Enzymology- An overview-1

Enzymes- An introduction

- Biologic (organic catalysts) polymers that catalyze the chemical reactions.

- Enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.

- With the exception of catalytic RNA molecules, or ribozymes, enzymes are proteins.

- In addition to being highly efficient, enzymes are also extremely selective catalysts.

-Thermolabile, site specific, with a high turn over number compared to the inorganic catalysts.

IMP

Characteristics of the enzymes

Enzymes are both intracellular and extracellular catalysts

Some are globular proteins and few are RNA-based molecules

Some enzymes need coenzymes or cofactors

Enhance the speed of biochemical reactions

Forms enzyme-substrate complex

Lowers the activation energy

Active site contains less hydrophobic amino acids

Produces product using specific substrate

Sensitive to temperature, pH, and substrate concentration

Required in very less amount compared to chemical catalyst

Active site contains 3 to 12 amino acids

Enzymes can be recycled or reused

Enzymes are larger than substrate

Function can be inhibited by inhibitors

Nomenclature of enzymes

-In most cases, enzyme names end in **–ase**

-The common name for a hydrolase is derived from the substrate

Urea: remove -a, replace with **-ase** = urease

uric acid = uricase

Lactose: remove - **ose**, replace with - **ase** = lactase

enzyme that works on histidine = histidinase

enzyme that works on argnine = argninase

- Other enzymes are named for the substrate and the reaction catalyzed

Lactate dehydrogenase

this enzyme is working on lactic acid and cause dehydrogenation (oxidation) and it will be converted to pyruvic acid

Pyruvate decarboxylase

this enzyme is working on pyruvic acid and cause the removal of carbon dioxide

- Some names are historical - no direct relationship to substrate or reaction type

Catalase

Pepsin in stomach

Chymotrypsin

Trypsin

in intestines

Classification of Enzymes

- Enzyme Commission (EC) – according to **International Union of Biochemistry and Molecular Biology (IUBMB)**

- Each enzyme was given 4 digit numbers [1.2.3.4]

1st indicates to which class does the enzyme belongs to

1st one of the 6 major classes of enzyme activity

2nd the subclass (type of substrate or bond cleaved)

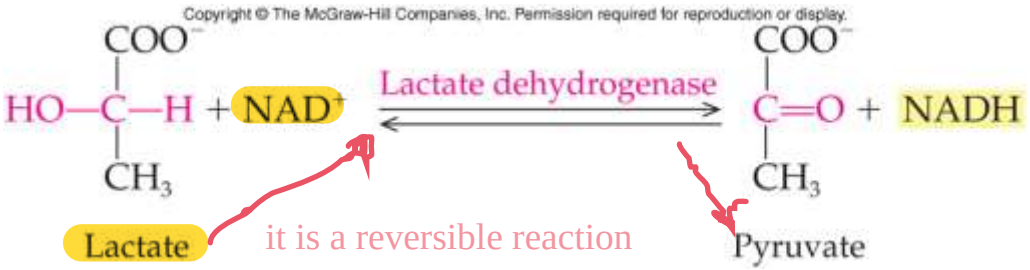
3rd the sub-subclass (group acted upon, cofactor required, etc...)

order for adding the enzyme in the EC by oldest to newest

4th a serial number… (order in which enzyme was added to list)

1- Oxidoreductases (**EC.1**) catalyze redox reactions, such as (Alcohol dehydrogenase [EC 1.1.1.1])

