Enzyme

I. Introduction

Enzymes are biological catalysts-i. e., substances of biological origin that accelerate chemical reactions. The orderly course of metabolic processes is only possible because each cell is equipped with its own genetically determined set of enzymes. It is only this that allows coordinated sequences of reactions (metabolic pathways). Enzymes are also involved in many regulatory mechanisms that allow the metabolism to adapt to changing conditions. Almost all enzymes are proteins. However, there are also catalytically active ribonucleic acids, the *"***ribozymes***"*.

For all enzymatic processes the rate of the reaction depends upon the concentration of the enzyme and its substrates, other conditions like temperature and pH being constant.

I. Enzyme classes

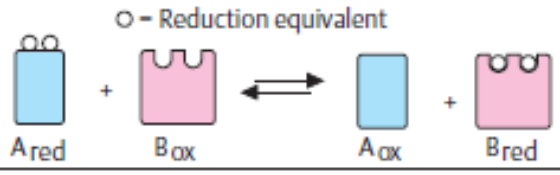
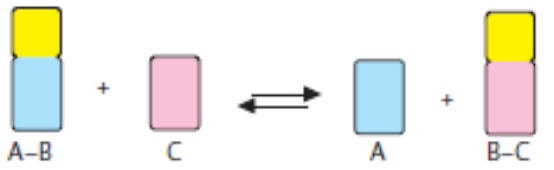
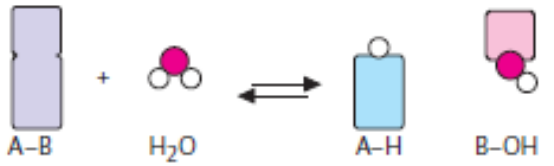
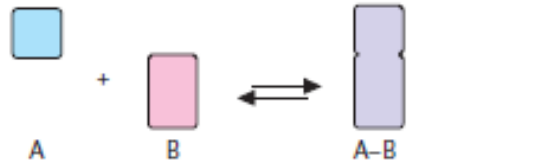
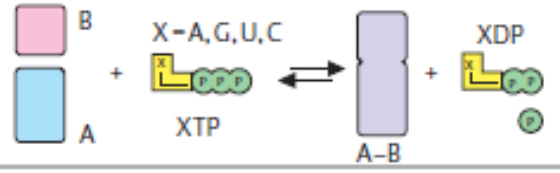
More than 2000 different enzymes are currently known. A system of classification has been developed that takes into account both their reaction specificity and their substrate specificity. Each enzyme is entered in the Enzyme Catalogue with a four-digit Enzyme Commission number (**EC number**). The first digit indicates membership of one of the six **major classes**. The next two indicate subclasses and subsubclasses. The last digit indicates where the enzyme belongs in the subsubclass. For example, lactate dehydrogenase has the EC number 1.1.1.27 (class 1, oxidoreductases; subclass 1.1, CH–OH group as electron *donor*; sub-subclass 1.1.1, NAD $(P)^+$

as electron acceptor). Enzymes with similar reaction specificities are grouped into each of the six major classes (Table 3):

1. **The oxidoreductases** (class 1) catalyze the transfer of reducing equivalents from one redox system to another.
2. **The transferases** (class 2) catalyze the transfer of other groups from one molecule to another. Oxidoreductases and transferases generally require coenzymes.
3. **The hydrolases** (class 3) are also involved in group transfer, but the acceptor is always a *water molecule.*
4. **Lyases** (class 4) often also referred to as "synthases" catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing.
5. **The isomerases** (class 5) move groups within a molecule, without changing the gross composition of the substrate.
6. **The ligases** (class 6) also referred to as "synthetases" the ligation reactions catalyzed by ligases are energy-dependent and are therefore always coupled to the hydrolysis of nucleoside triphosphates.

Table 3: Enzyme classes.

