ENZYME INHIBITION

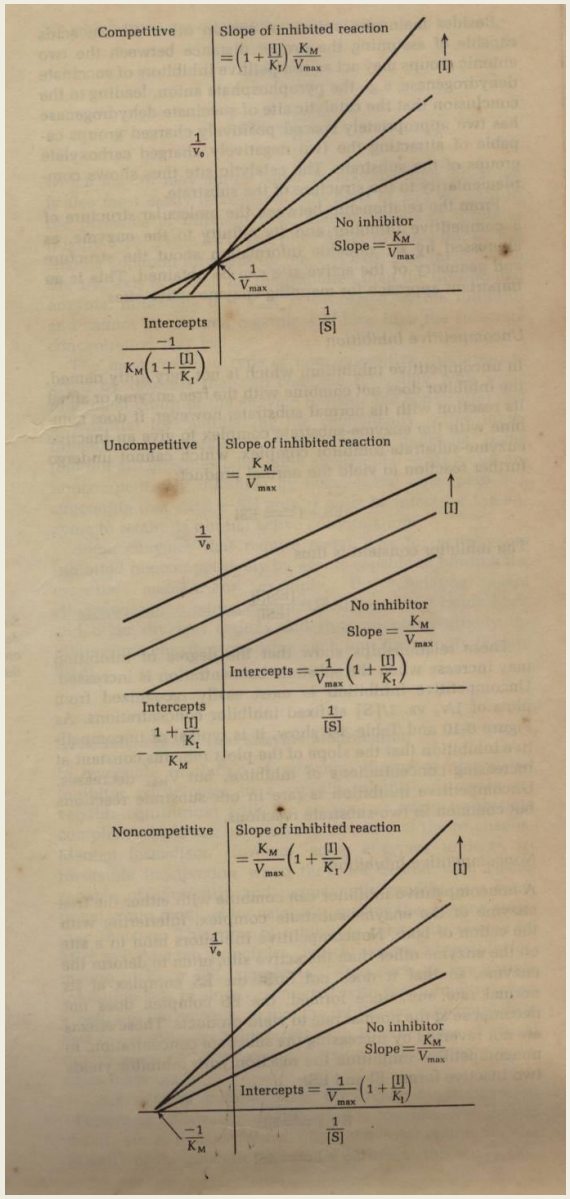
- Enzymes catalyze virtually every process in the cell. The catalytic activity of certain enzymes is altered by certain inorganic and organic molecules called modifiers. Those molecules which increase the enzyme activity are called **activators** (Positive modifiers) and those which decrease the enzyme activity are called **inhibitors** (Negative modifiers).
- 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.

Types of Enzyme Inhibitions

Reversible	Irreversible
1. Enzymes do follow Michaelis- Menten rate equation [hence Lineweaver-Burk plot also] and exhibit Rectangular hyperbolic curve when [V] is plotted against [S].	1. Enzymes usually do not follow Michaelis-Menten rate equation [hence Lineweaver-Burk plot also] and exhibit Sigmoidal curve when [V] is plotted against [S].
2. A reversible inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme.	2. 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 noncovalent in case of this type modification of enzymes which are commonly called as Regulatory enzymes also.
3. Three general types of inhibition are distinguished depending on three factors : (i) Whether the inhibition is or is not overcome by increasing the concentration of the substrate. (ii) Whether the inhibitor binds at the active site or at allosteric site. (iii) Whether the inhibitor binds with the free enzyme only, or with the enzyme-substrate complex only, or with either of the two.	3. Two general types of inhibition / modulation are distinguished depending on two factors : (i) Catalytic activity is modulated through the noncovalent binding of a specific metabolite at a site on the protein other than the catalytic site – Allosteric enzyme. (ii) Catalytic activity is interconverted between active and inactive forms by the action of other enzymes – Covalently modulated enzymes.

