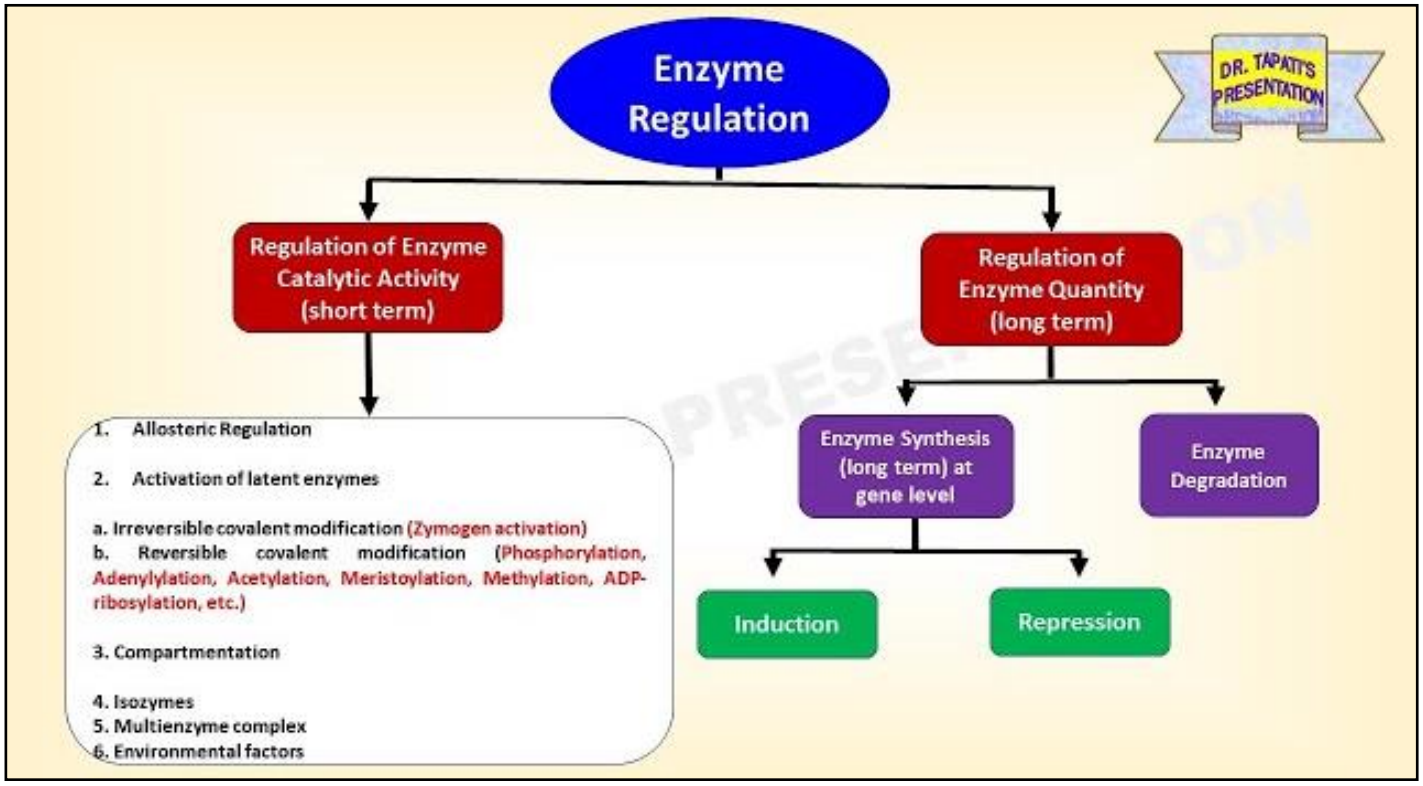
Allosteric enzymes

Introduction

To prevent a deficiency or accumulation of biomolecules, certain cellular mechanisms are based on controlling the activity of enzymes involved in metabolic pathways.

In the case of Michaelis kinetics, enzyme activity is regulated simply by substrate concentration. However, this type of control is insufficient in a metabolic process.

As a result, other means are used in vivo to regulate activity, particularly that of "key" enzymes in metabolic pathways. These are enzymes with multiple binding sites that are dependent on each other.



1. Definition :

1.1. Allostery :

The term allostery, introduced by Monod and Jacob, comes from *allos* = other and *stereos* = site or space. Allostery therefore means ***different site***.

