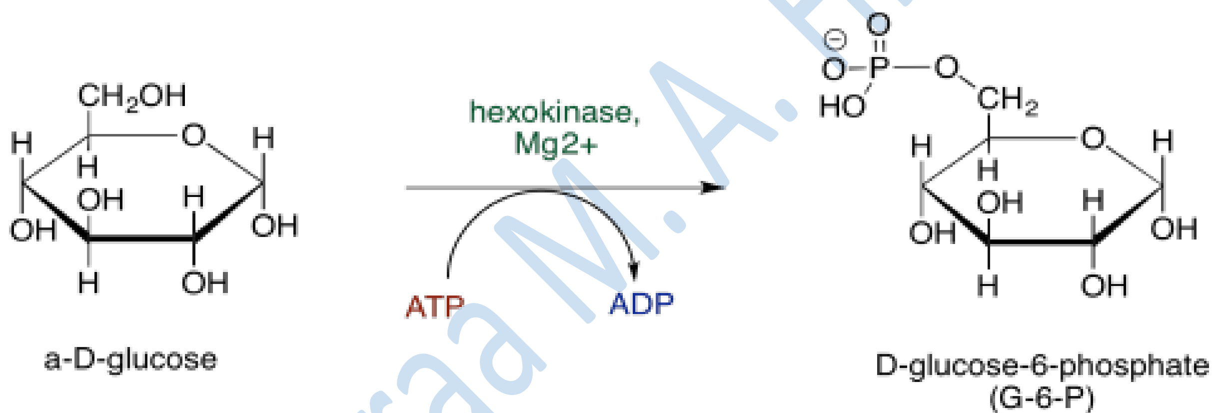
Regulation of Enzyme Activity:

Regulation of enzyme activity is essential for metabolism

1- Substrate-Level Control

The substrates and products of each enzyme-catalyzed reaction interact directly with the enzyme to regulate it.
A kinetics study shows that increased substrate concentrations accelerate reactions until enzyme saturation.

As an example, consider the first step in glycolysis (see Chapter 13)—the phosphorylation of glucose to yield glucose-6-phosphate (G6P):



Hexokinase catalyzes this reaction and is inhibited by its product, glucose-6-phosphate (G6P). If any subsequent steps in glycolysis are blocked, G6P will accumulate and bind to hexokinase. This results in the inhibition of hexokinase and slows down further production of G6P from glucose.

In many cases, the reaction product binds the enzyme's active site and acts as a competitive inhibitor. Hexokinase is an interesting example because the product (G6P) can act as a competitive inhibitor (by binding to the enzyme's active site) and an uncompetitive inhibitor (by binding at another site on the enzyme).

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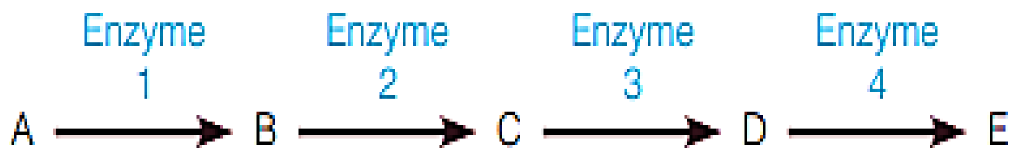
2- Feedback Control

Feedback control is important in the efficient regulation of complex metabolic pathways.

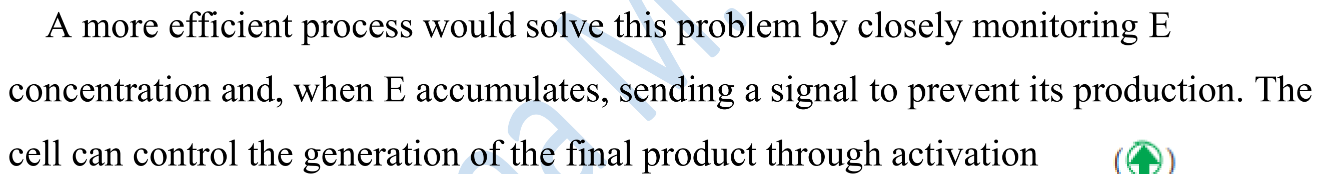
The metabolic pathways resemble assembly lines. The simplest metabolic assembly line looks like this:



Where **A** represents the initial reactant or raw material (crud), **B**, **C**, and **D** represent intermediate products, and **E** represents the final product.

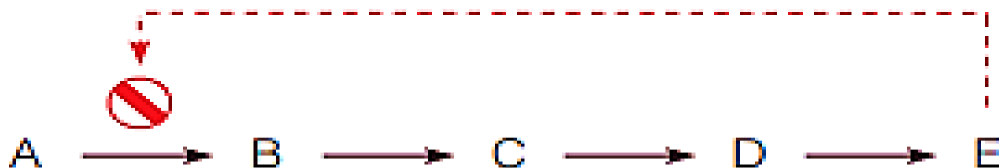
This pathway's end product **(E)** may be employed in another path, and **A** may be used in other processes. Consider that E's utilization slows. As previously pathway, E would accumulate, and A would be consumed. But this process is inefficient.

