Kinetics of allostearic enzyme
UNIT-3
Lecture-3

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Allosteric regulation of enzyme activity

- Allostery: 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.
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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.

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.
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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
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- **There are two Models for Allosteric Regulation**
- • Concerted (conceptually simple and often effective)
- • Sequential (probably correct but difficult to prove)
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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)
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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.

Simple Version of the Concerted Model

- This approximate model implies that the substrate does not bind to the inactive state. This must be a gross simplification but it explains the principle.
- Interestingly, it accounts for a lot of enzymatic behavior (it is the simplest model). It cannot explain negative cooperativity.
- For the transition R T Where L is the allosteric constant for the native enzyme Rate Equations for the Simplified Concerted Model • Where: T state is inactive, kR, and L are the same for all species. n is the number of protomers, kR is the intrinsic enzyme-substrate dissociation const. • This simple equation provides a simple kinetic model. • Allosteric regulators affect the value of "L"
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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.
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Regulation allosterique

- Exemple: La phosphofructokinase, enzyme-clé de la glycolyse fructose-6-phosphate + ATP => fructose-1,6-bisphosphate + ADP La cinétique est coopérative pour le fructose-6-phosphate, mais pas pour l'ATP, à basses concentrations. A partir de 0.5 mM, l'ATP est un inhibiteur allostérique (agissant sur un autre site que le site catalytique où il est un substrat).Activateurs allostériquesde la phosphofructokinase: ADP, AMP, cAMP, fructose-2,6-bisphosphate, etc (selon l'organisme). Ils se fixent tous au même site allostérique et l'empêchent l'ATP d'avoir son effet inhibiteur.
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Feed-Back Inhibition

- Feed-back inhibition is a common feature of complex biosynthetic pathways.
- It prevents the accumulation of unwanted intermediates and allows regulation of the level of important metabolites.
- • Because the substrate and final product of the pathway are generally chemically different, this demands that the final product bind at a different site relative to the substrate of the allosteric enzyme.
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Thanks