ENZYMES

3rd Week

1) Mechanisms of Enzyme Catalysis

-Covalent Catalysis

-Catalysis by proximity

-Acid-base catalysis

-Metal ion catalysis

2) Regulation of Enzymes

-Allosteric Regulation

-Reversible covalent modification

-Proteolytic breakdown

-Enzyme concentration

-isoenzymes (isozymes)

MECHANISMS OF ENZYME CATALYSIS

1) Covalent Catalysis: The active site of many enzymes contains catalytic residues that make transient covalent bonds with the substrate molecule.

Glycopeptide transpeptidase, chymotrypsin, and many different enzymes use this catalysis mechanism.

MECHANISMS OF ENZYME CATALYSIS

2) Catalysis by Proximity

- In reactions where more than one substrate is present, the substrates must be close enough to participate in the reaction and be in the proper orientation.

- The microenvironment of the active site allows the substrate molecules to collide with each other with sufficient energy.

MECHANISMS OF ENZYME CATALYSIS

3) Acid-Base Catalysis

- At the active site, hydrogen ion transfer can take place to form strong nucleophiles.
- Thus, the stabilization of the charged groups and the transition state is achieved.

Histidine aa in the enzyme chymotryipsin contributes to the formation of strong nucleophiles by contributing to hydrogen transfer.

MECHANISMS OF ENZYME CATALYSIS

4) Metal ion catalysis

Metals such as iron, copper, zinc are used as cofactors by many enzymes. These metals are positively charged and therefore help:

The formation of nucleophilic groups,
Stabilization of the transition state,
Ensuring that the substrate is kept within the active site

EXAMPLES FOR ENZYME CATALYSIS MECHANISMS

Before giving examples of catalysis mechanisms of enzymes, let's first get to know these basic enzymes

Proteases:

Serine proteases (digestion, blood clotting, reproduction, immunity, ... Trypsin, chymotrypsin, elastase, prothrombin, ..)
Cysteine proteases (programmed cell death, bone modeling, immunity, ...caspases, papain, cathepsin, ..)
Aspartyl proteases (digestive, ... renin, pepsin, ..)
Threonine proteases
Metalloproteases

Why cleaving the peptide bond?
Digestion
Regulation of various biological molecules and pathways

Regulation of Enzymes

1. Allosteric Regulation: An enzyme can be regulated by binding of various modulators to the allosteric site.

2. ***Reversible covalent modification:*** Various groups reversibly bind to the enzyme to control its activity. Ex: Protein kinases, protein phosphatases

3. Proteolytic cleavage: *Many enzymes are synthesized in an inactive form (zymogen, pro-enzyme) and they are activated by*

4. Regulation of the enzyme concentration: The concentrations of the enzymes can be transcriptionally regulated depending on the conditions in which the enzyme is present.

5. Isoenzymes (isozymes): These enzymes allow the regulation of various metabolic pathways.

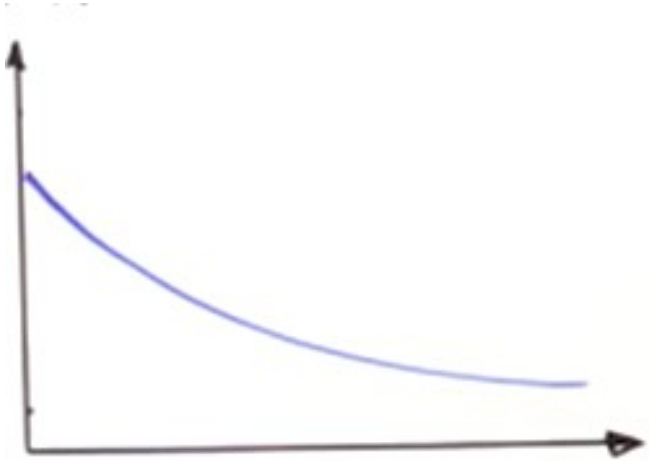
1) ALLOSTERIC REGULATION

►Some enzymes have regulatory regions, called allosteric regions, other than active regions.
►Various modulator groups bind to these regions and these groups act as regulators by inhibiting or activating the activity of the enzyme.
►This kind of regulation is called allosteric regulation.
►Cooperativity is an important interaction of allosteric enzymes.

AN EXAMPLE FOR ALLOSTERIC REGULATION

(Regulation of aspartate transcarbamoylase)

Formation of carbamoyl aspartate



CTPconcentration

Product of the reaction that begins with ATCase binds to ATCase to inhibit its activity (feedback inhibition).
Since structure of CTP is different from the structures of the substrates, CTP is called as an allosteric inhibitor.

AN EXAMPLE FOR ALLOSTERIC REGULATION
(Regulation of aspartate transcarbamoylase)

- Most allosteric enzymes, such as ATCase, follow the sigmoidal curve. This curve differs from typical Michaelis Menten kinetics.

- The reason that allosteric enzymes follow this curve is that the substrate binding to one active site unit of the enzyme changes the substrate binding affinity to the other active site units.

- This type of behavior is called cooperativity. In cooperative interaction, binding of the substrate to an active site changes the substrate affinity of other active sites through interaction between the subunits.

AN EXAMPLE FOR ALLOSTERIC REGULATION

(Regulation of aspartate transcarbamoylase)

At high substrate concentration, binding of the substrate to one of the catalytic sites is transferred by cooperative interactions between the catalytic subunits while inducing substrate binding to other units, while the ATCase enzyme at a low substrate concentration is in a tight and tense configuration (T state) that does not support substrate binding. In this case, the ATCase enzyme is in a loose-relaxed conformation (R state).