A more efficient process would solve this problem by closely monitoring E concentration and, when E accumulates, sending a signal to prevent its production. The cell can control the generation of the final product through activation



or inhibition of a critical step in the pathway.

It would be most efficient to slow down the first step, converting A to B ($A \rightarrow B$). So, the "machine" should be regulated by the concentration of E.

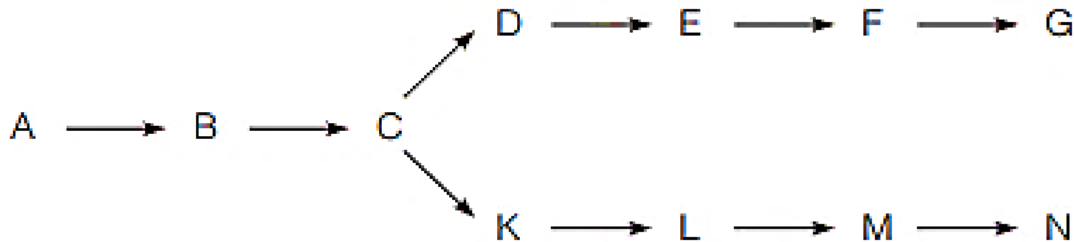


This type of feedback control is called feedback inhibition because an increase in the concentration of E leads to a decrease in its production rate.

Note that inhibiting the first step prevents unwanted utilization of A and accumulation of E.

3- Substrate is fed into two pathways

Other metabolic situations require more complicated patterns in which activation and inhibition may be useful. For example, consider a slightly more complex case in which ***A is fed into two pathways***, which leads to two products needed in roughly equivalent amounts. Then, a scheme like the following emerges:



To control the pathways so that **G and N** keep in balance, high concentrations of G might inhibit the C → D enzyme or activate C → K enzyme. Conversely, high concentrations of N might inhibit C → K enzyme or activate the C → D enzyme.

An example of this kind of control is found in the synthesis of the purine and pyrimidine monomers that go into making DNA, because approximately equal quantities of all four deoxyribonucleotides are required for DNA replication.

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4- Allosteric Enzyme

Allosteric enzymes show cooperative substrate binding and can respond to a variety of inhibitors and activators

A special class of enzymes capable of allosteric regulation. The term **allosteric** is derived from **Greek words** meaning **"other structure,"** that regulator structures can be very different from either the substrate or the direct product.

Allosteric enzymes are frequently multisubunit proteins with multiple active sites. They exhibit **cooperativity** in substrate binding (**homoallostery**) and regulation of their activity by other **effector** molecules (**heteroallostery**).

An example of allosteric control of protein function. It is a hemoglobin. It is a four-subunit protein with four binding sites for its **"substrate,"** oxygen. Oxygen **"substrate"** binding is **cooperative** and is influenced by other molecules and ions. The basic ideas presented for the analysis of hemoglobin function apply equally well to allosteric enzymes.

Cooperativity

Cooperativity: If an enzyme (protein) has more than one ligand-binding site, there is a possibility that these sites will interact with each other through the binding process. This is termed **cooperativity.**

Cooperativity includes:

1- Positive cooperativity: Occur when the binding of one molecule of a substrate of ligand **increases the affinity of the enzyme (protein) for other molecules of the same or different substrate or ligand**.

If the binding of the first molecule of the substrate increases the affinity of the enzyme for the substrate, the second step of the binding process will be faster than it is in a situation where there is no interaction between the binding sites.

2- Negative cooperativity: Occurs when binding one molecule of a substrate or ligand **decreases the affinity of the enzyme (protein) for other molecules of the**

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same or different substrate or ligand. The second step of the binding process is slower than if there were no interactions between the binding sites.

3- Homotropic cooperativity: This occurs when the binding of one molecule of **a substrate affects the binding to the enzyme (protein) of subsequent molecules of the same substrate or ligand** (i.e., the binding of one molecule of A affects the binding of further molecules of A).

4- Heterotropic cooperativity: Occurs when the binding of **one substrate molecule influences the binding of a different substrate or ligand to the enzyme** (i.e., the binding of one molecule of A impacts the binding of B).

