BIOMOLECULES

ENZYMES

ENZYMES

History of cellular enzymes

- An enzyme may be defined as "a biomolecule that enhances the rate of biochemical reactions but does not affect the nature of final product".
- They are produced by living cells only.
- Enzymes are **biocatalysts**.
- Enzymes are proteinaceous substances.
- There are some nucleic acids that behave like enzymes. These are called ribozymes. **E.g., RNA enzymes (RNase).**

Characteristics of enzymes

(1) Almost all enzymes are proteins. Though, there are some nucleic acids that behave like enzymes. These are called ribozymes.

- L_{19} RNAase (a ribozyme) was discovered by T.Cech from rRNA of a protozoan, Tetrahymena thermophila.
- RNAase P or Ribonuclease P (a ribbzyme) was & covered by Altman from a prokaryotic cell.

(2) Enzymes are colloidal substances, which are macromolecules of amino acids and are synthesised by ribosomes under genetic control.

(3) Encyme can be depicted by a line diagram. An enzyme like any protein has -

(a) Primary structure : Amino acid sequence of the protein. Its lack active sites.

(b) Secondary structure : It is a helical structure which also lack active sites.

(c) Tertiary structure : In this structure backbone of the protein chain folds upon itself, the chain criss-crosses itself and hence many crevices or pockets are made such pockets represent active sites.

The catalytic structured most ofthe enzymes are tertiary and globular.

(d) Quarternary structure : Represented by, isoenzymes and active sites are present.

(4) Active site : An active site of an enzyme is a crevice or pocket into which the substrate fits. Thus enzymes through their active site, catalyse reactions at a high rate.

(5) Enzymes are very specific to their substrate or reactions. They are required in very small amount to catalyse a reaction. Catalytic power of an enzyme depends upon –

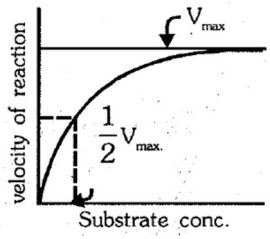
(a) Turn over number (b) Km constant

(a) Turn over number : It is the number of substrate molecules converted into products per unit time by a molecule of enzyme. Thus, catalytic power is directly proportional to turn over number. Carbonic anhydrase enzyme is considered as the fastest enzyme.

Enzyme	Turnover number
Carbonic anhydrase	306 lakh/minute
Catalase	50 lakh/minute
Flavoprotein	50/minute
Lysozyme	30/minute

(b) Km constant: This was coined by Michaelis and Menten. It is the concentration of substrate at which rate of reaction attains half of its maximum velocity.

$$K_m = \frac{1}{2} V_{max.}$$



Catalytic power of an enzyme id inversely proportional to its Km value.

(6) Enzyme increase the rate of reaction several times by lowering down activation energy.

(7) Catalytic power of an enzyme remains same even outside the living system.

(8) Enzymes when not in use, represent inactive form, called zymogen or pro-enzyme. Pepsinogen is an inactive form of pepsin, similarly trypsinogen is an inactive form of trypsin.

Enzyme (Biocatalyst)	Inorganic catalyst
Enzymes are thermo-sensitive and get damaged at high temperatures (say above 40°C)	They work efficiency at high temperatures and high pressures.

However enzymes isolated from organisms who normally live under extremely high temperatures (eg. hot vents and sulphur springs). are stable and retain their catalytic power even at high temperature. Thermal stability is thus an important quality of such enzymes isolated from thermophilic organisms. e.g. tag polymerase.

Uncatalysed reaction versus catalysed reaction

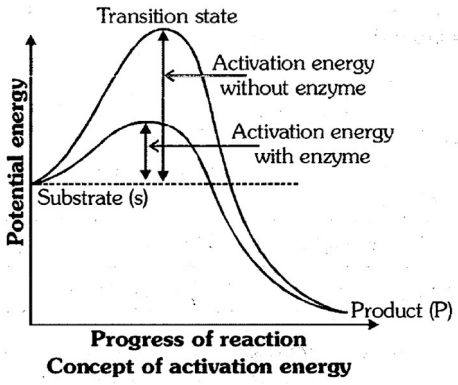
$$\underset{\text{Carbon di oxide}}{CO_2} + H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} \underset{\text{Carbonic acid}}{H_2CO_3}$$

- In the absence of an enzyme this reaction is very slow with about 200 molecules of H_2CO_3 being formed in an hour. However in the presence of enzyme carbonic anhydrase inside cytoplasm the reaction speeds dramatically with about 6,00,000 molecules being formed every second. The enzyme has accelerated the reaction rate by about 10 million times.

