ENZYMOLOGY 1

Introduction

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Enzymology 1

- **Definition**: Biologic (organic catalysts) polymers that catalyze the chemical reactions.
- **Nature**: Enzymes are proteins except catalytic RNA molecules (Ribozymes) (Ribozymes: short segment of RNA molecule which act as enzyme in **processing RNA molecules** (From immature to mature RNA))
- **Characteristics**:
  i. Enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction. Throughout their life span
  ii. Highly efficient.
  iii. Extremely selective catalysts. Specific
  iv. Thermolabile. (Enzymes are affected by the change of temperature when the temperature increases the enzymes will denatured)
  v. Site specific. (Enzymes found in cytoplasm differ from those found in organelles or membrane)
  vi. High turnover number compared to inorganic catalysts and other organic catalysts
  (Turnover number: the number of substrate molecules that can be converted by one molecule of catalyst into product molecule in a unit time)
- **Inorganic Catalysts such as Ni, Pb, Pt.**
  - Thermostable
  - Lower turnover number

Nomenclature of enzymes (In most cases, enzyme names end in –ase)

1. The common name for a hydrolase is derived from the substrate.
   - Urea: remove -a, replace with -**ase** = urease
   - Lactose: remove - **ose**, replace with - **ase** = lactase
2. Other enzymes are named for the substrate and the reaction catalyzed.
   - Lactate dehydrogenase
   - Pyruvate decarboxylase
3. Some names are historical - no direct relationship to substrate or reaction type. (Digestive Enzymes)
   Catalase, Pepsin, Chymotrypsin, Trypsin

Classification of Enzymes

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

- Each enzyme was given 4-digit numbes [1.2.3.4]

1st one of the 6 major classes of enzyme activity

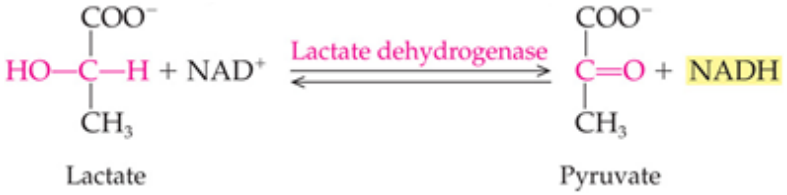
2nd the subclass (type of substrate or bond cleaved)

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

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

The 6 Magor Classes are:

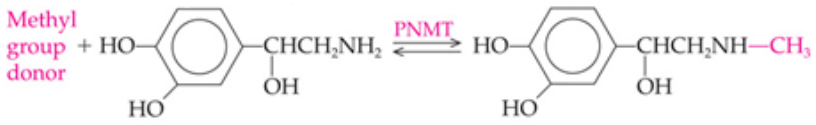
1) Oxidoreductases (EC.1) catalyze redox reactions.
   such as (Alcohol dehydrogenase [EC 1.1.1.1])
   - Reductases
   - Oxidases



Lactate Pyruvate

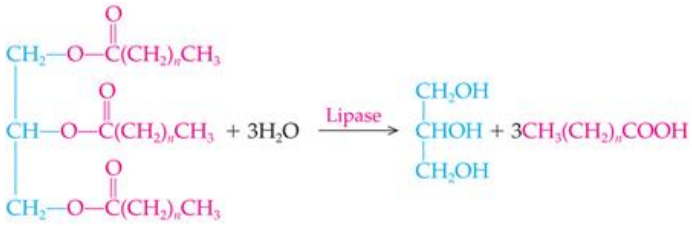
Note1: In biological systems oxidation and reduction reactions take place together.
Note2: Because these reactions change the pH, they require H^+ Carriers (NAD/FAD).