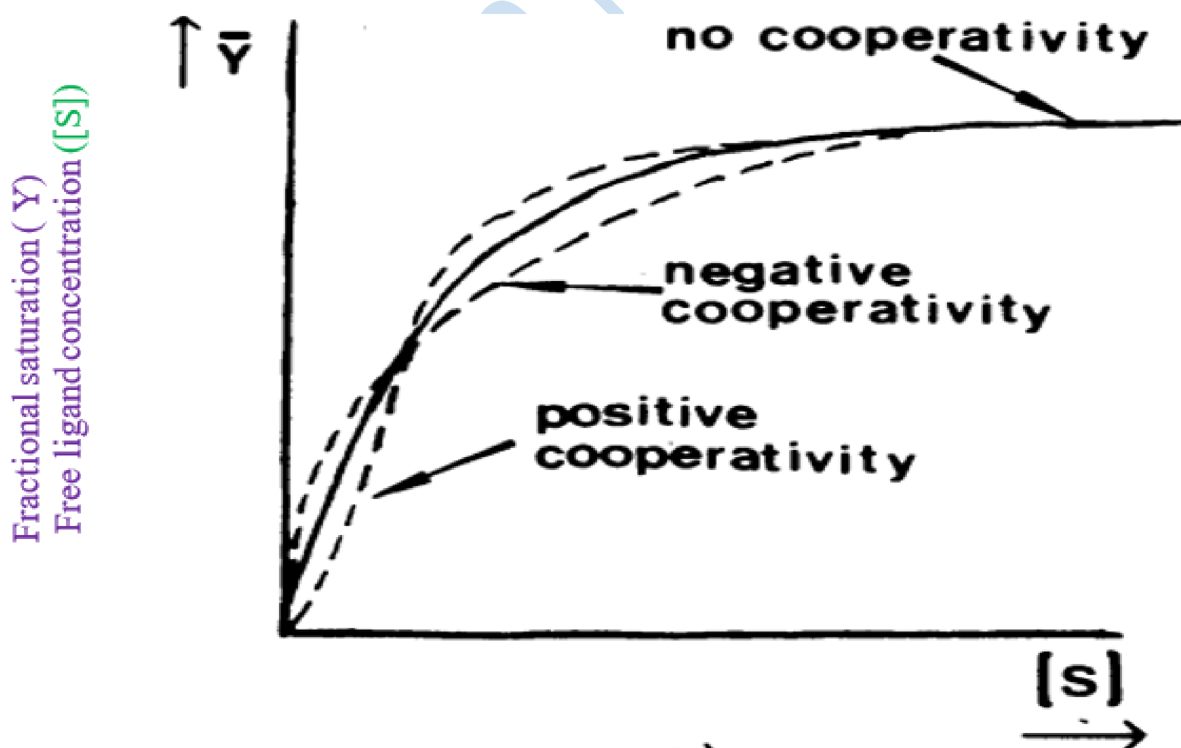
Cooperative effects may be positive and homotropic, positive and heterotropic, negative and homotropic or negative and heterotropic.

Allosteric inhibition (section 8.2.7) is an example of negative heterotropic cooperativity

and

allosteric activation an example of positive heterotropic cooperativity.

As shown in Figure bellow:



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K-series enzymes and V-series enzymes.

Allosteric-controlled enzymes can be classified into **K-series enzymes** and **V-series enzymes.**

K-series and V-series enzymes are two classifications of enzymes based on their kinetics and the nature of their inhibition.

1- K-series enzymes:

K-series enzymes follow Michaelis-Menten kinetics, which describe the rate of enzymatic reactions as a function of substrate concentration. In this model, the relationship between substrate concentration ([S]) and reaction velocity (V)) is hyperbolic.

The term *Km* has no real meaning for an allosteric enzyme; a more appropriate term is **($S_{0.5}$),** which is the substrate (ligand) concentration required to produce 50% saturation of the enzyme (protein).

For a K-series enzyme, (**$S_{0.5}$**) substrate, i.e., the substrate concentration required to half-saturate the enzyme, varies with the concentration of the modifier.

- ❖ Allosteric inhibitors *increase the sigmoidal nature of the binding curve for substrate; thus,* they decrease the fractional saturation of an enzyme with its substrate at low and moderate concentrations, decreasing the value of *v0* under these conditions (Fig. 13.1).
- ❖ Allosteric activators, tend to increase the hyperbolic nature of the substrate binding curve. In each case, the degree of allosteric effect depends on the concentration of modifier, but the value of *V*max is not affected.

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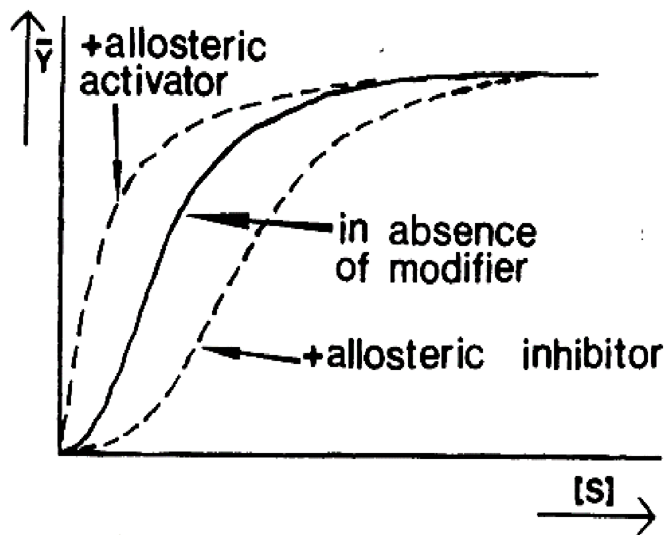

Fig. 13.1 — Effect of allosteric activators and inhibitors on the binding of a substrate to a K-series enzyme, at fixed concentrations of modifier and enzyme.

