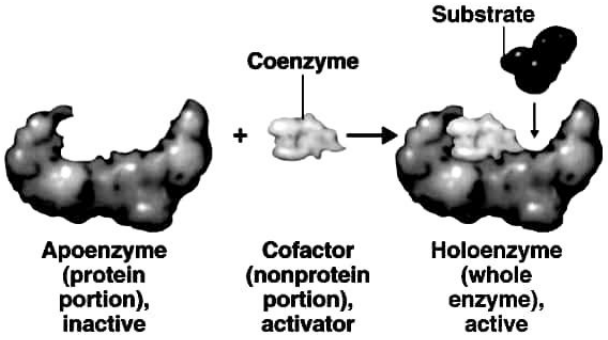
- Extracellular Enzymes: Certain enzymes leave the cells and function outside them. They are called the extracellular enzymes or exoenzymes. They mainly include the digestive enzymes, for example: salivary amylase, gastric pepsin, pancreatic lipase secreted by the cells of the salivary glands, gastric glands and pancreas respectively. Certain enzymes function in the blood. In microbes some enzymes function outside the organism. The enzymes retain their catalytic action even after extraction from the cells. Rennet tablets containing the enzyme rennin from the calf's stomach are used to coagulate milk protein caseinogen for cheese (casein) formation. Their best example is observed in insectivorous plants which secretes enzymes for digestion of insects caught by their leaf segments. The scutellum of cereals also produces some exoenzymes for absorbing nourishment from the endosperm.

Structure of Enzyme

Structurally, enzymes are proteinaceous in nature. They may be exclusively made up of proteins and may contain another molecule. Accordingly, enzymes have been divided into two types:

- Simple Protein Enzymes
- Conjugated Enzymes

- Simple Protein Enzymes: They are wholly made up of proteins. Example: Urease, amylase, pepsin, trypsin, etc.



- Conjugated Enzymes: Coenzymes are made up of protein part apoenzyme and non protein part named as a cofactor. The two together constitute the complete enzyme or holoenzyme. Both apoenzyme and cofactor remain unchanged at the end of a chemical reaction. Apoenzyme is denatured by heat while the cofactor is heat stable.

Cofactors are of 3 types:

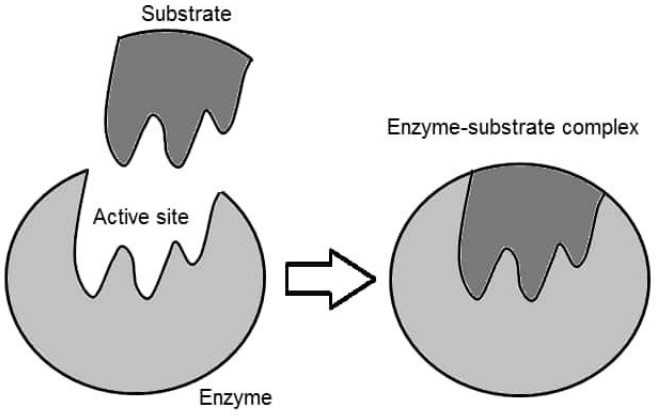
- Coenzymes: Coenzymes are Non-protein organic groups which readily separate from apoenzymes. They are generally made up of Vitamins. Coenzymes function in a group transfer reaction. They can pick up and release hydrogen. The two reactions require different apoenzymes. Example: TPP (Thiamine pyrophosphate), Co-A, THF (Tetrahydrofolic acid), FMN (Flavin mononucleotide), FAD (Flavin adenine dinucleotide) and NAD (nicotinamide adenine dinucleotide).
- Prosthetic Group: They are attached firmly to the apoenzymes. They function like coenzymes as carrier of the certain groups. Example: Pyridoxal phosphate, Biotin. Unlike coenzymes prosthetic group require single enzyme for group transfer.
- Inorganic Cofactor: They are also called as a metal activators. They are metallic inorganic cations which are loosely held to the enzyme. Example: Mn^{+2}, Fe^{+2}, Co^{+2}, Zn^{+2}, Ca^{+2}, K^{+1}, Mg^{+2}.
    - Oxidation-reduction reaction by acting as electron carrier. Forming link between substrate and enzyme.
    - Removal of end products and inhibitors.
    - Change of surface charge of the enzyme protein.

Active Site

Enzymes being proteinaceous are very big molecules as compared to reactant and substrate molecules. The whole surface of an enzyme is a not active in catalyzing a reaction. Only a small portion of an enzyme is active, it is called active site of an enzyme. An active site is that area of enzyme which is capable of attracting and holding of particular substrate molecules for making or breaking of their bonds.