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 (From donor ATP to acceptor)
   - Transmethylases; such as: PNMT transfer a methyl group (From donor SAM to an acceptor)

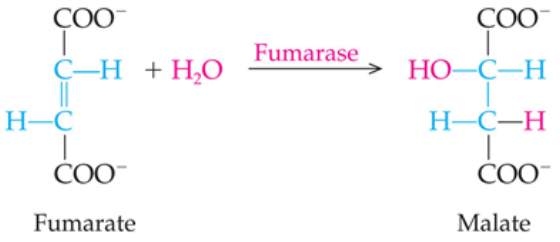


Norepinephrine Epinephrine

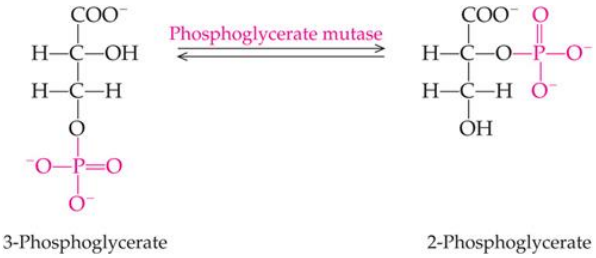
3) Hydrolases (**EC.3**) cleave bonds by adding water
   such as (Alkaline phosphatase [EC 3.1.3.1])
   - Phosphatases
   - Peptidases
   - Lipases



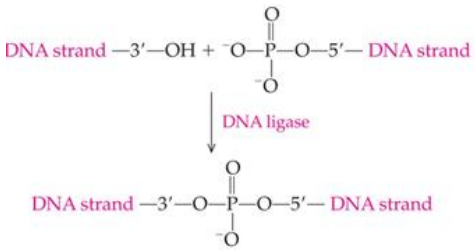
4) Lyases (**EC.4**) catalyze removal of groups to form double bonds or the reverse break double bonds.
   such as (Pyruvate decarboxylase [EC 4.1.1.1])
   - Decarboxylases
   - Synthases
   - Fumarase (Catalyze the addition of H_2O to fumarate to convert it to malate)
     Note: The adding of water here is to convert the state of the bond and without the cleave of the molecule like hydrolases (double→single)



5) Isomerases (**EC.5**) catalyze intramolecular rearrangements. (Change the position of some groups intramolecularly)
   such as (Alanine racemase [EC 5.1.1.1])
   - Epimerases
   - Mutases
   - Racemases



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



E.g. DNA ligase is ligase that repairs single-stranded discontinuities in double stranded DNA molecules.

E.g. T4 DNA ligase is the most-commonly used in laboratory research, it can ligate either cohesive or blunt ends of DNA, oligonucleotides

Potentially confusing enzyme nomenclature: synthetase (requires ATP), synthase (no ATP required); phosphatase (uses water to remove phosphoryl group), phosphorylase (uses Pi to break a bond and generate a phosphorylated product); dehydrogenase (NAD+/FAD is electron acceptor in redox reaction), oxidase (O2 is acceptor but oxygen atoms are not incorporated into substrate), oxygenase (one or both oxygens atoms are incorporated).

Active site (Catalytic site):

- **Definition**: The site to which the substrate is binding to the enzyme
- **Shape**: Takes the form of a cleft or pocket
- **Location**: at any region of an enzyme and takes up a relatively small part of the total volume of an enzyme
- **Active site characteristics**
  A. Should have 3D configuration
  B. Should be complementary to the binding site of the substrate
  C. Should contain highly reactive groups
     - The most highly reactive groups of amino acids
       a. Hydroxy containing AAs: Serine, Threonine, Tyrosine
       b. Modified Hydroxy containing AAs: Hydroxyproline, Hydroxyleucine
       c. Acidic AAs: Glutamate, Aspartate
       d. Imidazole containing AAs: Histidine
       e. Sulfhydryl containing AAs: Cysteine

