Enzyme

MODULE-05

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Catalysis

The process by which a substance speeds up a chemical reaction without being consumed in the process.

(or)

catalysis is the process of increasing the rate of a chemical reaction by adding a substance known as a catalyst.

eg - the making of soap, the fermentation of wine to vinegar, starch break down into sugar (glucose).

1812 - Russian chemist Gottlieb Kirchhof.
↳ study the behavior of starch in boiling water.

$$\text{Starch} \xrightarrow{\Delta} \text{no change.}$$

$$\text{Starch} \xrightarrow[\Delta]{\text{conc } H_2SO_4} \text{Glucose.}$$

catalysis

- Homogenous Cat. Rxn.
  - catalyst and substance in same phase.
  - eg - solid - solid, liquid - liquid
- Heterogeneous.
  - catalyst in different phase.
  - solid - liquid
  - ... etc.

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Enzyme / Biocatalyst

An enzyme is a biological catalyst and is almost always a protein. It speeds up the rate of a specific chemical reaction in cell.

↳ sugar cube $\xrightarrow[\text{water}]{\text{normal}}$ breaks and give energy in a year.

↳ All enzymes are proteins which are high molecular weight macromolecules.

↳ Enzyme may consists of a single poly-peptide chain or a cluster of polypeptide chains.

↳ Polypeptide chain is made up of number of amino acid units linked by peptide bonds.

↳ The arrangement of enzymes is specific for a particular enzyme and determines the properties of the enzyme.

↳ $(-NH_2)$ — polypeptide chain — $(-COOH)$

$(-NH_2)$ ⇓ (amino) ⇓ Biosynthesis.

$(-COOH)$ ⇓ (carboxyl).

↳ polypeptide chains are linked by disulphide $(-S-S-)$ bridges.
└ connect polypeptide chain.

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⇒ Active site

An enzyme has a distinct cavity or cleft in which the substrate is bound is called active site.

Substrate:- specific compound acted upon by an enzyme.

eg. $CO_2 + H_2O \xrightarrow[\text{anhydrase}]{\text{carbonic}} H_2CO_3$ (carbonic acid).

→ without carbonic anhydrase – 200 molecules/hour.
" with carbonic anhydrase – 6 lakh molecules/sec.
↓
present in cytoplasm.

⇒ Nature of enzyme Action:–

① Active site
Substrate $\xrightarrow[\text{enzyme}]{\text{combined}}$ product.

② When substrate binds to enzyme, It forms enzyme-substrate complex (ES). This complex formation is a transient phenomenon.

③ When substrate binds to the active site of an enzyme a new structure of the substrate called Transition state.

(P T O).

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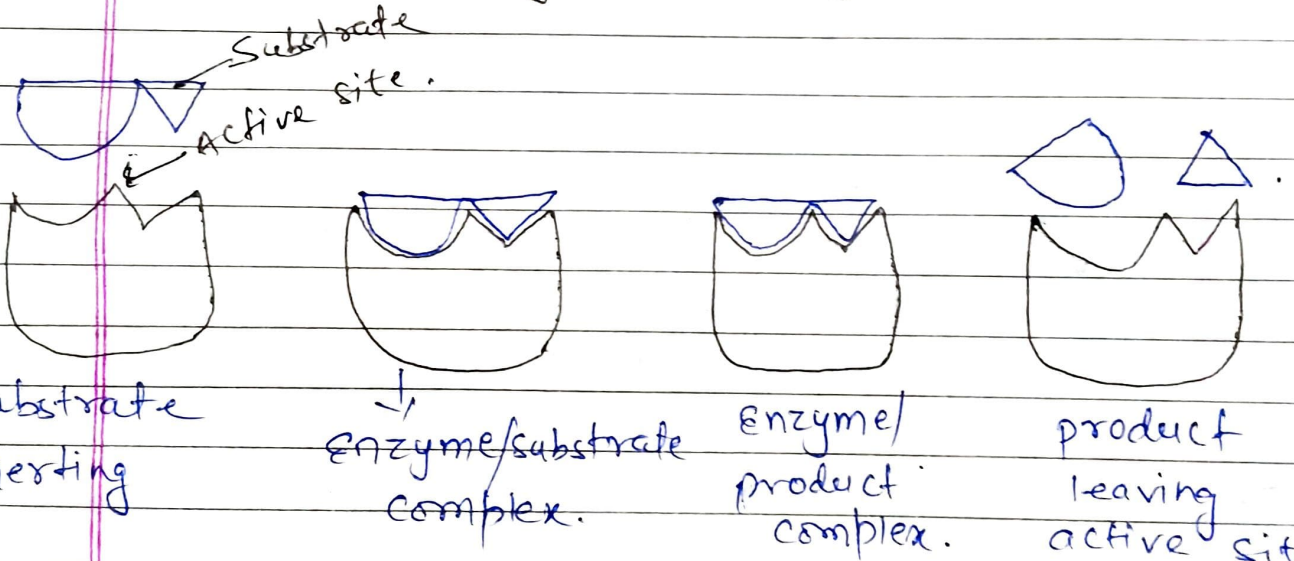
③ The molecules of substrate undergo chemical changes, breaking or making of bonds and finally the product is formed and is released from the active sites.