How do enzymes bring about such-high rates of chemical conversions ?

- The chemical or metabolic conversion refers to a reaction. The chemical which is converted into a product is called a substrate. Hence enzymes i.e. proteins with three dimensional structures including an active site to convert a substrate (S) into a product (P) Symbolically, this can be depicted as:

$$S \rightarrow P$$



Concept of activation energy

- They-axis represents the potential energy content. The x-axis represents the progression of the structural transformation or states through the 'transition state'. In above graph 'P' is at a lower level than 'S', thus this reaction is 1m exothermic reaction. (No need to supply energy in order to form the product.) However, whether it is an exothermic or spontaneous reaction or an endothermic or energy requiring reaction, the 'S' has to go through a much higher energy state or transition state. ·
- "The difference in average energy content of 'S' from that of this transition state is called activation energy".
- Enzymes eventually bring down this energy barrier making the transition of 'S' to 'P' more easy. All changes (ES complex, EP complex) occured during transition state are transient and unstable.

Nature of enzyme action

- Each enzyme (E) has a substrate (S) binding site in its molecular structure so that a highly reactive enzyme- substrate complex (ES) is produced. This complex is short-lived and dissociates into its product(s) P and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP). The formation 'of the ES complex is essential for catalysis.

Enzyme + Substrate ⇌ EPC → Enzyme + Product

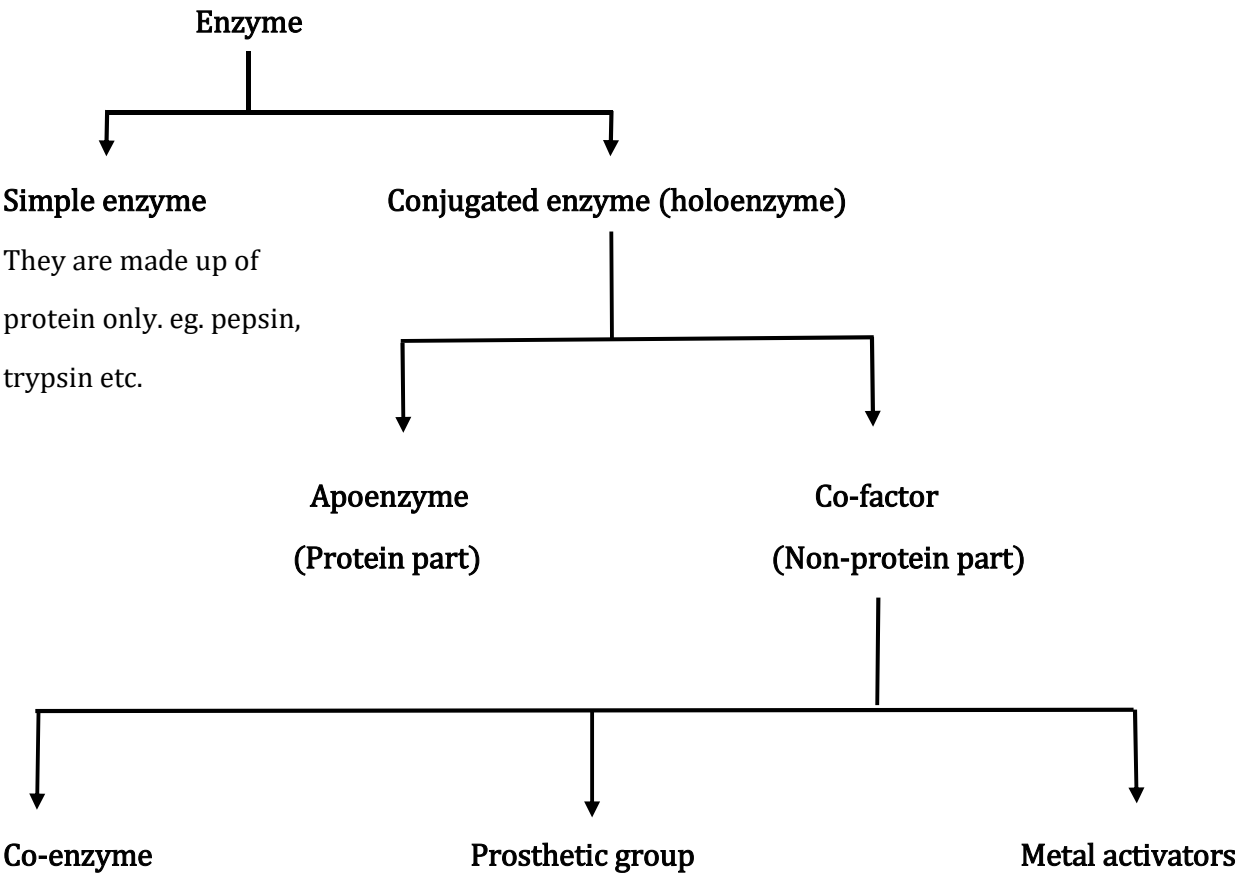
The catalytic cycle of an enzyme action can be described in the followirig steps:

