COURSE MSc (BIOTECHNOLOGY) III SEM

PAPER CODE: MBT-304

PAPER TITLE: ENZYMOLOGY AND ENZYME TECHNOLOGY

Allosteric enzyme

By:

DR. ANNIKA SINGH

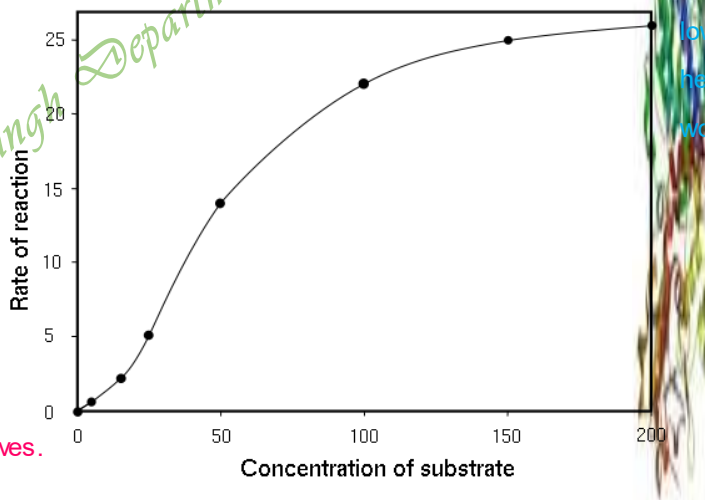
DEPARTMENT OF BIOTECHNOLOGY

INSTITUTE OF BIOSCIENCE AND BIOTECHNOLOGY

Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

Allosteric enzyme

- Enzyme whose activity is modified by the noncovalent binding of an allosteric effector at a site other than the active site. This binding mediates conformational changes, altering its catalytic or binding properties.
- Allosteric enzymes are an exception to the Michaelis -Menten model.
- They do not obey the Michaelis -Menten kinetics but instead have sigmoidal kinetics.
- Since allosteric enzymes are cooperative , a sigmoidal plot of V_0 versus [S] results:
- The binding of the substrate to an enzyme's active site affects the binding of substrate to other active sites.
- This property of cooperativity accounts for the sigmodial (S) curve of V_0 versus the concentration of substrate.
- Sigmoidal plot results from the combination of the T and R state curves.



Allosteric enzyme

Properties of Allosteric Enzymes

There are distinct properties of Allosteric Enzymes that makes it different compared to other enzymes.

(1) **Allosteric enzymes do not follow the Michaelis-Menten Kinetics.** This is because allosteric enzymes have multiple active sites. These multiple active sites exhibit the property of cooperativity, where the binding of one active site affects the affinity of other active sites on the enzyme; it is these other affected active sites that result in a sigmoidal curve for allosteric enzymes.

(2) **Allosteric Enzymes are influenced by substrate concentration** . For example, at high concentrations of substrate, more enzymes are found in the R state. The T state is favorite when there is an insufficient amount of substrate to bind to the enzyme. In other words, the T and R state equilibrium depends on the concentration of the substrate.

(3) **Allosteric Enzymes are regulated by other molecules.** This is seen when the molecules 2,3-BPG, pH, and CO_2 modulates the binding affinity of hemoglobin to oxygen. 2,3-BPG reduces binding affinity of O_2 to hemoglobin by stabilizing the T state. Lowering the pH from physiological pH=7.4 to 7.2 (pH in the muscles and tissues)favors the release of O_2. Hemoglobin is more likely to release oxygen in CO_2 rich areas in the body.

Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

Allosteric enzyme

- The special property of Allosteric enzymes is that it contains an allosteric site on top of its active site which binds the substrate.
- The binding of a nonsubstrate molecule to the allosteric site functions to influences the activity of the enzyme.
- In influencing the activity, it can either enhance or impair the activity of the enzyme. Another important property of allosteric enzymes is that it also contains many polypeptide chains with multiple active and allosteric sites.
- The nonsubstrate molecules that bind at the allosteric sites are called allosteric modulators.

