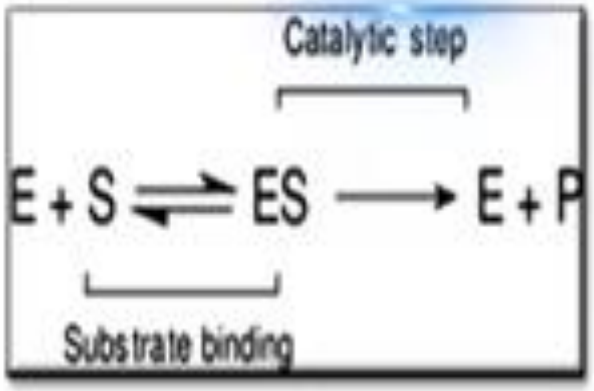
Introduction to Enzymes

An enzyme is a **biocatalyst**, which enhances the rate of thermodynamically favourable biological reactions to several thousand to million folds.

Enzymes are highly specialized catalysts with extraordinary catalytic power and also with remarkable specificity, catalysing almost all cellular reactions. Therefore they are known as the basis of life.

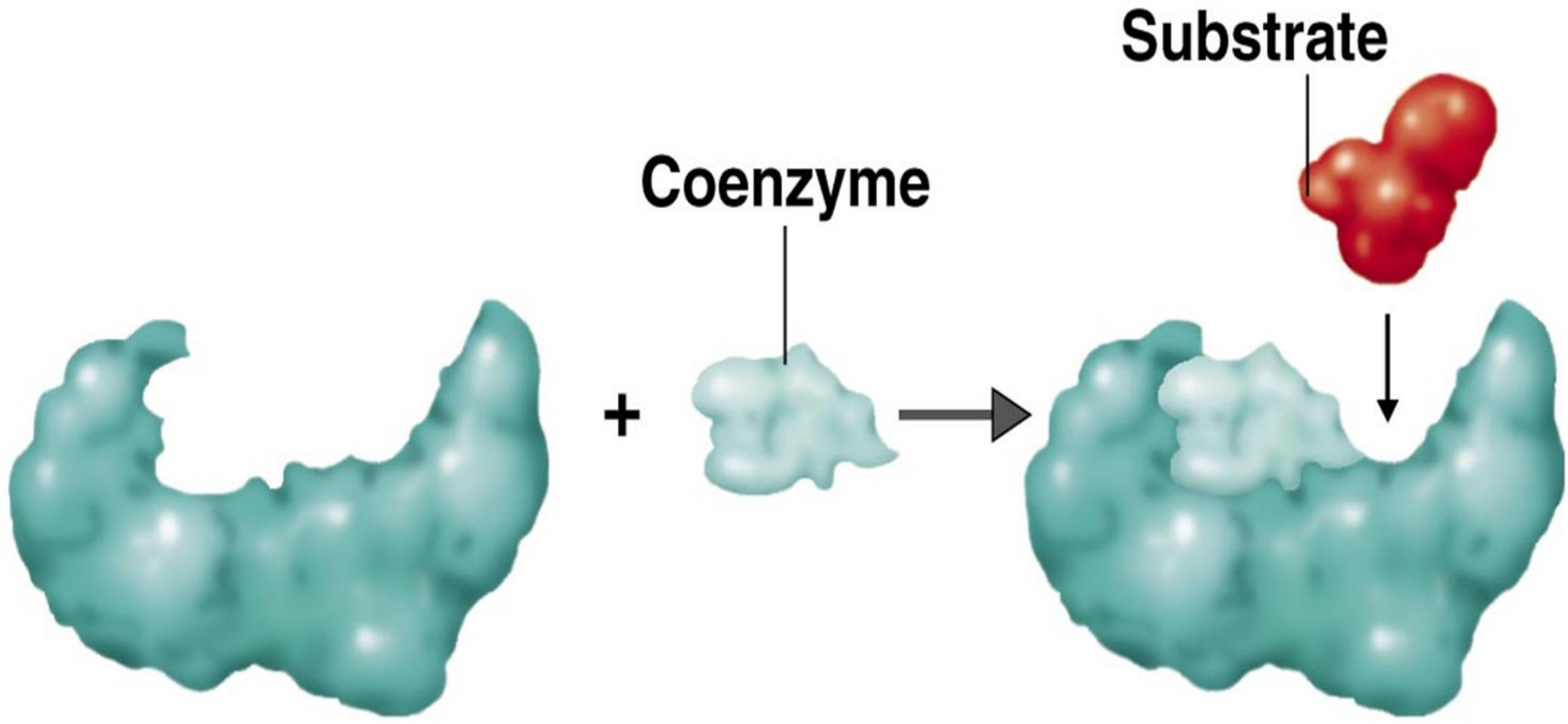
- All enzymes are proteins which act as biological catalysts.
- They catalyse the rate of biochemical reactions occurring in various vital life processes.
- The enzymes, during biocatalysis, themselves do not undergo any chemical change but are regenerated at the end of the reaction.
- The substances on which the enzymes act to yield products are called as "Substrates".