1. First the substrate binds to the active site of the enzyme. fitting into the active site.
2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.

3. The active site of the enzyme, now in close proximity of the substrate breaks or form the chemical bonds of the substrate and the new enzyme-product complex is formed.
4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

COFACTORS

- Enzymes are composed of one or several polypeptide chains. However, there are a number of cases in which non-protein constituents called cofactors are bound to the enzyme to make the enzyme catalytically active. In these instances the protein portion of the enzymes is called the apoenzyme and non protein portion is called the cofactor.
- Three kinds of cofactors may be identified : co-enzymes, prosthetic groups and metal ions.



- They are organic compounds.
- They are loosely bound to the apoenzyme.
- Their association with apoenzyme is only transient (usually occurring during the course of catalysis).
- Most of them are vitamin derivatives.

- They are organic compounds.
- They are tightly bound to the apoenzyme.
- In peroxidase and catalase, haem is the prosthetic group and it is a part of the active site of the enzyme.

- They form coordination bonds with side chains at the active site and at the same time form one or more coordination Bonds with the substrate.

IMPORTANT EXAMPLES OF COENZYMES AND METAL ACTIVATORS :

Examples of co-enzymes	Examples of metal activator
Co. I (NAD/DPN) : Derivative of niacin	Fe^{++} : Cytochrome c oxidase, peroxidase, aconitase
Co. II (NADP/TPN) : Derivative of niacin	Cu^{++} : Cytochrome c oxidasde, tyrosinase
FAD : Derivative of riboflavin	Zn^{++} : Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase
FMN : Derivative of riboflavin	Mg^{++} : Hexokinase, glucokinase, pyruvate kinase, PEPcase, RuBisCO
TPP : Derivative of thiamine	K^{+} : Pyruvate kinase
Co. R : Derivative of ubiquinone	Mn^{++} : arginase, ribonucleotide reductase, decarboxylase
Co. R : Derivative of biotin	Mo : Nitrogenase complex, nitrate reductase
Co. A : Derivative of panthothenic acid	Se : Glutathione peroxidase

CLASSIFICATION AND NOMENCLATURE OF ENZYMES

- Most of these enzymes have been classified into different groups based on the type of reactions they catalyse.
- Enzymes are divided into 6 classes each with 4-13 subclasses and named accordingly by a four-digit number.

1. **Oxidoreductases/dehydrogenases**

   These enzymes catalyse **oxidation reduction** reactions, usually involving the transfer of hydrogen atoms or ions from one molecule to another. Eg., Alcohol dehydrogenase, cytochrome oxidase

   $$S \text{ reduced} + S' \text{ oxidised} \longrightarrow S \text{ oxidised} + S' \text{ reduced.}$$

2. **Transferases**

   These enzyme catalyse the transfer of a specific group (e.g., amino, methyl, acyl, phosphate) from one kind of molecule to another. **e.g.:** $S - G + S' \longrightarrow S + S' - G$

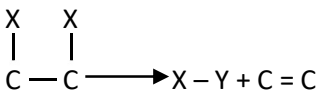
   Ex. Phosphotransferase, Peptidyl transferase etc.

