Al-Rasheed University College
Department of Medical Laboratory Technologies
Second class
Biochemistry

Lecture 2
Enzymes (continued)

By
Dr. Rusul H. Hamza

Effect of Inhibitors

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called **inhibitor**. Two general classes

1. Reversible inhibitor
2. Irreversible inhibitor.

Reversible Inhibitor

- Reversible inhibitors bind to enzymes through **noncovalent** bonds.
- Activity of the enzyme is restored fully when the inhibitor is removed.

Types of reversible inhibitors are:

i. Competitive inhibitor

ii. Noncompetitive inhibitor

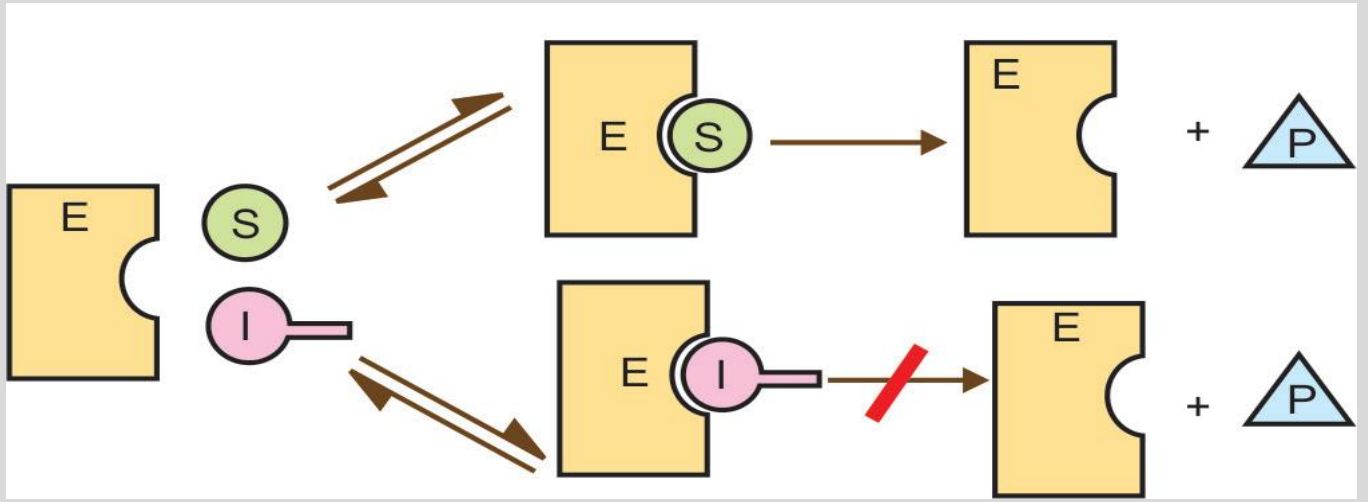
iii. Uncompetitive inhibitor.