Allosteric enzyme

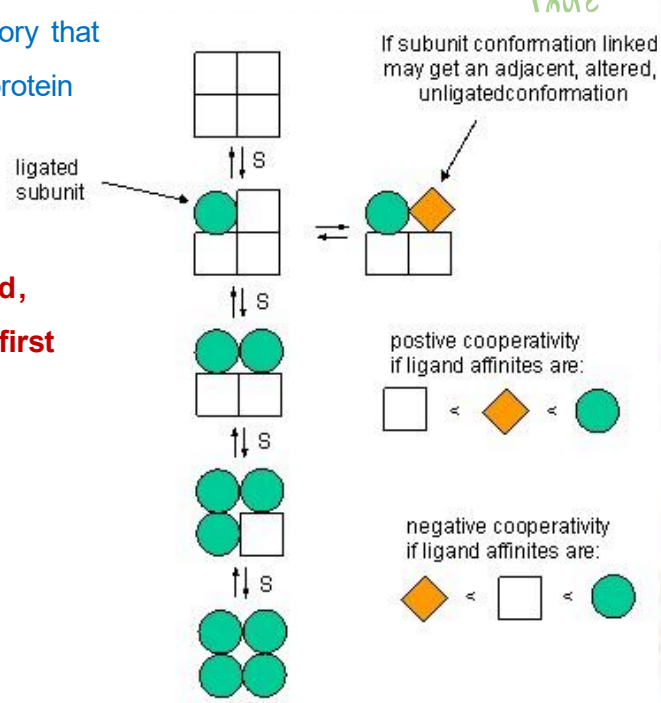
- **Monod-Wyman-Changeux model**(**MWC model**, also known as the **symmetry model**) describes allosteric transitions of proteins made up of identical subunits.
- It was proposed by Jean-Pierre Changeux based on his PhD experiments and described by Jacques Monod , Jeffries Wyman, and Jean-Pierre Changeux .

1. An allosteric protein is an oligomer of protomers that are symmetrically related
2. Each protomer can exist in (at least) two conformational states, designated T and R; these states are in equilibrium whether or not ligand is bound to the oligomer.
3. The ligand can bind to a protomer in either conformation. Only the conformational change alters the affinity of a protomer for the ligand. The regulators merely shift the equilibrium toward one state or another.

- One crucial feature of the model is the dissociation between the binding function (the fraction of protein bound to the regulator), and the state function (the fraction of protein under the activated state)

Allosteric enzyme

- The **sequential model** (also known as the **KNF model**) is a theory that describes cooperativity of protein subunits



- **KNF named after Koshland, Némethy and Filmer, who first suggested the model**

Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

The KNF model follows the structural theory of the induced fit model of substrate binding to an enzyme. **Koshland**

A slight change in the conformation of an enzyme improves its binding affinity to the transition state of the ligand, thus catalyzing a reaction.

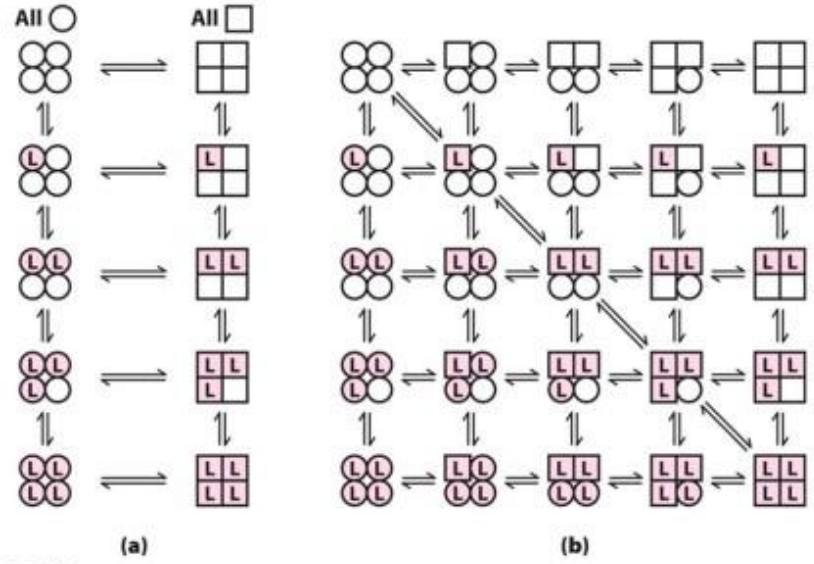
This follows the KNF model, which models cooperativity as the changing conformation of the ligand binding site upon ligand binding to another subunit.

