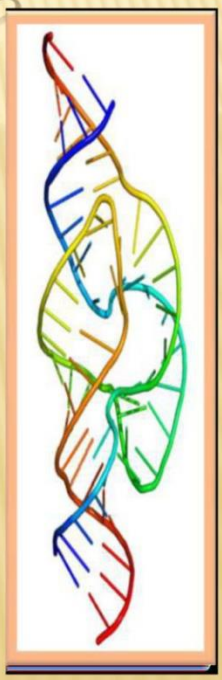
ENZYMES

INTRODUCTION TO ENZYMES

- Are the Bio-Catalysts which increase the velocity of biological reaction and remains unchanged at the end of the reaction.
- They are present in the cytoplasm of all cells.
- Most enzymes are three dimensional *globular proteins.*
- Some special RNA species also act as enzymes and are called *Ribozymes e.g.* hammerhead ribozyme.



Hammerhead enzyme

METABOLIC ACTIVITIES

- A part of metabolism which take place in the body of an organism.
- It is the phenomenon which includes set of chemical reaction which are essential for a living organism to maintain its life.
- A significant amount of energy may be released during reaction or may be stored in form of energy.

PURPOSE OF METABOLISM

- 3 Main purpose of metabolism are:-

1) Conversion of food to energy to run cellular processes.

2) Conversion of food /fuel to building blocks for proteins, lipids, nucleic acids & carbohydrates.

3) Elimination of nitrogenous waste.

CLASSIFICATION OF METABOLIC REACTIONS

- It is classified into two types:-

a) Catabolic:- Breaking down of Compounds.
e.g. Breaking down of glucose.

b) Anabolic:- Building up of Compounds.
e.g. Proteins, Carbohydrates etc.

WHAT IS CATALYST?

- It is a substance that is not consumed by the chemical reaction, but acts to lower its activation energy to speed up chemical reaction.
- It can be recovered chemically unchanged at the end of reaction.
- Increasing the rate of chemical reaction by introducing catalyst is known as catalysis.

5 TYPES OF ENZYMES

- AMYLASE:- Produced in Mouth (Breakdowns large starch molecules into smaller sugar molecules).
- PEPSIN:- Produced in Stomach (Breakdowns proteins into amino acids).
- TRYPSIN:- Produced in Pancreas (Breakdowns proteins).
- PANCREATIC LIPASE:- Produced in Pancreas (Used to break up fats).
- DEOXYRIBONUCLEASE & RIBONUCLEASE:- Produced in Pancreas (Breaks bond in nucleic acid like DNA & RNA).

CHEMICAL NATURE OF ENZYMES

- On the basis of chemical nature, it is classified into two types:-

  a) Simple Enzymes:- Composed of Amino Acids only e.g. protein.

  b) Conjugated Enzymes:- Possess a non protein group known as cofactor along with some protein part known as Apoenzyme.
- The complete biologically active conjugated enzyme is known as Holoenzyme.

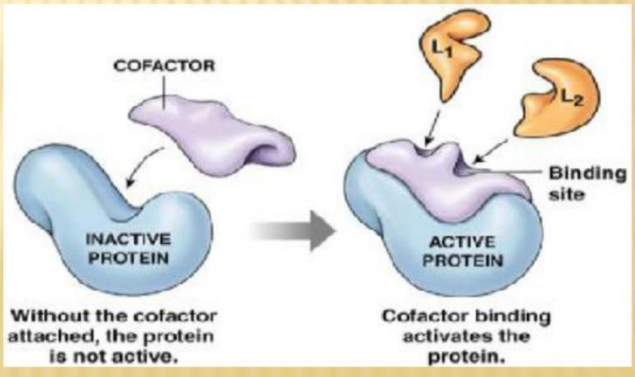
  Cofactor + Apoenzyme = Holoenzyme

WHAT ARE CO-FACTORS?

