Regulation of Enzyme Activity

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The Theme of This Lecture

Regulation of Enzyme Activity at Protein Level.

1. Covalent modification.
2. Noncovalent (allosteric) regulation
3. Protein degradation (will not be considered).
Why regulate?

Regulation at Different Levels

- Translational regulation
- Covalent modification
- Allosteric regulation
- Protein degradation
- DNA RNA

In the cell, enzymes do not work alone but often work together in groups. These sets of reactions are called metabolic pathways. Given the fact that enormous amount of energy and resources are dedicated for each pathway to carry out different metabolic functions, the cells have to regulate the activities of the enzymes very precisely.

Regulation will allow the changing needs of the cell to meet its energy and resource demands. If a product is available in excess, it could then divert the resources to other needy reactions. If a product is needed, it could activate the pathway to produce more of the biomolecule that is needed.

Thus regulation is the process by which cells can turn on, turn off, or modulate the activities of various metabolic pathways.

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- Why regulate?
250,000 fold. This represents an amplification of 25 mmoles of glucose -1- phosphate per gram of muscle.

Approximately 0.1 umoles of adrenalin per gram of muscle will trigger the syntheseis of ATP Cyclic AMP.

Amplification of enzyme activity

And get the ATP from Glucose-6-P for running!!

You see the lion.
You are afraid!!
Start running and get the ATP from glucose-6-P for running!!

Amplification of enzyme activity
Four kinds of regulation of enzyme activity:

1. **Regulation of enzyme activity**
2. **Allosteric regulation**
3. **Proteolytic activation** (irreversible covalent modification)
4. **Stimulation and inhibition by control proteins**

- **Proteolytic activation** is irreversible. Once the protein is activated, the process cannot be reversed.

**Four Kinds of Regulation**
Examples of Proteolytic Activation

- Zymogen activation.
- Blood clotting.
- Complement activation.
- Prophenoloxidase activation.
- Inactive hormones to active hormones.

Activation mechanism of Chymotrypsin
Reversible Covalent Modification

Examples of reversible covalent modifications
- Phosphorylation - dephosphorylation
- Adenylation - deadenylation

A single trigger rapidly switches a whole pathway on or off.

Reversible covalent modification - Phosphorylation

At the same time glycogen synthase activity is shut off by phosphorylation.

While running, glycogen phosphorylase activity is enhanced by phosphorylation.

While running, glycogen phosphorylase activity is enhanced by phosphorylation.
Regulation - covalent modification

ATP ADP

KINASE

PHOSPHATASE

ACTIVE ENZYME INACTIVE ENZYME

H₂O Pi

PHOSPHORYLATION AND DEPHOSPHORYLATION OF SERINE (THREONINE AND TYROSINE)

Stimulation and inhibition by control proteins

Activation of Protein Kinase by cyclic AMP

Activation of Protein Kinase by cyclic AMP

Phosphorylation and Dephosphorylation

Regulation - covalent modification
Allosteric proteins show sigmoidal kinetic behavior.

Normal Protein

Michaelis-Menten Kinetics

Allosteric Protein

Sigmoidal Kinetics

For ATCase, ATP is an allosteric activator and CTP is an allosteric inhibitor.

Effect of activator and inhibitor:
- Activator decreases sigmoidity
- Inhibitor increases sigmoidity

ATCase, ATP is an allosteric activator. CTP is an allosteric inhibitor.
Allosteric enzymes

- Do not exhibit Michaelis Menten type kinetics.
- Show sigmoidal kinetic behavior.
- Must undergo drastic conformational changes in a subunit that may or may not be transmitted to other subunits.
- Binding of a ligand causes conformational or subunit interaction.
- The sigmoidal graph arises due to cooperativity interaction (can be an activator or an inhibitor).
- If it is a different ligand, it is called heterotropic homotropic interaction.
- If the substrate itself is regulator, it is called allosteric regulation.

Allosteric regulation

- If the enzyme is non-allosteric enzymes, then they are larger and more complex subunits.
- Usually possess catalytic and regulatory subunits.
- Upon binding of modulatory ligand, must undergo drastic conformational changes.
- Show sigmoidal kinetic behavior.
- Do not exhibit Michaelis Menten type kinetics.

Allosteric enzymes
Desensitization

ATCase treated with mercurials do not exhibit allosteric kinetics.

This desensitization is caused by the separation of catalytic and regulatory subunits by the reaction with mercurials.

End Product Inhibition

If F is available by some chance, the pathway should be shut off.

End product inhibition provides this regulation.
Concerted model or symmetry model

The enzyme exists only in two states: T (taut or tensed) and R (relaxed). Substrates and activators have great affinity for the R state, while inhibitors have higher affinity for the T state. Ligands affect the equilibrium between the T and R states, which must be conserved while going from one state to the other.

Heterotropic interactions could be either positive or negative. Positive cooperativity is possible, which means that only binding of the same ligand to a second subunit assists the binding of the same ligand to a third subunit always.

The two states are T (taut or tensed) and R (relaxed). The enzyme exists only in two states, with symmetry conserved.

Concerted model or symmetry model

While going from one state to the other, ligands affect the equilibrium between T and R states. Inhibitors have higher affinity for T state, while substrates and activators have great affinity for R state. Heterotropic interactions could be either positive or negative.
Sequential model

Ligand binding alters the conformation of the enzyme. While going from one state to the other, symmetry need not be conserved.

Binding of ligand to a subunit alters the conformation of only that subunit. This alteration is transmitted to other subunits by subunit interaction. Therefore, multiple states are possible. Binding of ligand to one subunit may help or hinder its binding to the other subunit. That is both positive and negative homotropic interaction are possible in this model.

Heterotropic interactions could be either positive or negative. Binding of ligand to one subunit may help or hinder its binding to the other subunit - that is both positive and negative heterotropic interaction are possible in this model.

Substrate

Sequential model
In the Symmetry model only positive homotropic interaction is allowed. [S binding always helps another S binding]

Heterotropic interactions could be positive or negative. [S and I binding is negative heterotropic interaction and S and A binding is positive heterotropic interaction].

In the Sequential model, both homotropic and heterotropic interactions could be positive or negative. [That is even S binding to one subunit could inhibit the S binding to the next subunit (negative homotropic interaction is possible here)].

Aspartate transcarbamoylase

The S binding to the next subunit (negative homotropic interaction) is allowed. [S binding always helps another S binding].

In the Symmetry model only positive homotropic interaction is possible here. [S and S binding is positive heterotropic interaction].
For ATCase, CTP is an allosteric inhibitor. ATP is an allosteric activator.