Regulation of enzymatic activity

More than one enzymes are involved in metabolic pathways and either one or more enzyme can increase or decrease the rate of reaction in response to certain signals and therefore adjust the rate of the entire metabolic sequences. In this way the regulatory enzymes allow the cell to meet changing needs for energy and for biomolecules required in growth and repair.

The regulation of enzymes occurs in following ways

1. **Allosteric regulation (Activation and Inhibition)**
2. **Activation of latent enzymes**
3. **Compartmentation of metabolic pathways**
4. **Control of enzyme synthesis**
5. **Enzyme degradation**
6. **Isoenzymes**

1. **Allosteric Regulation**

One of the enzymes possess additional sites, where certain substances binds and modulate the catalytic activity of the enzymes. Such substances are called **allosteric modulators** or **allosteric effectors** (small metabolites and cofactors) and the enzymes that undergo modulation is called **allosteric enzymes**.

- The modulators for allosteric enzymes may be inhibitory or stimulatory.
- Regulatory enzymes for which substrate and modulator are identical are called **homotropic** enzymes, their effect is always positive.
- When the modulator is a molecule other than the substrate, the enzymes is said to be **heterotropic,** their effect is either positive or negative**.**
- Allosteric enzymes generally have one or more regulatory or allosteric, sites for binding the modulator.
- Each regulatory site is specific for its modulator.

Classes of allosteric enzymes

Enzymes that are regulated by allosteric mechanism are referred to as allosteric enzymes. They are divided into two classes based on the influence of allosteric effector on **Km** and **Vmax**.

K-class of allosteric enzymes.

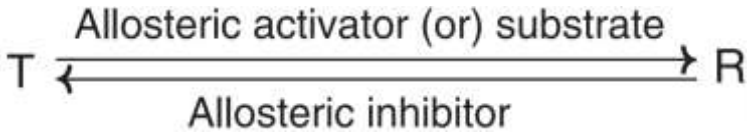
The effector **changes the Km** and not the Vmax. Double reciprocal plots, similar to competitive inhibition are obtained e.g., phosphofructokinase.

V-class of allosteric enzymes

The effector **alters the Vmax** and not the Km. Double reciprocal plots resemble that of non-competitive inhibition e.g., acetyl CoA carboxylase.

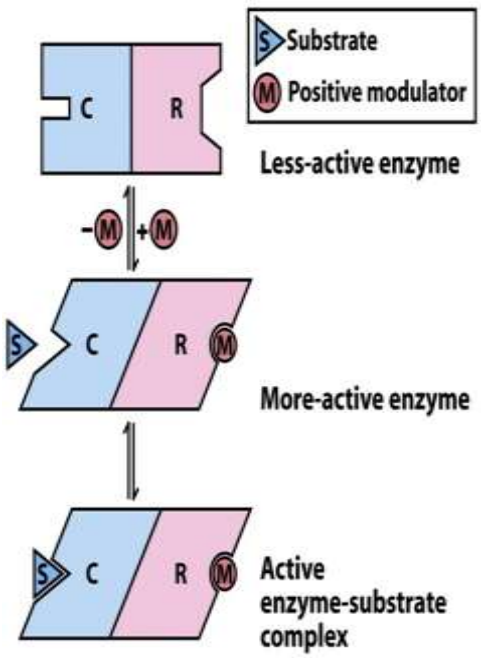
Allosteric effectors bind reversibly and non-covalently to allosteric site of the allosteric enzymes and brings about a conformational change in the active site of the enzyme. Binding of the allosteric effectors leads to inhibition or activation of the catalytic activity.

The allosteric enzymes exist in two conformational states – the T (tense or taut) and the R (relaxed). The T and R states are in equilibrium.