- Reductases
- Oxidases



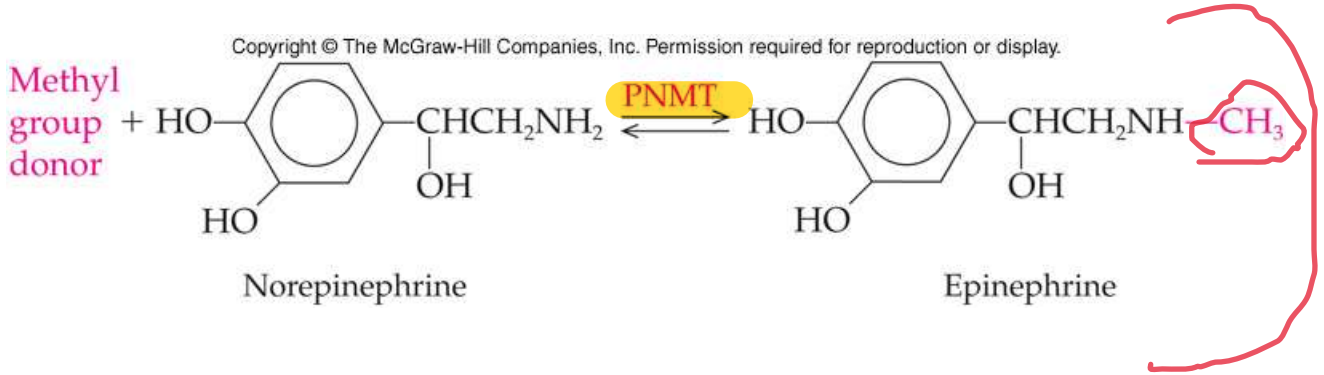
it is a reversible reaction
cuz like the cell gets ride of the hydrogen ions it may also sometimes need the hydrogen to join the pyruvic acid to form lactic acid

2- Transferases (**EC.2**) transfer a group from one molecule to another, such as (Hexokinase [EC 2.7.1.2])

-Transaminases catalyze transfer of an amino group

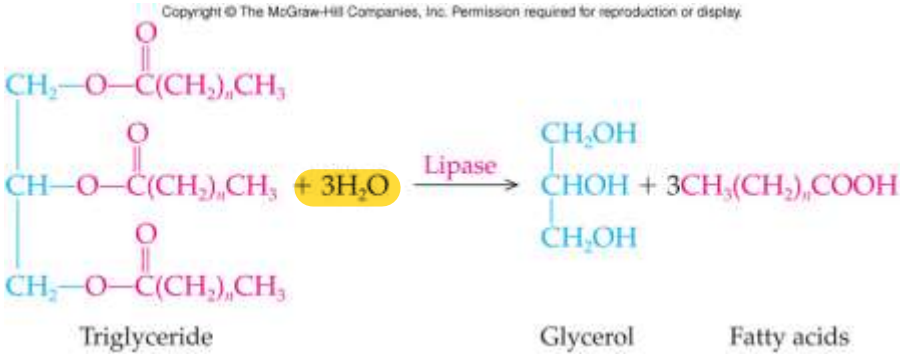
- Kinases transfer a phosphate group

methyl transferases act upon this reaction to give epinephrine



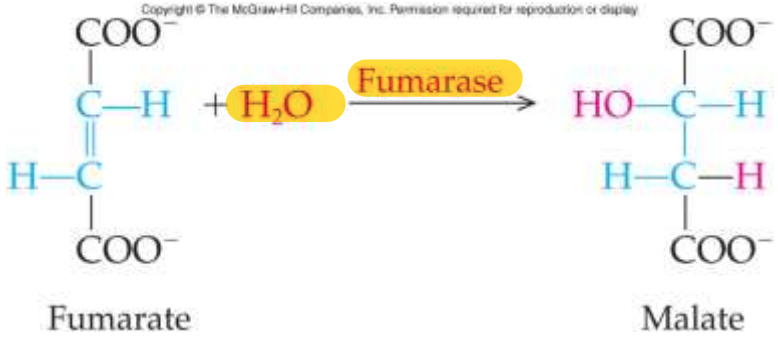
3- Hydrolases (**EC.3**) cleave bonds by adding water, such as
(Alkaline phosphatase [EC 3.1.3.1])

- Phosphatases
- Peptidases
- Lipases



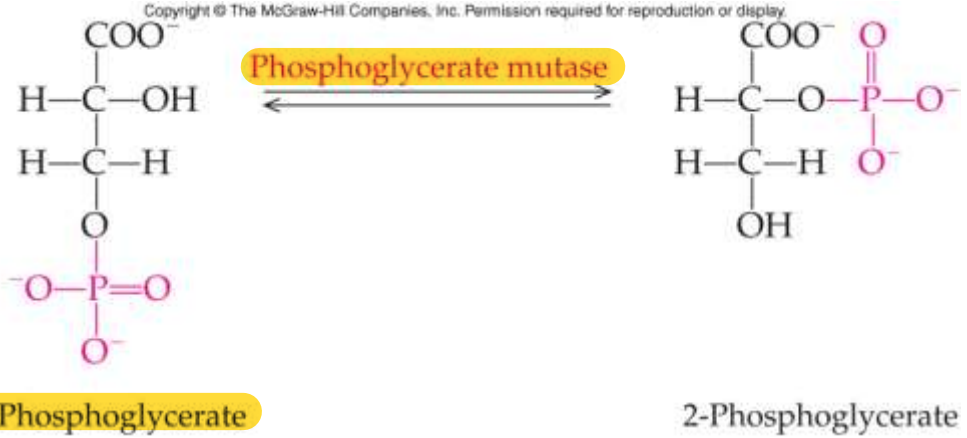
4- Lyases (**EC.4**) catalyze removal of groups to form double bonds or the reverse break double bonds, such as
(Pyruvate decarboxylase [EC 4.1.1.1])