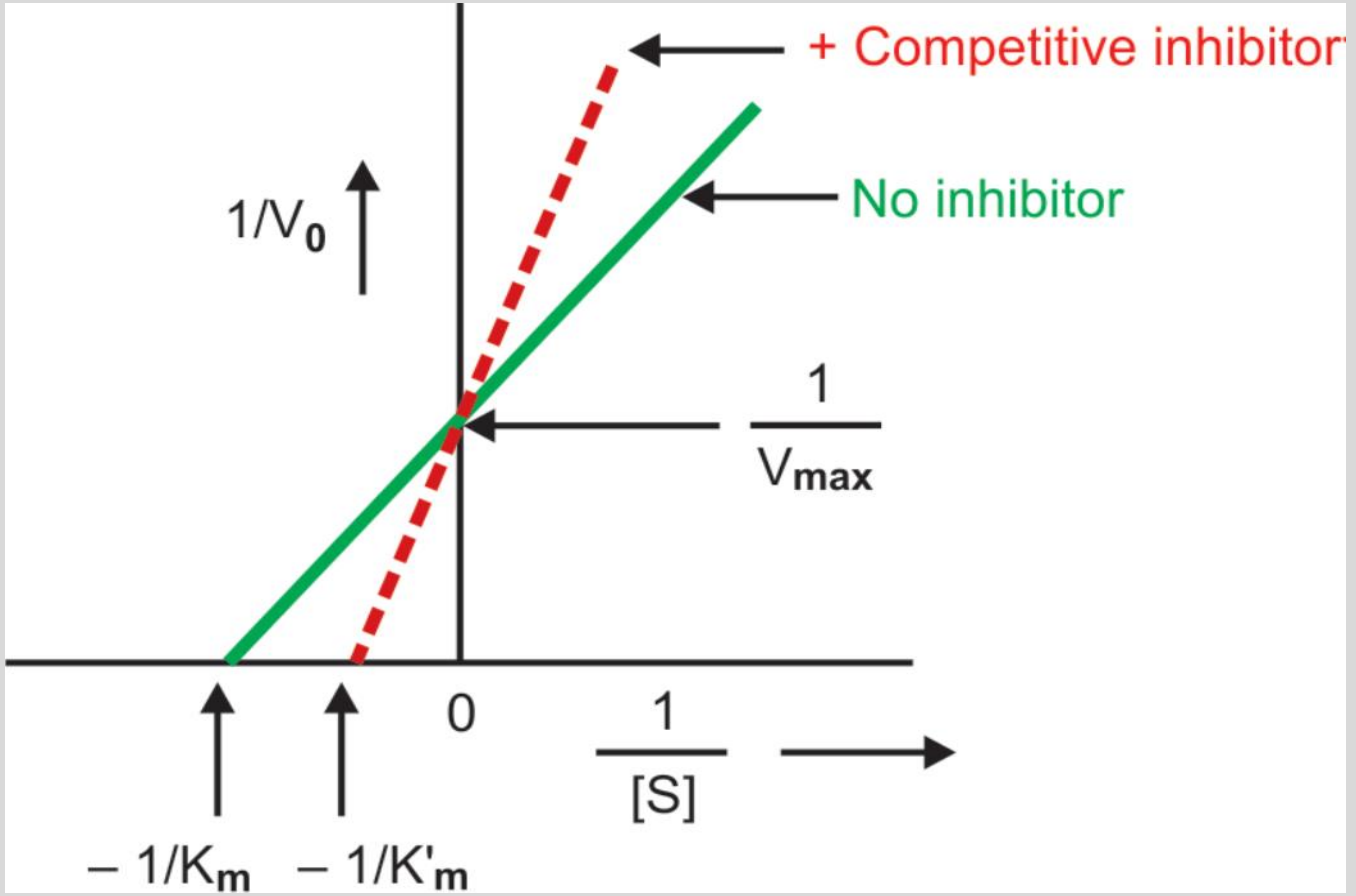
Reversible inhibitors:
Competitive inhibitor

- The chemical structure of the inhibitor (I) **closely resembles** that of the substrate (S) and binds to the enzyme at the active site, forming an EI complex rather than ES-complex.

- When both the S and inhibitor are present, they compete for the same binding site on the enzyme.