- Enzymes that are synthesised within the cell are intracellular/endoenzymes. Extracellular/exo enzymes are those secreted from the cells into the environment

They are divided into two general categories: **Simple enzymes**, which consists entirely of amino acids and **Conjugated enzymes**, contains a non-protein group called a **cofactor**, which is required for biological activity.

Removal of the cofactor from a conjugated enzyme produces a simple enzyme, called an **apoenzyme**, which generally is biologically inactive. The complete, biologically active conjugated enzyme (simple enzyme plus cofactor) is called **holoenzyme**.



Apoenzyme (protein portion), inactive

Cofactor (nonprotein portion), activator

Holoenzyme (whole enzyme), active

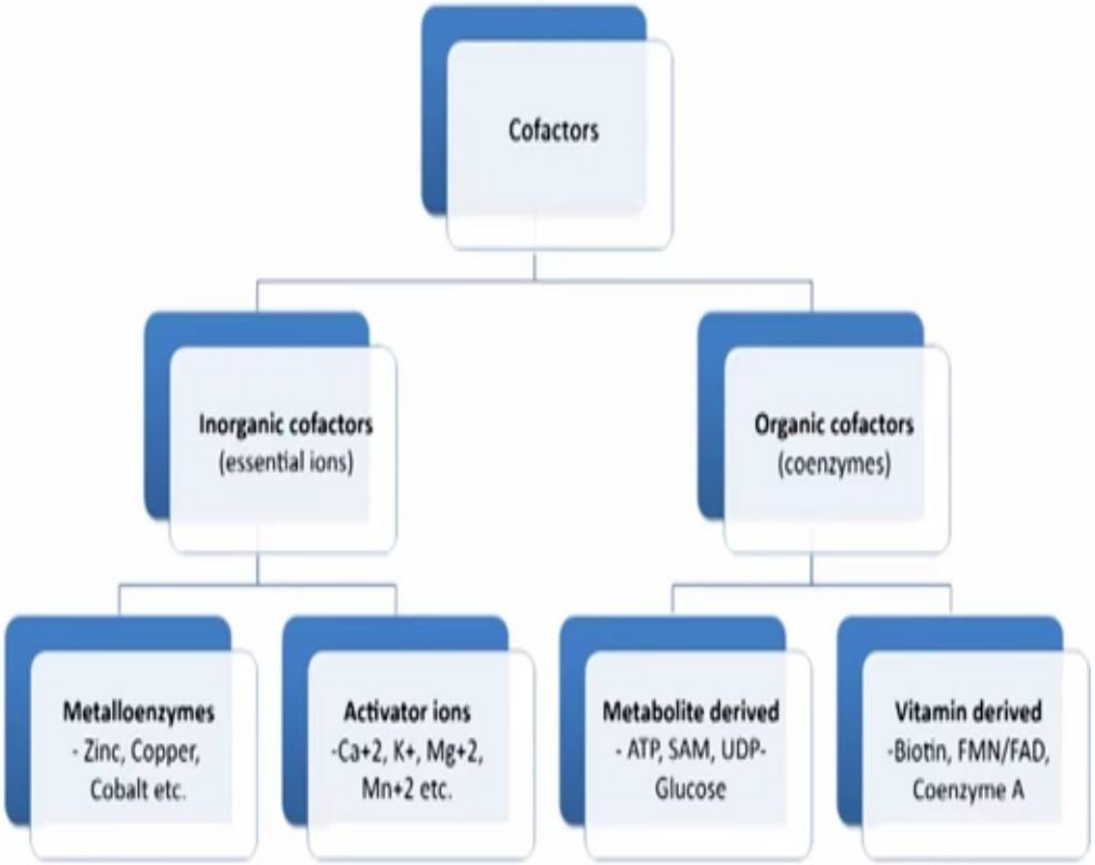
A **cofactor** can be linked to the protein portion of the enzyme either covalently or non-covalently.