- Decarboxylases
- Synthases



5- Isomerases (**EC.5**) catalyze intramolecular rearrangements, such as (Alanine racemase [EC 5.1.1.1])

- Epimerases
- Mutases



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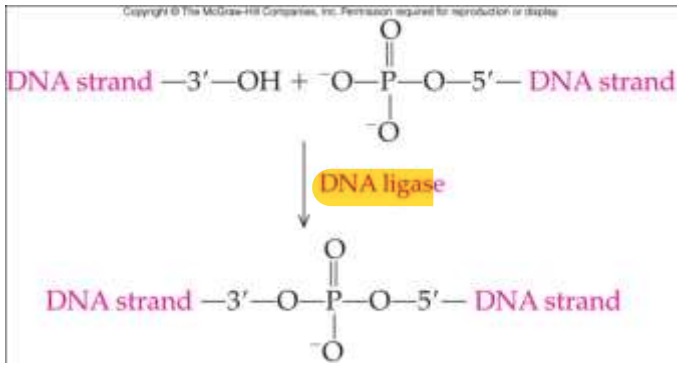
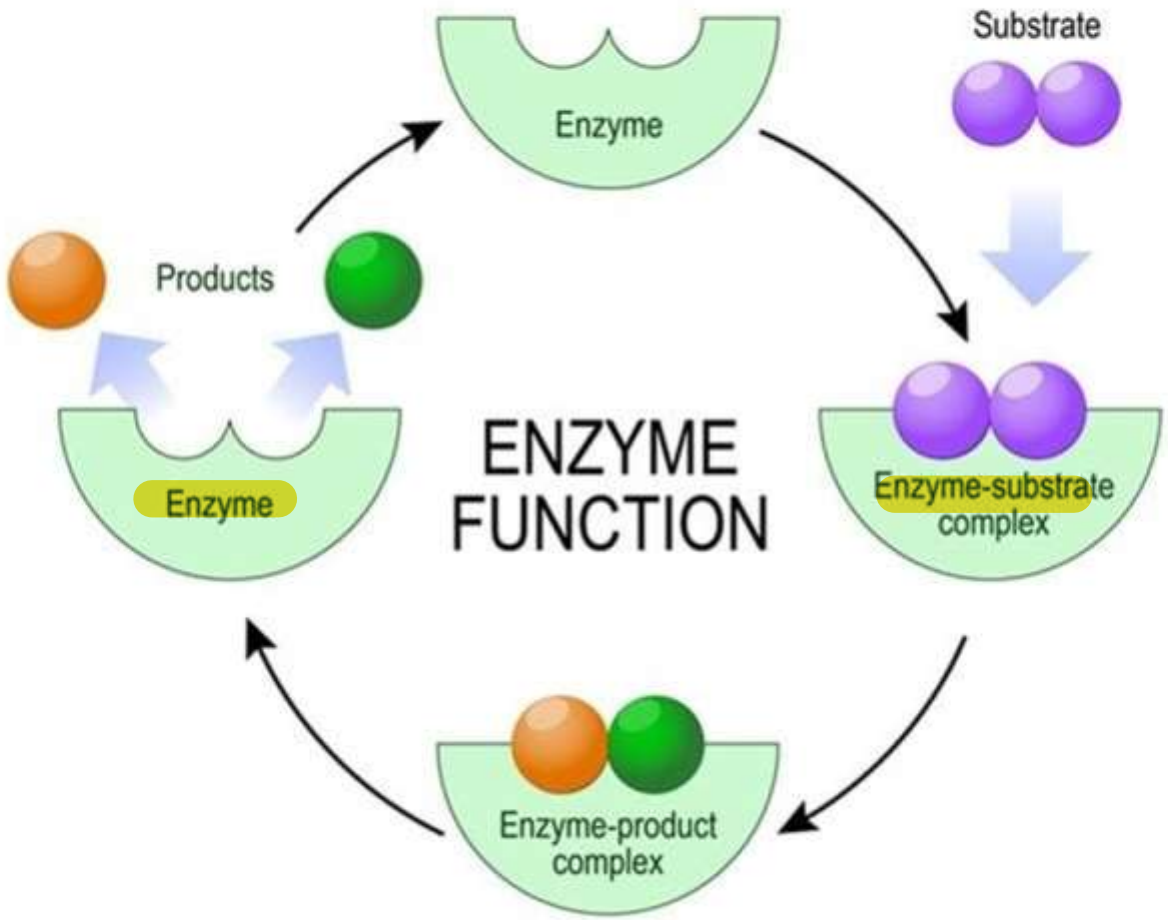
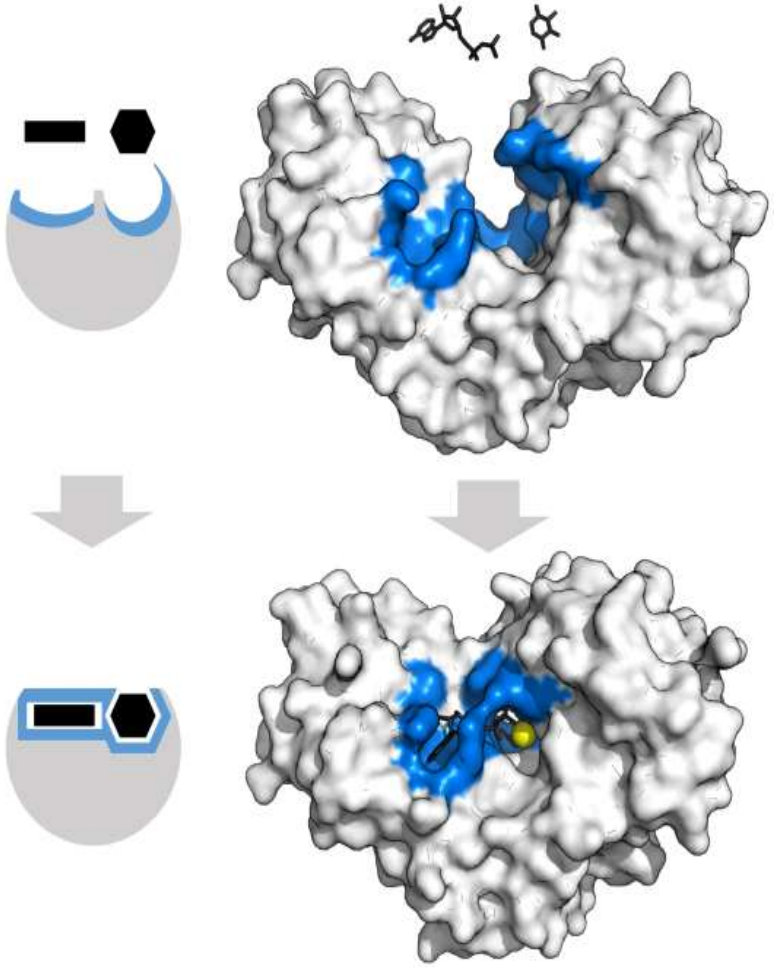
6- Ligases (**EC.6**) catalyze a reaction in which a C-C, C-S, C-O, or C-N bond is made or broken, such as (Isoleucine-tRNA ligase [EC 6.1.1.5])




Substrate
Enzyme
Products
Enzyme
ENZYME
FUNCTION
Enzyme-substrate
complex
Enzyme-product
complex

Active site

- Takes the form of a cleft or pocket like a gate on the surface of the enzyme
- Takes up a relatively small part of the total volume of an enzyme
- Substrates are bound to enzymes by multiple weak attractions
- The specificity of binding depends on the precisely defined arrangement of atoms in an active site
- The active sites of multimeric enzymes are located at the interface between subunits and recruit residues from more than one monomer