⇒ How do enzymes catalyze R·xn



* $E + S \rightleftharpoons ES \longrightarrow EP \longrightarrow E + P$.

ES - Complex is essential for catalysis.

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Factors affecting Enzyme activity

Enzymes are proteins with tertiary structure. Any changes in tertiary structure would affect the action of ~~catalys~~ enzymes.

(1) Temperature

↳ Optimum Temperature ⟶ Highest enzyme activity.

↳ Enzyme activity declines both above and below the optimum temperature.

↳ At low temperature, enzymes become temporarily inactive.

↳ At high temperature there is a loss of enzyme activity due to protein denaturation.

↳ At higher temperature K.E of molecules in an enzyme becomes strong to break the weak hydrogen bond present in tertiary structure of enzyme resulting in loss of catalytic activity. This change in structure is called denaturation of enzyme.

↳ Once an enzyme denatures, it remains inactive as temperature is lowered down.

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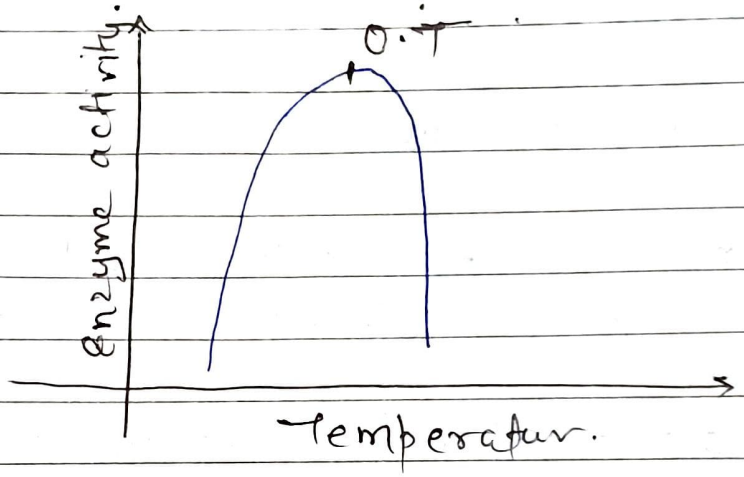
↳ Optimum temperatures for human enzymes is (35-40)°C

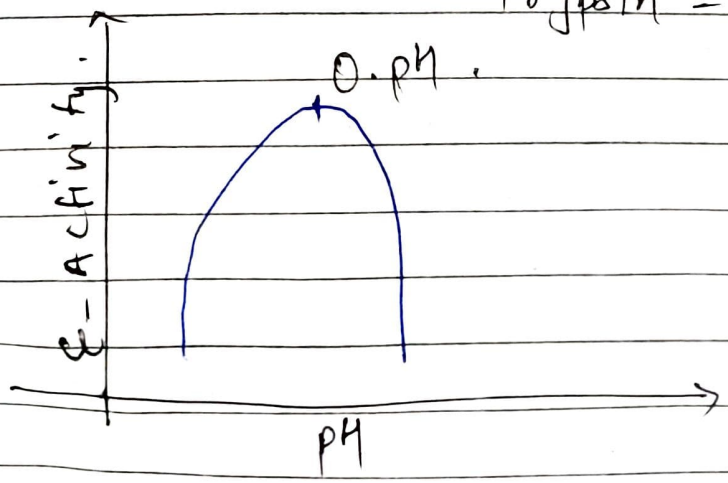
↳ The enzyme activity decreases with decrease as well as increase in temperature and stops at 0°C and above 80°C.



