ENZYMOLOGY - II

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Processes at the active site

1- **Catalysis by proximity**: for the molecules to react they must come within bond-forming distance of one another. When an enzyme binds substrate molecules at its active site, it creates a region of high local substrate concentration.

Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another.

Function of the Enzyme here is to prevent the bimding, only it made them close to eachother and made region of high local substrate concentration.

Processes at the active site

1- **Catalysis by proximity**:

- So we come to summarize that:

1. There would be no reaction unless the molecules are in bond forming distance.
2. The enzyme would cause crowdness of the substrate molecules at the active site which would cause pushing of the substrate molecules in the active site and then it would finally lead to proximity of the reactive groups.
3. This crowdness would also cause orientation of the reactive groups into the active site and the binding site of the substrate.

- If there is NO stabilization for the reaction it won't be carried out efficiently

Processes at the active site

2- **Acid base catalysis**: the ionizable functional groups of aminoacyl side chains of prosthetic groups contribute to catalysis by acting as acids or bases.

- General acid catalysis involves partial proton transfer from a donor that lowers the free energy of the transition state.

- General base catalysis involves partial proton abstraction from an acceptor to lower the free energy of the transition state.
- They have an important role in the formation of bonds between the enzyme and substrate and stabilization of substrate in the active site to reach the transition state.

3- **Catalysis by strain**: enzymes that catalyze the lytic reactions involve breaking a covalent bond typically bind their substrates in a configuration slightly unfavorable for the bond that will undergo cleavage.

- The substrate binds on the active site of the enzyme in a slightly unfavourable bond, which would lead to loss of covalent bonds

4- **Covalent catalysis**: accelerates reaction rates through transient formation of enzyme-substrate covalent bond and for more stabilization.

Three stages in covalent catalysis:

1- Nucleophilic reaction between enzyme and substrate
2- Electrophilic withdrawl of electrons from substrate
3- Elimination reaction (reverse of stage 1)

4- Covalent catalysis cont:

- The less the energy of activation is needed, the easier for the molecules of substrates in the enzyme to be in the transition state and converted into products.

- The faster the process of removal of the electrons was, the bigger numbers would be in favor of the molecules of the substrate and the reactive groups in the active site of the enzyme in the ionized state.

5- Metal Ion catalysis

- Two classes of metal ion dependent enzymes:

1- Metalloenzymes contain tightly bound transition metal ions (Fe2+, Fe3+, Cu2+, Zn2+, Mn2+) and if removed there would be no reaction.

- The enzyme would never react without the precense of metal ion

2- Metal-activated enzymes loosely bind metal ions (alkali or alkaline metal including Na+, K+, Mg2+ and Ca2+)

- The metal ion isn’t essential part of the enzyme
- So the reaction can be carried out efficiently without them.

- Metal ions enhance catalysis in three major ways:

1- Binding to and orienting substrates for reaction as Mg2+ binding to ATP

2- Mediating redox reaction through changes in oxidation state such as reduction of O2 to H2O through electron transfer etatilicaf ot sdael hcihw (noitazinoi) (snortcele fo lavomeR) fo trap evitca eht ni spuorg evitcaer eht neewteb noitcaretni eht .etartsbus fo etis gnidnib dna emyzne eht

3- Electrostatic stabilization or shielding of negative charges as Mg2+ binding to ATP

6- Electrostatic catalysis

- Enzymes seem to arrange active site charge distributions to stabilize the transition states of catalyzed reactions
- Substrate binding generally excludes water from an enzyme active site generating a low dielectric constant within the active site
- Electrostatic interactions are stronger
- pka's can vary by several pH units due to proximity of charged groups

- Alternative form of electrostatic catalysis: several enzymes as superoxide dismutase apparently use charge distributions to guide polar substrates to their active sites

Enzyme Specificity

- In general, there are four distinct types of specificity:

1- Absolute specificity: the enzyme will catalyze only one reaction.
2- Group specificity: the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups
3- Linkage specificity: the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure
4- Stereo chemical specificity: the enzyme will act on a particular steric or optical isomer.

Cofactors

- Cofactors can be subdivided into two groups: metals and small organic molecules

-small organic molecules are derivatives of Vitamins (Vit.B)

- Cofactors that are small organic molecules are called coenzymes. WEAK
- Most common cofactor are also metal ions.

- If tightly bound, the cofactors are called prosthetic groups. STRONG

- Loosely bound Cofactors serve functions similar to those of prosthetic groups but bind in a transient, dissociable manner either to the enzyme or to a substrate

Prosthetic groups

- Tightly integrated into the enzyme structure by covalent or non-covalent forces. e.g.;
  Pyridoxal phosphate)Vit. B)6
  Flavin mononucleotide(FMN)Vit.B)2
  Flavin adenine dinucleotide(FAD) Vit.B)2
  Thiamin pyrophosphate (TPP)) Vit.B)1
  Biotin)Vit.B)7
  Metal ions – Co, Cu, Mg, Mn, Zn

- Metals are the most common prosthetic groups

Role of metal ions

- Enzymes that contain tightly bound metal ions are termed – Metalloenzymes

- Enzymes that require metal ions as loosely bound cofactors are termed as metal-activated enzymes

- Metal ions facilitate:
  - Binding and orientation of the substrate
  - Formation of covalent bonds with reaction intermediates
  - Interact with substrate to render them more electrophilic or nucleophilic

Coenzymes

- They serve as recyclable shuttles—or group transfer agents—that transport many substrates from their point of generation to their point of utilization.

- The water-soluble B vitamins supply important components of numerous coenzymes.