3. **Hydrolases**

   These enzyme catalyse the hydrolysis of organic foods i.e., the breakdown of large molecules by addition of water. Most of the hydrolysing (digestive) enzymes are located in lysosomes. e.g., all digestive enzymes such as lipases (digest the stored food material of castor seeds).

4. **Lyases :**

   These enzymes catalyse the breakage of specific covalent bonds and removal of groups without hydrolysis e.g., fumerases, carboxylases, aminases, histidine decarboxylase (splits C–C-bond of histidine, forming CO_2 and histamine).

5. **Isomerases**

These enzymes catalyse the rearrangement of molecular structure to form isomers. e.g., phosphohexose isomerase (phosphoglucomutase) acts on glucose 6-phosphate to form fructose 6-phosphate (both C_6 compounds)

6. **Ligases or Synthetases**

These enzymes form bonds and join two molecules together, using energy supplied from the breakdown of ATP, e.g., DNA ligase is used to repair breaks in DNA molecules.

Mechanism of Enzyme Action

- Energy is required to bring the inert molecules into the activated state.
- The amount of energy required to raise the energy of molecules at which chemical reaction can occur is called **activation energy.**
- Enzymes act by decreasing the activation energy so that the number of activated molecules is increased at lower energy levels.
- If the activation energy required for the formation of the enzyme-substrate complex is low, many more molecules can participate in the reaction than would be the case if the enzyme were absent.

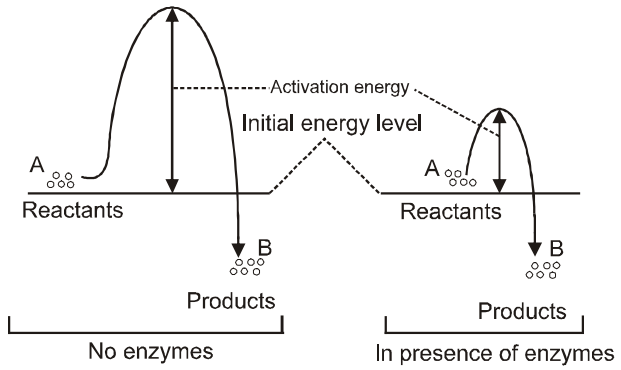
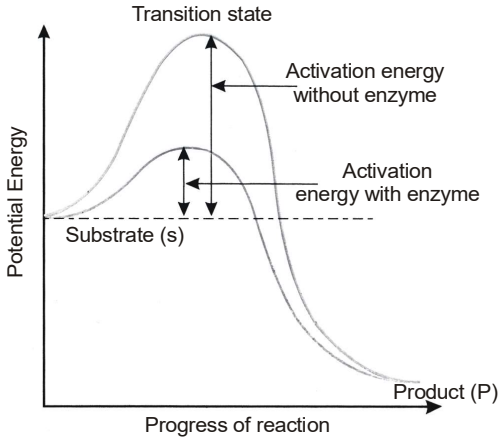


Fig : Showing that activation energy of an enzyme catalysed reaction is lower than that of an uncatalysed reaction

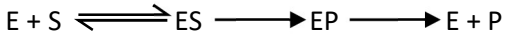


Concept of activation energy

Nature of Enzyme Action

- Each enzyme (E)has a substrate (S)binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced.

- This complex is short-lived and dissociates into its product (P) and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP).
- The formation of the ES complex is essential for catalysis.



The catalytic cycle of an enzyme action can be described in the following steps:

1. First, the substrate binds to the active site of the enzyme, fitting into the active site.
2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.
3. The active site of the enzyme, now in close proximity of the substrate breaks the chemical bonds of the substrate and the new enzyme- product complex is formed.
4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

There are two theories to explain the mode of action of enzymes.