Competitive	Uncompetitive	Noncompetitive
1. The inhibitor can combine with the free enzyme in such a way that it competes with the normal substrate for binding at the active site. It is also called as Substrate analogue Inhibition. 2. An enzyme-inhibitor complex is formed [EI], analogous to the enzyme-substrate complex [ES]. Higher substrate concentration can lower the rate of inhibition. 3. The degree of inhibition depends on the relative concentrations of the substrate and the inhibitor. 4. Example : Enzyme – Succinate Dehydrogenase Substrate – Succinate Inhibitors – Malonate,Glutarate, Oxalate etc. 5. Kinetics : Slope $[K_m/V_{max}]$ is changed; Intercept on ordinate $[1/V_{max}]$ is not changed.	1.The inhibitor does not combine with the free enzyme or affects its reaction with its normal substrate; however, it does combine with the enzyme-substrate complex. 2. An inactive enzyme- substrate-inhibitor complex [ESI] is formed here which cannot undergo further reaction to yield the normal product. 3. The degree of inhibition may increase when the substrate concentration is increased. 4. Example : Rare in one-substrate reaction; but common in Bi-substrate reaction. 5. Kinetics : Slope $[K_m/V_{max}]$ is not changed; Intercept on ordinate $[1/V_{max}]$ is changed.	1. The inhibitor can combine with either the free enzyme or the enzyme-substrate complex, interfering with the action of both. Inhibitors bind to a site on the enzyme other than the active site. 2. Inhibitors often to deform the enzyme, so that these do not form the [ES] complex at its normal rate and once formed, the [ES] complex does not decompose at the normal rate to yield products. Two inactive complexs, [ESI] & [EI] are formed. 3. The degree of inhibition is not reversed by increasing the substrate concentration. 4. Example : Metal ion-requiring enzymes can be inhibited by chelating agents like EDTA. 5. Kinetics : Slope $[K_m /V_{max}]$ is changed; Intercept on ordinate $[1/V_{max}]$ is also changed.

Double-reciprocal plots showing the effect of competitive, uncompetitive, and noncompetitive inhibition of enzyme



IRREVERSIBLE ENZYME INHIBITION

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 multienzyme 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 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 show a typical kinetics **which do not appear to follow the classical Michaelis-Menten rate equation. Some allosteric enzymes show sigmoidal plots of [V] vs. [S], Whereas others show non-rectangular hyperbolic curves.**

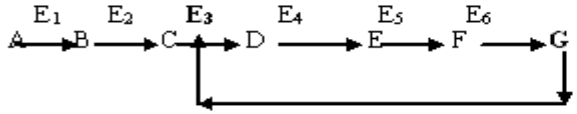
- Several models have been proposed to explain the mechanism of allosteric regulation.

- The **symmetry model** proposes that the allosteric enzyme molecule occurs in only one of two possible conformations, active and inactive[J.Monod et al.].

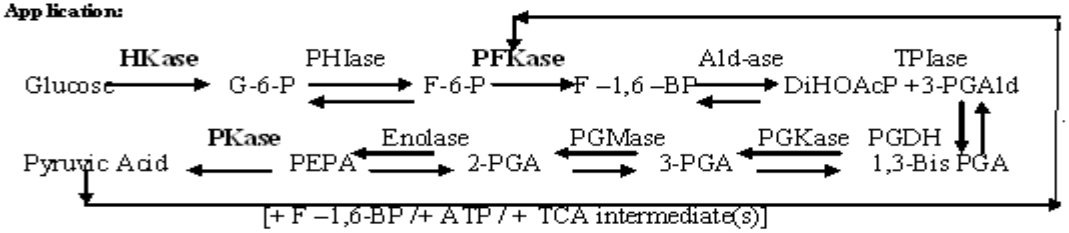
- The **sequential model** postulates that the subunit changes their conformation in sequence, not simultaneously, so intermediate states of differing catalytic activity occur [D.E. Koshland].

Allosteric inhibition

Scheme:



Application:

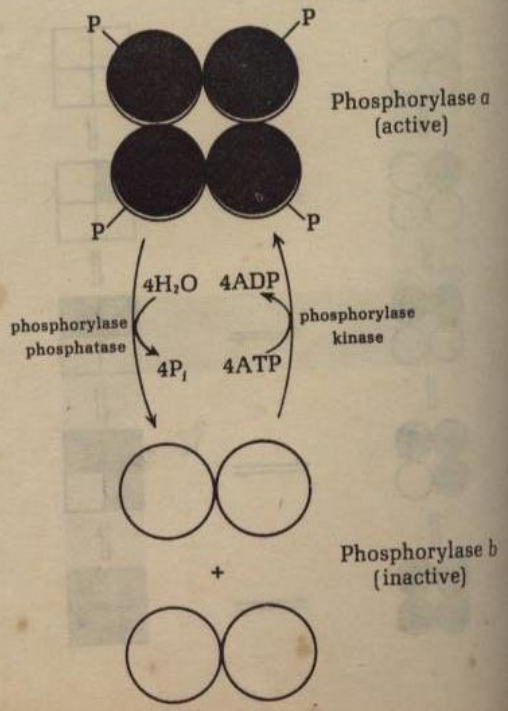


Covalently Modulated Regulatory Enzymes

- A class of regulatory enzyme undergoes interconversion 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 enzymatic 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.

Modulation of glycogen phosphorylase activity by covalent modification. The active form of the enzyme, phosphorylase a, a tetramer, is enzymatically dephosphorylated by phosphorylase phosphatase to yield the inactive phosphorylase b, a dimer. By the action of phosphorylase kinase, phosphorylase a is regenerated by the phosphorylation of four specific serine residues at the expense of ATP.



Covalently modulated regulatory enzyme