1.2. Allosteric enzymes :

Allosteric enzymes are oligomeric enzymes that have the ability to change their spatial structure. This property is called "allosteric transition," which occurs following interaction with ligands (substrate, activator, or inhibitor), each of which binds to its specific site. This binding causes a change in enzyme activity.

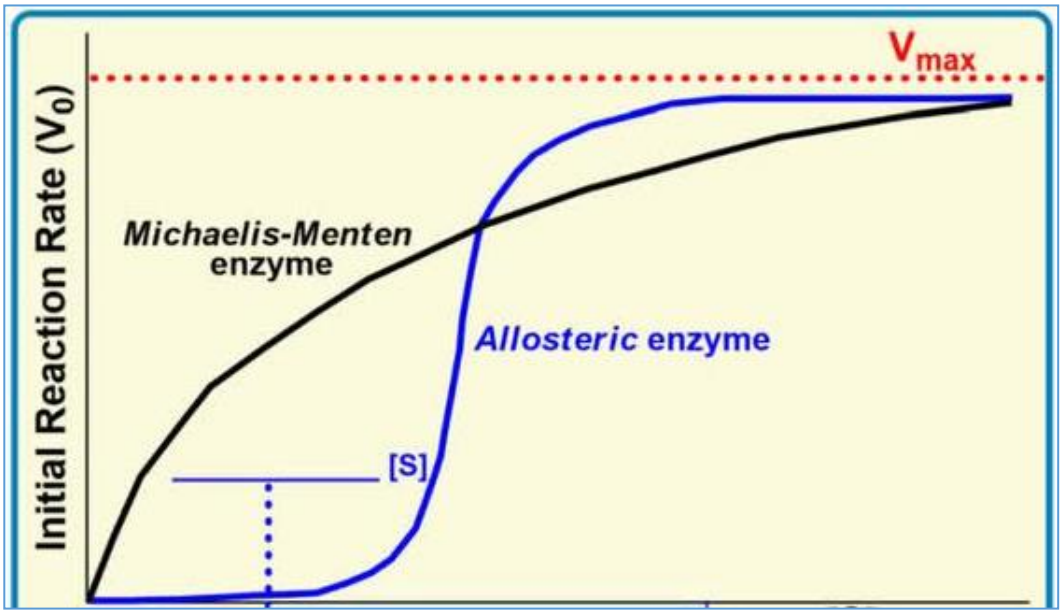
2. Classification :