Two essential assumptions guide the KNF model:

- The protein exists in a single state of either low or high affinity for the ligand, when not bound to the ligand
- Upon ligation of a binding site, a conformational change is produced in that region of the protein. Changing this region of the protein may influence the conformation of nearby binding sites on the same protein, thus changing their affinity for the ligand.
- In negative cooperativity, affinity goes from high to low, while in positive cooperativity, affinity goes from low to high.

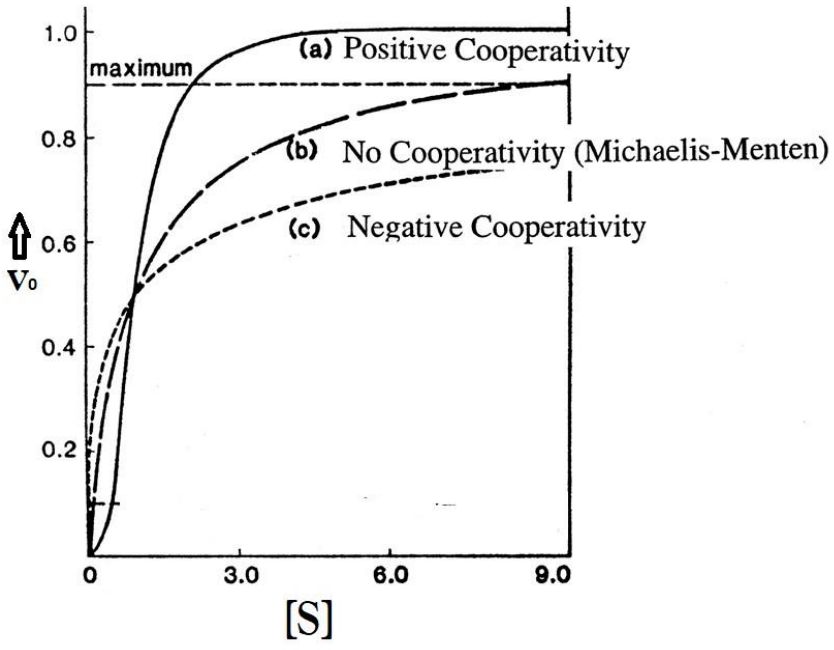
Allosteric enzyme

Models for cooperative binding of oxygen to hemoglobin
The concerted model versus the sequential model



Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

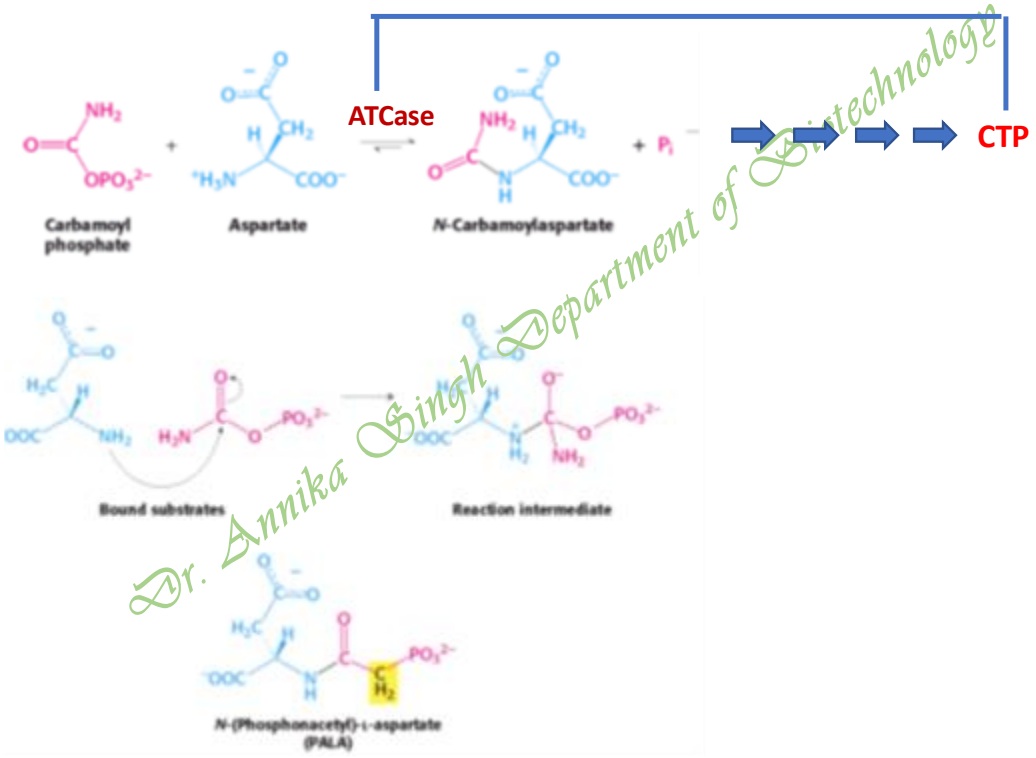
Allosteric enzyme



Aspartate Trascarbamoylase

- Aspartate trascarbamoylase is an allosteric enzyme
- The enzyme catalyzes the first step in the synthesis of pyrimidines.
- The enzyme functions to catalyze the condensation of aspartate and carbamoyl phosphate to form N carbamoylaspartate and orthophosphate.
- The enzyme ultimately catalyzes the reaction that will yield cytidine triphosphate (CTP).
- This allosteric enzyme is unique in that for high products of the final product CTP, the enzyme activity is low.
- However, for low concentrations of the final product CTP, the enzymatic activity is high.
- The allosteric nature is thus represented as the CTP molecule has a odd configuration or shape that is unlike the substrates.
- Rather than binding to the active site, CTP binds to the allosteric site.
- Thus, CTP functions as an allosteric inhibitor decreasing the enzymatic activity of the enzyme.

Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

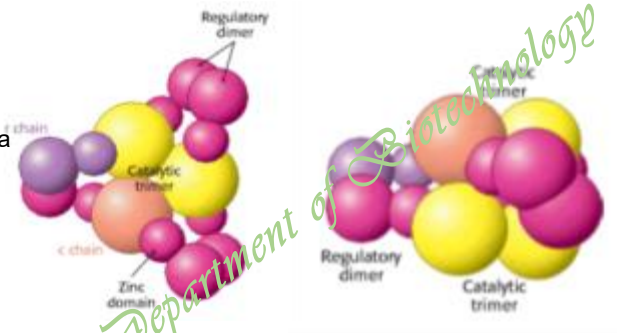


Dr. Annika Singh Department of Biotechnology

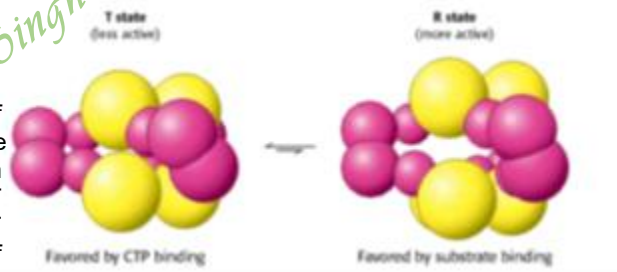
Structure of ATCase

The quaternary structure of aspartate transcarbamoylase as viewed from the top.