C. The enzyme classes

Class	Reaction type	Important subclasses
1 Oxidoreductases		Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases		C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases		Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")		C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases		Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")		C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

II. Theories of Active Site

In 1894, Fischer proposed that the substrate fits into the active site of the enzyme as a key fits into the lock. Because of this model, the theory is known as lock and key theory of enzyme action (Figure 13).

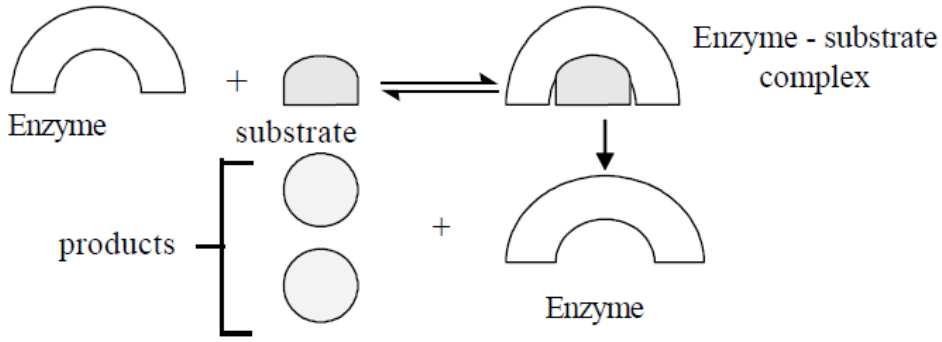


Figure 13: lock and key theory of Fischer.

According to lock and key theory, there are exact functional groups and structural features in the enzyme into which substrate molecule must fit. The region of the enzyme that complexes with the substrate is called **active site or catalytic site**. The theory cannot be applied for all the enzymatic reactions because in some reactions the substrate molecules and the active site are not structurally similar to fit in with each other. Moreover, in certain cases the catalytic activity is observed even though a fit is impossible.

Later, lock and key theory was modified by Koshland in 1963 in the form of 'induced fit mechanism'. The essential feature of this theory is the flexibility of the enzyme active site. In Fischer model, the active site is presumed to be a rigid preshaped structure to fit the substrate, while in the induced fit model the substrate induces the conformational change in the

enzyme (Figure 14), so that the substrate and active site come close to each other in such a way that the substrate fits the active site in a more convenient manner.

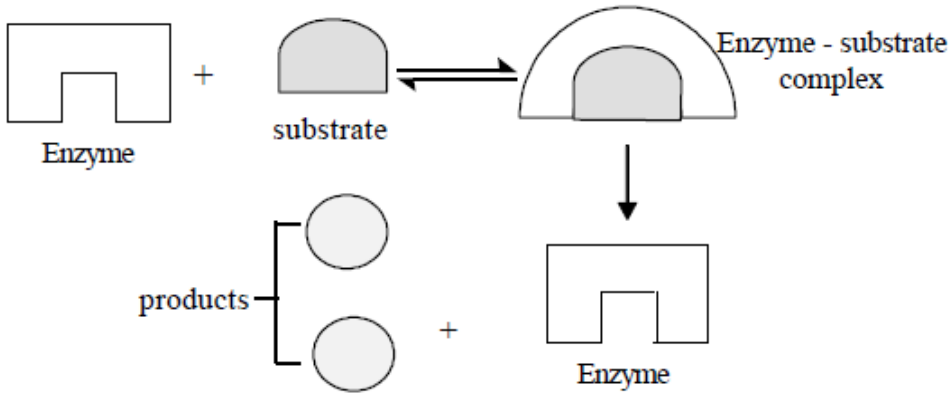


Figure 14: Induced fit model of Koshland.

The active site on the enzyme molecule exerts a binding force on the substrate molecule by hydrophilic and hydrophobic catalytic groups. Enzyme substrate complexes are formed by multiple bonding i.e., covalent, electrostatic and hydrogen bonding with the substrate. The functional groups at the active site are arranged in a definite spatial manner so that the ES complex formation is favorable.