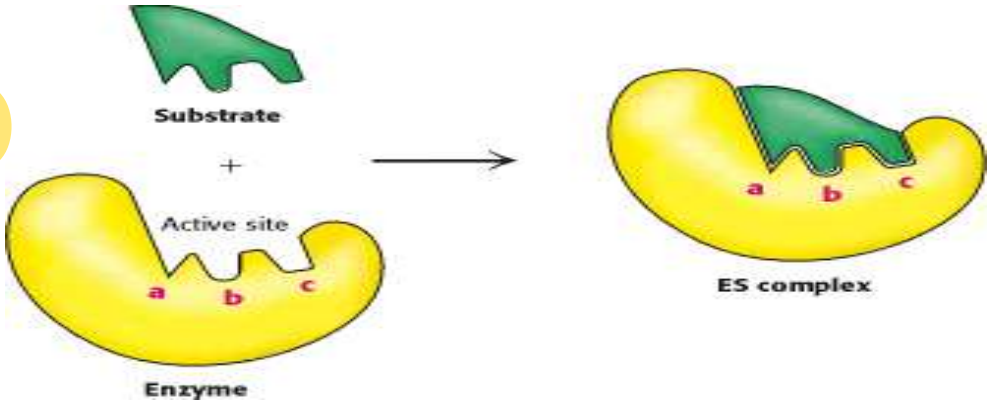


Enzyme substrate binding

-Two models have been proposed to explain how an enzyme binds its substrate: the lock-and –key model and the induced-fit model.

- Lock-and-Key Model of Enzyme-Substrate Binding, in this model, the active site of the unbound enzyme is complementary in shape to the substrate.

-"lock and key model" **accounted for the exquisite specificity of enzyme-substrate interactions**, the implied rigidity of the enzyme's active site failed to account for the dynamic changes that accompany catalysis.



Induced-Fit Model of Enzyme-Substrate Binding

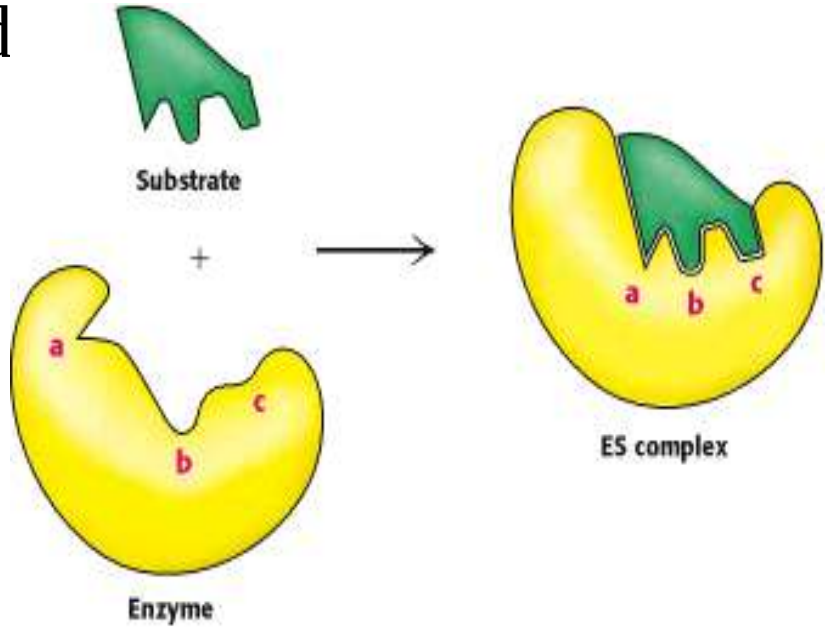
- In this model, the enzyme changes shape on substrate binding.

this model is a flexible model that changes shape so that the substrate can fit

-The active site forms a shape complementary to the substrate only after the substrate has been bound.

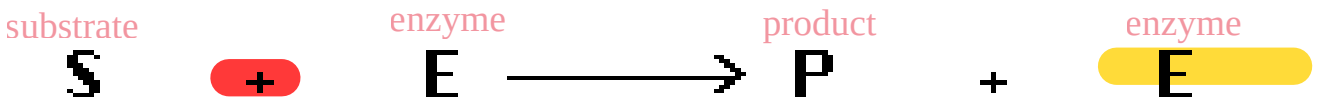
hormones induce the change in the active site

- When a substrate approaches and binds to an enzyme they induce a conformational change, a change analogous to placing a hand (substrate) into a glove (enzyme).