The schematic drawing at the right is a simplified representation of the relationships between subunits. A single trimer [catalytic (c) chains, shown in orange and yellow] is visible; in this view, the second trimer is hidden behind the one visible. (B) A side view of the complex.



The R State and the T State Are in Equilibrium. Even in the absence of any substrate or regulators, aspartate transcarbamoylase exists in an equilibrium between the R and the T states. Under these conditions, the T state is favored by a factor of approximately 200.



Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

This enzyme also has separate regulatory and catalytic subunits on separate polypeptide chains.

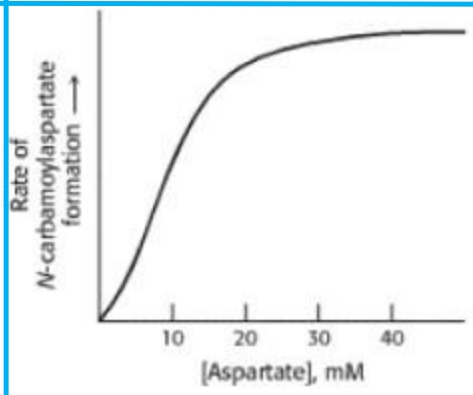
There are instances though when CTP concentrations remain high and cells in the body need more enzyme. This is when a different allosteric molecule

ATP functions to attach to the allosteric site and functions as enzyme activator enhancing the activity of the enzyme.

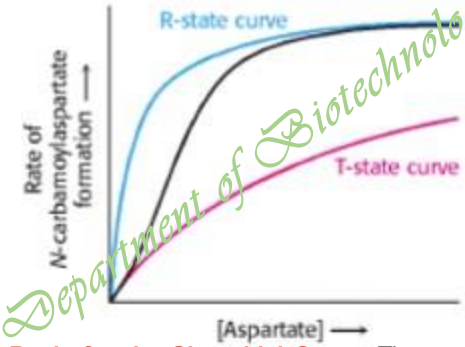
Thus, even with high concentrations of CTP, the enzyme activity could be enhanced because of ATP, which also acts on the allosteric site.

This example explains the benefits of allosteric control and the ability allosteric enzymes to adapt to various conditions of the environment.

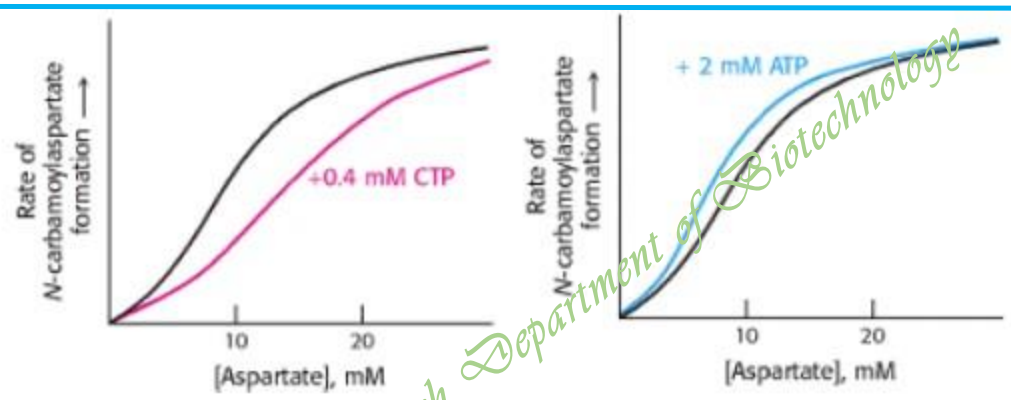
This is particularly helpful for cells because there are occasions when the cell requires an allosteric activator like "ATP" to enhance the enzyme even when it is inhibited due to high amounts of product. (CTP) The aspect of feedback inhibition is represented as well as high amounts of product acts to inhibit the action of the enzyme acting in a inhibitory manner.