- Co-factor is the non protein molecule which carries out chemical reactions that can not be performed by standard 20 amino acids.
- Co-factors are of two types:

  a) Organic co-factors

  b)Inorganic cofactors



INORGANIC CO-FACTORS

- These are the inorganic molecules which requires the proper activity of enzymes (i.e. Metal ion activator).
- Examples: k^{+} , Fe^{+2} ,Cu^{+2}

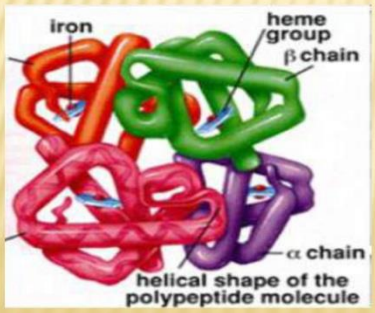
ORGANIC CO-FACTORS

- These are the organic molecules which requires the proper activity of enzymes (i.e. Coenzymes & Prosthetic group).
- Coenzymes:- Non protein part which remains loosely bound or attached to the enzyme.
- Prosthetic group:- Organic group that are tightly associated with protein part of enzymes.

TYPES OF ORGANIC CO-FACTORS

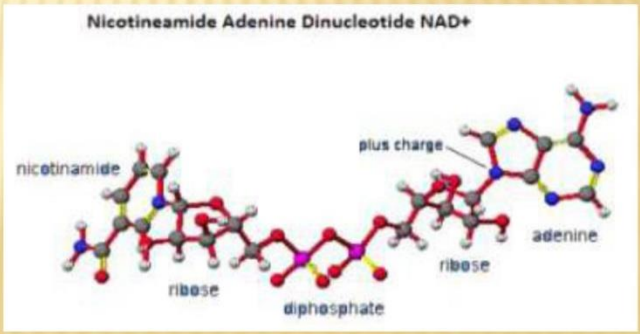
Prosthetic Group

- A prosthetic group is a tightly bound organic cofactor e.g. Flavins, heme groups and biotin.



Coenzyme

- A coenzyme is loosely bound organic co-factor e.g NAD^+

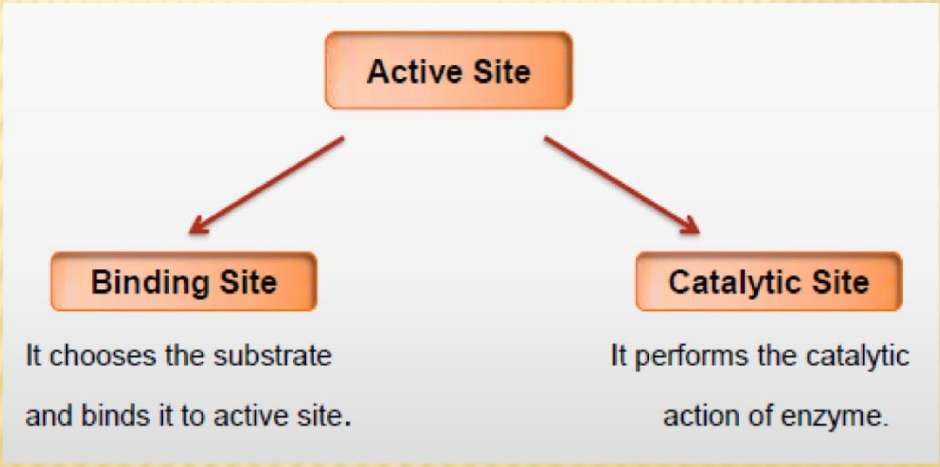


STRUCTURE OF ENZYMES

- The active site of an enzyme is the region that binds substrates, co-factors and prosthetic groups and contains residue that helps to hold the substrate.
- Active sites generally occupy less than 5% of the total surface area of enzyme. Active site has a specific shape due to tertiary structure of protein.
- A change in the shape of protein affects the shape of active site and function of the enzyme.

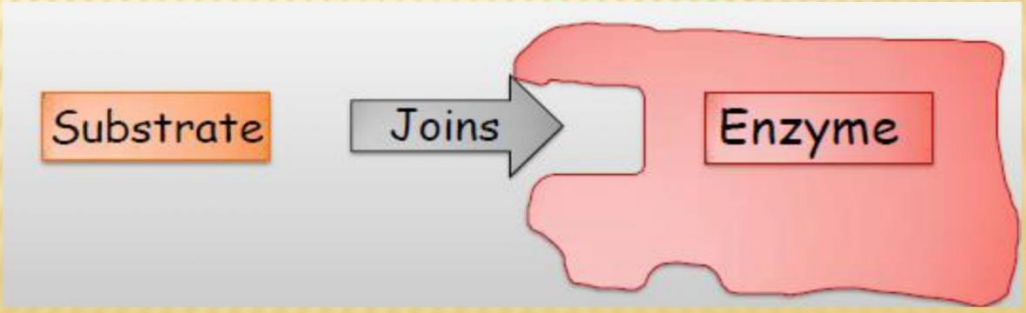
ACTIVE SITE

- Active site can be further divided into:



SUBSTRATE

- The reactant in biochemical reaction is termed as substrate.
- When a substrate binds to an enzyme it forms an enzyme substrate complex.