2- V-series enzymes:

In V-series enzymes, the presence of a modifier results in a change in Vmax but not in the apparent Km (or $S_{0.5}$) for the substrate.

V-series enzymes typically exhibit a change in Vmax in the presence of a modifier, whether an inhibitor or an activator. Without altering the apparent Km (Michaelis constant).

In other words, the presence of the modifier affects the maximum rate of enzyme catalysis (Vmax) but does not change the enzyme's affinity for the substrate (as measured by Km).

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Homoallostery

Homoallosteric effects (cooperative substrate binding). We contrasted binding by the single-subunit protein myoglobin with binding by the multisubunit hemoglobin. Myoglobin gives a hyperbolic binding curve (Figure 7.7); hemoglobin, with its cooperative binding, gives a sigmoidal curve (Figure 7.10d). We find the same contrast when we compare the V vs. [S]

The curve of a single-site enzyme obeying Michaelis–Menten kinetics differs from that of a multisite enzyme, showing cooperative binding (Figure 11.44a). The same applies to an enzyme that binds substrate cooperatively. The enzyme shows low activity at low substrate concentration, suggesting poor substrate binding. However, as substrate levels increase and more substrate is bound, the enzyme becomes more active because it binds substrate more readily to the last sites to be filled (see Figure 11.44b).

We imagine this happening, as with hemoglobin, because as more substrate is bound, the enzyme undergoes a transition from a **lower-affinity state (T state) to a higher-affinity state (R state), which has a lower KM.**

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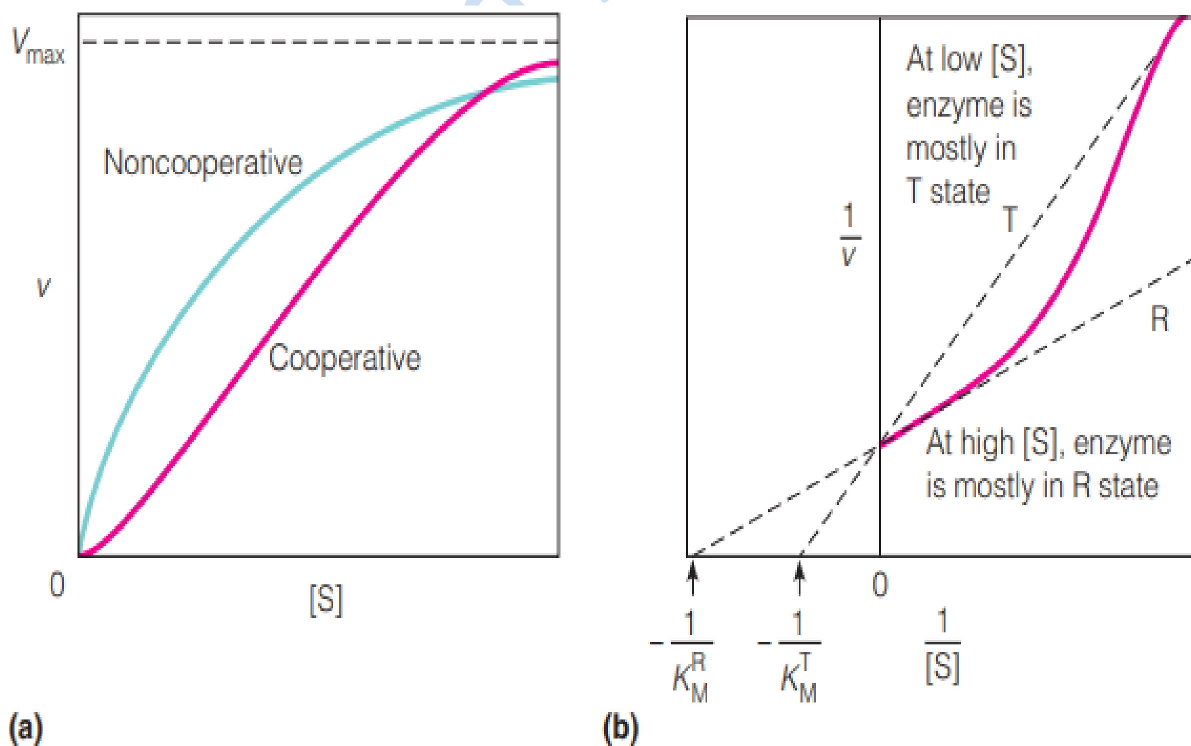

FIGURE 11.44 Effect of cooperative substrate binding on enzyme kinetics.