Mechanism of Action of Enzymes

- Enzymes are catalysts and increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are neither used up in the reaction nor do they appear as reaction products.
- The basic enzymatic reaction can be represented as follows:



- Where E represents the enzyme catalyzing the reaction, S the substrate, the substance being changed, and P the product of the reaction.
- The mechanism of action of enzymes can be explained by two perspectives:

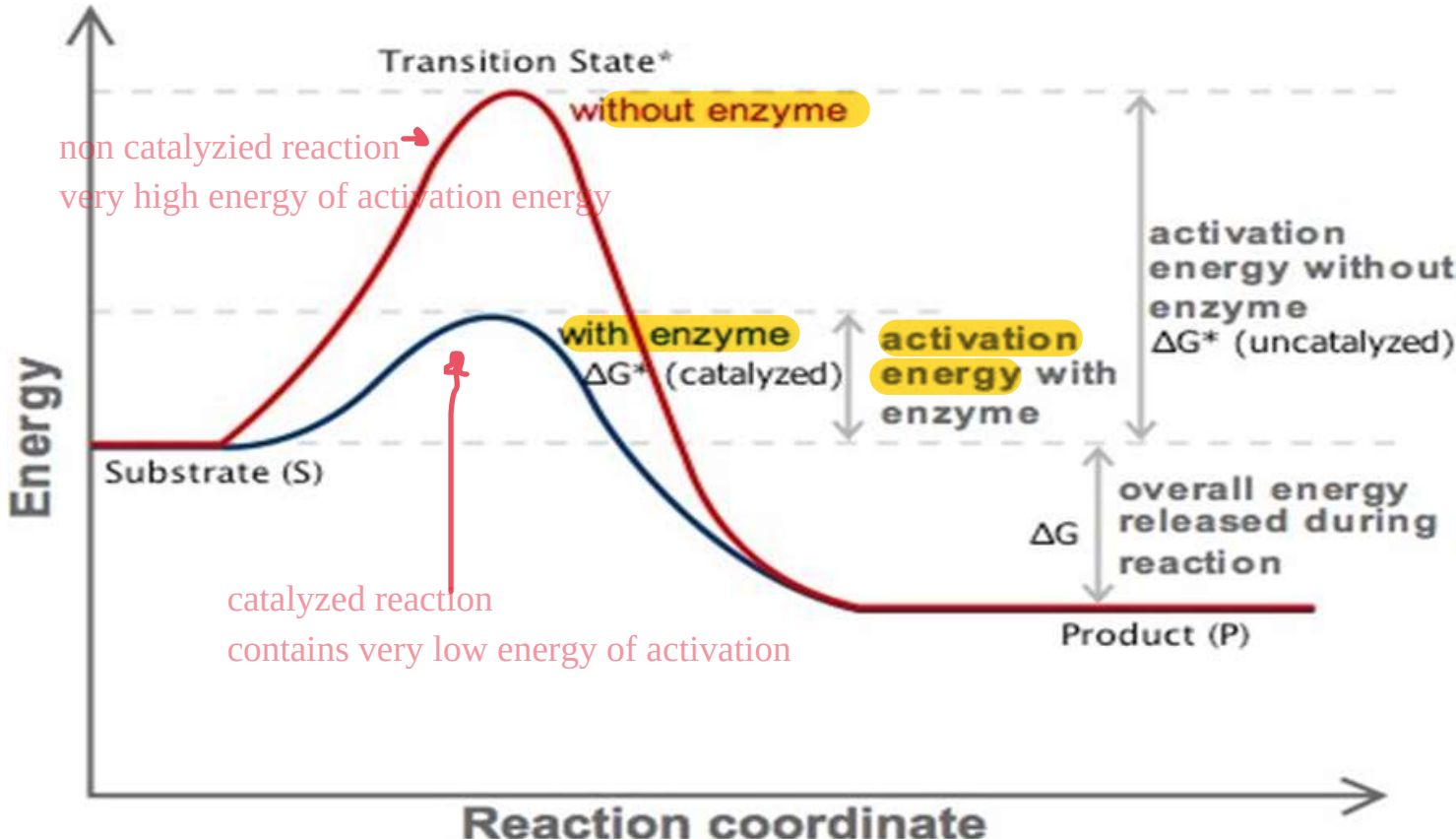
1- Thermodynamic changes

2- Processes at the active site

Thermodynamic changes

- All enzymes accelerate reaction rates by providing transition states with a lowered ΔG F for formation of the transition states.
- The lower activation energy means that more molecules have the required energy to reach the transition state.

the diff is in
-energy of activation
-timing



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.