- Chemical moieties transported by coenzymes include hydrogen atoms or hydride ions, methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides (dolichol).

Diagnostic significance of enzymes

1- Enzymes can act as diagnostic markers of underlying diseases .

2- Enzymes can also act as reagents for various biochemical estimations and detections

Enzymes as diagnostic markers

1- **Functional plasma enzymes** (Plasma derived enzymes):

- Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood.

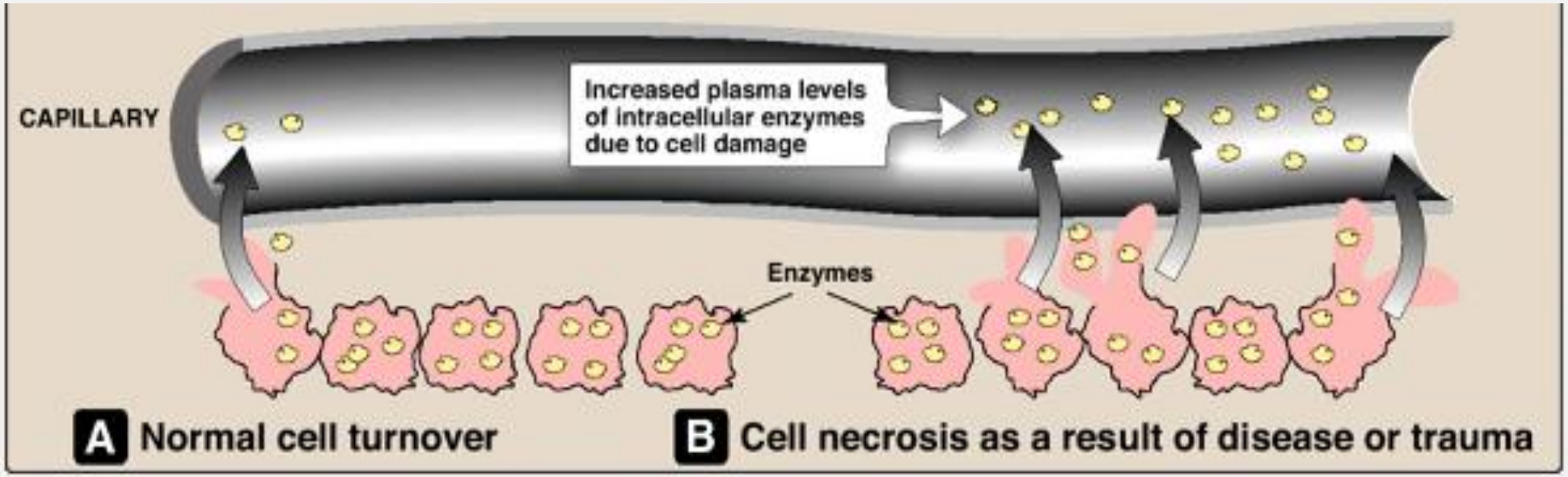
Examples of these functional plasma enzymes include lipoprotein lipase, pseudo cholinesterase, and the proenzymes of blood coagulation and blood clot dissolution .The majority of these enzymes are synthesized in and secreted by the liver.

2- **Nonfunctional plasma enzymes** (Cell derived enzymes):

- Plasma also contains numerous other enzymes that perform no known physiologic function in blood.

-These apparently nonfunctional plasma enzymes arise from the routine normal destruction of erythrocytes, leukocytes, and other cells.

-Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes.



Isoenzymes (Isoenzymes)

- Are homologous enzymes that catalyze the same reaction but have differences in enzymatic properties.
- Often different isoenzymes are found in different locations in a cell or in different organs/tissues of an organism.
- They are from different polypeptide chains that coded by different genes and so, they are affected by different activators and different inhibitors in different tissues.

e.g.:

Lactate dehydrogenase isoenzymes,

- The enzyme interconverts lactate and pyruvate (LDH)
- Humans have two isoenzymic chains for lactate dehydrogenase: LDH (M) found in muscle and LDH (H) found in heart.
- M is optimized to work under anaerobic conditions and H optimized to work under aerobic conditions.

-There are 5 different isoenzymes.
-The relative ratio of the isoenzymes depends on the location in the organism as well as the developmental stage.

Isoenzyme	Tissue origin
LDH1 (H4)	Cardiac and kidney
LDH2 (H3M)	Cardiac, kidney, brain and RBCs
LDH3 (H2M2)	Brain, lung and WBCs
LDH4 (HM3)	Lung, skeletal muscle
LDH5 (M4)	Skeletal muscle and liver

CK/CPK Isoenzymes

- There are three Isoenzymes.
- Measuring them is of value in the presence of elevated levels of CK or CPK to determine the source of the elevation.
- Each isoenzyme is a dimer composed of two protomers 'M' (for muscles) and 'B'(for Brain).
-These isoenzymes can be separated by, electrophoresis or by ion exchange chromatography.

Isoenzyme	Electrophoretic mobility	Tissue of origin	Mean % in blood
MM(CK3)	Least	Skeletal muscle Heart muscle	97-100%
MB(CK2)	Intermediate	Heart muscle	0-3%
BB(CK1)	Maximum	Brain	0%

ENZYME KINETICS

- It is the field of biochemistry concerned with the quantitative

- measurement of the rates of enzyme-catalyzed reactions and the study of the factors affecting these rates.

- The rate of a chemical reaction is described by the number of molecules of reactant(s) to be converted into product(s) in a specified time period which is dependent on the concentration of the chemicals involved in the process and on rate constants that are characteristic of the reaction.

THANK YOU