What physiological function does sigmoidal kinetics fulfill?

- Enzymes obeying sigmoidal kinetics can regulate substrate levels to relatively constant levels
- Substrate can easily accumulate up to the critical level at which the enzyme is inactive at lower [S], allowing [S] to increase up to [S]c, as shown in Figure 11.45.
- However, any further increase of the substrate [S] leads to a greatly increased enzyme activity so that the substrate will be more rapidly consumed.
- Although natural allosteric enzymes rarely exhibit curves as sigmoidal as in Figure 11.45, the principle remains: Multi-subunit enzymes help maintain substrate-level control.

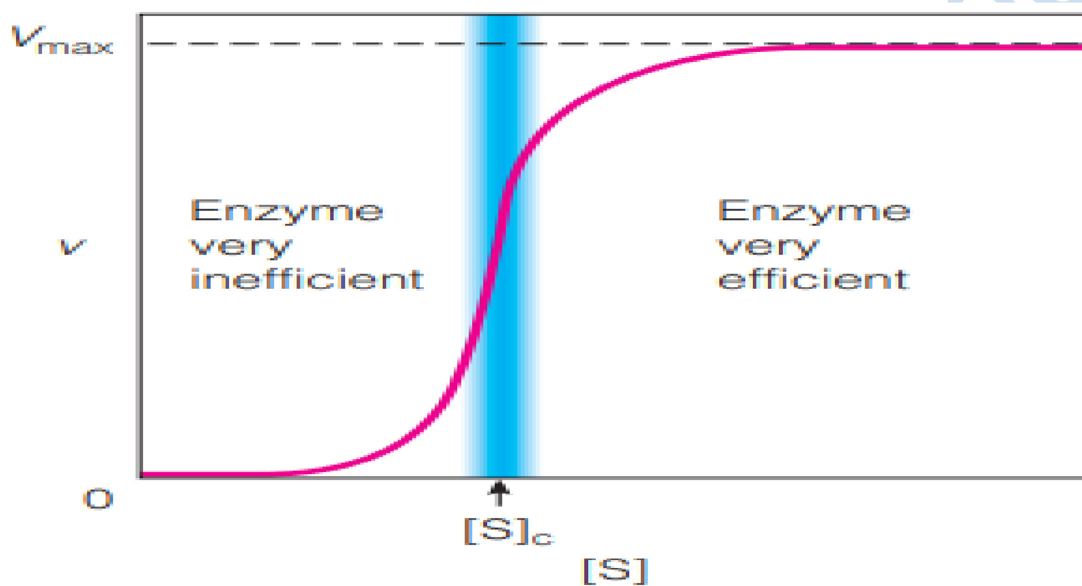

FIGURE 11.45 Effect of extreme homoallostery. The v vs. [S] curve. At concentrations below the enzyme is almost inactive; above this concentration, it is very active

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Heteroallostery

The major allosteric control is through heteroallosteric effectors, which may be either inhibitors or activators. The activation and inhibition of enzymes by allosteric effectors are the keys to feedback control.

Suppose an enzyme molecule exists in two conformational states (T and R) that differ in the strength with which the substrate is bound or in the catalytic rate. In that case, its

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kinetics can be controlled by any substance that alters the binding equilibrium with the protein. **Allosteric inhibitors shift the equilibrium toward T, and activators shift it toward R as shown in** Figure 11.46, The V vs. [S] curve is sigmoidal without activation or inhibitors. Activators shift the system toward the R state; inhibitors shift the system toward T state. [S]c represents the homeostatic concentration range for S. Some enzymes are regulated by multiple inhibitors and activators, allowing subtle metabolic control.