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** to lower 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.

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.

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

Three stages in covalent catalysis:

1- Nucleophilic reaction between enzyme and substrate

2- Electrophilic withdrawal of electrons from substrate

3- Elimination reaction (reverse of stage 1)

5- Metal Ion catalysis

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- Two classes of metal ion dependent enzymes:

1- Metalloenzymes contain tightly bound transition metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+})

2- Metal-activated enzymes loosely bind metal ions (alkali or alkaline metal including Na^{+}, K^{+}, Mg^{2+} and Ca^{2+})

- Metal ions enhance catalysis in three major ways:

1- Binding to and orienting substrates for reaction as Mg^{2+} binding to ATP 3 bridges form

2- Mediating redox reaction through changes in oxidation state such as reduction of O_2 to H_2O through electron transfer

3- Electrostatic stabilization or shielding of negative charges as Mg^{2+} 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.

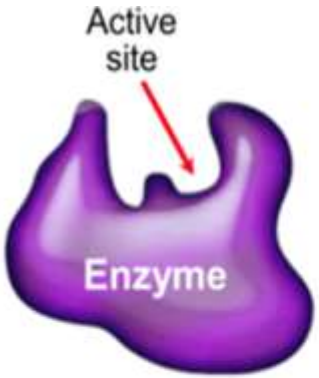
- Some enzyme require cofactors to be active.
- Cofactors are a non-protein components of the enzyme.
- Organic Molecules (Coenzymes)
- Inorganic ions e.g., Ca2+ , Zn2+ (Prosthetic group)
- Cofactors may be:

tightly bound

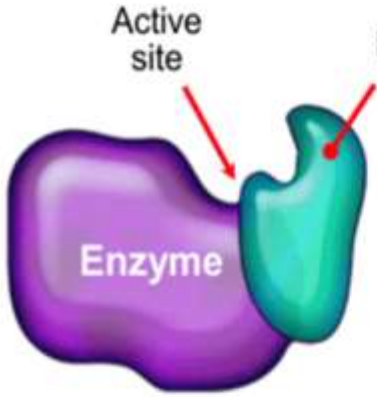
1- The Permanently attached cofactors, are called Prosthetic group (such as a vitamin, sugar, or lipid or inorganic such as a metal ion)

loosely bound

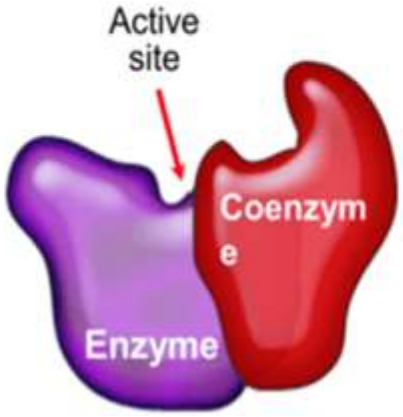
2- Temporarily attached cofactors are called coenzyme, its detach after a reaction and may participate in the reaction with other enzyme.



Enzyme is protein only
Example: lysozyme



Enzyme + prosthetic group
Example:
flavoprotein + FAD



Enzyme + coenzyme
Example:
dehydrogenases + NAD

Cofactors

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

- Cofactors that are small organic molecules are called coenzymes.

- Most common cofactor are also metal ions.

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

- 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 — derivated from vitamin D6
  - Flavin mononucleotide (FMN) — derivated from riboflavin vitamin b2
  - Flavin adenine dinucleotide (FAD) — derivated from riboflavin vitamin b2
  - Thiamin pyrophosphate (TPP) — derivated from vitamin b1
  - Biotin — derivated from vitamin b7
  - Metal ions – Co, Cu, Mg, Mn, Zn

- Metals are the most common prosthetic groups

Coenzymes

- Very often vitamins
- 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).