Some cofactors are simple metal ions and other cofactors are complex organic groups, which are also called **coenzymes**. Cofactors which are tightly associated with the protein covalently or non-covalently are called **prosthetic group**.

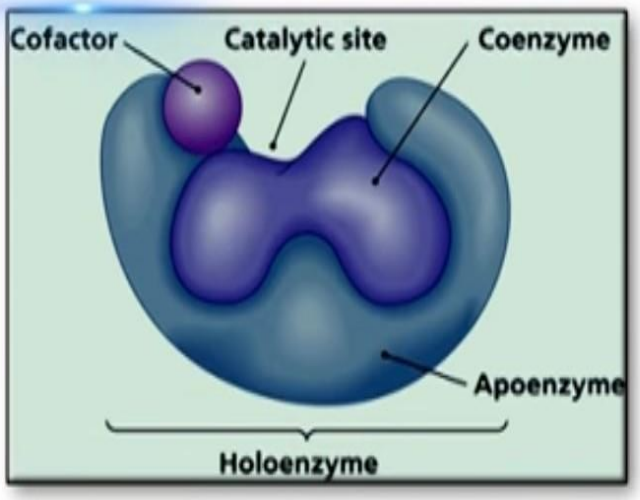
What are cofactors?

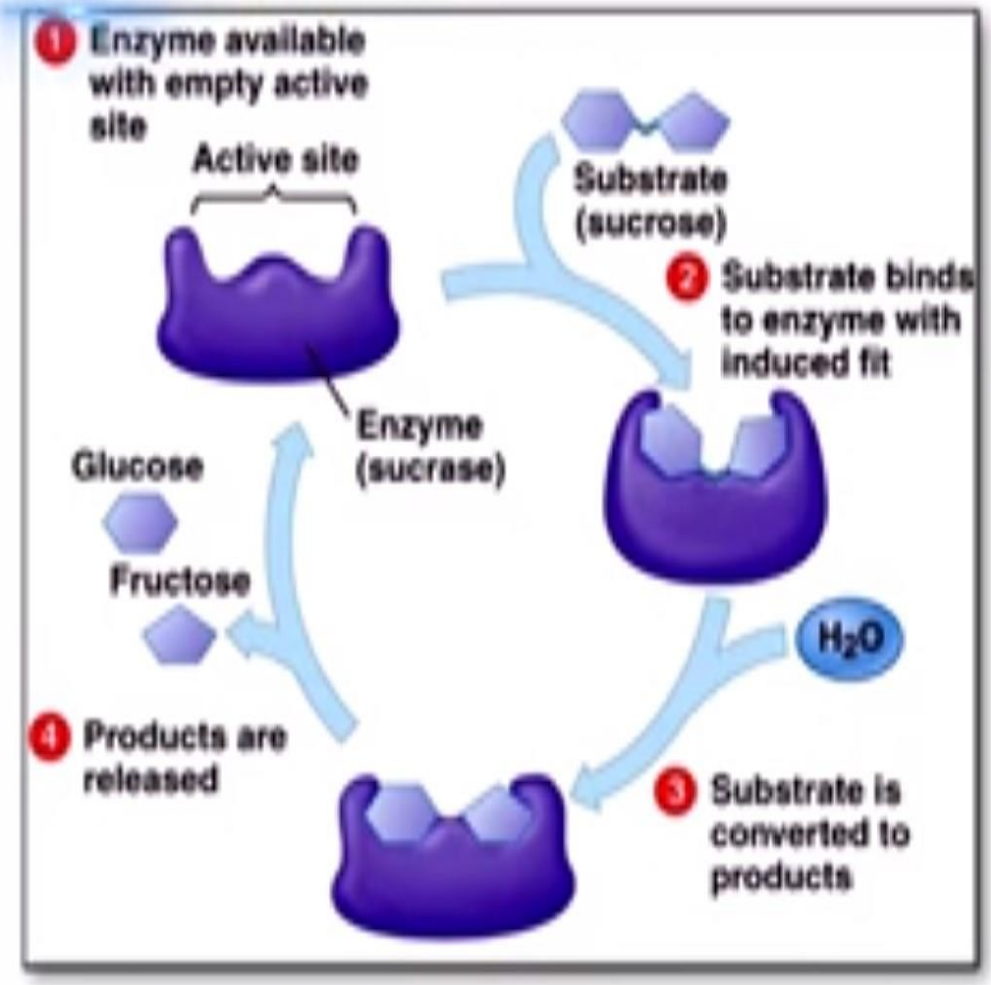
- Cofactors are the non-protein components of an enzyme.
- A cofactor may be an organic or inorganic molecule.
- An inorganic cofactor is called an essential ion, whereas, an organic cofactor is called a coenzyme.
- A cofactor required in fixed quantity for reaction to occur is called a co-substrate.