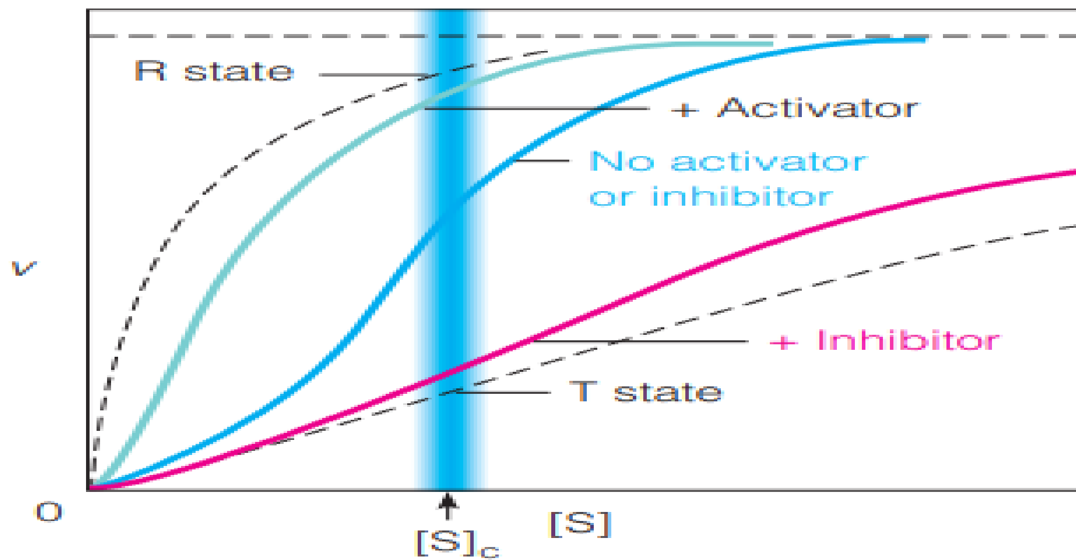

FIGURE 11.46: Heteroallostery (Hetero-allosteric control of an enzyme). [S]c represents the homeostatic concentration range for S.

An Example of an Allosteric Enzyme

Aspartate Carbamoyltransferase:

An excellent example of allosteric regulation is provided by the aspartate carbamoyltransferase (also known as aspartate transcarbamoylase or ATCase), a key enzyme in pyrimidine synthesis (Chapter 22).

As described in Fig 11.47, ATCase is at a crossing point in biosynthetic pathways.

Glutamine, glutamate, and aspartate are also used in protein synthesis, but once aspartate has been carbamoylated to form N-carbamoyl-L-aspartate (CAA), the molecule is committed to pyrimidine synthesis.

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Thus, the enzyme (ATCase) that controls this step must be sensitive to pyrimidine needs.

In bacteria like E. coli, the activity of ATCase is regulated to respond to this need. This enzyme is inhibited by cytidine triphosphate (CTP) and activated by ATP. Both responses make physiological sense; when CTP levels are already high, more pyrimidines are not needed.

On the other hand, high ATP indicates a purine-rich state (indicating a requirement for higher pyrimidine synthesis) and an energy-rich cell condition where DNA and RNA synthesis will be active.

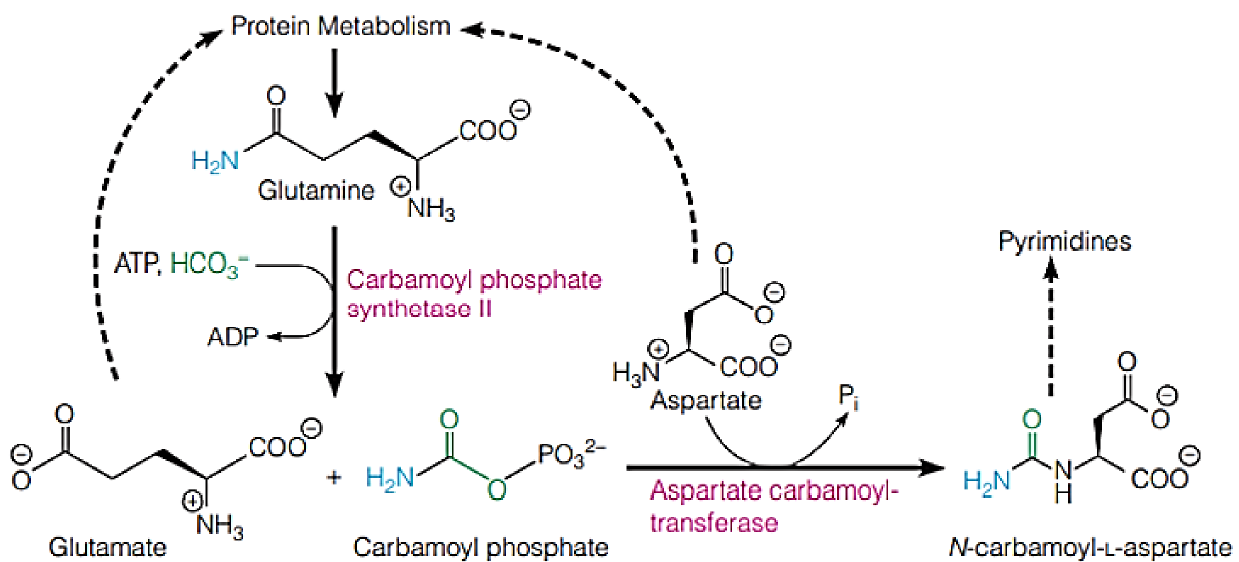

FIGURE 11.47 Control points in pyrimidine synthesis.