MECHANISM OF ENZYME ACTION

HOW DO ENZYMES CATALYSE REACTION?

- All enzymes are chemically protein which are composed of amino acids.
- The particular shape of enzymes are due to the folding pattern which is obtained due to fixed sequence of amino acid sequence.
- Pockets are formed in enzymes due to folding of polypeptides chains.
- Enzyme (E) binds to Substrate molecule(S) at these sites to form enzymes substrate complex(ES).

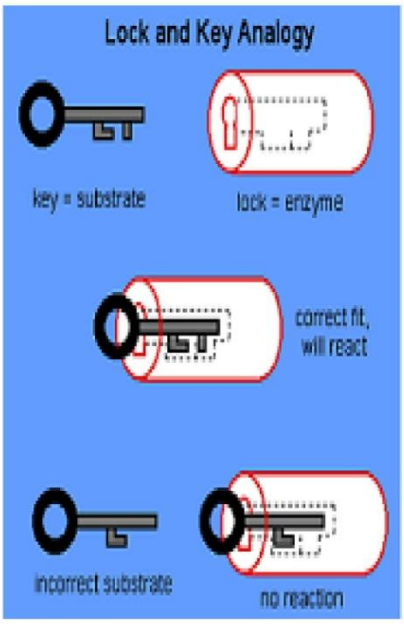
HOW DO ENZYMES CATALYSE REACTION?

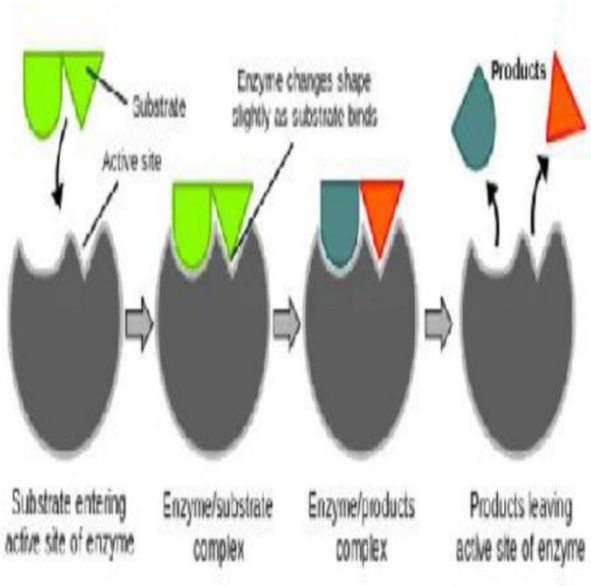
- The ES thus obtained is a complex substance which is unstable, highly reactive.
- It further converts into Product (P) releasing the Enzyme unaltered.
- $E + S \rightleftharpoons ES \rightleftharpoons P + E$
- This concept was proposed by Michaelis & Menten in 1913.

LOCK AND KEY THEORY

- In 1894, German chemist Emil Fischer proposed the **lock and key theory**, which states that enzymes have a specific shape that directly correlates to the shape of the substrate.
- Substrates fit into an enzyme the way a **key** fits into a **lock.**
- Only those substrates that exactly fit into the enzyme can be catalyzed.

LOCK AND KEY MODEL OF ENZYME ACTION





ASSUMPTIONS:-

- Substrate diffuses into the active site and binds to it – the substrate must be a specific shape to fit into the enzyme
- The substrate forms temporary bonds with the AA's in the enzyme to produce an **ENZYME – SUBSTRATE COMPLEX**
- The reaction takes place and the bonds in the substrate are broken – in this case, the larger molecule has been broken down into smaller molecules. An **ENZYME – PRODUCT COMPLEX** has formed
- The products unbind from the active site and diffuses out. The enzyme is ready to be used again.

INDUCED FIT MODEL

- The **induced-fit model**, proposed by Daniel Koshland in 1958.
- He states that when the active site on the enzymes makes contact with the proper substrate, the enzyme molds itself to the shape of the molecule.