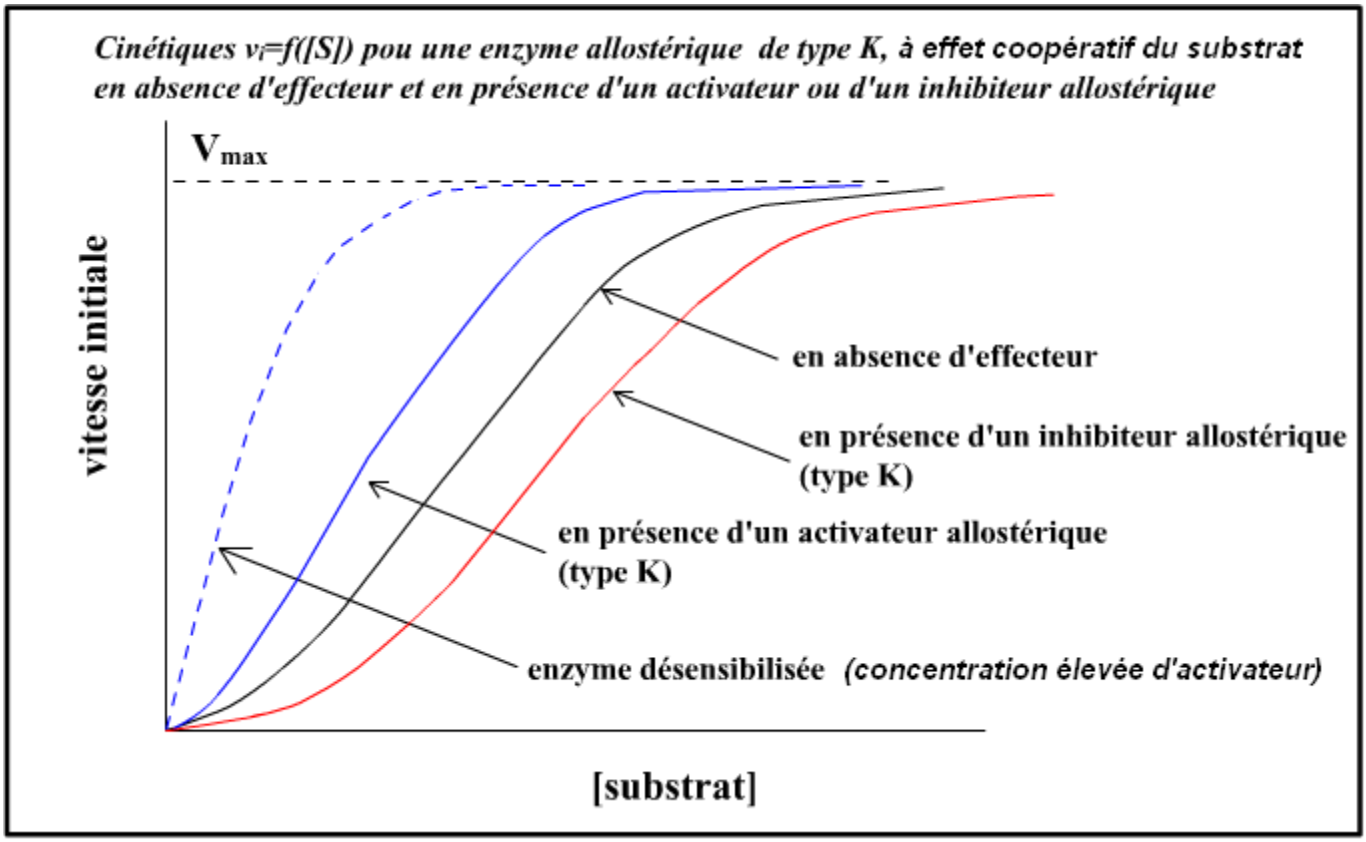
According to Monod, Wyman, and Changeux (1965), there are three groups of allosteric enzymes :

- ***Homotropic enzymes :*** The substrate in this group also acts as a modulator, accelerating enzyme activity.
- ***Heterotropic enzymes :*** These enzymes are stimulated or inhibited by regulatory substances other than the substrate; these are effectors.
- ***Homo-heterotropic enzymes :*** In this group, the enzymes have the substrate and other molecules as effectors.

3. Cooperative association :

- ✓ Cooperative association is observed in oligomeric enzymes with multiple binding sites. In fact, the binding of a substrate molecule to one of the active sites increases or decreases the affinity of the other sites for accepting a new substrate molecule.
- ✓ Positive cooperativity corresponds to an increase in affinity, while negative cooperativity corresponds to a decrease in affinity.
- ✓ Cooperativity requires that the enzyme molecule be composed of subunits capable of interacting with each other.
- ✓ The binding of the substrate to the active site of one of the subunits causes a change in electrical charges, a structural or conformational change that spreads to nearby subunits and is capable of altering the affinity of its active site.
- ✓ The interactivity of the subunits causes kinetic behavior that no longer follows the Michael model.





Measuring the initial rate as a function of the variation in substrate concentration gives a sigmoid curve that differs from the Michaelis-Menten curve.

Thus :

- ✓ The presence of an allosteric activator reduces the shape of the sigmoid curve, decreasing the value of K and therefore increasing the affinity of the enzyme for its substrate. The allosteric activator shifts the equilibrium towards the R form (relaxed form), which is the positive heterotropic effect.
- ✓ However, when the concentration of the activator is excessive, the curve returns to the shape of a hyperbole. The cooperative effect is eliminated and the kinetics become Michaelis-Menten.
- ✓ The presence of an allosteric inhibitor increases the value of K, decreasing the enzyme's affinity for its substrate. The allosteric inhibitor shifts the equilibrium towards the T form (tensed form), which is the negative heterotropic effect. The presence of an inhibitor accentuates the sigmoid shape.

4. Allosteric transition :

The regulatory enzyme has at least two functional sites :

- The active site adapted to the substrate.

- The allosteric site whose spatial conformation is adapted to the allosteric effector.

When the allosteric effector binds to the allosteric site, it causes a very slight reversible structural change in the entire enzyme protein : this is the *allosteric transition*, which has the direct effect of modifying the kinetics of the reaction.

