ALLOSTEARIC ENZYME UNIT-3

Presented By
Dr. Mamta Rathore
Teaching Associates
Department of Agricultural Biochemistry
C.S.Azad University of Agriculture &Technology,Kanpur

- Allosteric regulation of enzyme activity Allostery: Key Point Binding of a ligand at a site different from the active site modulates the activity.
- This behavior extends well beyond the normal use of the word “allostery” which is often used to discuss cooperative interactions.
- The molecular basis for allostery provides insight into many regulatory mechanisms.
- That which has been learned by studying allosterically regulated enzymes/proteins has profoundly influenced our understanding of cooperativity and enzyme regulation in general.
-

- Allostery vs. cooperativity • The terms allostery and cooperativity are confusing
- . • Allostery strictly refers to influence of activity by a distant site.
- • Cooperativity indicates that the occupancy of one site in a multisubunit enzyme influences the binding on the others.
- This is a form of allostery, but is only one manifestation of a general phenomena.
- • Unfortunately allostery had become almost exclusively associated with the behavior of multi-subunit enzymes.
-

- Kinetic Signature of Cooperativity in Enzymes PFK-1 sigmoïde LDH hyperbole • Multisubunit enzymes that exhibit cooperativity show a sigmoidal initial velocity curve in contrast to the hyperbolic curve for independent subunits. Double reciprocal plot
-

- Kinetic Consequences of Allosteric Effectors on Cooperative Enzymes This is the traditional view of feed-back inhibition and regulation in “allosteric” enzymes.
-

- Types of Regulation • Homotrophic (or: homotropic) responses: This refers to allosteric modulation of enzyme activity by substrate molecules. This necessarily must occur in multisubunit enzymes. • Heterotrophic (or heterotropic) responses: This refers to regulation by non-substrate molecules or combinations of non-substrate and substrate molecules. • Allosteric regulation can be positive or negative.
-

- Allosteric regulation of enzyme activity E1 E2 E3 E4 A ---------▫ B ---------▫ C ---------▫ D ---------▫ Z Based on genetic data obtained in the 1940 Negative feed-back: the product of a metabolic pathway inhibity the first step
-

- Allosteric regulation of enzyme activity • Homotropic effect • (POSITIVE or NEGATIVE COOPERATIVITY) • Subunit interactions are essential 2 type of systems a. systems V (regulation of Vmax) very unusual! b. systems K (régulation de l'affinité) b. Heterotropic effect (allosteric effectors) Act on the cooperativity
-

- Allosteric regulation of enzyme activity E4 + S ▫ E4S KD1 E4S + S ▫ E4S2 KD2 E4S2 + S ▫ E4S3 KD3 E4S3 + S ▫ E4S4 KD4 KD1 =KD2 =KD3 =KD4 Equal affinity; no cooperativity KD1 >KD2 >KD3 >KD4 Increased affinity; positive cooperativity KD1 <KD2 <KD3 <KD4 Decreased affinity; negative cooperativity
-