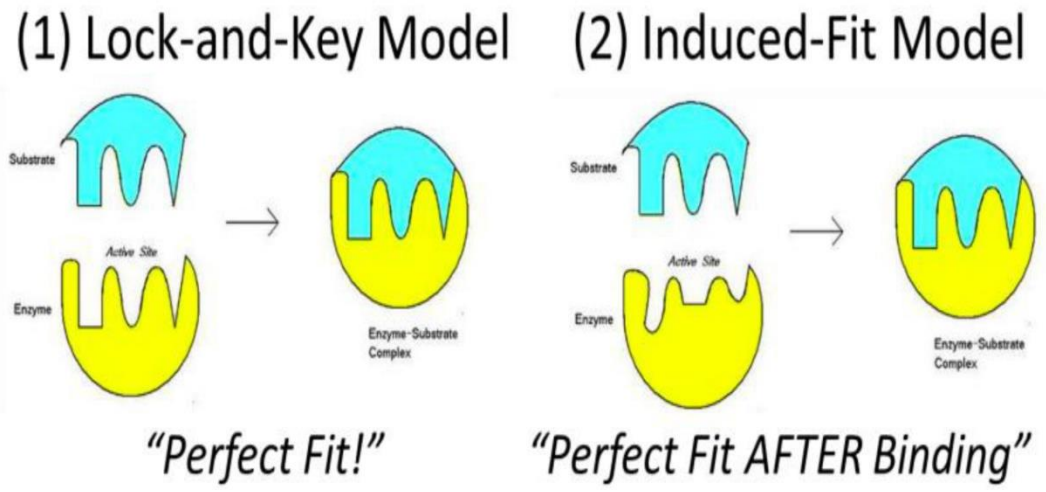
ASSUMPTIONS:-

- Structure of active sites of an enzymes are not rigid but are flexible which can change binding of substrate to it.
- Induces the enzymes to alter the active site in order to make it fit for substrate.
- Substrate fits into this site forming an enzyme substrate complex.
- After completion of reaction products and enzymes are released.

COMPARISON

INDUCED FIT MODEL	LOCK AND KEY MODEL
A model for enzyme-substrate interaction in which the active site of the enzyme does not completely fit to the substrate	A second model for enzyme-substrate interaction in which the active site of the enzyme completely fits with the substrate
Suggested by Daniel Koshland in 1958	Suggested by Emil Fischer in 1894
The active site of the enzyme does not completely fit with the substrate	The active site of the enzyme precisely fits with the substrate
The active site of the enzyme has to undergo a conformational change in order to improve binding	Describes the specificity of the active site of the enzyme to a particular substrate
The active site of the enzyme contains two components	The active site of the enzyme contains a single entity
There is a separate catalytic group in the enzyme	There is no separate catalytic group in the enzyme
The active site of the enzyme is not static	The active site of the enzyme is static
A transition state develops before the reactants undergo changes	No transition state
Catalytic group weakens the substrate bonds either by the nucleophilic or electrophilic attack	No weakening of the substrate bonds
Describes the mechanism of nonaction over competitive inhibitors	Describes the specificity of the active site of the enzyme to a particular substrate

COMPARISON OF LOCK AND KEY MODEL WITH INDUCED FIT MODEL



PRINCIPLE OF ENZYME CATALYSIS

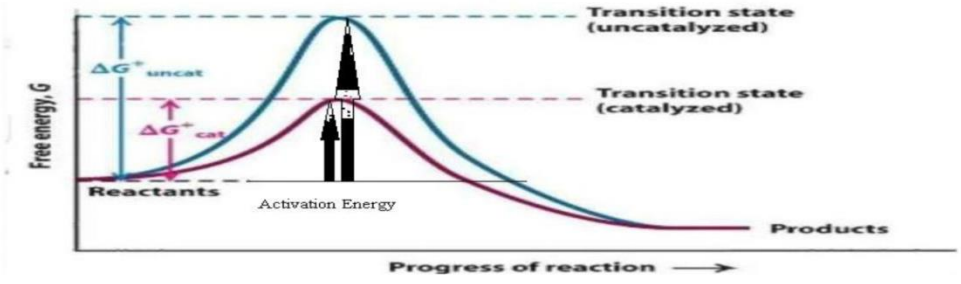
- The catalytic efficiency of enzymes is explained by two perspectives.