Many enzymes require non proteinous group called as **coenzymes** for their maximal activity. The enzymes requiring coenzymes for their activity also possess sites for the attachment of coenzymes. The complexes formed in such cases are known as enzyme-substrate-coenzyme complexes.

Certain enzymes require a metal ion, in addition to coenzyme for their full activity. These metallic ions are called positive modifiers of enzyme activity. Examples of such enzymes include alcohol dehydrogenase, peroxidase, catalase and xanthine oxidase etc., which contain sites for binding metal ions. The removal of metal from these enzymes often results in partial or total loss of enzymatic activity. These enzymes are otherwise called as **metallo enzymes**. The common metallic ions required for enzymatic activity are K^+, Cu^+, Mg^{2+}, Ca^{2+} etc.

III. Enzyme Inhibitor

The rates of enzyme catalysed reactions are decreased by specific inhibitors. Inhibitors are compounds that combine with enzymes and prevent enzyme and substrate from forming ES complex. The toxicity of many compounds such as hydrogen cyanide and hydrogen sulphide results from their action as enzyme inhibitors. Many drugs also act to inhibit specific enzymes. Thus, knowledge of enzyme inhibitors is vital to understand drug action and toxic agents.

Compounds which convert the enzymes into inactive substances and then adversely affect the rate of enzyme catalysed reactions are called as enzyme inhibitors. Such a process is known as enzyme inhibition. Two broad classes of enzyme inhibitors are generally recognized. They are reversible and irreversible inhibitors. This depends on whether the inhibition can be reversed or not.

A. Reversible enzyme inhibition

A reversible enzyme inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme. Three general types of reversible inhibition is observed: competitive, noncompetitive and un-competitive, depending on the following factors.

1. Whether the inhibition is over come or not by increasing the concentration of the substrate.
2. Whether the inhibitor binds with the active site or site other than the active site (allosteric site).
3. Whether the inhibitor binds with the free enzyme only or with the enzyme substrate complex only or with either of the two.

a. Competitive Inhibition

Competitive inhibitors can combine reversibly with the active site of enzyme and compete with the substrate for binding with the active site. If the site is occupied by the inhibitor it is unavailable for the binding of the substrate (Figure 15). The competitive inhibitor always resembles the structure of the substrate. In some cases competitive inhibitors are exact structural analogues of the substrates. The combination of a competitive inhibitor (I) with enzyme (E) can be written in the same manner as combination with substrate, although the inhibitor cannot be chemically transformed to products.

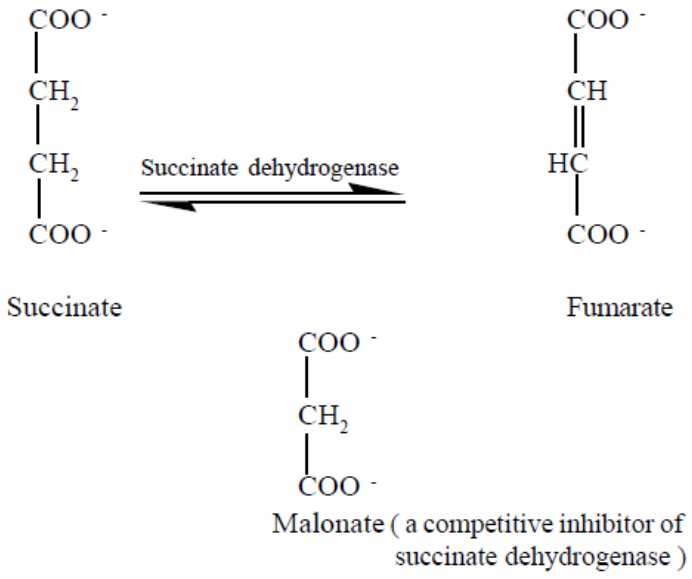
$$E + I \rightleftharpoons EI$$

$$K_i = [E]\,[I] / [EI]$$

K_i is equal to the dissociation constant for the enzyme – inhibitor complex EI.