5. The three-dimensional conformations of allosteric enzymes :

Allosteric enzymes have two possible quaternary structures :

- ***The inactive conformation is called T (Tense)*** because the interprotomeric bonds are strong. The T state has lower catalytic activity and low affinity for the substrate. It binds the inhibitor(s). When there is little substrate, the enzyme remains in the T form.
- ***The active conformation is called R (Relaxed)*** because the interprotomeric bonds are weak. The R state has stronger activity and a high affinity for the substrate. It binds the substrate(s) and activator(s). When there is a lot of substrate, the enzyme changes to the R form.

The substrate binds rapidly to the R form and much more slowly to the T form. Due to interactions between the different subunits, the entire enzyme can switch between the T and R conformations. The result of this allosteric behavior is a sigmoidal curve of rate as a function of substrate concentration.

6. Allosteric systems :

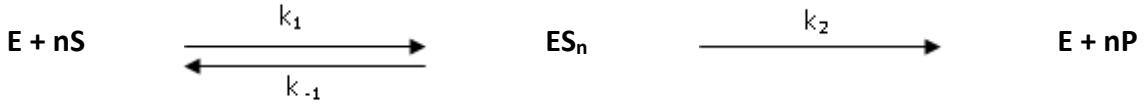
Three types of allosteric enzymes can be distinguished according to the effects exerted on them by the homotropic and heterotropic effectors that affect them. Thus, we distinguish between :

- ***Enzymes in the K system***, where the effector only modifies the apparent affinity (relative to Km) of the enzyme for the substrate. The R and T forms have the same maximum velocity. The substrate and effector all have different affinities for the R and T forms of the enzyme. This results in a consistently sigmoidal binding pattern. This is the most common system.
- ***Enzymes in the V system***, where the effector only modifies the maximum speed (Vmax) of the reaction. In this system, the substrate has the same affinity for both R and T forms of the enzyme. This results in hyperbolic substrate binding. Effectors A and I have different affinities for each R and T form, resulting in sigmoidal binding. The R and T forms differ in their catalytic activities (VmaxR differs from VmaxT).
- ***Enzymes in the mixed system***, where the effector modifies both parameters Km and Vmax.

7. Hill's equation :

A.V. Hill characterized the cooperative behavior of oligomeric enzymes in 1910.

Given an oligomeric enzyme with **n** active sites, the reaction scheme is as follows :



The law of conservation allows us to write that : $[E_t] = [E] + [ES_n]$

The reaction rate is : $v = k_2 [ES_n]$

In a stationary state, we have : $k_1 [E] [S]^n = k_{-1}[ES_n] + k_2 [ES_n]$

The Michaelis-Menten constant is : $Km = k_{-1} + k_2 / k_1$

so : $Km = [E] [S]^n / [ES_n]$, from which : $[E] = Km [ES_n] / [S]^n$

By replacing [E] with its value in the conservation equation :

$[E_t] = Km [ES_n] / [S]^n + [ES_n] = [ES_n] (Km/[S]^n + 1)$ from which $[ES_n] = [E_t] / (Km/[S]^n + 1)$

We have : $v = k_2 [ES_n]$ so $v = k_2 [E_t] / (Km/[S]^n + 1) = Vmax / (Km/[S]^n + 1)$

The formula for speed becomes :

$$\mathbf{v / Vmax = [S]^n / Km + [S]^n}$$ this is ***Hill's equation***

Also : $v (Km + [S]^n) = Vmax [S]^n$

Or even : $v Km = [S]^n (Vmax – v)$ and by rearrangement : $\mathbf{(v /Vmax – v) = [S]^n / Km}$

The logarithmic form is : $\log (v /Vmax – v) = n \log [S] – \log Km$

We know that at half saturation : $v = Vmax / 2$ so $\log (v /Vmax – v) = 0$

$\log Km = n \log [S]_{0,5}$ from which $\mathbf{\log (v /Vmax – v) = n \log [S] - n \log [S]_{0,5}}$

Hill's graphical representation :