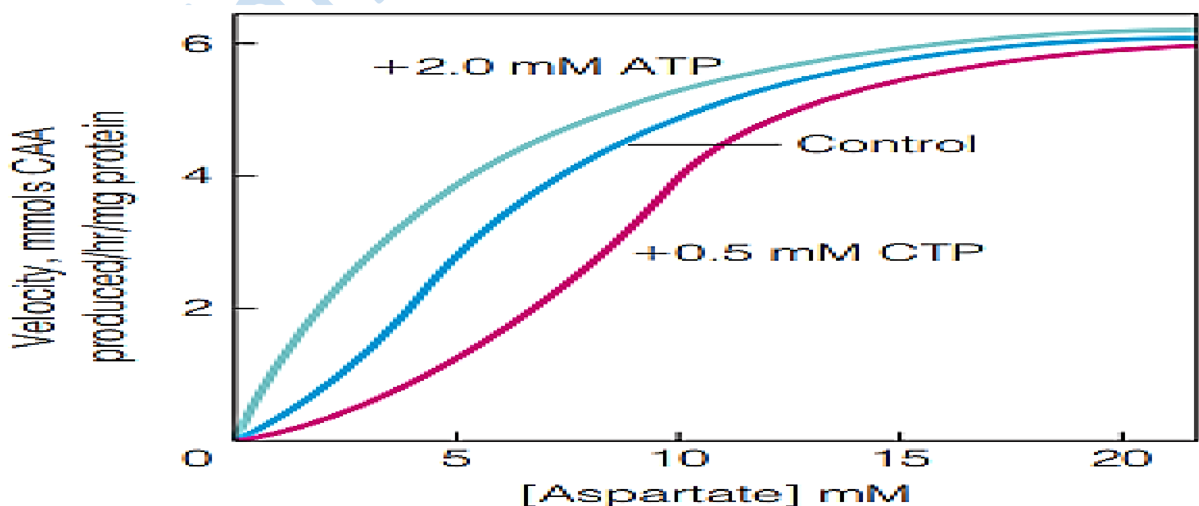

FIGURE 11.48: Regulation of aspartate carbamoyltransferase by ATP and CTP.

In mammals, the carbamoyl phosphate synthetase II is inhibited by UDP, UTP, CTP, dUDP, and UDP-glucose. These compounds all inhibit the binding of the ATP substrate.

Like most allosteric enzymes, ATCase is a multi-subunit protein. Figure 11.49 a. The of ATCase has six catalytic subunits distributed in two levels. These six (catalytic) subunits combine with six regulatory subunits. So it appears that pairs of regulatory subunits link catalytic subunits at the two levels.