Thermodynamic changes

Processes at the active site

THERMODYNAMIC CHANGES

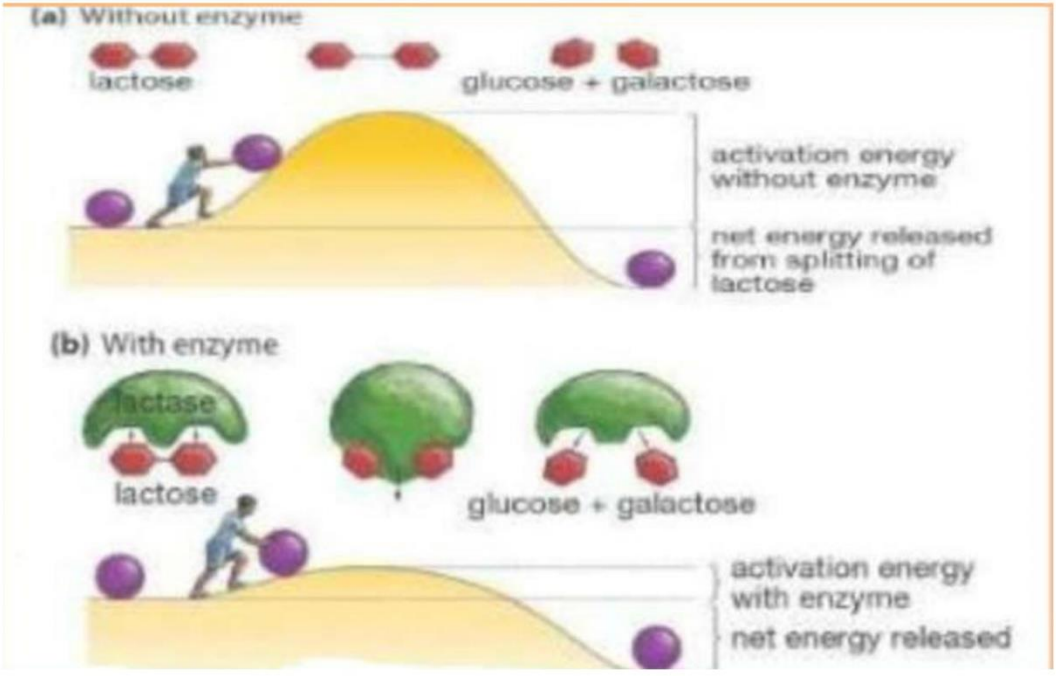
- All chemical reactions have energy barriers between reactants and products.
- The difference in transitional state and substrate is called *activational barrier*.
- Substrate binds with enzymes at active sites which reduces activation energy and promote catalyst.



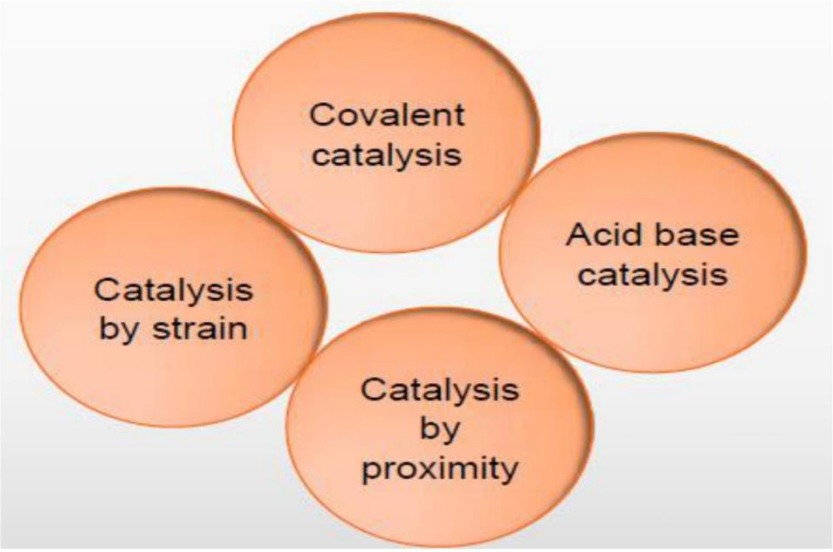
THERMODYNAMIC CHANGES

- Formation of ES increases the concentration of substrate molecule which results the movement of reaction in forward direction.
- Function of enzyme is to hold the substrate molecules in suitable orientation for reaction.
- During reaction the substrate pass through a intermediate forms. Enzymes accelerate reaction rates by forming transitional state having low activational energy.
- Reaction rate is increased. The total energy of the system remains the same and equilibrium state is not disturbed.