Types of cofactors



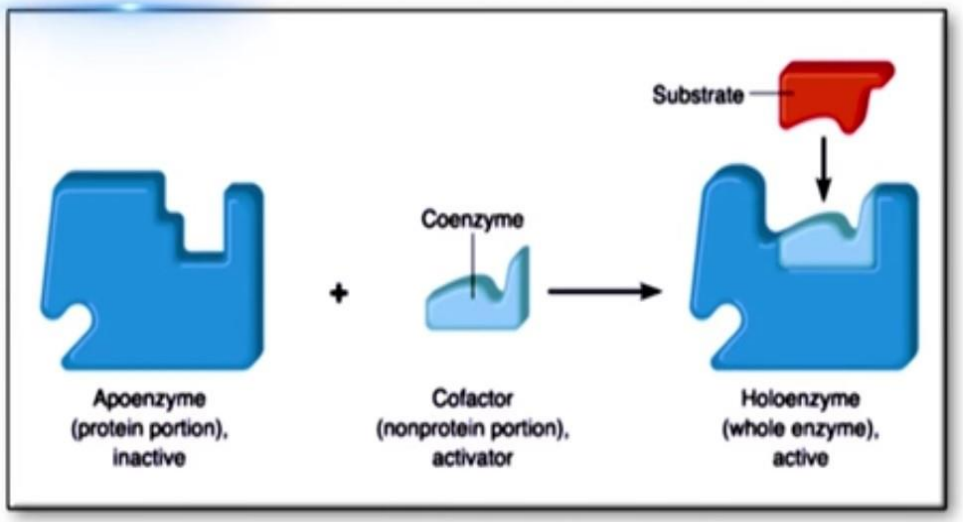
- Apoenzyme = Enzyme – Cofactor
- Holoenzyme = Enzyme + Cofactor
- A reaction requires many components in correct amount and arrangement. These components include - the enzyme, its substrate and co-substrate, the enzyme cofactor.




1 Enzyme available with empty active site
Active site
Enzyme (sucrase)
Substrate (sucrose)
2 Substrate binds to enzyme with induced fit
H_2O
3 Substrate is converted to products
4 Products are released
Glucose
Fructose

Mechanism of action

- Cofactors prepare the enzyme's active centre or catalytic site for catalysis.
- Cofactors provide additional reactive groups to the enzymes and thereby aid in catalysis.



Discovery and History of Enzymes

1833: **Payne and Persoz** found that that an alcohol precipitate of a malt extract contained a substance that converted starch into sugar. This was the first discovery of an enzyme and they named it diastase.

1850: **Louis Pasteur** observed that ferment of sugar into alcool by yeast is catalysed by ferments (later named enzymes), which are always associated with the yeast cells.

1876: **W.F. Kuhne** coined the term enzyme (Greek, which means ‘in yeast’) since the fermenting ability was associated with the yeast.

1894: **Emil Fischer** performed some classical studies on carbohydrate metabolizing enzymes in which he demonstrated the specificity shown by an enzyme for its substrate. On the basis of his experiments, Fischer proposed the **lock and key hypothesis** to describe the interaction of enzyme with substrate.

1897: **Edward Buchner** succeded in extracting the set of enzymes from the yeast cells in active form and demonstrated for the first time the conversion of sugar into alcohol in vitro.