the DR did not mention about memorizing this table and asked to understand it

Important Prosthetic Groups and Coenzymes

they are not only made of metal ions

Prosthetic Group	Enzymes/ Proteins
Zn^{++}	Carbonic anhydrase , Alcohol dehydrogenase
Fe^{+++} or Fe^{++}	Hemoglobin, Cytochromes, ferrodoxin
Cu^{++} or Cu^{+++}	Cytochrome oxidase
K^{+} and Mg^{++}	Pyruvate Phosphokinase

derivatives

Coenzymes	Vitamins
Nicotinamide adenine dinucleotide (NAD^{+}) or nicotinamide adenine dinucleotide phosphate ($NADP^{+}$)	vitamin B_3 (niacin)
Flavin mononucleotide (FMN^{+}) or flavin adenine dinucleotide(FAD^{+})	vitamin B_2 (riboflavin)
Pyridoxal phosphate	vitamin B_6 (pyridoxine)
Coenzyme A	Pantothenic Acid

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

2 types

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** .

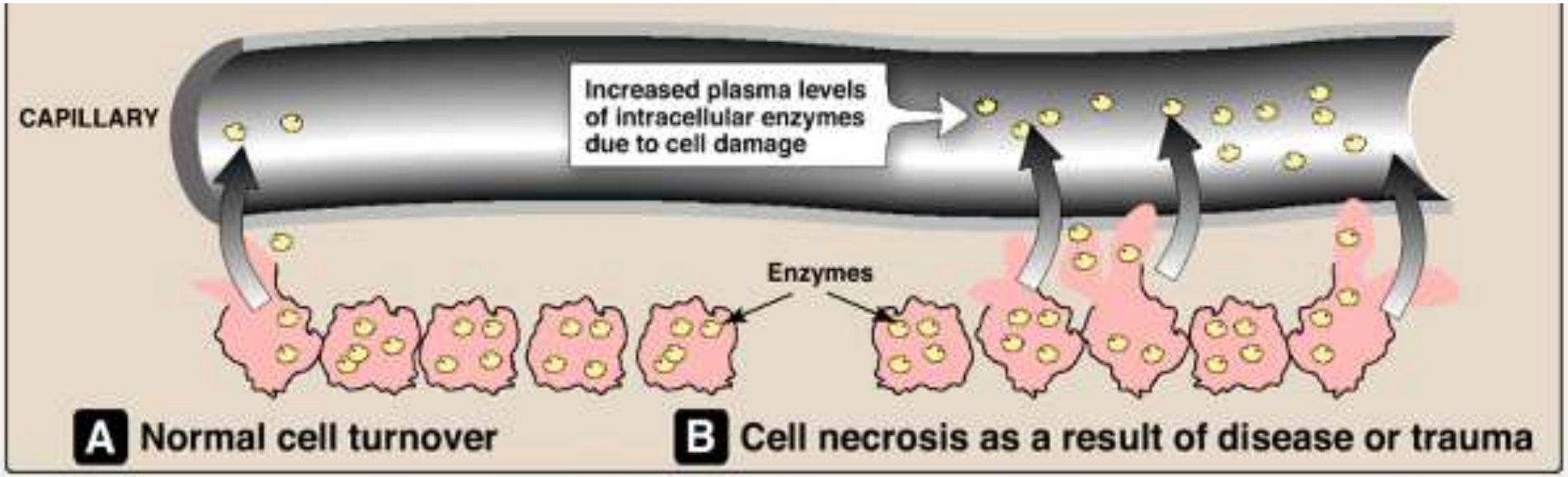
The majority of them are synthesized in and secreted by liver.

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.



2 types of enzymes that are used in the diagnosis of diseases

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) have only H polypeptide	Cardiac and kidney
LDH2 (H3M) have 3 H and 1 M polypeptide	Cardiac, kidney, brain and RBCs
LDH3 (H2M2) 2 H and 2 M	Brain, lung and WBCs
LDH4 (HM3) 1 H and 3 M	Lung, skeletal muscle
LDH5 (M4) 4 M	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 (speed)	Tissue of origin	Mean % in blood
MM(CK3)	Least (2 M polypeptide chains)	Skeletal muscle Heart muscle	97-100% are the most in number and have the least mobility
MB(CK2)	Intermediate (1 M and 1 B)	Heart muscle	0-3%
BB(CK1)	Maximum (2 B polypeptide chains)	Brain	0%

M have a greater mass than B

he skipped it

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.