Factors affecting enzyme activity

The activity of an enzyme can be affected by a change in the conditions which can alter the tertiary structure of the protein. These include :

(1) Temperature

(2) pH

(3) Change in substrate concentration

(4) Inhibitor

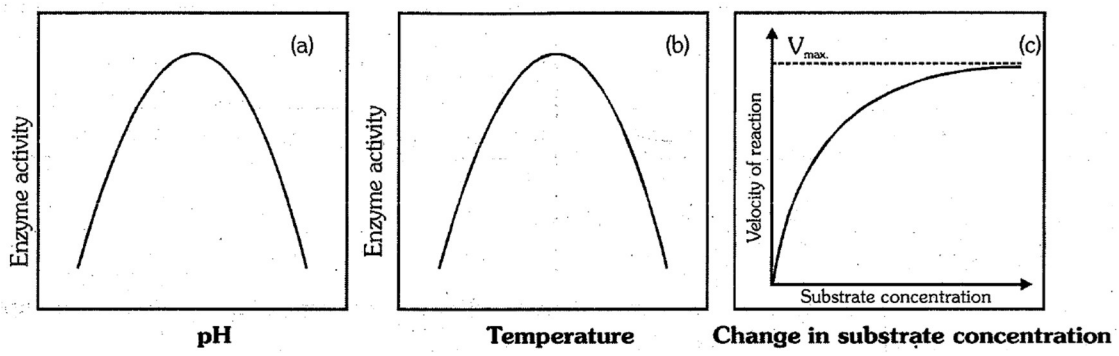
(1) Temperature:

Enzymes generally function in a narrow range of temperature. Each enzyme shows its highest activity at a particular temperature called the optimum temperature. Activity declines both below and above the optimum value. Low temperature preserves the enzyme in a temporarily inactive state whereas high temperature enzymatic activity because proteins are denature by heat.

A general rule of thumb is that rate doubles or decreases by half for every 10°C change in either direction. Thus value of Q_{10} for enzymatic activities is 2.

(2) pH :

Enzymes generally function in a very narrow range of pH. Each enzyme shows its highest activity at a particular pH called the optimum pH. Activity declines both below and above the optimum value.



(3) Change in substrate concentration :

With the increase in substrate concentration. the velocity of the enzymatic reaction rises at first. The reaction ultimately reaches· a maximum velocity (V_{max}).

This velocity is not exceeded by any further rise in concentration of the substrate.

Reason : The enzyme molecules are fewer than the substrate molecules and after saturation of these enzyme molecules, there are no free enzyme molecules to bind with the additional substrate molecules.

Enzyme Inhibition

- Enzyme inhibitor is defined as a substance which bind with the enzyme and bring about a decrease in catalytic activity of that enzyme.
- The inhibitor may be organic or inorganic in nature.
- There are three broad categories of enzyme inhibition:

1. Reversible inhibition
2. Irreversible inhibition
3. Allosteric inhibition

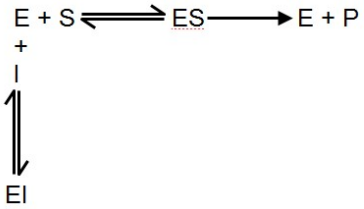
1. Reversible Inhibition

- The inhibitor binds non-covalently with enzyme and the enzyme inhibtion can be reversed if the inhibitior is removed.
- The reversible inhibition is further sub-divided into

I. Competitive inhibition II. Non-competitive inhibition

I. Competitive inhibition

- The inhibitor (I) which closely resembles the real substrate (S) is regarded as a **substrate analogue**.
- The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis.
- As long as the competitive inhibitor holds the active site, the enzyme is not available for the substrate to bind.
- During the reaction, ES and EI complexes are formed as shown below:

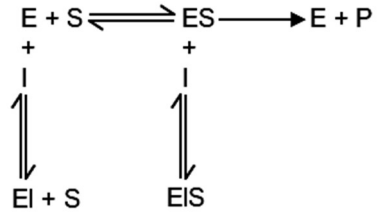


- In competitive inhibition, the **K_m value increases** whereas **V_{max}** remains **unchanged**
- The enzyme succinate dehydrogenase (SDH) is a classical example of competitive inhibition with succinic acid as its substrate.