1926: **J.B. Sumner** (Cornell University, USA) isolated, purified and also successfully crystallized the enzyme urease from jack beans. He found that the urease crystals are purely made of proteins and hence reported that enzymes are nothing but proteins. But his conclusions were opposed vehemently by the well known German biochemist **Richard Willstater**, who insisted that enzymes are nothing but low molecular weight organic compounds and the proteins crystals were found in the urease preparation could be impurities.

1930: **John Northrop and his colleagues from Rockfeller University, USA** crystallized pepsin and trypsin and found that they were also proteins crystals. Received Nobel Prize in 1935.

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

1964: **R.B. Merrifield** and his group paved the way for laboratory synthesis of enzymes for the first time (tailor made synthetic enzymes called **Synzymes.** The first enzyme which was assembled on a solid phase matrix was the Ribonuclease, which contains 124 amino acids.

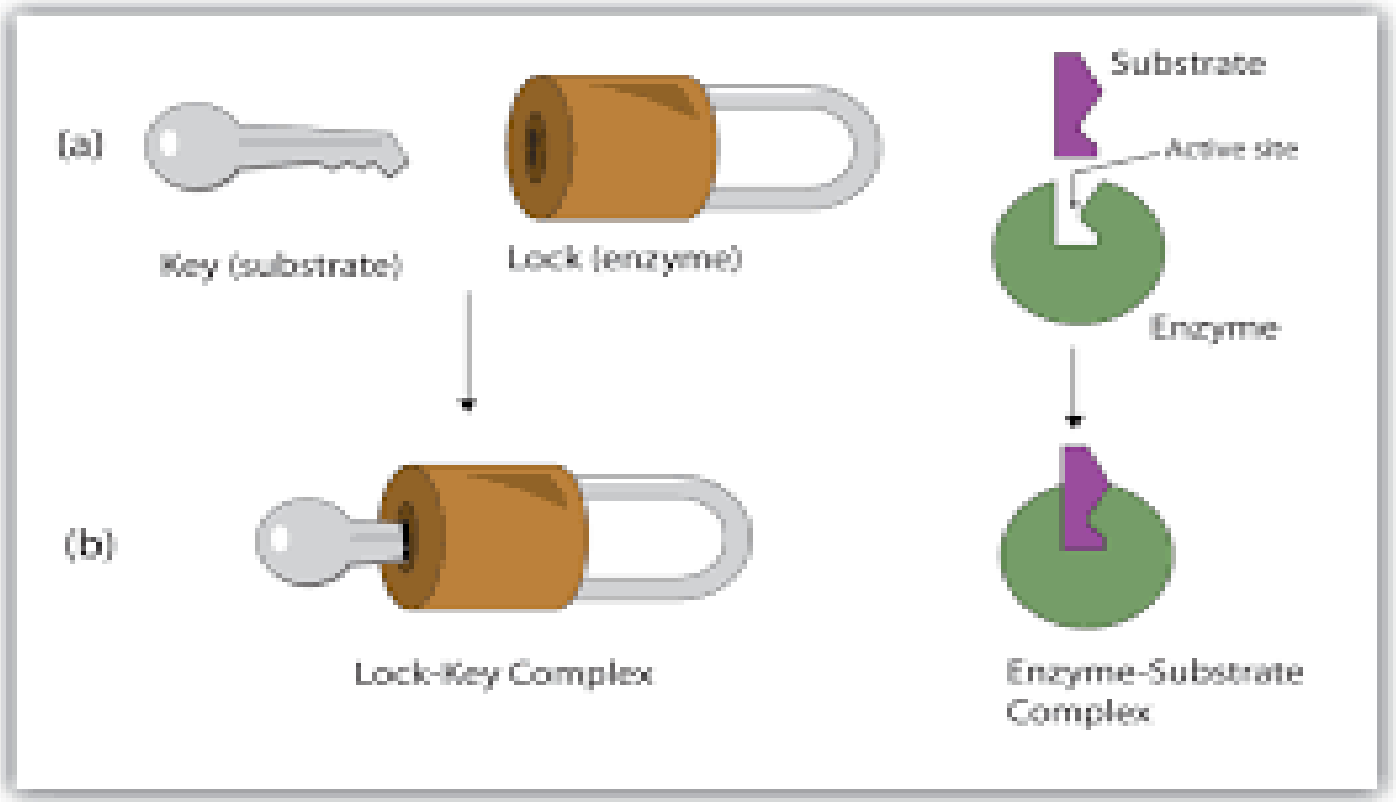
1965: Lysozyme was the first enzyme for which the X-ray structure was determined at high resolution by **David Phillips**.