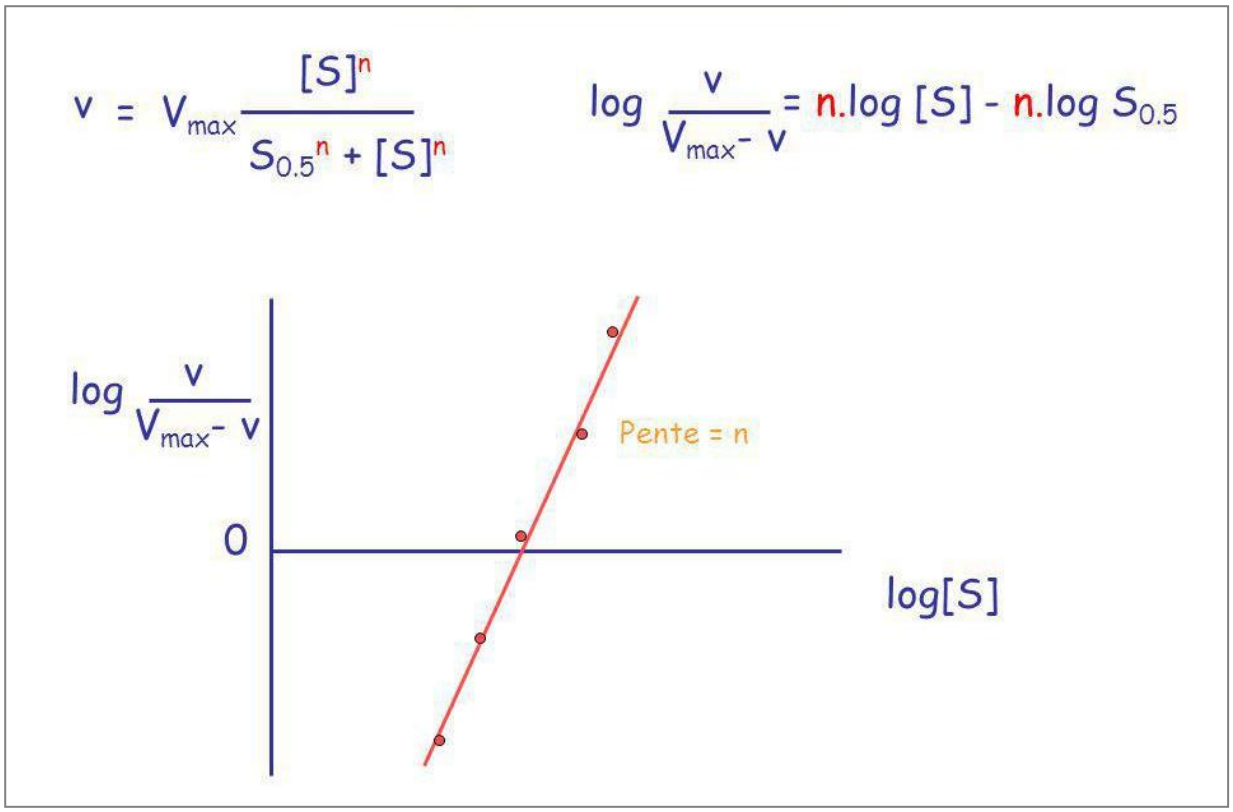
The plot of **log (v / Vmax – v) = f(log [S])** is a straight line with slope n and intercept at the origin **(– log Km)** and intercept on the x-axis **(log Km / n).**

Hill's graphical representation is used to determine the degree of cooperativity. n is called the Hill number or Hill coefficient (nH). It takes into account the degree of cooperativity, and the higher its value, the greater the cooperativity.

The absence of cooperativity corresponds to n = 1, i.e., Michaelis kinetics. Allosteric enzymes give a value different from 1 :