- ❖ Special arrangement of reactive groups in the active site of the enzyme will define the special 3D arrangement of the active site
- ❖ The specificity of binding depends on the precisely defined arrangement of atoms in an active site
- ❖ Q: Which of the following amino acids participate the most in the active? (Serine, Threonine, Tyrosine, Hydroxyproline)
  Answer: Serine Why??
  Tyrosine: used in producing hormones and melanin
  Threonine: essential amino acid
  Hydroxyproline: modified amino acid
  Serine: nonessential, nonmodified, sufficient amount in cells
- ❖ Substrates are bound to enzymes by multiple weak attractions, in order to stabilize the substrate in the active site
  Types of bonds (interactions):

  a- Hydrogen bonding
  b- Electrostatic interaction
  c- Hydrophobic interaction
  d- Disulfide bonding (strongest)
- ❖ The active sites of multimeric enzymes (4ry Structure) are located at the interface between subunits and recruit residues from more than one monomer, thus the substrate is more stabilized because the substrate form bonds between more than one subunit

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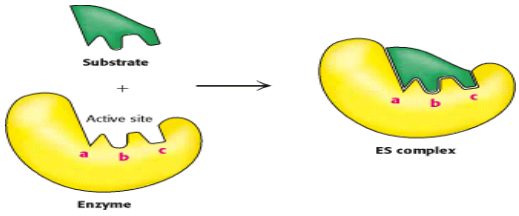
Enzyme substrate binding

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

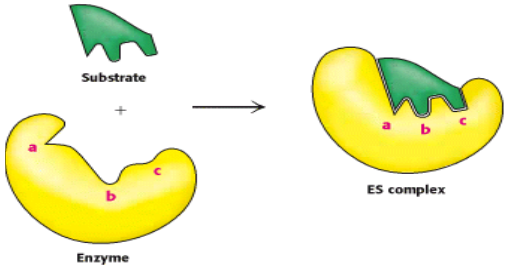
Lock-and-Key Model of Enzyme-Substrate Binding

- 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. (الترجمة: هذ النموذج يعد مثال على الدقة في التخصص وتميز الروابط)
- 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

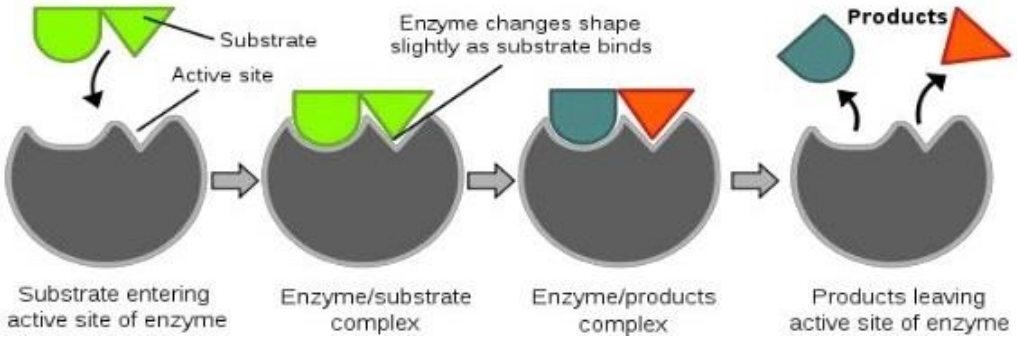
- In this model, the enzyme changes shape on substrate binding.
- The active site forms a shape complementary to the substrate only after the substrate has been bound.
- When a substrate approaches and binds to an enzyme they induce a conformational change, a change analogous to placing a hand (substrate) 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:

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

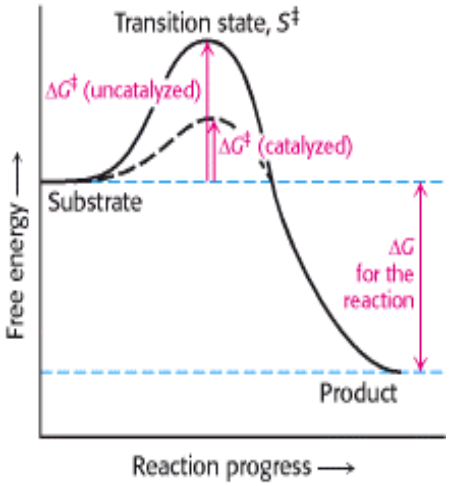


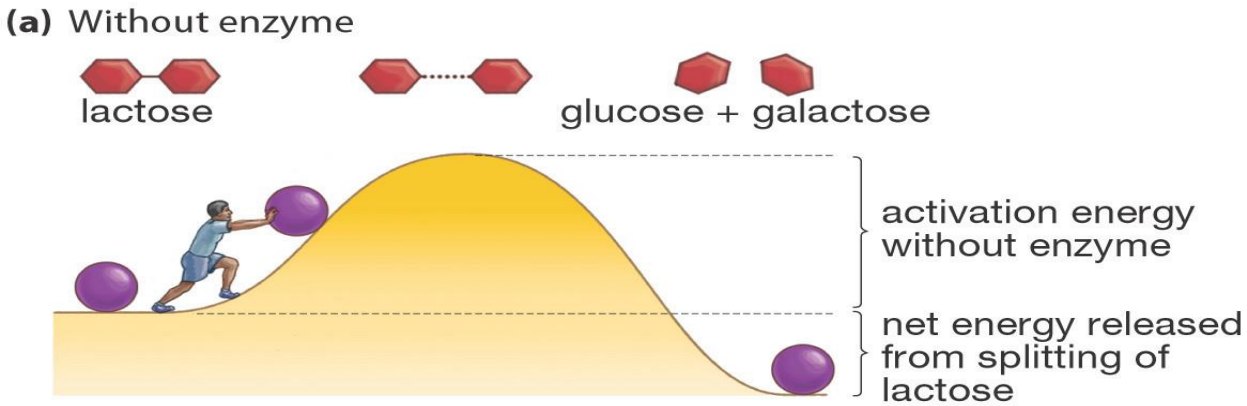
- Where E represents the enzyme catalyzing the reaction, S the substrate, the substance being changed, and P the product of the reaction.
- The mechanism of action of enzymes can be explained by two perspectives:

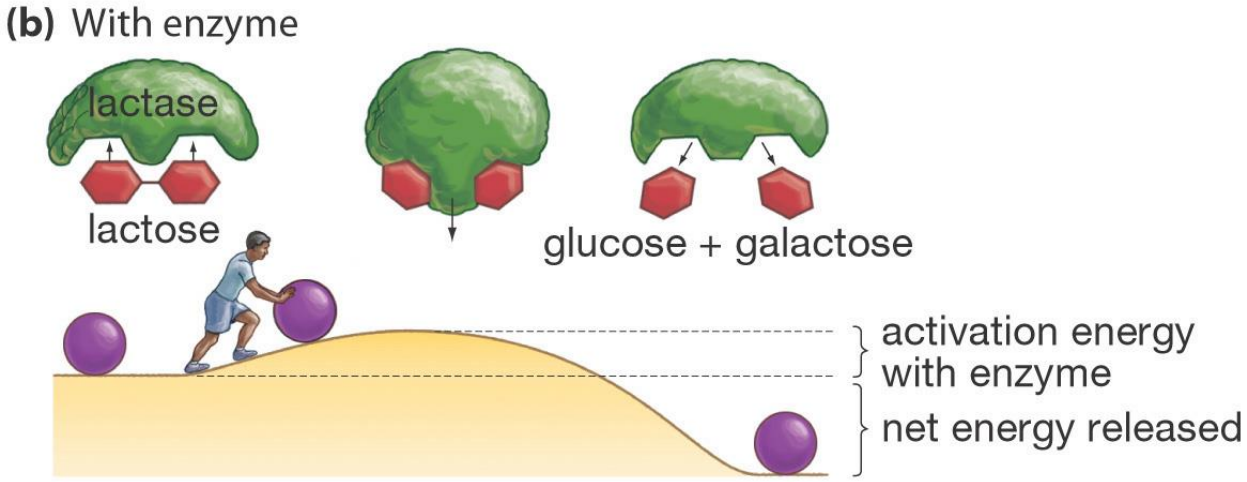
  1- Thermodynamic changes
  2- Processes at the active site

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.
- Note: Activation energy (ΔG F): Energy needed to be supplied in order to reach transition state







Processes at the active site (How does enzymes speed up reactions)