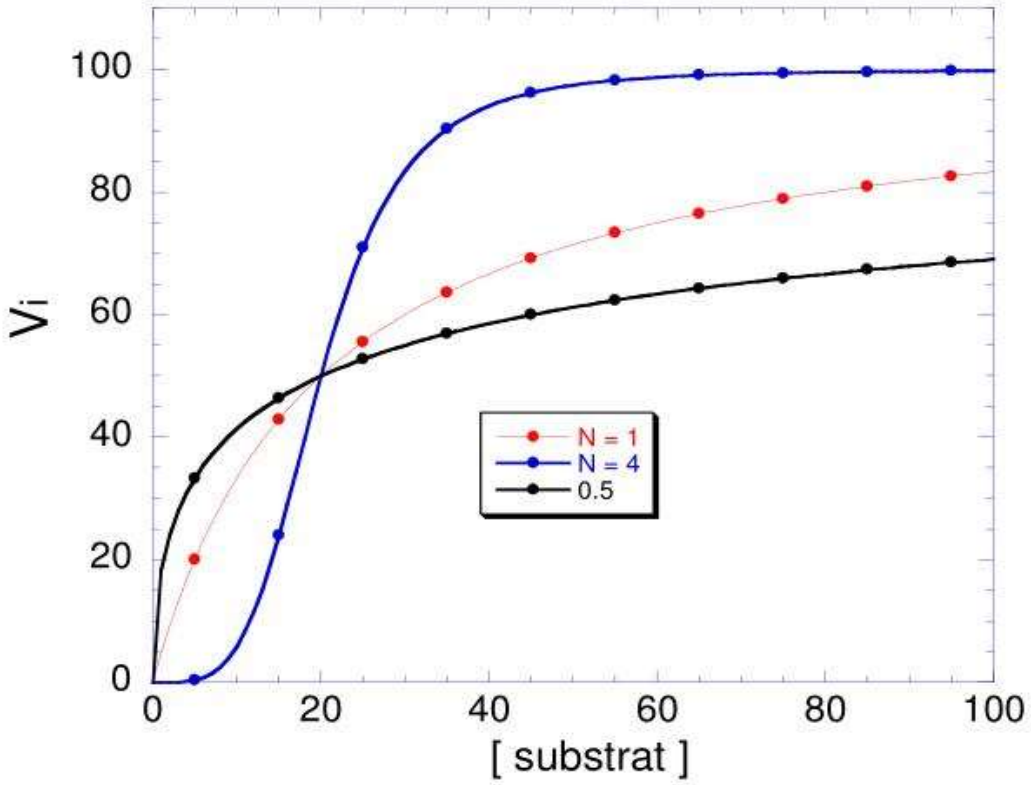
Allosteric regulation of enzyme activity

Empirical Hill equation

$$V = \frac{V_{max}[S]^N}{K_{0.5}^N + [S]^N}$$

Michaelis equation

$$V = \frac{V_{max}[S]}{Km + [S]}$$



- Allosteric regulation of enzyme activity Hill plot The empirical Hill equation v*K0.5N + v*[s]N = Vmax *[s]N v*K0.5N = Vmax *[s]N - v*[s]N v*K0.5N = [s]N * (Vmax – v) v /(Vmax – v) = [s]N K0.5N Log(v /(Vmax – v)) = [s]N K0.5N log(v /(Vmax – v)) =Nlog [s] + Nlog K0.5 Plot log(v /(Vmax – v) in fonction of log[s]: slope N
-

- The concerted mechanism Hypothesis: conformationnal changes in proteins Enzyme studied: PFK-1 of E. coli Jean-Pierre Changeux (1936-) Jacques Monod (1910-1976) Genetist PhD student (at that time) Jeffries Wyman (1901–1995) Protein biochemist (thermodynamic coupling)
-

The concerted mechanism

Hypothesis: conformationnal changes in proteins
Enzyme studied: PFK-1 of *E. coli*

Jacques Monod (1910-1976)
Genetist

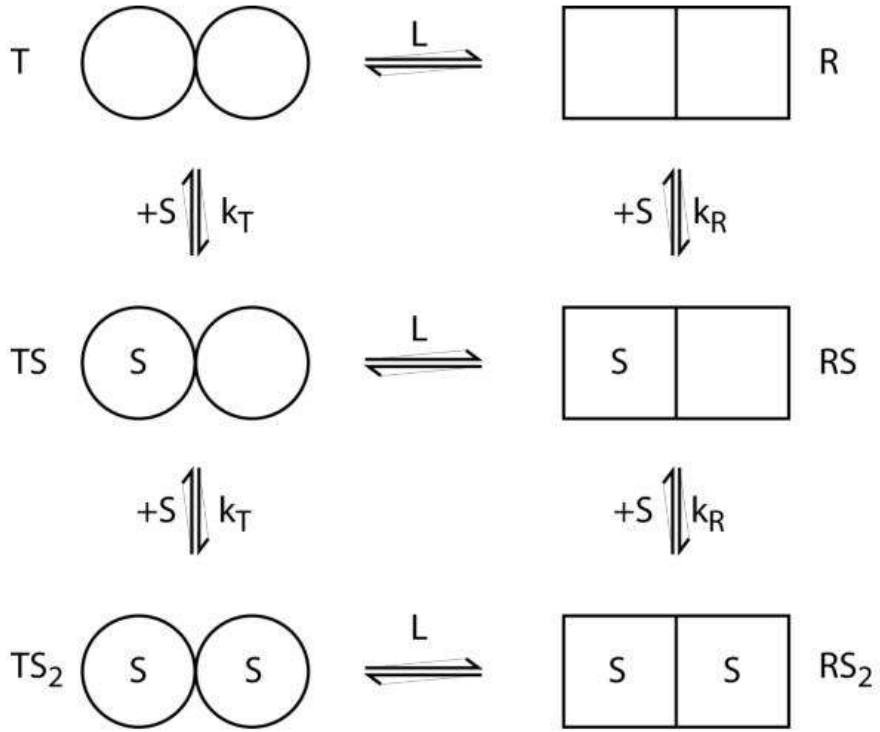
Jeffries Wyman (*1901–1995*)
Protein biochemist
(thermodynamic coupling)