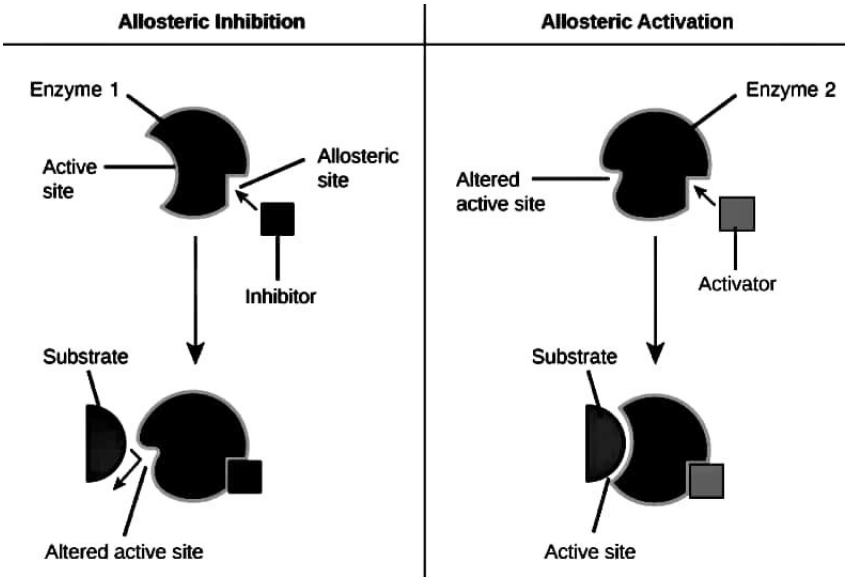
Properties of Active Site

- An enzyme may have one to several active site.
- An active site occupies only a very small area as compared to the size of the enzyme.
- Active sites are in the form of cleft or crevices.
- Each active site has a specific grouping of amino acids which is due to the tertiary or quaternary structure of protein part of the enzyme.
- Besides active site certain enzymes possess sites for the attachment with regulatory substances or modulators like activators and inhibitors.



Allosteric Enzymes

These are the enzymes which have two or more receptor sites which are non-overlapping and stereo-specifically different. The site other than active site are called as allosteric or regulatory site. The latter can attract different substances which produce reversible change in enzyme structure. The phenomena is called allosteric transition.



The substances that bring about allosteric transition are called allosteric transition elements. They are of two types:

- Inhibitors
- Activators

An activators binds with the allosteric site of enzyme. It brings about conformational change in active site so as to make it operational.

An inhibitor bind to allosteric site and brings about change in active site that is unable to bind with substrate.

Isozymes

Isozymes are also called isoenzymes. Isozymes are multiple molecular forms of an enzyme which have a similar substrate specificity. Over 100 enzymes have been found to possess isozymes. For example: α-Amylase of wheat endosperm has 16 isozymes, Lactic dehydrogenase has 5 isozymes in human. Isozymes forming enzymes possess a quaternary structure and are made up of two or more polypeptides called monomers. Isozymes become different because of change in number of various monomers constituting them.

Zymogens

They are inactive enzyme precursor which after some modification or activation give rise to enzymes. Many enzymes are synthesized in zymogen state. They are changed to the reactive state at particular PH, presence of substrate or after some special treatment.

Activation Energy

Most of the chemical reactions do not start automatically because molecules of the reactant have an energy barrier to become reactive. The minimum energy required to overcome the energy barrier is called activation energy.

Activation energy increases the rate of vibration of molecules. The increased vibrations allow more and forceful collisions between them. The reactive site can come together for the start of chemical reaction. Substances receiving the activation energy and hence capable of reacting are said to reach a state called Transition State.

Enzymes reduce the activation energy required for chemical reaction. Enzymes are able to lower the activation energy by bringing the reactants together over their surface. This overcomes the forces of repulsion and allow reactive sites to take part in chemical reaction. Besides lowering the activation energy enzymes also bring about-