1962 & 1967: **Arber and Geller** groups discovered restriction enzymes and ligases. Paved the way for the new branch of biology –Biotechnology.

1986: The belief that '**All enzymes are proteins but all proteins are not enzymes'** was shattered by **Alexander Rich and Thomas Cech's** group discovered that certain RNA molecules also exhibited catalytic properties like enzymes. Those self-splicing 'Ribonucleic acid enzymes are called **Ribozymes**.

1996: Site- directed mutagenesis technique developed by **M. Smith** for precisely manipulating the genes of any enzyme even at one nucleotide level and study its effect on the properties of the new mutant enzyme.

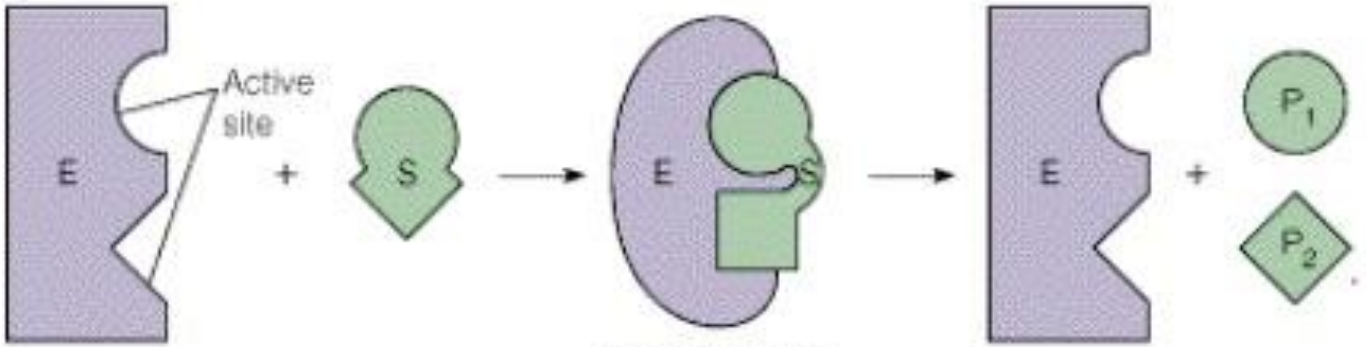
The lock and key hypothesis states that the substrate fits perfectly into the enzyme, like a lock and a key would.
This is in contrast with the induced fit hypothesis, which states that both the substrate and the enzyme will deform a little to take on a shape that allows the enzyme to bind the substrate.



Induced fit hypothesis

✓proposed in 1958 by Daniel E. Koshland, Jr.: 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

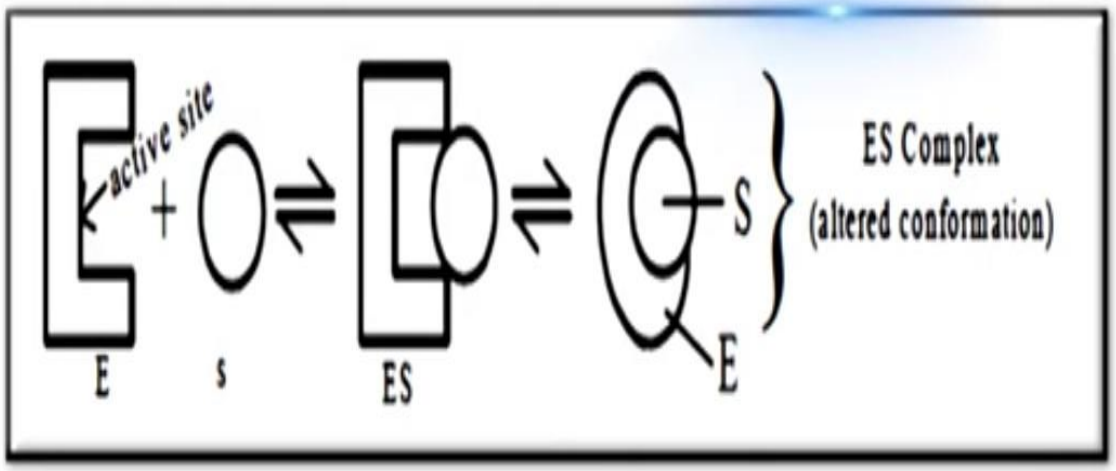


Induced fit model

Active site of enzymes

- An active site is that part of an enzyme that directly binds to a substrate and carries a reaction.