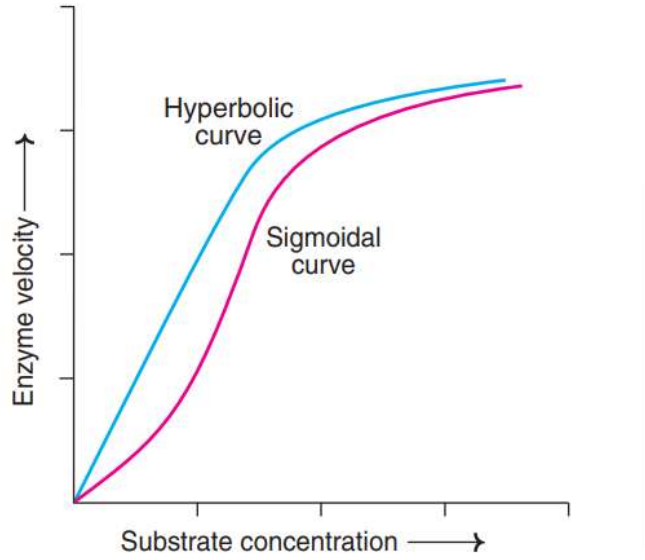
Allosteric inhibitors favour **T** state whereas activators and substrates favour **R** state. The substrate can bind only with the R form of the enzyme. The concentration of enzyme molecule in the R state increases as more substrate is added, therefore the binding of the substrate to the allosteric enzyme is said to be cooperative.

Subunit interactions in an allosteric enzyme, and interactions with inhibitors and activator. In many allosteric enzymes the substrate binding site and the modulator binding site(s) are on different subunits, the catalytic (C) and regulatory (R) subunits, respectively. Binding of the positive (stimulatory) modulator (M) to its specific site on the regulatory subunit is communicated to the catalytic subunit through a conformational change. This change renders the catalytic subunit active and capable of binding the substrate (S) with higher affinity. On dissociation of the modulator from the regulatory subunit, the enzyme reverts to its inactive or less active form.



The Kinetic properties of Allosteric enzymes diverge from Miehaelis-menten Behaviour

The relationship between V_0 and the [S] of Allosteric enzymes is different from the Michealis-Menten kinetics. Allosteric enzymes also exhibit saturation when substrate concentration is sufficiently high but, they produce **sigmoidal saturation curve** rather than producing hyperbolic curve. On the sigmoidal saturation curve we can find the value of substrate concentration when the V_0 is half but we cannot refer it *K*m, because the enzyme does not follow the hyperbolic Michaelis-Menten relationship. Sigmoid kinetic behaviour generally reflects cooperative interactions between protein subunits.

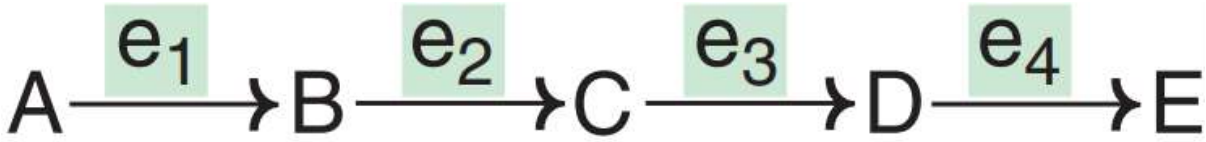


Effect of substrate concentration on allosteric enzyme (red line-sigmoidal curve) in comparison with normal enzyme (blue line-hyperbolic curve).

Feedback regulation

Feedback inhibition or end product inhibition is a specialised type of allosteric inhibition necessary to control metabolic pathways for efficient cellular function. In the process of **feedback inhibition**, the end product of a metabolic pathway acts on the key enzyme regulating entry to that pathway, keeping more of the end product from being produced.

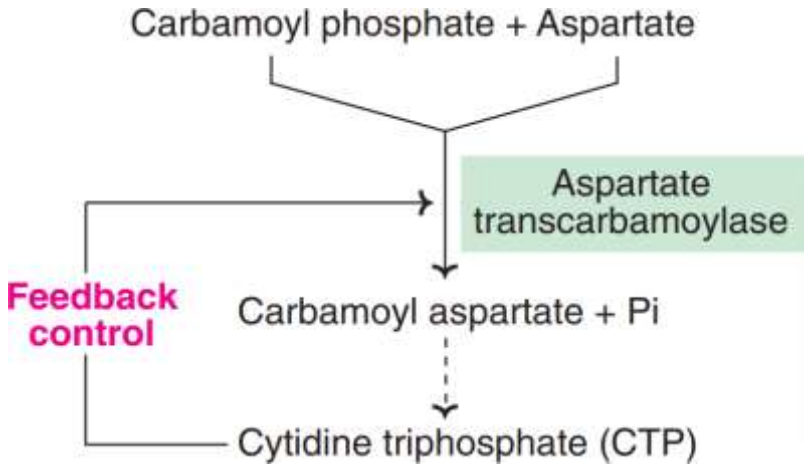
The process of inhibiting the first step by the final product, in a series of enzyme catalysed reactions of a metabolic pathway is referred to as feedback regulation. Look at the series of reactions given below



A is the initial substrate, B, C, and D are the intermediates and E is the end product, in a pathway catalysed by four different enzymes (e1, e2, e3, e4). The very first step (A or B by the enzyme e1) is the most effective for regulating the pathway, by the final end product E. This type of control is often called negative feedback regulation since increased levels of end product will result in its (e1) decreased synthesis.