$n > 1$ positive cooperativity

$n < 1$ negative cooperativity



8. *Theoretical models of allostery :*

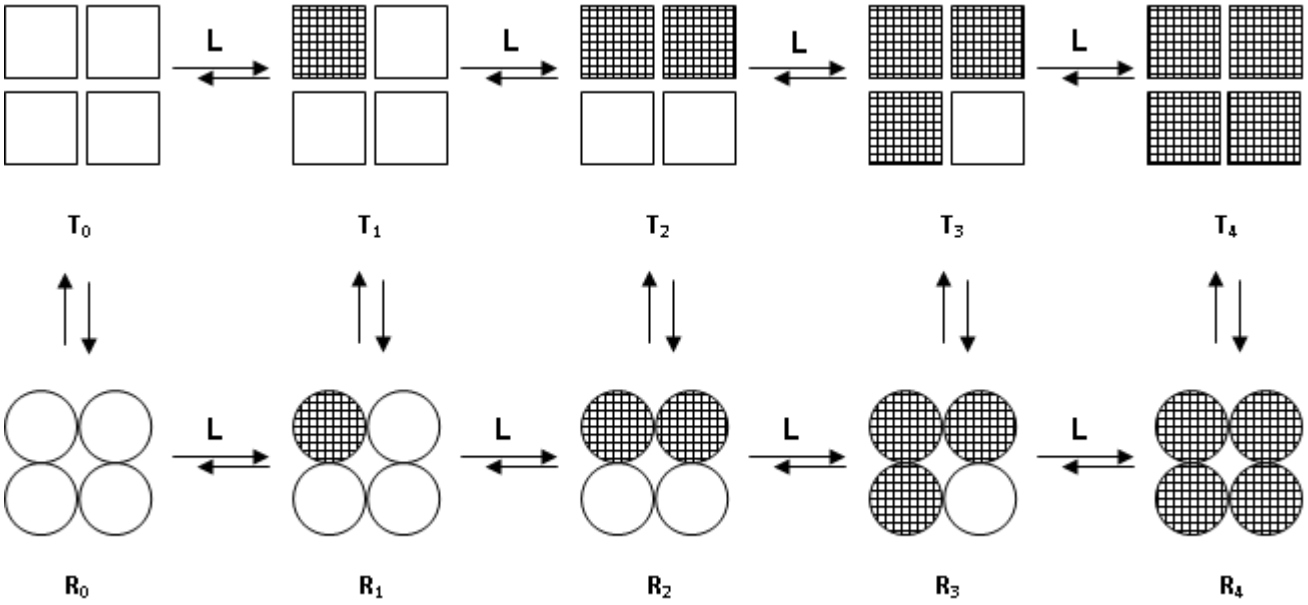
Several theories have been proposed to explain how allosteric enzymes work, particularly conformational change. To do this, researchers have adopted two models :

1. The concerted model (symmetry model) proposed by Monod, Wyman, and Changeux.
2. The sequential model proposed by Koshland, Nemethy, and Filmer.

8. 1. Monod model (concerted, symmetric, or MWC) :

Proposed in 1965 by Monod, Wyman, and Changeux, this model requires the following hypothesis :

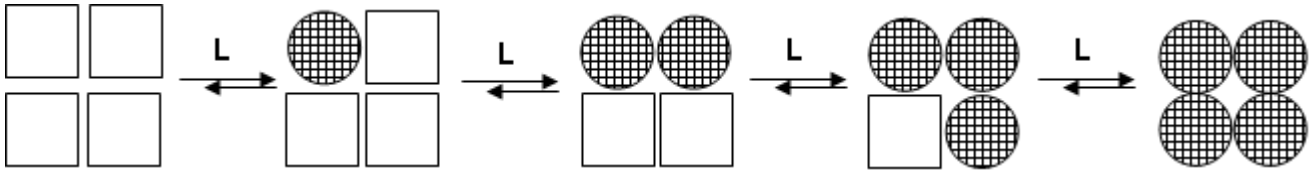
- The enzyme has two conformational states, **R** (relaxed) and **T** (tense), which differ in their tertiary and quaternary structures.
- The two forms, which differ in their affinity for the substrate and their maximum speed, pre-exist when any ligand binds.
- When a transition from the **R** form to the **T** form occurs, the subunits remain symmetrical. This means that the enzyme adopts a single form ; there is no hybrid form.



8. 2. Koshland model (sequential or KNF) :

Proposed in 1966 by Koshland, Nemethy, and Fischer, this model derives from the hypothesis of induced adaptation of an enzyme to its substrate :