- Particular amino acids residue is present in the active sites that leads to catalytic action, and promotes the formation or degradation of bonds. These are called as 'catalytic' or 'active' amino acids or 'catalytic residues'.



Factors that influence enzyme activity

- Enzyme activity is influenced by several factors such as:
  - pH
  - Temperature
  - Substrate concentration
  - Metal ions
  - Inhibitors
  - Enzyme concentration

Effect of pH

- Each enzyme has a particular pH where its activity is maximum. This pH is known as **optimal pH.**

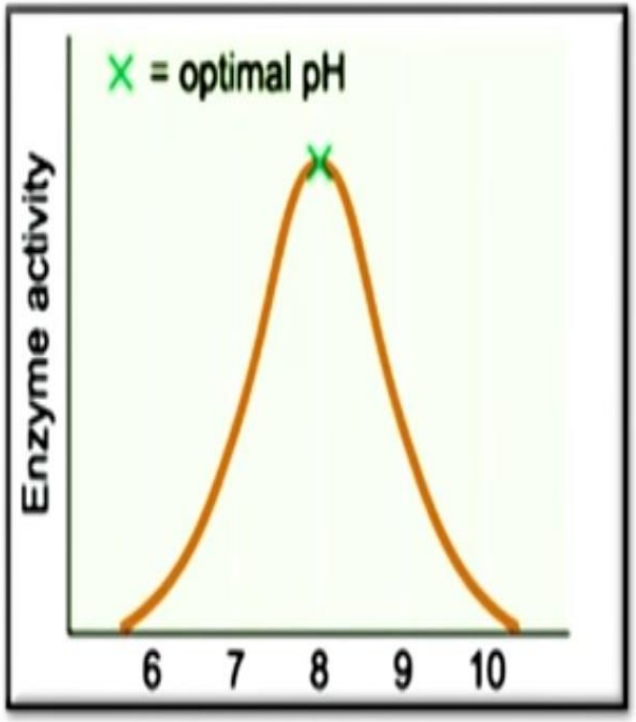


Figure 3 The pH activity relationship

Effect of Temperature

- Each enzyme also has a particular temperature at which is activity is maximum. This temperature is often referred to as the **optimum temperature.**
- The optimum temperature of most enzymes is found to be 37°C.

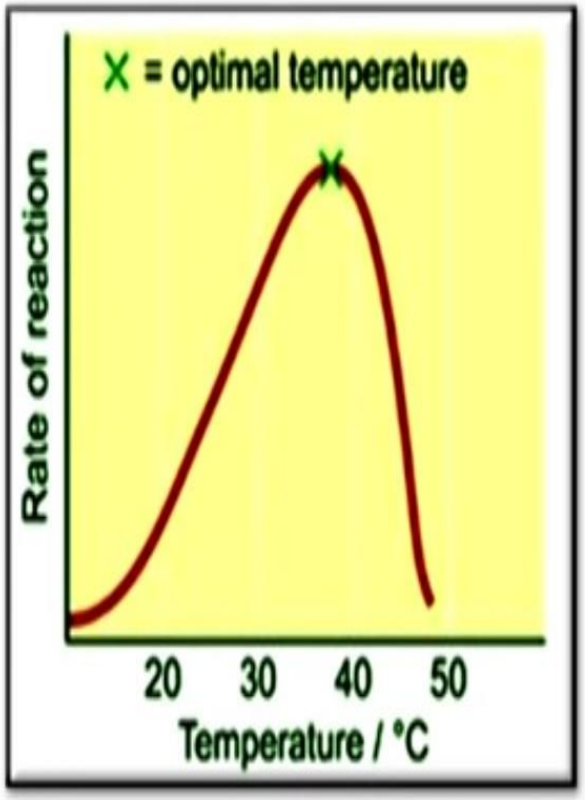


Figure 4 The temperature activity relationship

Effect of concentration of the substrate

- Rate of the reaction increases proportionally with increase in the concentration of substrate.
- At a particular substrate concentration, all active sites are saturated with substrate molecules. Further increase in concentration of substrate therefore does not lead to any increase the reaction rate