The degree of inhibition depends upon the relative concentration of the substrate and the inhibitor. It also depends on the relative affinity of inhibitor towards enzyme active site. Thus, by increasing the substrate concentration we can decrease the degree of inhibition keeping inhibitor concentration at constant level.

The classic example is the inhibition of succinate dehydrogenase by malonate and other dicarboxylic acids. Succinate dehydrogenase is a member of the group of enzymes catalyzing the Krebs tricarboxylic acid cycle.



It catalyzes the removal of two hydrogen atoms from the two

methylene carbon atoms of succinate. Succinate dehydrogenase is inhibited by malonate, which resembles succinate in having two ionized carboxyl groups.

Many micro organisms like bacteria synthesize the vitamin folic acid from para-aminobenzoic acid. Sulphanilamide and other sulfa drugs are structural analogs of para-aminobenzoic acid. So, sulfa drugs act as competitive inhibitor and occupy the active site of some bacterial enzyme catalyzing this reaction. When this reaction is affected, it blocks the folic acid biosynthesis which is essential for the growth of micro organisms, ultimately results in the death of the micro organisms. Thus, many sulfa drugs act as antibiotics.

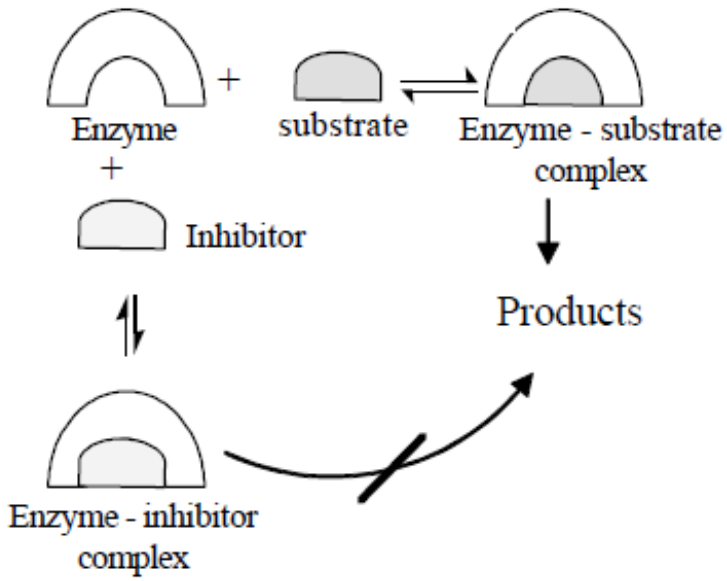


Figure 15: Competitive inhibition.

b. Un-competitive Inhibition

This type of inhibition occurs when an inhibitor combines reversibly only with ES to form ESI which cannot yield the products.

$$ES + I \rightleftharpoons ESI$$

$$K_i = [ESI] / [ES][I]$$

K_i = dissociation constant of ESI complex.

An un-competitive inhibitor also binds at an allosteric site and the binding takes place only in enzyme substrate complexes and not with the free enzyme molecule (Figure 16).