ATCase Displays Sigmoidal Kinetics. A plot of product formation as a function of substrate



Basis for the Sigmoidal Curve. The generation of the sigmoidalcurve by the property of cooperativity can be understoodby imaginingan allostericenzyme as a mixture of two Michaelis-Menten enzymes, one with a high value of *K* m that correspondsto the T state and another with a low value of *K* m that correspondsto the R state. As the concentration of substrateis increased,the equilibriumshifts from the T state to the R state, which resultsin a steeprise in activity with respectto substrateconcentration



Effect of CTP on ATCase Kinetics. Cytidine triphosphate (CTP) stabilizes the T state of aspartatetranscarbamoylase, making it more difficult for substrate binding to convert the enzyme into the R state. As a result, the curve is shifted to the right, as shown in red.

Effect of ATP on ATCase Kinetics. ATP is an allosteric activator of aspartatetranscarbamoylase because it stabilizes the R state, making it easier for substrate to bind. As a result, the curve is shifted to the left, asshown in blue.

Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

Allosteric Modulation

- **Positive allosteric modulation** An example of allosteric activation or positive modulation is seen in cytosolic IMP-GMP specific 5'-nucleotidase II (cN-II), where the affinity for substrate GMP increases upon GTP binding at the dimer interface.
- **Negative allosteric modulation** can be seen is between ATP and the enzyme phosphofructokinase within the negative feedback loop that regulates glycolysis.
- Phosphofructokinase (PFK) is an enzyme that catalyses the third step of glycolysis: the phosphorylation of fructose-6-phosphate into fructose 1,6-bisphosphate. PFK can be allosterically inhibited by high levels of ATP within the cell.
- When ATP levels are high, ATP will bind to an allosteoric site on phosphofructokinase , causing a change in the enzyme's three -dimensional shape.
- This change causes its affinity for substrate (fructose-6-phosphate and ATP) at the active site to decrease, and the enzyme is deemed inactive.
- This causes glycolysis to cease when ATP levels are high, thus conserving the body's glucose and maintaining balanced levels of cellular ATP.
- In this way, ATP serves as a negative allosteric modulator for PFK, despite the fact that it is also a substrate of the enzyme.

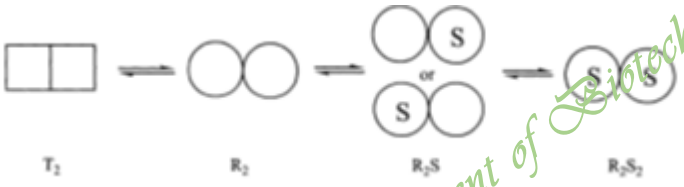
Dr. Annika Singh Department of Biotechnology

Types Of Allosteric Modulator

- **Homotropic** A homotropic allosteric modulator is a substrate for its target enzyme as well as a regulatory molecule of the enzyme's activity.
- It is typically an activator of the enzyme. For example, O_2 and CO are homotropic allosteric modulators of hemoglobin.
- GMP molecule in IMP/GMP specific 5' nucleotidase, binding of one GMP molecule to a single subunit of the tetrameric enzyme leads to increased affinity for GMP by the subsequent subunits as revealed by sigmoidal substrate versus velocity plots.
- **Heterotropic** A heterotropic allosteric modulator is a regulatory molecule that is not the enzyme's substrate. It may be either an activator or an inhibitor of the enzyme.
- For example, H^+, CO_2, and 2,3-bisphosphoglycerate are heterotropic allosteric modulators of hemoglobin.
- GTP molecule in IMP/GMP specific 5' nucleotidase, binding of GTP molecule at the dimer interface in the tetrameric enzyme leads to increased affinity for substrate GMP at the active site indicating towards K-type heterotropic allosteric activation.
- some allosteric proteins can be regulated by both their substrates and other molecules. Such proteins are capable of both homotropic and heterotropic interactions.

Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

Monod-Wyman -Changeux Equation



$$R_2 \rightleftharpoons T_2 \quad \text{(equilibrium constant } L\text{)}$$
$$R_2 + S \rightleftharpoons R_2S \quad \text{(intrinsic dissociation constant } K_R\text{)}$$
$$R_2S + S \rightleftharpoons R_2S_2 \quad \text{(intrinsic dissociation constant } K_R\text{)}$$

$$\therefore \text{Fractional saturation } Y = \frac{[R_2S] + 2[R_2S_2]}{2([R_2] + [R_2S] + [R_2S_2] + [T_2])}$$

$$= \frac{[R_2S] + 2[R_2S_2]}{2([R_2] + [R_2S] + [R_2S_2] + L[R_2])}$$

Dr. Annika Singh Department of Biotechnology

Monod-Wyman -Changeux equation

- **Monod-Wyman-Changeux equation** for a protein consisting of *n* protomers, each with a binding site for the substrate or ligand (S)
- According to this equation, the greater the value of *L*, the more sigmoidal a plot of *Y* against [S].
- If *L* = 0, a hyperbolic curve is obtained.
- A hyperbolic curve is also obtained, for a monomeric protein, i.e. where *n* = 1, and where the substrate can bind equally well to the R and the T conformation.
- **n = 1: non-cooperative binding**
- **n > 1: positive cooperative binding**
- **n < 1: negative cooperative binding**

$$Y = \frac{\left(\frac{2[R_2][S]}{K_R} + \frac{2[R_2][S]^2}{(K_R)^2}\right)}{2([R_2] + \frac{2[R_2][S]}{K_R} + \frac{[R_2][S]^2}{(K_R)^2} + L[R_2]} = \frac{\frac{[S]}{K_R}\left(1+\frac{[S]}{K_R}\right)}{L+\left(1+\frac{[S]}{K_R}\right)^2}$$

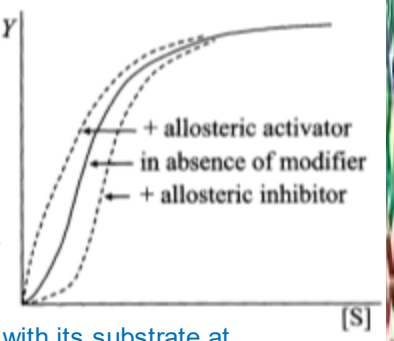
$$Y = \frac{\frac{[S]}{K_R}\left(1+\frac{[S]}{K_R}\right)^{n-1}}{L+\left(1+\frac{[S]}{K_R}\right)^{n}}$$

Chhatrapati Shahu Ji Maharaj University, Kanpur
ENZYMOLOGY AND ENZYME TECHNOLOGY
Dr ANNIKA SINGH
DEPARTMENT OF BIOTECHNOLOGY

K-series or V-series allosteric enzymes

K-series enzymes are those where the presence of the modifier changes the binding characteristics of the enzyme for the substrate but does not affect the *V* max of the reaction. Example PFK1

- ***S0.5*** : which is the ligand concentration required to produce 50% saturation of the protein.
- For a K-series enzyme, ($S_{0.5}$) i.e. the substrate concentration required to half-saturate the enzyme, varies with the concentration of modifier.
- Allosteric inhibitors, by increasing the value of *L*, 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 substrate concentrations, decreasing the value of v0 under these conditions.
- Allosteric activators, on the other hand, tend to increase the hyperbolic nature of the substrate binding curve



- **V-series enzymes are those where the presence of a modifier results in a change in the *V* max but not in the value of the apparent *Km* (or $S_{0.5}$) for the substrate .**
- The binding curve (and Michaelis-Menten plot) for the substrate at constant modifier concentration is a rectangular hyperbola, but the binding curve for the modifier itself
- is sigmoidal.
- This can be explained, according to the MWC model, if the substrate can bind equally-well to the R- and T-forms of the enzyme, but the reaction catalysed by the R-form is faster than that catalysed by the T-form.
- V-series enzymes are much less common than K-series enzymes, examples include fructose-l,6- bispbosphatase, of which AMP is an allosteric inhibitor, and pyruvate
- carboxylase, activated by acetyl-CoA.