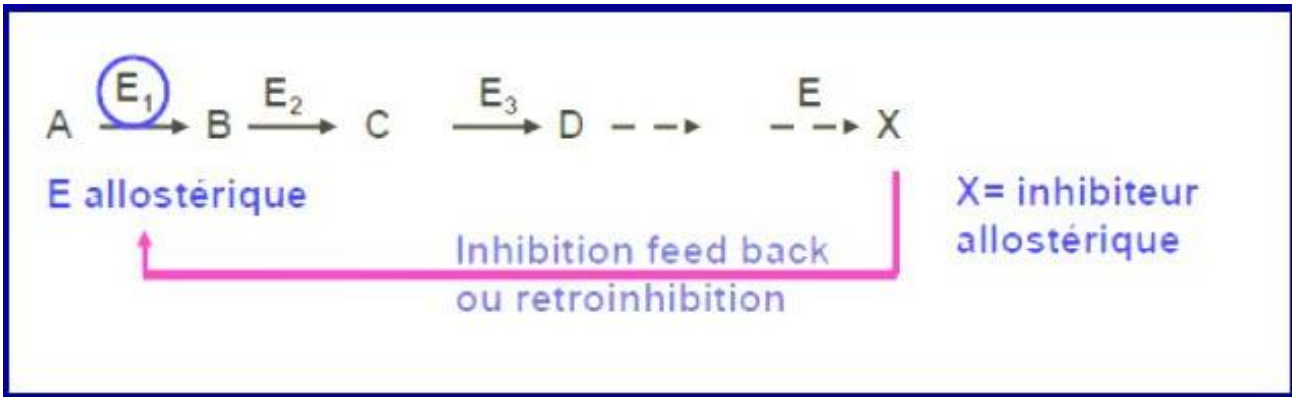
- In the absence of a ligand, the enzyme protein exists in only one form (R or T).
- Under the effect of substrate binding, the conformational change is sequential with the appearance of a hybrid form.



9. Allosteric regulation :

In metabolic pathways such as glycolysis, certain enzymes are allosteric :

- ✓ They are weakly active when there is little substrate.
- ✓ If the amount of substrate increases, their activity increases even faster and prevents the accumulation of substrate.
- ✓ The allosteric effector is usually the last or one of the last metabolites in the chain of reactions in which the enzyme acts. When the effector accumulates, it means that the cell no longer needs this metabolite, which in turn inhibits an allosteric enzyme governing one of the early stages of the metabolic chain (retroinhibition).



10. Desensitization of allosteric enzymes :

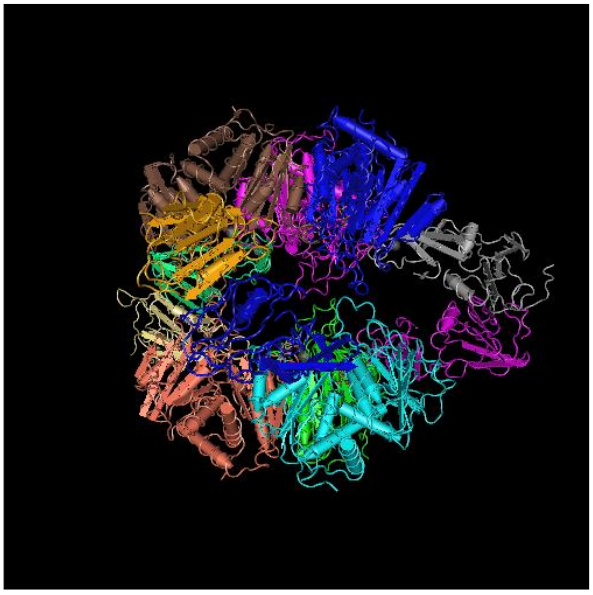
Treatment of an allosteric enzyme with physical agents (heating) or chemical agents (urea, mercury derivatives) results in a loss of the enzyme's sensitivity to allosteric effectors.

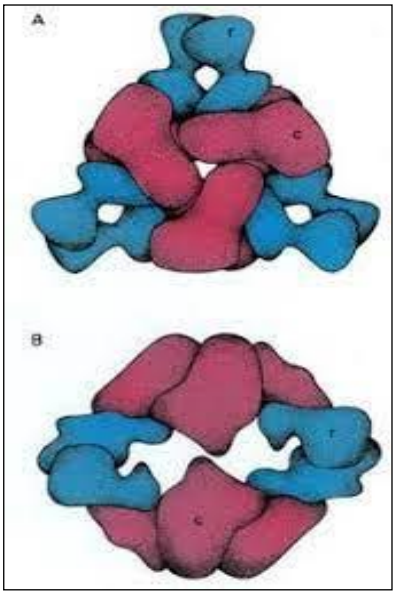
However, the enzyme's activity persists. Only the allosteric site is destroyed. This results in a loss of cooperativity and the kinetics become hyperbolic.