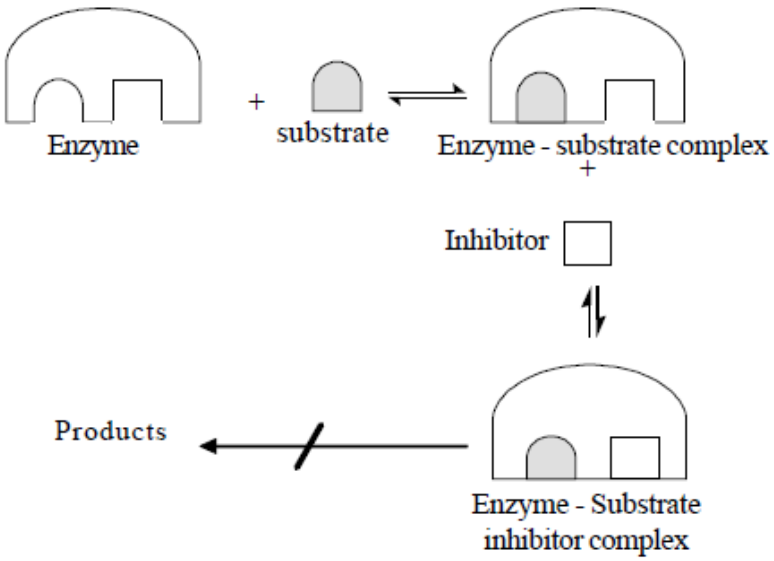


Figure 16: Uncompetitive inhibition

c. Non-competitive Inhibition

In this type of inhibition no competition occurs between the substrate and the inhibitor and the inhibitor has no structural resemblance with the

substrate and it binds with the enzyme at a place other than the active site. Since I and S may combine at different sites, formation of both EI and ESI complexes take place (Figure 17). The enzyme is inactivated when inhibitor is bound, whether the substrate is present or not. Non competitive inhibition in contrast to competitive inhibition cannot be overcome by increasing substrate concentration. For example various heavy metal ions such as Ag^{2+}, Hg^{2+}, Pb^{2+} inhibit the activity of a variety of enzymes. Urease can be inactivated by any one of these metal ions.

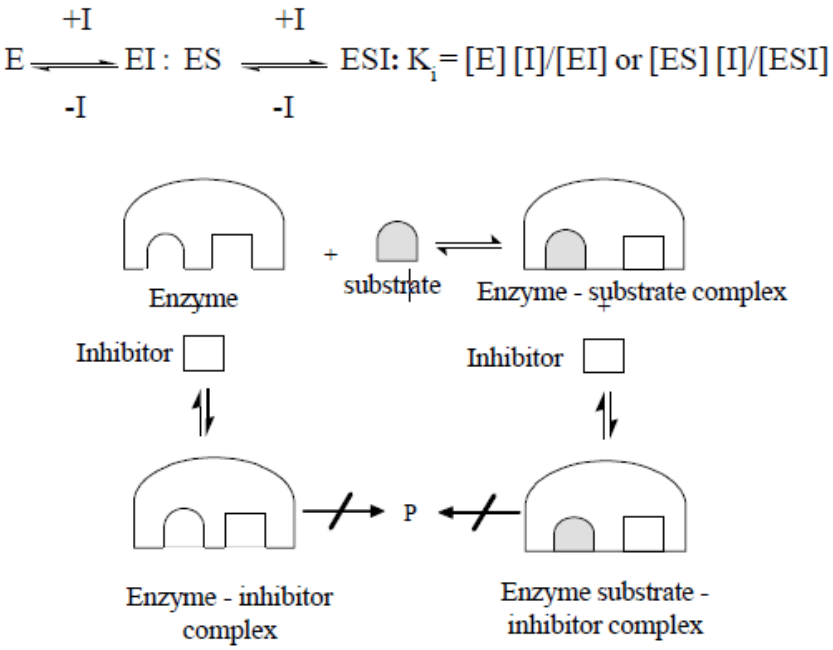


Figure 17: Noncompetitive inhibition.

B. Irreversible enzyme inhibition

Irreversible inhibitors are those that combine with or destroy a functional group on the enzyme that is essential for its activity. The irreversible inhibitor dissociates very slowly from its target enzyme because it becomes very tightly bound to its active site, thus inactivating the enzyme

molecule. The bonding between the inhibitor and the enzyme may be covalent or noncovalent.

Examples of irreversible inhibition

1. Alkylating agents such as iodoacetamide, irreversibly inhibit the catalytic activity of some enzymes by modifying cysteine and other side chains.
2. Organo phosphorous compounds such as diisopropyl phosphoflouridate are potential irreversible inhibitors of enzymes that have active seryl residues at their catalytic sites.

Study Questions

1) Compare between competitive and non-competitive Inhibition.

2) Define the following:

- Ribozymes
- Enzyme Inhibitor
- Catalytic site

3) State the enzyme classes and explain any two.

References

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