This is a real cellular economy to save the cell from the wasteful expenditure of synthesizing a compound which is already available within the cell.

Aspartate transcarbamoylase (ATCase) is a good example of an allosteric enzyme inhibited by a feedback mechanism. ATCase catalyses the very first reaction in pyrimidine biosynthesis. Carbamoyl phosphate undergoes a sequence of reactions for synthesis of the end product, CTP. When CTP accumulates, it allosterically inhibits the enzyme aspartate transcarbamoylase by a feedback mechanism.



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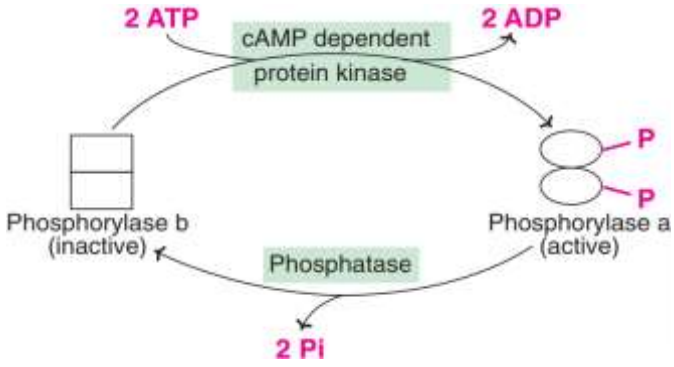
2. Activation of latent enzymes

Latent enzymes are inactive in the beginning but they are activated by certain modifications like break down of one or more peptide bonds (e.g. chymotrypsinogen, pepsinogen and

plasminogen), phosphorylation and dephosphorylation of specific amino acid residues, oxidation and reduction of disulphide bonds (reversible covalent modification).

Example 1. Proenzymes –namely chymotrypsinogen, pepsinogen and plasminogen, are respectively – converted to the active enzymes chymotrypsin, pepsin and plasmin by the breakdown of one or more peptide bonds.

Example 2. Glycogen phosphorylase is a muscle enzyme that breaks down glycogen to provide energy. This enzyme is a homodimer (two identical subunits) and exists in two interconvertible forms. Phosphorylase b (dephospho enzyme) is inactive which is converted by phosphorylation of serine residues to active form phosphorylase A.



Example 3. Glycogen synthase, acetyl CoA carboxylase, HMG CoA reductase are active in dephosphorylated state and become inactive when phosphorylated.

3. Compartmentation

Enzymes are often stored in a compartment for example in a particular organelle. Enzymes are compartmentalized because they are needed for specific processes at a place where they act. By compartmentalization they can readily find their substrate. For example lysosomal enzymes are found in lysosome. The pH of the lysosome is 5 which is best for the activity of the lysosomal enzymes whereas the pH of the cytosol is not suitable for the activity of lysosomal enzymes.

4. Control of Enzyme Synthesis

The synthesis of the enzymes are regulated by the genes by their induction and repression. The process by which the genes are induced to synthesize the enzyme is called induction and by repression the synthesis of enzymes are repressed by the genes.

5. Enzyme degradation

In general, the key and regulatory enzymes are most rapidly degraded. If not needed, they immediately disappear and, when required, they are quickly synthesized. Though not always true, an enzyme with long half-life is usually inactive in its catalytic activity.

6. Isoenzymes

Isozymes are defined as multiple molecular forms of an enzyme demonstrating similar or identical catalytic properties. Multiple forms of the same enzyme will also help in the regulation of enzyme activity, many of the isoenzymes are tissue-specific. Although isoenzymes of a given enzyme catalyse the same reaction, they differ in Km, *Vmax* or both. e.g. isoenzymes of LDH and CPK.