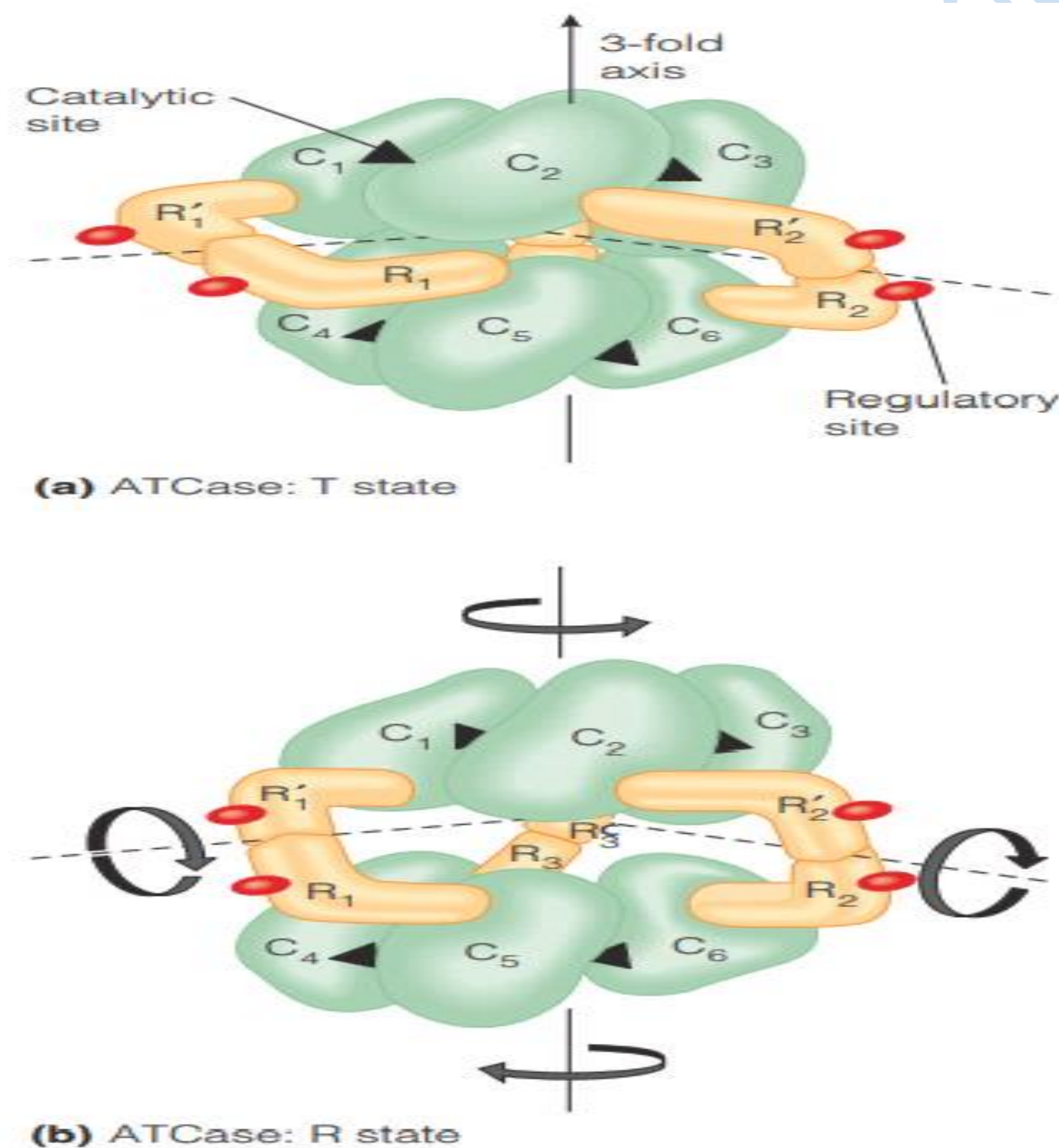

FIGURE 11.49 Quaternary structure of aspartate carbamoyltransferase (ATCase).

The structure of ATCase's catalytic and regulatory subunits (Figure 11.50)

- The active region sits between the aspartate-binding and carbamoyl-phosphate-binding - sites of the catalytic subunit.
- The regulatory subunit has two parts: zinc and allosteric. The first binds a structurally essential zinc atom; the second has an ATP/CTP binding site.
- ATP and CTP compete for the same site. Therefore, the ratio of ATP to CTP in the cell regulates the activity of ATCase.

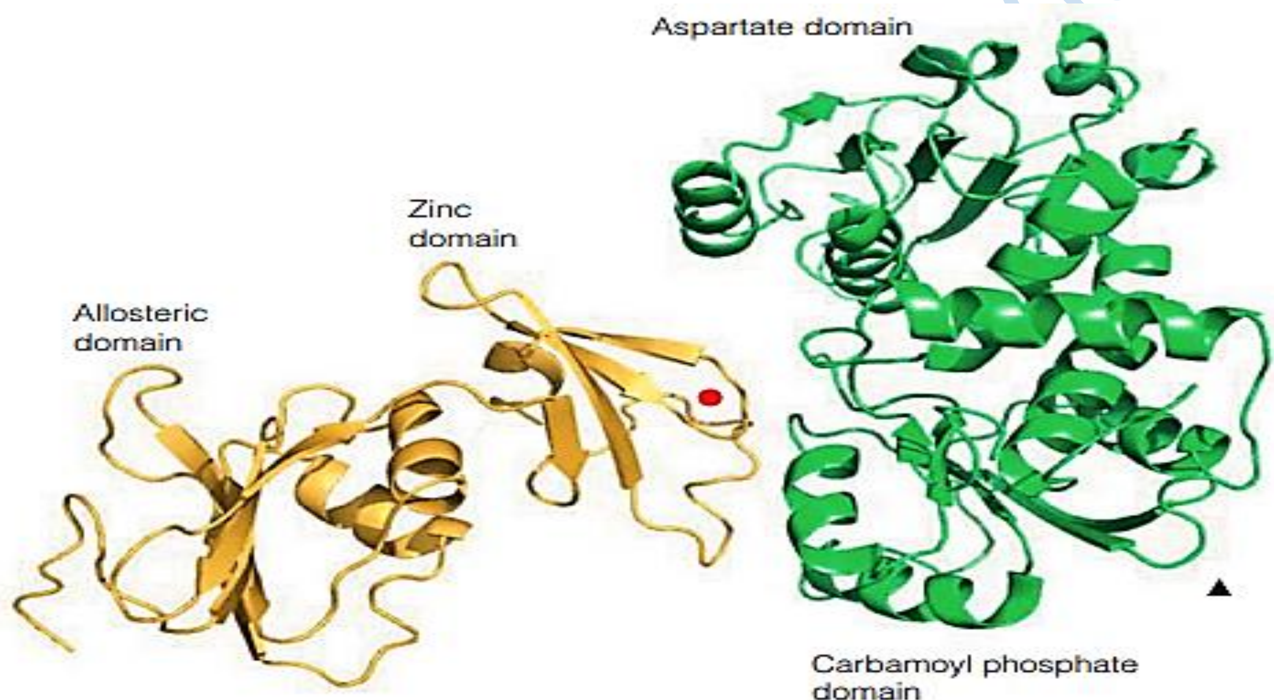

FIGURE 11.50 The detailed structureof ATCase.

Note: The ability of organisms to control metabolism in subtle and complex ways through allosteric enzymes should now be obvious; even so, this type of regulation is insufficient for all requirements.

We now move on to covalent modification, a fundamentally different regulator mechanism.

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