- Proximity of reactants (Closeness of reactants)
- Proper orientation of substrate molecules so that they enter the transition state.
- Distortion of the substrate.
- Provide protons or accept protons for the reaction.
- Formation of covalent complexes with the substrate molecules to assist them in undergoing change. For example:

$$R—X + H_2O \rightarrow \text{No Reaction}$$

$$R—X + E—OH \rightarrow R—OH + E—X$$

$$E—X + H_2O \rightarrow H—X + E—OH$$

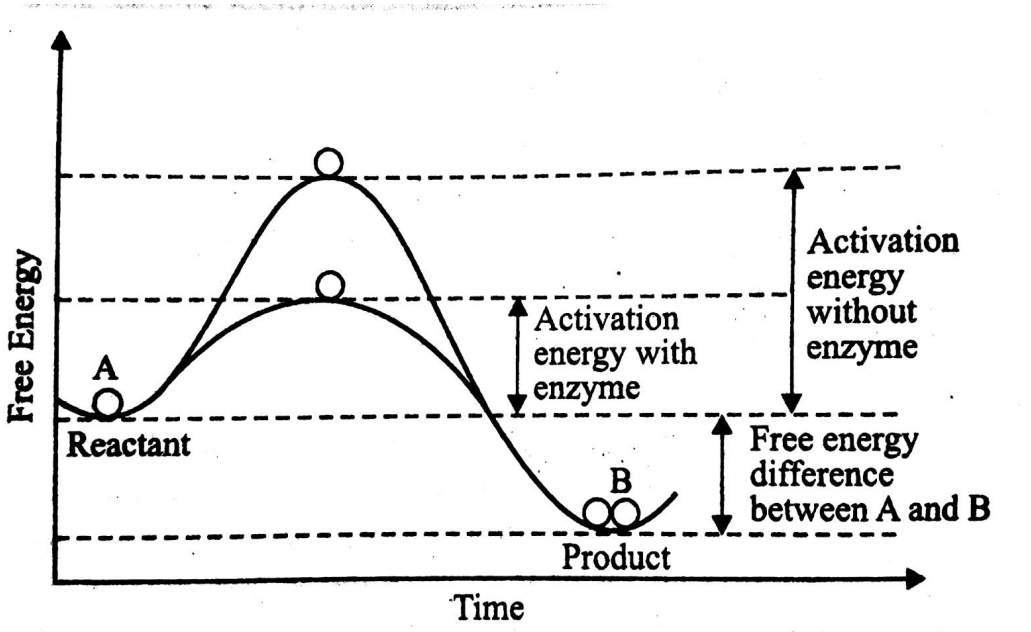


FIGURE **Activation energy requirement of noncatalysed and enzyme catalysed reactions.**

Energy Kinetics

Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying and enzyme kinetics in this way can reveal the catalytic mechanism of the enzyme, its role in metabolism, how its activity controlled, and how a drug or an agonist might inhibit the enzyme. Enzymes are usually protein molecules that manipulate other molecules - the enzyme's substrate - these target molecule bind to an enzyme's active site and are transformed into products through series of steps known as the enzymatic mechanism.

$$E + S \rightleftharpoons ES \rightleftharpoons ES^* \rightleftharpoons EP \rightleftharpoons E + P$$

These mechanisms can be divided into single substrate and multiples substrate mechanism. Kinetic studies on enzymes that only bind one substrate, such as triose-phosphate isomerase, aim to measure affinity with which enzyme bind with this substrate and turnover rate, when enzymes bind multiple substrate, such as dihydrofolate reductase, enzyme kinetics can also show the sequence in which these substrate bind and the sequence in which products are released.

Knowledge of the enzyme's structure is helpful in interpreting Kinetic data. For example: the structure can suggest how substrates and products bind during catalysis, what changes occur during the reaction, even the role of particular amino acid residues in the mechanism.

Not all biological catalysts are protein enzymes, RNA based catalyst such as ribozymes and ribosomes are essential to many cellular functions, such as RNA splicing and translation. The main difference between ribozymes and enzymes is that RNA catalyst are composed of nucleotides, whereas enzymes are composed of amino acids. Ribozymes are also perform a more limited set of reactions, although there reaction mechanism and kinetics can be analysed and classified by the same methods.

