ENZYME INHIBITION (ALLOSTERIC, COVALENT MODULATION AND ISOZYMES)

Enzyme inhibition

Enzymes catalyze almost every process in the cell. The catalytic activity of certain enzymes is altered by certain inorganic and organic molecules called **modifiers**. Those molecules which increase the enzyme activity are called **activators** (Positive modifiers) and those which decrease the enzyme activity are called **inhibitors** (Negative modifiers). The terminology 'enzyme inhibition' itself means inhibition or decrease of enzymes or some processes related to its production or any other enzyme activity. During the reaction, the active sites of enzymes are filled up with these substances (inhibitors) instead of substrate molecules. Thus the activity of the enzyme is lost. The inhibitors raise the activation energy required for the reaction.

Compounds which convert the enzymes into inactive substances and thus adversely affect the rate of enzyme-catalyzed reaction are called **enzyme inhibitors**. Such a process is known as **enzyme inhibition.** Two broad classes of enzyme inhibitions are generally recognized: **Reversible** and **Irreversible**, depending on whether the enzyme-inhibitor complex dissociates rapidly or very slowly.

Irreversible inhibition results from the formation of a stable enzyme inhibitor (EI) complex which results in complete inhibition of the enzyme, eg. Inhibition of 'SH' enzyme' (Sulfhydryl group) by iodoacetamide, the inhibition of Xanthine oxidase by CN-, and inhibition of cholinesterase by nerve gases.

Reversible Inhibitor

A reversible inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme.
Reversible inhibition is of three types – **competitive**, **non-competitive** and **uncompetitive inhibition**.

i) **Competitive Inhibition**:

This type of inhibition depends on the fact that the inhibitors competes with the true substrate for the 'active site' of the enzyme. The inhibition is relieved by increasing the substrate.
The most important example of competitive inhibition is the inhibition of succinic dehydrogenase by malonic acid. The molecular structure of succinic acid is very similar to malonic acid. Because of this similarity in structure, the enzyme can react with both to form complexes. However, only the enzyme-succinic acid complex decomposes to yield a reaction product.

$$E + I \underset{}{\overset{Ki}{\rightleftharpoons}} EI$$

Where E is the enzyme, I is inhibitor (Malonic acid) Ki inhibition association constant and EI enzyme inhibitor complex.

ii) **Non-competitive inhibition**

In non-competitive inhibition, the inhibitor reacts with the enzyme to reduce catalytic activity without preventing the formation of enzyme substrate complex. The affinity of the enzyme for substrate is not reduced, but the maximum velocity of the reaction is reduced, eg. Fluoride ions inhibit enolase and thiocyanate ions inhibit fumerase. In both the cases the inhibition cannot be overcome by increasing the concentration of the substrate.

iii) **Uncompetitive Inhibition**:

In case of uncompetitive inhibition, the inhibitor is thought combine with forms of the enzyme, but they do not combine actually with the substrate (eg. Enzyme-substrate complex). These forms of enzymes are known as 'substrate non-combining' forms and cannot then be converted back into the 'substrate-combining' forms of the enzyme. This type of inhibition is not relieved by increasing the concentration of the substrate. Uncompetitive inhibition is quite common in multi-substrate reactions, but is rare in reactions involving single substrate.

Apart from these, there is another inhibition viz. **Allosteric Inhibition**. In case of allosteric inhibition, the inhibitor, is structurally quite different from the substrate. It is bound at a site other than the active site of the enzyme. This binding of the inhibitor alters the conformation of the coenzyme protein and thereby prevents it from binding to the substrate. Since the inhibitors bind at a site other than the active site of the enzyme they are called allosteric effectors or determinants and the sites to which they bind, allosteric sites (allows = other). The whole phenomenon is also called as allosteric effect or feedback inhibition and it is always reversible. The allosteric inhibition is of great physiological and biochemical importance.

(NB: Enzyme conformation refers to the specific, flexible three-dimensional shape of an enzyme, crucial for catalytic activity and substrate specificity.)

Irreversible Inhibitor

An 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 enzyme may be covalent or non-covalent in case of this type modification of enzymes which are commonly called as Regulatory enzymes also. Two general types of inhibition/ modulation are distinguished depending on two factors:

(i) Catalytic activity is modulated through the non-covalent binding of a specific metabolite at a site on the protein other than the catalytic site –**Allosteric enzyme**.

(ii) Catalytic activity is inter converted between active and inactive forms by the action of other enzymes –**Covalently modulated enzymes**.