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. (Microenvironment)
- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another.
- Note: This prosses is used by anabolism enzymes by which they increase the possibility of bond formation

2- Acid base catalysis:

- The ionizable functional groups of aminoacyl side chains of prosthetic groups (Glutamate, Aspartate, Imidazole ring of Histidine) contribute to catalysis by acting as acids or bases [[(Proton donor/acceptor) (Proton carriers)]]
- 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
- Importance of these ionizable groups:
  a) Stabilizing of the substrate in the active site (Ionizable → carry charge → cam make more interaction with the substrate → stabilize the substrate in the active site)
  b) Help in the function of the enzyme by acting as proton carrier groups

3- Catalysis by strain:

- Special property of the 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:

a. Nucleophilic reaction between enzyme and substrate
b. Electrophilic withdrawal of electrons from substrate
c. Elimination reaction (reverse of stage 1)

5- Metal Ion catalysis

- Two classes of metal ion dependent enzymes:
  1. Metalloenzymes contain tightly bound transition metal ions (Fe2+, Fe3+, Cu2+, Zn2+, Mn2+)
  2. Metal-activated enzymes loosely bind metal ions (alkali or alkaline metal including Na+, K+, Mg2+ and Ca2+)
- 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
  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 uses 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. (Specific substrate with a particular bond)

2- **Group specificity:** the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups
E.g. Transmethylase

3- **Linkage specificity:** the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure
E.g. Lipase which work on the ester bond of the TAG regardless fatty acids bonded

4- **Stereo chemical specificity:** the enzyme will act on a particular steric or optical isomer.
E.g. In our bodies enzymes works on L-amino acids and not D-amino acids

Dual specificity of enzymes: are enzymes which can act on two different substrates catalyzing the same reaction or act on two different substrates catalyzing two different reactions

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Cofactors

- **Terminology:**
  - **Cofactor:** A cofactor is a non-protein chemical that assists with a biological chemical reaction. Co-factors may be metal ions, organic compounds, or other chemicals that have helpful properties not usually found in amino acids. Some cofactors can be made inside the body, such as ATP, while others must be consumed in food.
  - **Prosthetic group:** is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins
  - **Coenzyme:** is an organic non-protein compound that binds with an enzyme to catalyze a reaction. (serve as recyclable shuttles—or group transfer agents—that transport many substrates from their point of generation to their point of utilization.)
- 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
- Cofactors can be subdivided into two groups: metals and small organic molecules
  - **Metal ions (Inorganic molecules):**
    - Enzymes that contain tightly bound metal ions (prosthetic group) are termed as **Metalloenzymes.**
    - Enzymes that require metal ions as loosely bound cofactors are termed as **metal-activated enzymes.**
    - Metal ions facilitate:
      a. Binding and orientation of the substrate
        - Metals facilitate the binding of the substrate to the active site by extending the repertoire of catalytic capabilities beyond those afforded

by the limited number of functional groups present on the aminoacyl side chains of peptides.

- Also, they rotate the substrate in order to bind correctly

b. Formation of covalent bonds with reaction intermediates

- Every metabolic pathway is consisting from more than one reaction, these pathways begin with substrates, passing through the reactions we got unstable molecules called intermediates and ending by products
- These unstable intermediates might flee from the pathway thus the metal ions covalently bind to it in order to keep it in

c. Interact with substrate to render them more electrophilic or nucleophilic

- Thus, the substrate molecule carry charges → keep the substrate molecule in the reactive form → stabilize the substrate in the active site → increase the rate of reaction

Organic molecules:

- Most cofactors that are small organic molecules are called coenzymes. (if it satisfies the circumstances)
- Tightly bounded organic molecules are prosthetic groups (some name it as coenzyme prosthetic groups)
- Other organic molecules that are not coenzymes such as ATP are considered loosely bounded cofactors (must be present in the medium surrounding the enzyme for catalysis to occur).