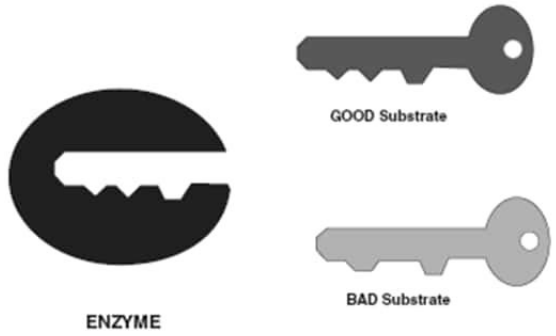
Mechanism of Enzyme Action

There are two theories about mechanism of enzyme action-

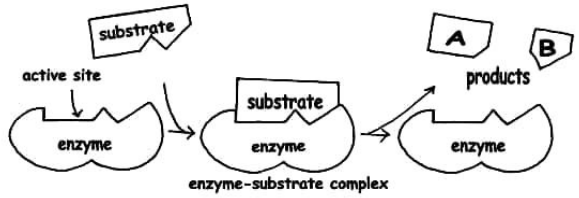
- Lock and Key Mechanism
- Induced Fit Mechanism

Lock and Key Mechanism

It was proposed by Emil Fischer in 1894. The active site and substrate molecule have complimentary geometrical shapes. It is similar to lock and key which have complementary geometrical shape in the region of their activity.



As the lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme. The active sites can bind to substrate molecules to form an intermediate compound called Enzyme Substrate Complex. This Complex undergoes chemical change and is changed into enzyme product complex.



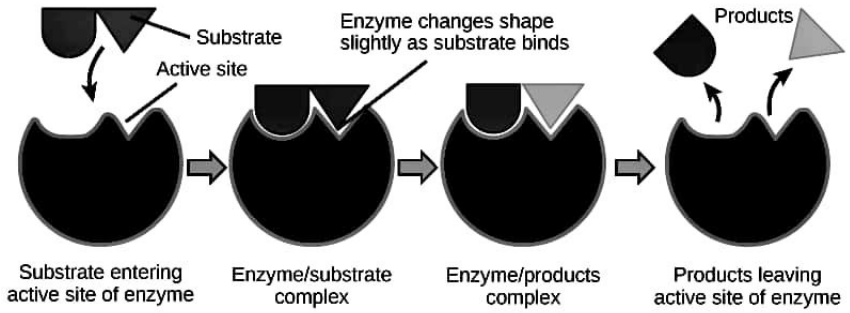
The products of the chemical reaction dislodge from the enzyme and enzyme become free for reuse.

Induced Fit Mechanism

According to this mechanism, active sites of an enzyme are not static but undergo conformational change. The active site possess two groups:

- Buttressing Group
- Catalytic Group

Buttressing group is meant for supporting the substrate while catalytic group breaks the substrate into the product. The substrate attaches itself to the buttressing group. The enzyme substrate complex brings about conformational change in active site so that the catalytic group comes to lie opposite substrate bonds to be broken. The substrate is held over the active site by hydrogen bonds. The catalytic group causes strain on substrate bonds.



The strain breaks the bond and products are formed. The products are released so that enzyme becomes free to attract another substrate molecule.

Enzyme Inhibition

Reduction or stoppage of enzyme activity due to the internal or external forces or chemicals is called Enzyme Inhibition. It may be Reversible or Irreversible and competitive or non-competitive.

- Reversible Inhibition: The reversible inhibition is that which can be overcome through withdrawal of inhibitor is temporary. Due to blocking of active site or binding of linkages required for the maintenance of the active site.
- Irreversible Inhibition: Irreversible inhibition is that type of which is of a permanent nature. Due to either change in enzyme confirmation or formation of a non-functional complex. i.e. slow to degrade.
- Competitive Inhibition: it is a type which is caused by blocking of active site of enzyme by a chemical. i.e. similar in structure to the substrate. Competitive inhibition is usually reversible.
- Non-Competitive Inhibition: It is a type which is caused by chemical, quite dissimilar to substrate. It may cause attraction of active sites, confirmation or structure of enzymes. Non-competitive inhibition may be Reversible or Irreversible.

Major Types of Enzyme Inhibition

- Protein Denaturation: Enzyme activity is a dependent upon the maintenance of tertiary structure of the protein molecule. The protein structure is destroyed by heat, high energy radiations and salts of heavy metals.

- Reversible Covalent Modification: It may convert an active enzyme into an inactive state and vice-versa.

- Irreversible Non-Competitive Inhibition: It is an Irreversible inhibition of an enzyme activity by presence of a substance that has no structural similarity with the substrate. The inhibitor destroys or combines irreversibly with the functional group of enzyme.

- For example: Cyanide inhibits the activity of cytochrome oxidase, Di-isopropyl fluorophosphate (DFP) inhibits several enzymes like Trypsin, Phosphoglucomutase, Kino Trypsin.

- Allosteric Modulation (Feedback Inhibition): It is a type of reversible, noncompetitive inhibition found in allosteric enzymes. The Inhibitor is low molecular intermediate product of a metabolic pathway having a chain of reactions involving a number of enzymes. It is therefore also called feedback inhibition. The Inhibitor is also called negative modulator.
    - Example: is stoppage of activity of a glucokinase by glucose-6-phosphate, the product of reaction catalyzed by it.