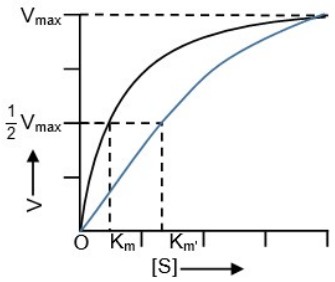
II. Non-competitive inhibition

- The inhibitor binds at a site other than the active site on the enzyme surface.
- This binding impairs the enzyme function.
- The inhibitor has no structural resemblance with the substrate.

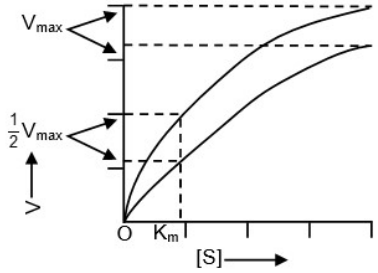
- The inhibitor generally binds with the enzyme as well as the ES complex.
- The overall relation in non-competitive inhibition is represented below:



- For non-competitive inhibition, the **K_m value is unchanged** while **V_{max} is lowered.**



Effect of competitive inhibitor on enzyme velocity



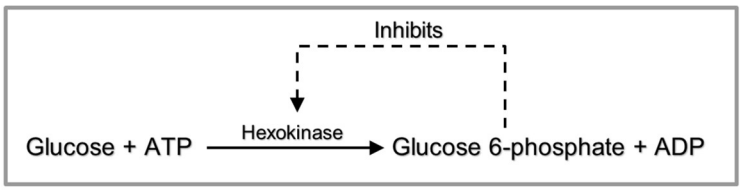
Effect of non-competitive inhibitor on enzyme velocity

2. Irreversible inhibition

- The inhibitors bind covalently with the enzymes and inactivate them, which is irreversible.
- These inhibitors are usually toxic substances.

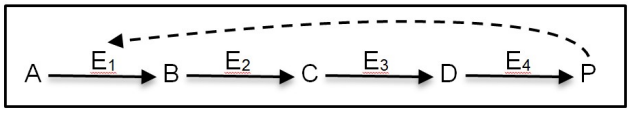
3. Allosteric inhibition (Modulation)

- Allosteric literally means 'another place'.
- Some inhibitors join an enzyme at a specific site and change the form of the active site meant for the substrate.
- These inhibitors are known as modifiers or modulators and the sites where they fit in are called allosteric sites.
- Modulators are of two types-positive (activators) and negative (inhibitors).



Feedback inhibition

- Feedback inhibition or **end product inhibition** is a specialized type of allosteric inhibition necessary to control metabolic pathways for efficient cellular function.
- In number of cases, accumulation of the final product of the reaction is capable of inhibiting the first step of reaction.
- The product P checks the activity of enzyme which converts A into B. It is quite useful mechanism because it checks the accumulation of products.



- The phenomenon in which the end product of a metabolic pathway can regulate its own production by inhibition of the enzymes of its own pathway is called **feed back inhibition** or negative feed back inhibition.

LOCK AND KEY HYPOTHESIS

- According to this hypothesis the enzyme and its substrate have a complementary shape.
- The specific substrate molecules are bound to a specific site of the enzyme molecule.
- The theory can be explained easily by the fact that a particular lock can be opened by a particular key specially designed to open it.
- Similarly enzymes have specific sites where only a particular substrate can be attached.
- The lock and key model accounts for enzyme specificity.

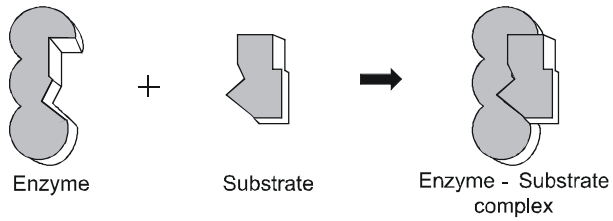


Fig : Lock and key model of enzyme action

Induced fit hypothesis:

- According to this view, active site is not rigid but static and it has two groups– buttressing group and catalytic group.
- Initially substrate bind to the buttressing group which induces the catalytic group to fit the substrate and catalytic group weakenes the bonds of reactant or substrate by electrophilic and nucleophilic forces.