➢ **Prosthetic groups**

Tightly integrated into the enzyme structure by covalent or non-covalent forces. e.g.;

- Pyridoxal phosphate (B6)
- Flavin mononucleotide (FMN) (B2)
- Flavin adenine dinucleotide (FAD) (B2)
- Thiamin pyrophosphate (TPP) (B1)
- Biotin (B7)
- Folate (B9)
- Metal ions – Co, Cu, Mg, Mn, Zn

Metals are the most common prosthetic groups

- **Coenzymes**
  - In order for an organic molecule to be cofactor it must satisfy some circumstances:
    - **a.** It must not bound tightly to enzymes
    - **b.** it serves as recyclable shuttle—or group transfer agent—that transport many substrates from their point of generation to their point of utilization.
  - Chemical moieties transported by coenzymes include hydrogen atoms or hydride ions (NAD, FAD), methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides (dolichol).
  - The water-soluble B vitamins supply important components of numerous coenzymes.
  - Unlike enzymes coenzymes are not specified for a current reaction

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Diagnostic significance of enzymes

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

E.g. Glucose oxidase enzyme is used to measure the blood glucose level

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

A. Functional plasma enzymes

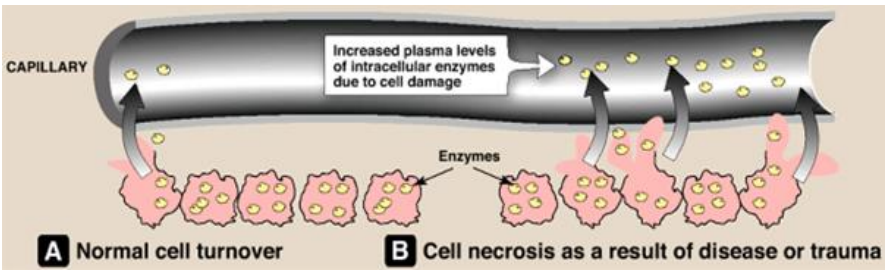
B. Nonfunctional plasma enzymes

A. 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.
- Thus, high concentration of them is normal whereas low concentration is abnormal
- 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.

B. 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 (apoptosis) 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.



SERUM ENZYME	MAJOR DIAGNOSTI C USE
Amylase	Liver and pancreatic disease
Acid phosphate	Prostate Cancer
Alkaline phosphatase	Liver or bone disease
Creatine phosphokinase	Myocardial infarction, muscle disorders
Lactate dehydrogenase	Myocardial Infarction, leukemia, anemia
Renin	Hypertension

	Nonfunctional plasma enzymes	Functional plasma enzymes
Substrates location	Intracellular	Blood plasma
Low concentrations	Normal	Abnormal
High concentrations	Abnormal	Normal

Isoenzymes (Isozymes)

- They are type of intracellular enzymes
- They are different forms of the same enzyme
- They have some similar characteristics:
  a) Catalyze the same reaction. b) Give the same products.
  c) Work on the same substrate.
- They differ in other characteristics:
  a) Their origin (Often different isozymes are found in different locations in a cell or in different organs/tissues of an organism).
  b) 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.
  c) Have differences in enzymatic properties. Such as: electrophoresis mobility, and molecular weight
- **Examples:**

1. **<u>Lactate dehydrogenase isozymes</u>**

- The enzyme interconverts lactate and pyruvate (LDH)
- Humans have two isozymic chains for lactate dehydrogenase:
  i. LDH (M) found in muscle
  ii. LDH (H) found in heart.
- M is optimized to work under anaerobic conditions.
  H is optimized to work under aerobic conditions.
- It is tetrameric molecule (Consist of 4 subunits) → There are 5 isozymes
- The relative ratio of the isozymes depends on the location and the developmental stage of the organism.