Jean-Pierre Changeux
(1936-)

PhD student (at that time)

- The concerted mechanism • Allosteric enzymes are composed of identical protomers that occupy equivalent positions in the enzyme.
- Each protomer contains a binding site for each specific ligand.
- • Each protomer can exist in only one of two states. The R (relaxed or high substrate affinity state) or T (taut or low substrate affinity state).
- • All protomers in enzyme molecule must be in either the R or T state. The R and T states are in equilibrium with each other.
- • The binding affinity of a specific ligand depends on the conformation of the enzyme (R or T) and not on the neighboring site occupancy.
-

The concerted mechanism

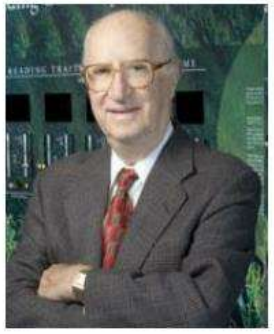


- A general set of equilibrium rate equations can be derived from this model.

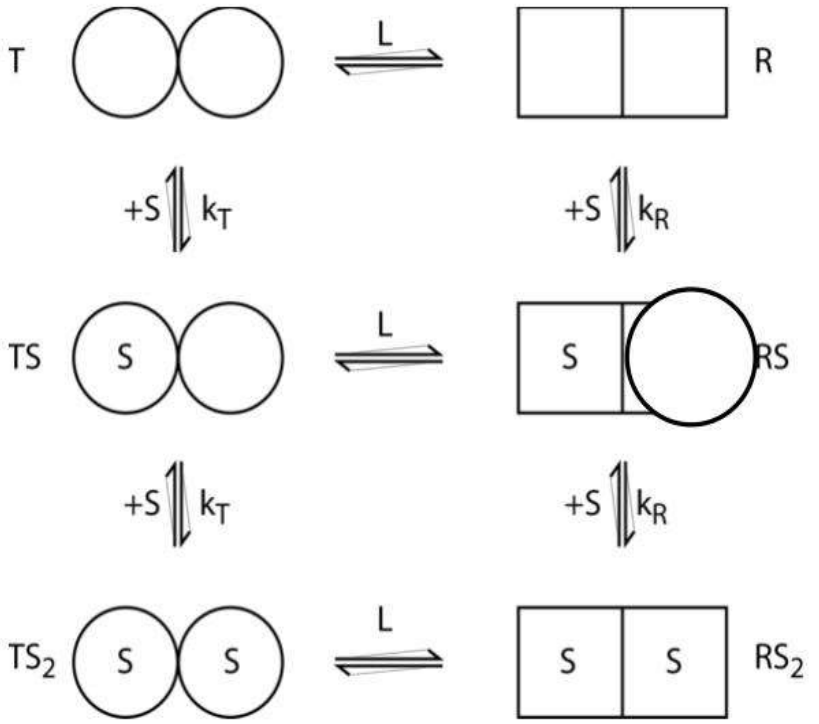
- Effect of Activator and Inhibitors on the Concerted Model • Allosteric effectors modify the apparent equilibrium constant for the T to R transition.
- In this approximation it is assumed that the inhibitor binds to the T state whereas the activator binds exclusively to the R state.
-

SEQUENTIAL MODEL FOR ALLOSTERIC REGULATION OF COOPERATIVE ENZYMES DANIEL E. KOSHLAND JR. (1920-2007)

- Sequential Model for Allosteric Regulation of Cooperative Enzymes



Daniel E. Koshland Jr. (1920-2007)



Thanks