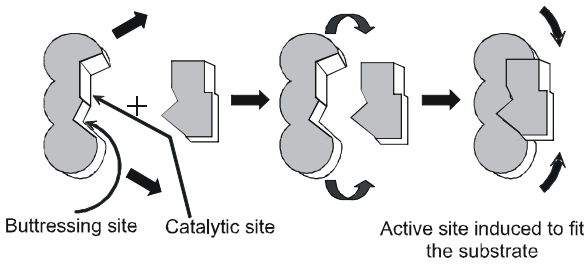


Fig : Induced fit model of enzyme action

Properties of enzymes

1. Chemical reactions

- Chemical compounds undergo two types of changes.
- A physical change simply refers to a change in shape without breaking of bonds. This is a **physical process**.
- Another physical process is a change in state of matter when ice melts into water, or when water becomes a vapour.
- However, when bonds are broken and new bonds are formed during transformation, this will be called a **chemical reaction**.
- For example, $Ba(OH)_2 + H_2SO_4 \rightarrow BaSO_4 + 2H_2O$ is an inorganic chemical reaction.
- Similarly, hydrolysis of starch into glucose is an organic chemical reaction.
- Rate of a physical or chemical process refers to the amount of product formed per unit time.
- It can be expressed as rate $= \frac{\delta p}{\delta t}$.
- Rate can also be called velocity if the direction is specified.
- Rates of physical and chemical processes are influenced by temperature among other factors.
- A general rule of thumb is that rate doubles or decreases by half for every 10ºC change in either direction.

- Catalysed reactions proceed at rates vastly higher than that of uncatalysed ones.
- When enzyme catalysed reactions are observed, the rate would be vastly higher than the same but uncatalysed reaction.
- For example, $CO_2 + H_2O$ H_2CO_3

Carbon dioxide water $\xrightleftharpoons{\text{Carbonic anhydrase}}$ carbonic acid

- In the absence of any enzyme this reaction is very slow, with about 200 molecules of H_2CO_3 being formed in an hour.
- However, by using the enzyme present within the cytoplasm called carbonic anhydrase, the reaction speeds dramatically with about 600,000 molecules being formed every second.
- The enzyme has accelerated the reaction rate by about 10 million times. The power of enzymes is incredible indeed.
- There are thousands of types of enzymes each catalysing a unique chemical or metabolic reaction.
- A multistep chemical reaction, when each of the steps is catalysed by the same enzyme complex or different enzymes, is called a metabolic pathway.
- For example.

Glucose Pyruvic acid

$C_6H_{12}O_6 + O_2$ $2C_3H_4O_3 + 2H_2O$

2. Colloidal nature

- All enzymes are colloidal in nature and thus provide large surface area for reaction to take place.

3. Catalytic properties

- Enzymes are active in extremely small amounts, e.g., one molecule of invertase can effectively hydrolyze 1,000,000 times its own weight of sucrose.
- One molecule of catalase is able to catalyze conversion of 5,000,000 molecules of hydrogen peroxide.

4. High efficiency

- The effectiveness of an enzymatic reaction is expressed in terms of its turn over number or catalytic centre activity, means number of substrate molecules on which one enzyme molecule acts in one minute.

5. **Molecular weight**
    - Enzymatic proteins are substances of high molecular weight. Bacterial ferredoxin one of the smaller enzymes has molecular weight of 6,000, where as pyruvic dehydrogenase one of the largest-has a molecular weight of 4600000.
6. **Specificity of enzyme**
    - Most of the enzymes are highly specific in their action.
    - A single enzyme will generally catalyze only a single substrate or a group of closely related substrates.
    - The active site possesses a particular binding site which complexes only with specific substrate. Thus, only a suitable substrate fulfils the requirements of active site and closely fixes with it.
7. **Reversibility of reaction**
    - The enzyme-controlled reactions are reversible.
    - The enzymes affect only the rate of biochemical reactions, not the direction. e.g., Lipase can catalyse splitting of fat into fatty acids and glycerol as well as synthesis of fatty acids and glycerol into fats.