1- LDH1 (H4)	cardiac and kidney	(Homotetrameric)
2- LDH2 (H3M)	cardiac, kidney, brain and RBCs	(Heterotetrameric)
3- LDH3 (H2M2)	brain, lung and WBCs	(Heterotetrameric)
4- LDH4 (HM3)	lung, skeletal muscle	(Heterotetrameric)
5- LDH5 (M4)	skeletal muscle and liver	(Homotetrameric)

2. **CK/CPK Isozymes**

- There are three Isoenzymes.
- Each isoenzyme is a dimer composed of two protomers (subunits) 'M' (for muscles) and 'B' (for Brain).
- These isoenzymes can be separated by, electrophoresis or by ion exchange chromatography. (M electrophoresis mobility < B electrophoresis mobility)

ISOENZYME	ELECTROPHORETIC MOBILITY	TISSUE OF ORIGIN	MEAN % IN BLOOD
MM(CK3)	Least (Heaviest)	Skeletal muscle Heart muscle	97-100%
MB(CK2)	Intermediate (Medium size/weight)	Heart muscle	0-3%
BB(CK1)	Maximum (Lightest)	Brain	0%

Note: Biomarkers should have certain characteristics:

- ❖ Highly sensitive: with least duration, the concentration of the biomarker is increased dramatically after the occurrence of the disease
- ❖ Highly specific: Only released from one organ

E.g. LH5 = diagnosis of liver disease
CK-MB = diagnosis of M.I
LDH3 = diagnosis of leukemia

ملاحظة: هذه صفحة هي عبارة عن جزئية ال cofactors نسخ عن المصدر الذي نقل منه الدكتور بهدف توضيح لا غير
المرجع: Harper's Illustrated Biochemistry 29th edition

PROSTHETIC GROUPS, COFACTORS, & COENZYMES PLAY IMPORTANT ROLES IN CATALYSIS

Many enzymes contain small nonprotein molecules and metal ions that participate directly in substrate binding or catalysis. Termed prosthetic groups, cofactors, and coenzymes, these extend the repertoire of catalytic capabilities beyond those afforded by the limited number of functional groups present on the aminoacyl side chains of peptides.

Prosthetic Groups Are Tightly Integrated into an Enzyme's Structure

Prosthetic groups are distinguished by their tight, stable incorporation into a protein's structure by covalent or noncovalent forces. Examples include pyridoxal phosphate, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), thiamin pyrophosphate, biotin, and the metal ions of Co, Cu, Mg, Mn, and Zn. Metals are the most common prosthetic groups. The roughly one-third of all enzymes that contain tightly bound metal ions are termed metalloenzymes. Metal ions that participate in redox reactions generally are complexed to prosthetic groups such as heme or iron-sulfur clusters. Metals also may facilitate the binding and orientation of substrates, the formation of covalent bonds with reaction intermediates (Co^{2+} in coenzyme B12), or interact with substrates to render them more electrophilic (electron-poor) or nucleophilic (electron-rich).

Cofactors Associate Reversibly with Enzymes or Substrates

Cofactors serve functions similar to those of prosthetic groups but bind in a transient, dissociable manner either to the enzyme or to a substrate such as ATP. Unlike the stably associated prosthetic groups, cofactors therefore must be present in the medium surrounding the enzyme for catalysis to occur.

The most common cofactors also are metal ions. Enzymes that require a metal ion cofactor are termed metal-activated enzymes to distinguish them from the metalloenzymes for which metal ions serve as prosthetic groups.

Coenzymes Serve as Substrate Shuttles

Coenzymes serve as recyclable shuttles—or group transfer agents—that transport many substrates from their point of generation to their point of utilization. Association with the coenzyme also stabilizes substrates such as hydrogen atoms or hydride ions that are unstable in the aqueous environment of the cell. Other chemical moieties transported by coenzymes include methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides (dolichol).

Many Coenzymes, Cofactors & Prosthetic Groups Are Derivatives of B Vitamins

The water-soluble B vitamins supply important components of numerous coenzymes. Several coenzymes contain, in addition, the adenine, ribose, and phosphoryl moieties of AMP or ADP. Nicotinamide is a component of the redox coenzymes NAD and NADP, whereas riboflavin is a component of the redox coenzymes FMN and FAD. Pantothenic acid is a component of the acyl group carrier coenzyme A. As its pyrophosphate, thiamin participates in decarboxylation of α-keto acids, and folic acid and cobamide coenzymes function in one-carbon metabolism.