- By Antimetabolites: Antimetabolites are substances which are analogous of a naturally occurring essential molecule. These inhibiting substances when are incorporated as errors in two proteins or nucleic acid inhibits the normal physiological process.
    - For example:
        - Normal Metabolite Analogue
        - Phenylalanine Fluorophenylalanine
        - Methionine Ethionine
        - Uracil 5-Fluorouracil

- Unresolved Inhibitors: The mechanism of inhibition of some substances is not clear though their general physiological effects are clearly define.For example: In oxidative phosphorylation, the inhibitor is 2,4-dinitrophenol (DNP) while Hill reaction is inhibited by substitute Urease and related compounds. Phosphorylation is inhibited by uncouplers which prevent the linking of inorganic phosphate with the ADP molecules.

Importance of Enzyme Inhibition

- Enzyme inhibition has a regulatory role on enzyme activity.
- Enzyme inhibitors are used to study metabolic pathway.
- Some inhibitors are used in controlling pathogenic activity. Ex: Sulpha drugs.
- Use of Inhibitors has shown the mechanism of enzyme action.

Factors Affecting Enzyme's Function

- **Temperature**

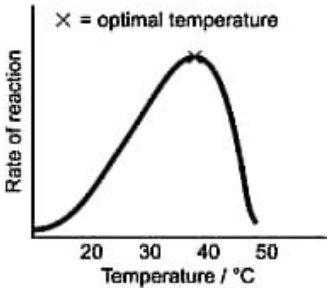
Enzymes function optimally at certain temperatures.

As temperature increases, kinetic energy increases and molecules are moving more, increasing the likelihood that enzyme and substrate molecules bump into each other, bind and react. Therefore, initially enzyme reaction rate increases with an increase in temperature.

But, if it gets too hot, the enzyme becomes "Denatured". It is the heat 'cooks' the protein. Since, it is denatured, the enzyme's three dimensional structure breaks down and it becomes misfolded

The enzyme's shape changes, therefore the three dimensional shape of its active site changes. Once the shape of the active site changes it cannot bind to the substrate anymore and the enzyme can't function anymore. Therefore, at higher temperatures, the enzyme's reaction rate decreases sharply.

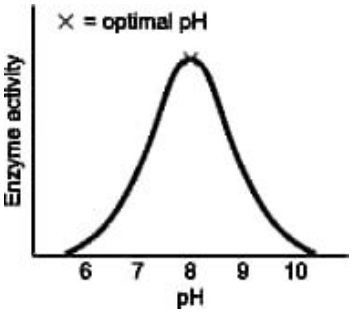
The optimal temperature for an enzyme is the temperature at which the engine "works best", for your body is a 98.6 degree fahrenheit (also known as 37°C).



- **PH (a measure of acidity)**

Enzymes function optimally at a certain PH-

  - Enzymes are extremely sensitive to changes in acidity.
  - Each enzyme works within quite a narrow pH range.
  - Changes in pH can make and break chemical bonds within the enzyme, changing the shape of the enzyme and therefore, its effectiveness. If the pH is too low (too acidic) or too high (too basic), the enzyme becomes “denatured”.The chemical bonds within the enzyme are rearranged and the enzyme become misfolded and the enzyme’s shape changes. The three dimensional shape of its active site changes and the active site can't bind to the substrate anymore. Thus, the enzyme cannot function anymore and the reaction rate decreases sharply.
  - The optimal pH for an enzyme is that pH at which the enzyme “works best”, and the rate of chemical reaction is highest.
  - The “optimal pH” for the most of the enzymes in your body is pH-8. There are exceptions such as the digestive enzymes of your stomach which function in an environment of pH- 3 to 4.



- **Concentration of Enzyme or Substrate**

When enzyme concentration is low, the reaction is slower. As enzyme concentration increases, the reaction is faster upto a point when the amount of substrate available becomes limiting. Similarly, when substrate concentration is low, the reaction is slower.

As the substrate concentration increases, the reaction is faster upto a point when the amount of enzyme available becomes limiting.

- **Turnover Number**

Most enzymes have high turnover number. A turnover number of an enzyme refers to the number of molecule of substances acted upon by one molecule of enzyme per minute. A molecule of enzyme catalyzed from cattle Liver decomposes 5,000,000 molecules of hydrogen peroxide in one minute at 0°C. The turnover number of Catalase is, thus, 5,000,000 at 0°C. High turnover numbers of enzymes explain their remarkable effectiveness even though they occur in a cell in a minute quantities.