AN EXAMPLE FOR ALLOSTERIC REGULATION
(Regulation of aspartate transcarbamoylase)

Homotropic effects --- the effect of substrates on allosteric enzymes
Heterotropic effects --- the effect of non-substrates on allosteric enzymes (for ex., effect of CTP and ATP on ATCase).

CTP is an allosteric inhibitor of ATCase. CTP binding stabilizes the T-state of the ATCase enzyme and inhibits ATCase activity.

ATP is the allosteric activator of ATCase. ATP binding stimulates the R-state of the ATCase enzyme and activates ATCase activity.

2) GERİ DÖNÜŞÜMLÜ (REVERSİBLE) KOVALAN MODİFİKASYON

Many proteins and enzymes are regulated by covalent modification. In this type of regulation, a functional group is added to the enzyme. The most common covalent modification is phosphorylation.

Why is phosphorylation so common as a covalent modification?

1) Phosphate groups increase the catalytic activity of the enzyme by giving the enzyme a negative charge.
2) Furthermore, the negatively charged oxygen atoms of the phosphate groups contribute to the enzyme-substrate interaction by making the H bond.
3) If ATP is abundant, the cell uses ATP rapidly.
4) Signal amplification: Enabling activated kinases, other kinases, and other enzymes and reaction pathways to stimulate signal transduction throughout the whole cell.
5) The cleavage of ATP results in a high level of energy availability and an exergonic reaction. Reversal of the reaction (in the kinase catalyzed state) is not preferred, as the stability will increase.
6) For this reason, the separation of phosphate groups from proteins is carried out by other group of enzymes called phosphatases.

AN EXAMPLE FOR REVERSIBLE COVALENT MODIFICATION

Protein kinase A phopsphorylates its substrates which is a form of covalent modification.

- Protein kinase A is responsible for the phosphorylation of many target enzymes and proteins, from serine or threonine residues.

- Protein kinase A undergoes allosteric regulation by cAMP (Figure).

3) REGULATION BY PROTEOLYTIC ACTIVATION

- Some enzymes are synthesized in an inactive form and the activity of these enzymes is regulated by proteolytic activation. Such precursor enzymes are termed pro-enzymes or zymogens. These enzymes are activated by proteases which break down them into one or several regions.

- Proteolytic activation does not use ATP energy, and such an activation occurs once throughout the life of the enzyme.

- Under what conditions does proteolytic activation occur, which enzymes undergo proteolytic activation?Sindirim enzimleri

1) Enzymes involved in blood clotting
2) Hormones
3) Enzymes involved in apoptosis
4) Collagen
5) Developmental and re-modeling

EXAMPLES FOR PROTEOLYTIC ACTIVATION

The enteropeptidase enzyme released from the small intestine mucosa leads to the release of the pancreatic proenzymes.

FINALISING PROTEOLYTIC ACTIVATION

- How is the activity of enzymes that have undergone proteolytic activation and completed function complete?

- -Pancreatic trypsin inhibitor → → → → Reversible inhibition of trypsin. Insufficient release of pancreatic trypsin inhibitor may lead to acute pancreatitis.

- -Alpha-1 anti-trypsin → → → An elastase inhibitor responsible for the destruction of anti-trypsin, elastin and collagen. The lack of this inhibitor leads to tissue damage and emphysema in the lungs.

4) REGULATION OF ENZYME CONCENTRATION

In conditions that require an increase or decrease in the activity of enzymes, the enzyme concentration varies with multiple biochemical mechanisms.

4) ISOENZYMES

- Enzyme activity can also be regulated using different forms of the same enzyme (isoenzymes).
- Isoenzymes:
- They have different amino acid sequences but catalyze the same reaction.
- They display different enzyme kinetics (km, Vmax, ...).
- They are regulated by different allosteric regulators.
- They are expressed from different genes.

EXAMPLES OF ISOENZYMES

Lactate dehydrogenase

H isozyme → → → found in the heart
It acts in the presence of high oxygen.

M isozyme → → → Striped cadaver is found
It functions in the presence of lower oxygen.

Blockage of blood flow in the heart during myocardial infarction (heart attack) damages the heart muscle and releases H isozyme of the enzyme to the blood through damaged cardiac muscle cells. For this reason, the presence of H isozyme of lactate dehydrogenase in blood is among the markers of heart attack and can be used for diasnosis.

Factors effecting enzyme activity

- pH,
- Temperature,
- Light and other physical parameters,
- Hormones and other biochemical parameters
- Enzyme concentration,
- Substrate concentration,
- Reaction products,
- Various ions

pH

The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH. For example, the pepsin enzyme, which has the strongest effect at pH 1-2, is not effective at neutral and alkaline environments. Trypsin with an optimal pH of 8 is not effective at acidic pH.

aspartate a-carboxyl ..... pH 4.5
lysine ε-amino ..... pH 9.5

Temperature

The rate of the enzymatic reaction also increases with temperature.
But once a certain temperature is exceeded, the enzymes become more denatured and lose their effect like other proteins.

Light and other physical conditions

For example, red and blue light enhance the effect of saliva amylase. UV light has an adverse effect. Even vigorous shaking of the enzyme solution may sometimes denature the enzyme.

Effect of Hormones and Other Biochemical Factors

Glutamate dehydrogenase enzyme consists of 4 subunits loosely attached. These can separate and cause the enzyme's activity to be lost. This alteration in enzyme structure can be brought about by various estrogenic, androgenic, and some other steroid pregnancy hormones.

Enzyme Concentration

The more enzyme molecules present in the environment the faster the reaction will be as long as there are sufficient substrates.

Substrate concentration

The more substrate molecules present in the environment the faster the reaction will be as long as there are sufficient amount enzymes.