(2) pH :—

↳ At optimum pH the activity of enzymes is max^m for most enzymes, the effective pH range is — 4-9. Beyond this limits denaturation of enzymes takes places.

eg— Optimum pH for pepsin = 2
Trypsin = 8.



(3) Concentration of substrate.

↳ Increase in substrate concentration, increase the velocity of enzymatic reaction.

↳ The Rxn soon reaches a max^m velocity (V_{max}) which is not exceed by further rise in Conc. of substrate.

(4) Product of concentration:—

Accumulation of the product of enzyme reaction lowers the enzyme activity.

Nomenclature And Classification of Enzymes:—

↳ Enzymes are generally named by adding -ase to the root.

↳ International Union of Biochemistry Report 1962 contains a scheme for enzyme classification. and divide into 6-groups.

(1) Oxireductases - >ehydrogenases:- Enzymes which catalyze oxidation reduction ~~reaction~~ Rxn involving transfer of electrons/H^+ from one molecule to another, In these reactions one compound is oxidized and the other is reduced.
eg - >ehydrogenase, Oxidase, reductase etc

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(2) Transferases:- These enzymes catalyse the transfer of specific group other than hydrogen from one substrate to another.

(3) Hydrolysis:- These enzymes catalyze the breakdown of larger molecules into smaller molecules with the addition of water.
eg- Amylase, lipase, maltase

$$\text{Sucrose} + H_2O \longrightarrow \text{Glucose} + \text{fructose}.$$

(4) Lyases:- These enzymes catalyze the substrate without the use of water.

eg- Decarboxylase, carbonic anhydrase, --etc.

(5) Isomerase:- These enzymes catalyse the rearrangement of molecular structure to form isomers.
eg-

$$\text{Glucose-6-phosphate} \overset{\text{Isomerase}}{\rightleftharpoons} \text{fructose-6-phosphate}.$$

(6) Ligases:- These enzymes catalyse covalent bonding of two substrate to form a large molecule. Use ATP as Energy source.
eg- phosphopenol pyruvate (PEP).

$$\underset{\text{pyruvic acid}}{\text{phosphopenol}} + CO_2 \xrightarrow[\text{PEP}]{\text{ATP}} \text{Oxaloacetic acid}.$$

Mechanism of Enzyme Action

↳ Two Hypotheses given by scientist to explain the mode of enzyme action.

(1) Lock - Key Hypothesis (Emil fischer - 1984).

↳ Enzyme & substrate molecules have specific geometrical shapes; which is similar to lock and key.

↳ The active site contains special groups $-NH_2$, $-COOH$, $-SH$ for establishing contact with the substrate molecules.

↳ After coming in contact with the active site of enzyme, the substrate molecules forms a complex called Enzyme - substrate complex.

↳ In enzym - substrate complex, the molecules of the substrate undergo chemical change and form products.

↳ The product no longer fits into the active site and escapes in surrounding medium, leaving the active site free to receive more substrates molecules.

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Step-①

Enzyme + Substrate ⟶ E-S complex.

Step-② E-S complex $\xrightarrow{\text{chemical changes}}$ Enzyme + End products

↳ This theory explains how a small concentration of enzyme can act upon a large amount of the substrate.

↳ Also explains how the enzyme remains unaffected at the end of chemical Rxn.

↳ Also explains how a substance having a structure similar to the substrate can work as a competitive inhibitor.

② Induced fit Hypothesis:- (Koshland - 1960)

↳ The active site of the enzyme doesn't initially exist in a shape that is complementary to the substrate but is induced to assume the complementary shape as the substrate becomes bound to the enzyme.