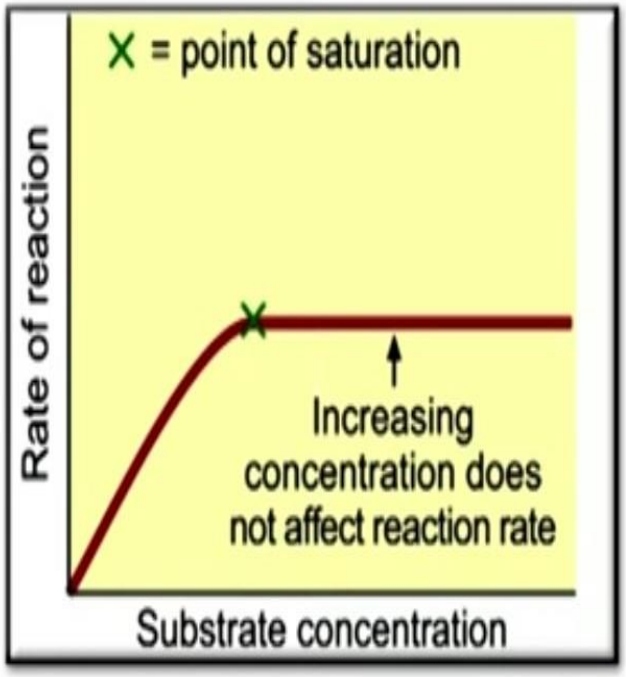


Figure 5 Substrate concentration - activity relationship

Effect of concentration of the enzyme

- Rate of an enzyme catalyzed reaction is directly proportional to the concentration of enzyme.
- At a fixed substrate concentration, all the substrate molecules are utilised completely. Even as the enzyme concentration is increased, there is no change in the reaction rate.

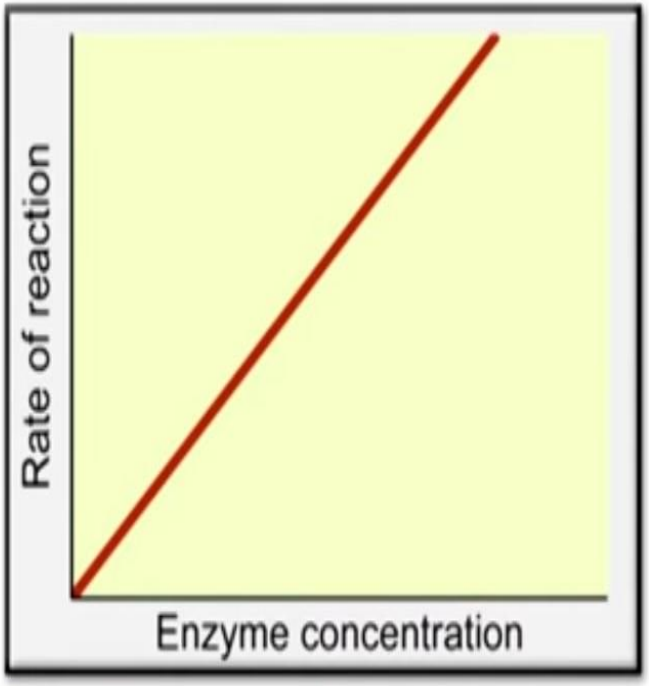


Figure 6 Enzyme concentration - activity relationship

- Enzymes which require metal ions for their activity or enzymes which contain metal ions in their structure are therefore known as **metalloenzymes.**

- Metal ions can be divalent , like Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} or monovalent such as Na^{+} and K^{+}.

- Cl^{-} ions are required for activity by amylases, Zn^{2+} ions are required by carbonic anhydrases for them to be catalytically active

Isoenzymes

- There are some enzymes that may exist in two or more forms.
- While these may differ in terms of physical, chemical as well as electrophoretic attributes, they have the same catalytic activity
- Examples of isoenzymes are lactate dehydrogenase which can exist in 5 different iso enzymic forms and catalyse the same reaction of conversion of lactate to pyruvate.