Allosteric enzymes

The allosteric enzymes are modulated by noncovalent binding of some specific metabolite. They usually catalyze the first or the most important reaction of a multi-enzyme sequence and are generally inhibited by the end product of the sequence which binds to a specific

regulatory or allosteric [Greek word: *allos*=other; *stereos*=solid/shape] site on the enzyme molecule.

Allosteric enzymes are usually irreversible under intracellular condition. They are usually much larger in molecular weight and more complex in configuration. Some of them are unstable at zero degree C; but stable at room/body temperature.

Allosteric enzymes may have positive [stimulatory] or negative [inhibitory] modulators. Allosteric enzymes having a single modulator are called monovalent and having multi modulators are called polyvalent.

Allosteric enzymes show two different types of control – heterotropic and homotropic. Heterotropic enzymes are stimulated/inhibited by an effector (modulator) molecule other than their substrate. Homotropic enzymes are modulated by their substrate itself. However, a large number of allosteric enzymes are of mixed homo-heterotropic type.

Allosteric enzymes are much larger and complex in structure. They generally consist of more than two subunits. The catalytic sites present on subunits are different from the modulator sites subunits to bind with the substrate increases substantially. Thus, conformational change is passed to all other subunits making them more or less likely to bind with substrate in presence of activator or inhibitor, respectively. Sequential model has also been able to incorporate negative cooperativity, which did not find any provision in concerted model.

Covalent Modulation

A class of regulatory enzyme undergoes inter conversion between **active** and **inactive** forms by **covalent modification** of some specific group in the enzyme molecule by other enzymes.

An example is Glycogen Phosphorylase, which is converted into its **inactive b form** by enzymeatic hydrolysis of its phosphorylated serine residues and dissociation of its tetrameric structure into a dimeric form. The latter can be converted back into active phosphorylase **a** by enzymatic phosphorylation.

NB: Elaboration of Covalent modulation (for better understanding)

Activity of enzymes is regulated is by reversible covalent attachment of a group such as *phosphoryl*, *adenylyl*, *adenosine*, *ribosyl*, etc. to specific amino acid residues of the enzyme protein. Covalent attachment of a protein such as *ubiquitin* may also alter the activity of an enzyme. The most significant group which alters the enzyme activity on being covalently attached is phosphoryl group. Phosphoryl group is generally attached reversibly to a specific *serine*, *threonine*, or *tyrosine* residue of the enzyme protein, resulting in alteration of structural and functional properties of the molecule. Since the group carries two negative charges, phosphoryl it will attract positively charged amino acids of the molecule while repelling amino acids with negatively charged side chains. As a result, conformation of the enzyme protein is altered. Phosphoryl group bound to the enzyme protein may also influence interaction with substrate molecule. Removal of phosphoryl group reverses the effect of phosphorylation. Phosphorylation and dephosphorylation of enzyme protein are catalyzed by protein kinases and phosphoprotein phosphatases, respectively.

Phosphorylation may result in activation or inhibition of enzyme activity which will depend on a particular enzyme. However, reverse will be true on dephosphorylation of that enzyme. One example is the regulation of pyruvate dehydrogenase activity, which is a component of pyruvate dehydrogenase complex and catalyzes conversion of pyruvate to acetyl-CoA.

Phosphorylation of pyruvate dehydrogenase makes it inactive, while removal of the phosphate group restores the active enzyme. Phosphorylation and dephosphorylation of the enzyme protein are carried out by ATP-dependent pyruvate dehydrogenase kinase and phosphopyruvate dehydrogenase phosphatase, respectively. Additionally, covalently modulated enzymes include enzymes in which protein conformation changes in response to reversible reduction and oxidation of sulphur containing groups of cysteine residues, which occurs in response to the redox status of the cell. Interconversion of sulfur-containing groups of the cysteine residues between dithiol (-SH SH-) and disulfide (–S-S-) is mediated by thioredoxin.

Isozymes:

Certain enzymes which are formed by genetical changes especially by the processes which form alleles and iso-alleles, are known as isoenzymes or isozymes. Thus isoenzymes are the enzymes performing the same function, but present in more than one molecular form within the same tissue or organism.

The isoenzymes show very small differences in the molecular structure with that of original enzyme. Physically and chemically the enzymes and isoenzymes are very similar and they catalyse the same reaction. For example, the enzyme lactic acid dehydrogenase, which catalyses the reaction of pyruvate to lactate, occurs in five different forms. All forms are known as isoenzymes and can be separated by electrophoresis.

(NB: Alleles and iso-alleles represent different levels of genetic variation that result in functional or structural changes in proteins, allowing organisms to adapt their metabolism to different conditions)