↳ An active site of an enzyme is a pocket into which the substrate fits

↳ According to this model, the enzyme and its active site is flexible and the active site of the enzyme contains two groups. —

(a) Buttressing Group:- use to support substrate.

(b) catalytic Group:- catalyzing the rxn when substrate comes in contact with the buttressing group.

Energy Kinetics

↳ The rate of the Rxn is dependent on the substrate concentration.

At low substrate concentration the rate of reaction (v) is proportional to substrate concentration.

As the substrate concentration is increased the velocity of Rxn falls and is no longer proportional to the substrate concentration.

further increase in substrate concentration, the rate of rxn becomes constant and independent of substrate concentration. The enzyme at this stage show the saturation effect.

↳ Rate of Rxn is limited by enzyme concentration.

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↳ The maximum rate of Rxn is characteristic of a particular enzyme at a particular concentration and is known as the maximum velocity (V_{max}).

↳ The substrate concentration that gives a rate that is halfway to V_{max} is called K_m. (Michaelis-Menten Constant).

↳ K_m is a useful measure of how quickly Rxn rate increases with substrate concentration.

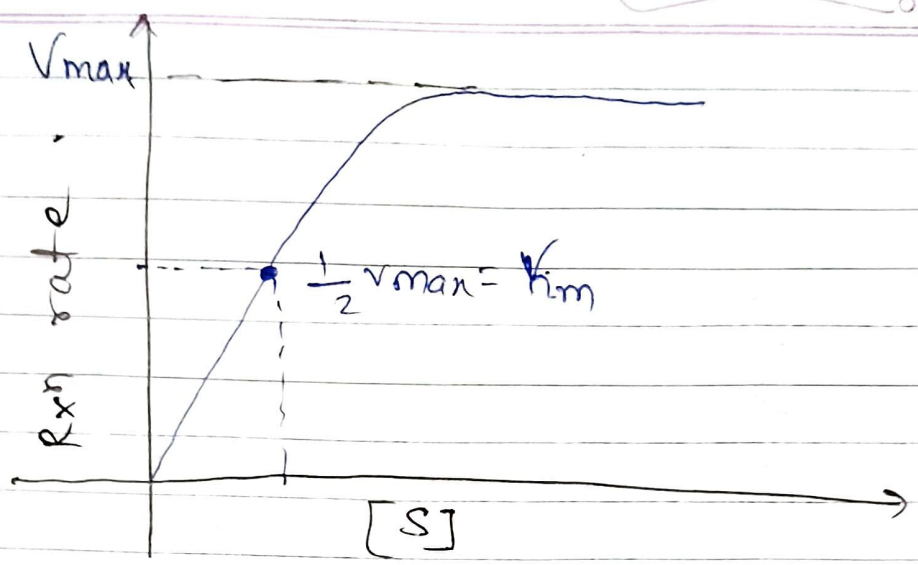
↳ K_m is also a measure of an enzyme affinity (tendency to bind) to its substrate.

* $K_m \propto \frac{1}{\text{enzyme affinity}}$.

↳ Michaelis and Menten give the theory of enzyme action & kinetic in 1913

$$V = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

V = Reaction velocity.
$[S]$ = Substrate concentration.
V_{max} = Max^m velocity.
K_m = Michaelis-Menten constant.