Example of an allosteric enzyme: aspartate transcarbamylase (ATCase)

Structure :

In *Escherichia coli*, aspartate carbamoyltransferase consists of 12 subunits with a total mass of 300 kDa : six catalytic subunits C of 34 kDa and six regulatory subunits R of 17 kDa. This C6R6 complex is organized into two catalytic trimers C3 and three regulatory dimers R2. The arrangement of these subunits allows this enzyme to exhibit very strong allosteric regulation with respect to its substrates.





Reaction :

Aspartate transcarbamylase is a transferase that catalyzes the reaction :

carbamyl-phosphate + L-aspartate ⇌ phosphate + N-carbamyl-L-aspartate.

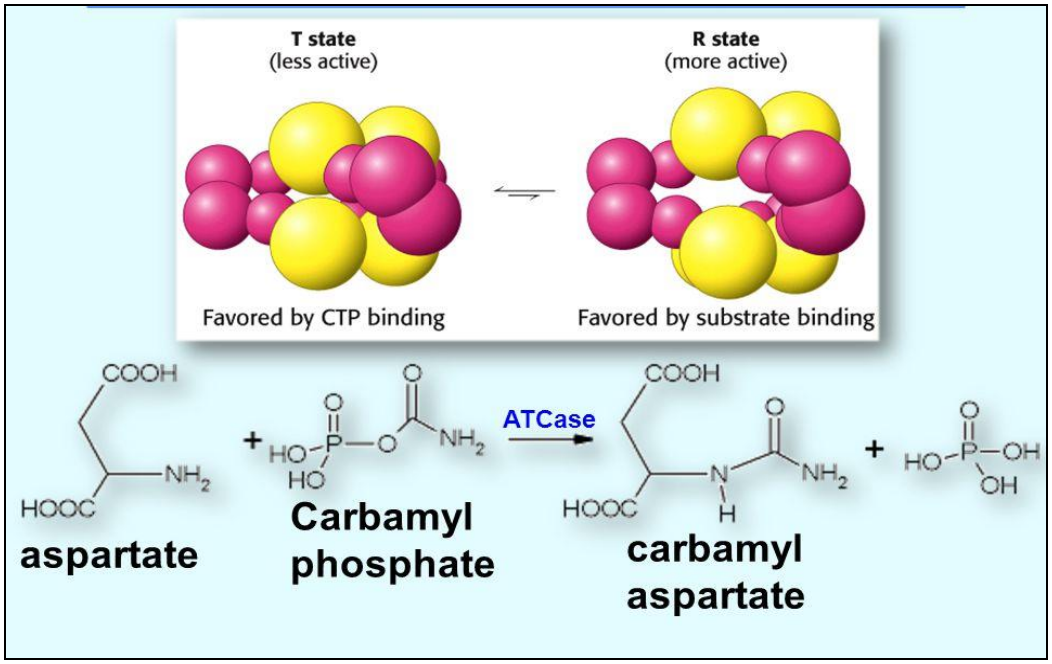
Thus, this enzyme has two catalytic substrates (aspartate and carbamoyl phosphate) and two effectors (ATP as an activator and CTP as an inhibitor).

Allosteric regulation:

This enzyme is involved in the first step of pyrimidine biosynthesis and does not follow Michaelis-Menten kinetics. It is an example of allosteric regulation at the initial stage of a metabolic pathway.

It switches between a tense state T with low affinity for substrates and low enzymatic activity, and a relaxed state R with high affinity and activity:

- ✓ The binding of substrates to the catalytic subunits shifts the equilibrium towards the R state, while the binding of CTP to the regulatory subunits shifts the equilibrium towards the T state.
- ✓ The binding of ATP to the regulatory subunits, on the other hand, shifts the equilibrium towards the R state.



Metabolic pathway :

The synthesis of pyrimidine bases is a metabolic pathway in the cytoplasm.

The pyrimidine synthesis pathway involves a first multi-enzyme complex that catalyzes the synthesis of dihydroorotate (complex A).

This product is oxidized by a dehydrogenase on the outer surface of the inner mitochondrial membrane, which transfers hydrogen to the mitochondrial respiratory chain.

A multienzyme (complex U) condenses orotate with 5-PRPP to form a nucleotide (orotidine monophosphate = OMP), which is then decarboxylated to UMP.

Activation of UMP leads to UTP, to which an amine function is added, originating from glutamine, resulting in CTP.