- **Enzyme Specificity**

One of the properties of enzymes that makes them so important as Diagnostic and Research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity, that is, they will catalyse only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

    - Absolute Specificity: The enzyme will catalyse only one reaction.
    - Group Specificity: The enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
    - Linkage Specificity: The enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
    - Stereochemical Specificity: The enzyme will act on a particular steric or optical isomer. Though enzymes exhibit great degree of specificity, cofactors may serve many apoenzymes.

Importance of Enzymes

- **Biological Function**

Enzymes serve a wide variety of functions inside living organisms. They are indispensable or signal transduction and cell regulation, often via kinases and phosphatases. They also generate movement, with myosin hydrolyze ATP to generate muscle contraction, and also transport cargo around the cell as a part of the

cytoskeleton. Other ATPases in the cell membrane are ion pumps involved in active transport. Enzymes are also involved in more exotic functions, such as a luciferase generating light in fireflies. Viruses can also contain enzymes for infecting cells, such as the HIV integrase and reverse transcriptase, or for viral release from cells, like the influenza virus neuraminidase. An important function of enzyme is in digestive system of animals. Enzymes such as amylases and proteases break down large molecules (starch or proteins respectively) into smaller ones, so they can be observed by the intestine. Starch molecules, for example, are too large to be absorbed by the intestine, but enzymes hydrolyze the starch chains into smaller molecules such as maltose and eventually glucose, which can then be absorbed. Different enzymes digest different food substances.

- **Metabolism**

Several enzymes can work together in a specific order, creating metabolic Pathways. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. After the catalytic reaction, the product is then passed onto another enzyme. Sometimes more than one enzyme can catalyse the same reaction in parallel, this can allow more complex regulation; with, for example, a low constant activity provided by one enzyme but an inducible high activity from a second enzyme. Without enzymes, metabolism would neither progress through the same steps and could not be regulated to serve the needs of the cell. Most central metabolic Pathways are regulated at a few key steps, typically through enzymes whose activity involves the hydrolysis of ATP, because, this reaction releases so much energy, other reactions that are thermodynamically unfavourable can be coupled to ATP hydrolysis, driving the overall series of Limited metabolic reactions.

- **Wine Manufacturing**

Papain is used in Brewing industry as a stabilizer for child proof beer, because it removes small amounts of protein that cause turbidity in chilled beer.

- **Cheese Making**

Since, long the animal renin (or rennet) is employed in making cheese. The enzyme rennet is obtained on a commercial scale from the 4th or true stomach of unweaned calves which are specifically slaughtered for this purpose.

- **Candy Making**

An enzyme, invertase helps preventing granulation of sugar in soft centred candies. Another enzyme, lactase prevents the formation of lactose crystals in the ice cream which would otherwise not allow the product seem sandy in texture.

- **Bread Whitening**

Lipoxygenase is used for whitening the bread.

- **Clarifying Fruit Juices**

The juices are clarified by adding a mixture of pectic enzymes which hydrolyze the pectic substances causing turbidity.

- **Correcting Digestion**

When the enzymes are present insufficiently in the body, certain digestive disorders come up. Pepsin, papain and amylase aid digestion in the stomach while pancreatic enzymes act in the duodenum.

- **Wound Healing**

Proteolytic enzymes from pig pancreas are used to alleviate skin diseases, bed sores and sloughing wounds. The enzymes commonly used for wound debridement are the proteases such as streptodornase, ficin and trypsin.

- **Dissolving Blood Clot**

The enzyme Urokinase which is manufactured from urine is being used efficiently in Japan in the treatment of a blood clot in brain, artery and other circulatory diseases.

- **Syrup Manufacturing**

These days immobilized glucose isomerase is being successfully used in the production of high Fructose corn syrup especially in the United States.

- **Diagnosing Hypertension**

A new method called radioimmunoassay procedure for diagnosing cases of hypertension has been developed by Bhabha Atomic research centre. In it, the activity of renin, a proteolytic enzyme secreted in the Kidneys, is calculated indirectly by measuring angiotensin-I which is formed by the action of renin. Renin act as part of a complex feedback mechanism for regulating blood volume and pressure.

- **Destaining Fabrics**

In dry cleaning, the stains due to glue, gelatin or starch are removed by employing certain enzymes, such as alcolase.