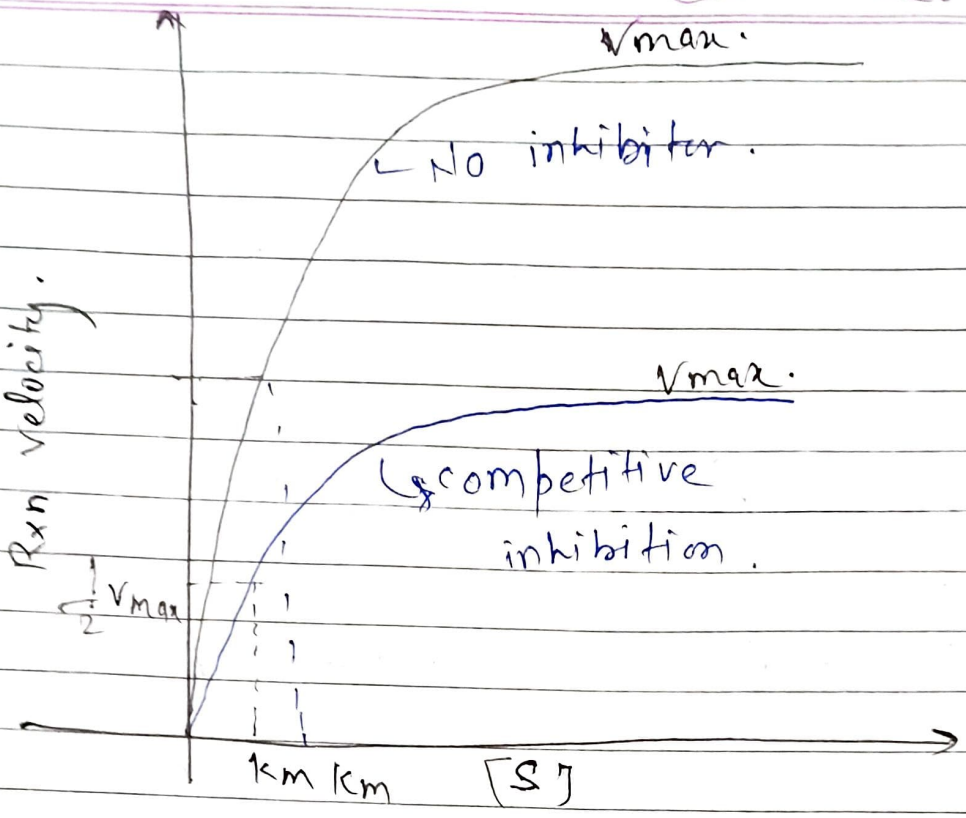
Inhibition of Enzyme Action :-

↳ Any substance which can diminish the velocity of an enzyme catalysed Rxn is called Inhibitor.
They act in 3-different ways.

(1) Competitive Inhibition :- This type of inhibition occurs when the inhibitor binds reversibly at the same site where substrate could normally bind and therefore competes with the substrate for that site.

↳ Effect on Vmax :- At high substrate concentration, the Rxn velocity reaches Vmax as observed in the absence of Inhibitor.

↳ Effect on Km :- In the presence of competitive inhibitor more substrate is needed to acheive $\frac{1}{2}$ Vmax.



(2) Non-competitive inhibition:- This inhibition is bought about by a substance which doesn't resemble the substrate in structure.