THERMO-DYNAMIC CHANGES OVERVIEW



PROCESSES AT THE ACTIVE SITE



COVALENT CATALYSIS

- Occurs when a transient covalent bond between enzymes and substrate is formed. Hydrolysis of compound takes as:-

WITHOUT COVALENT CATALYSIS:

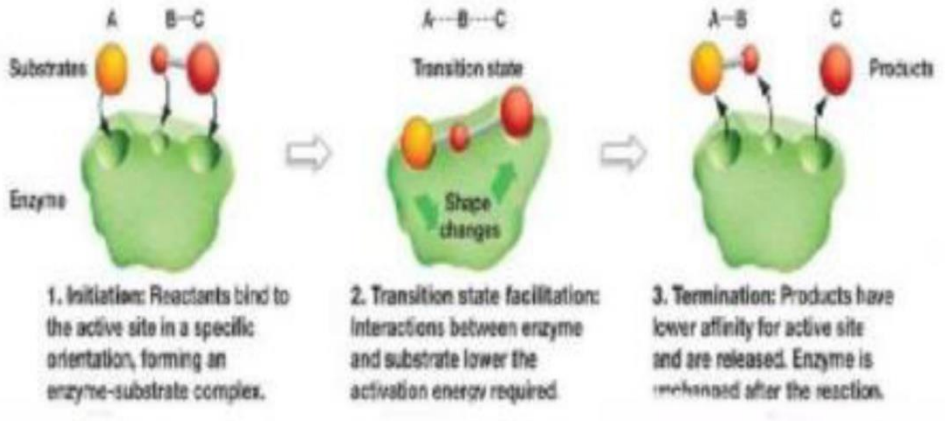
$$A—B \xrightarrow{H_2O} A + B$$

WITH COVALENT CATALYSIS:

$$A—B + X: \longrightarrow A—X + B \xrightarrow{H_2O} A + X: + B$$

- If the new pathway requires less activation energy and is faster it is followed.

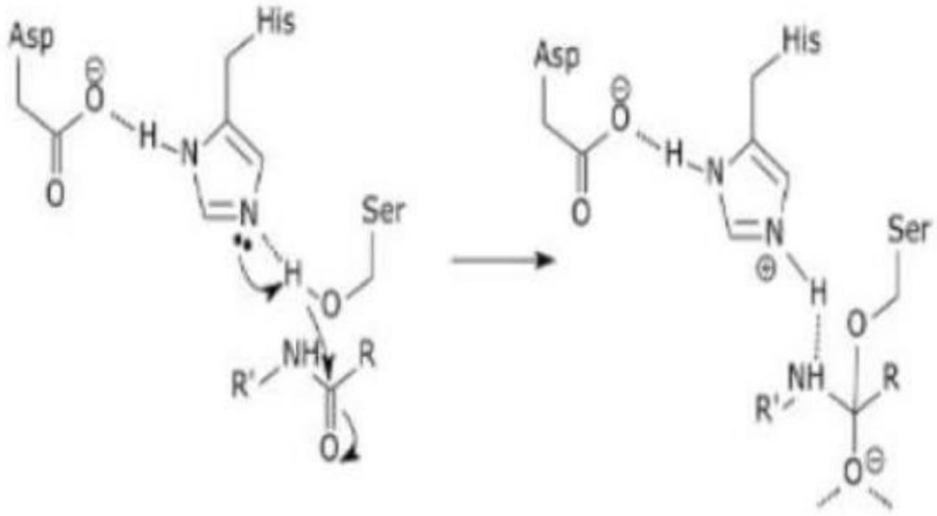
COVALENT CATALYSIS



ACID-BASE CATALYSIS

- Acceleration of a chemical reaction by the addition of an acid or a base, while itself not being consumed in the reaction.
- General acid - partial transfer of a proton from an acid lowers the free energy of the transition state.
- Rate of reaction increases with decrease in pH and increase in [Bronsted acid: a substance that gives up or donates hydrogen ions during a chemical reaction].

ACID-BASE CATALYSIS



FACTORS AFFECTING ENZYME ACTIVITY

- Major factors influencing the velocity of reactions are:-
  a) Concentration of Enzymes
  b) Concentration of substrates
  c) Temperature
  d) pH or hydrogen ion concentration
  e) Concentration of products