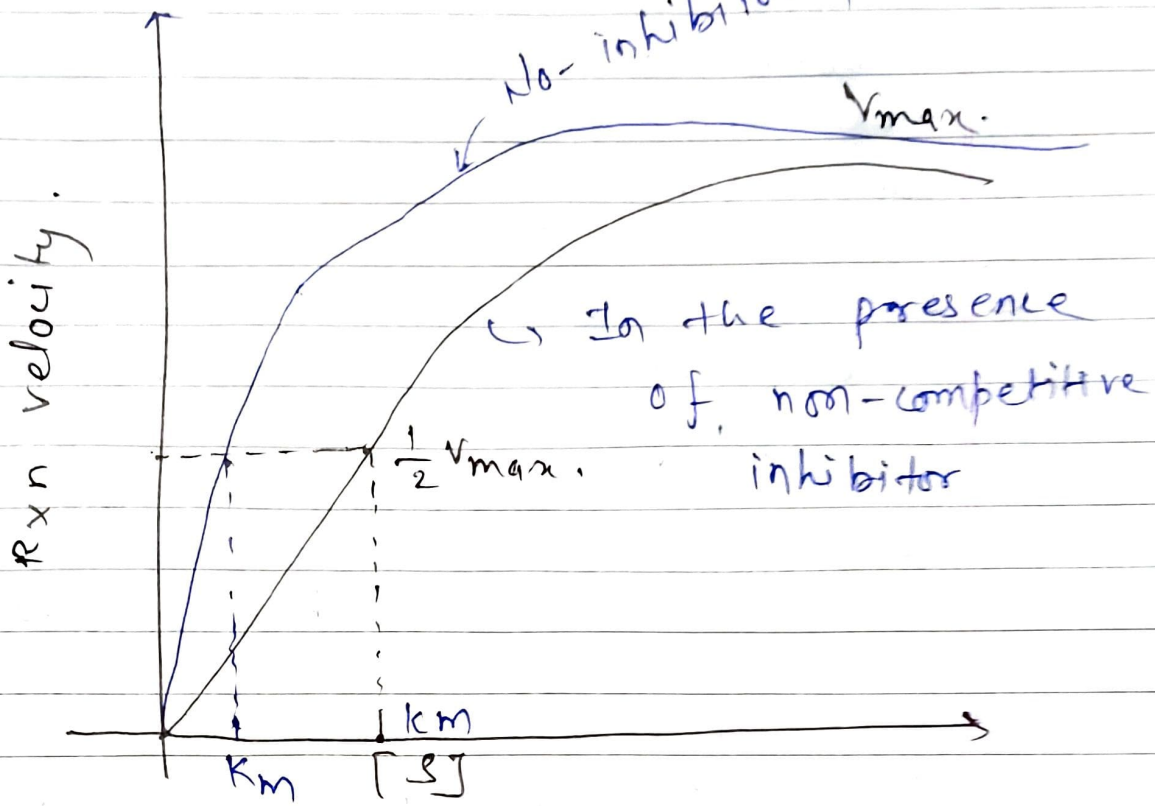
These inhibitor binds to the enzyme at some site other than the substrate binding site, thus no product is formed.

↳ Effect on Vmax:— Non-competitive inhibitors decrease the Vmax of the Rxn.

↳ Effect on Km:— Non-competitive inhibitors do not interfere with binding of substrate to enzyme.

Enzyme show same Km in presence and absence of non-competitive

inhibitors.



RNA catalysis

↳ Ribozymes are (RNA molecules that accelerate chemical Rxn). the enzyme that happen to made up RNA rather than protein.

↳ Two most important Rxn of the cells catalysed by RNA are —

① Splicing
② Viral replication.

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⇒ Application of Riboenzyme—

① Riboenzymes have been developed for the treatement of disease through gene therapy.

② Synthetic riboenzyme has been developed and entered clinical testing for HIV infection.

③ Riboenzyme have been designed to target the hepatitis C virus.

Importance of enzymes in Biology:—

① Biological uses

↳ These biological enzymes make the biochemical Rxn occur at ordinary temperature and also at quick pace.

eg—

One molecule of enzyme urease can break down 30,000 molecules of urea into CO_2 and ammonia in one second.

$$H_2C(=O)-NH_2 + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

Urea water Ammonia

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(2) Physiology

Enzymes present in stomach quickly and efficiently carry out the process of digestion. Also important for respiration, nerve pulse transmission, blood clothing, etc.

(3) Medical diagnosis

ELISA (Enzyme linked immunosorbent assay) is used for detecting disease like AIDS, Lyme disease.

(4) Medical Treatment

Enzyme Streptokinase is used for dissolving blood clot formed inside blood vessels.

(5) Genetic Engineering

Enzyme like ligases and endonucleases are used in genetic engineering.

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⇒ Some factor which enhance the activity of enzyme :—

① Cofactors :— small non-protein inorganic molecule that carries out Chemical Rxn.
eg - metal ions like iron & zince.

② Co-enzymes :— which are organic molecule that are non-proteins and mostly derivatives of vitamins soluble in water by phosphorylation.
eg - Thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD)., biotin, —etc.

③ Apo-enzyme :— Is an inactive form of enzyme taking the association of co-enzyme / cofactors.
Activation of the enzyme occurs upon binding of an organic or inorganic cofactor.

③ Holo-enzyme.

Apoenzyme + Cofactors = Holoenzyme.

eg - DNA polymerase, RNA polymerase which contain multiple protein subunits. ....