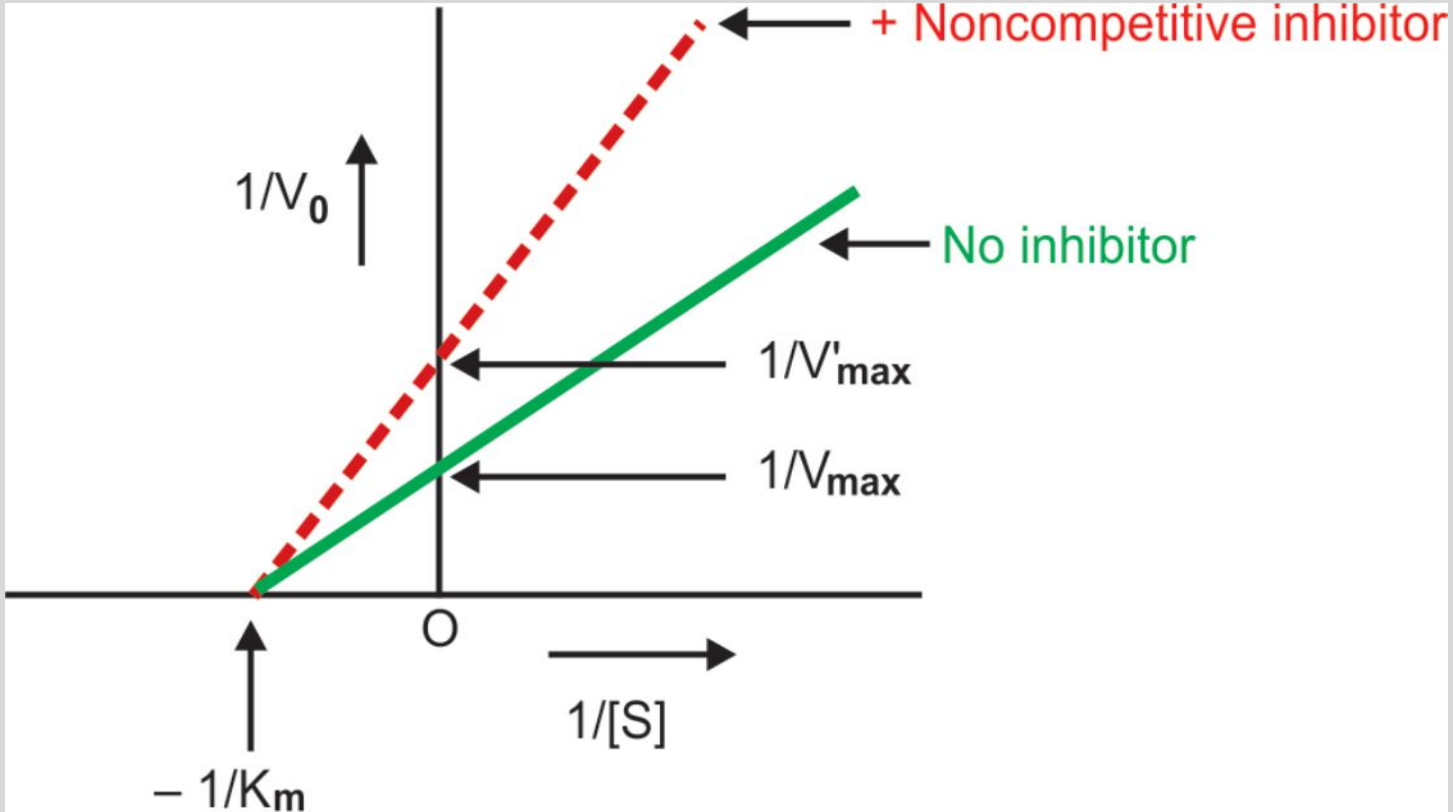
- Km increases
- Vmax remains unchanged

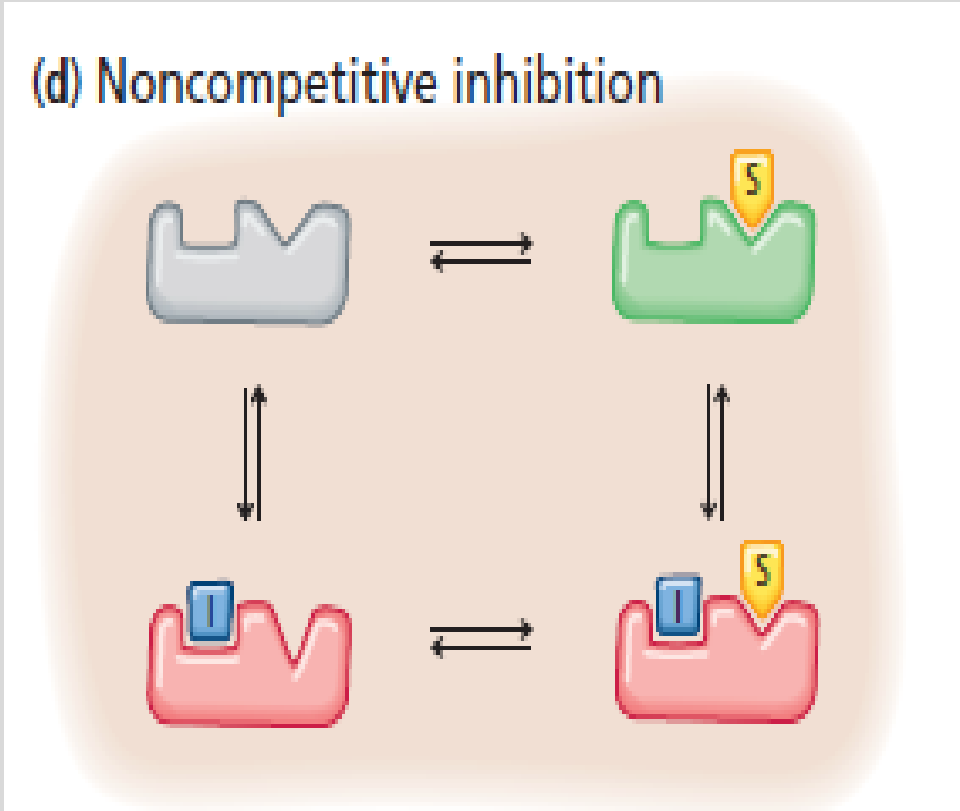




Reversible inhibitors Noncompetitive Inhibitors

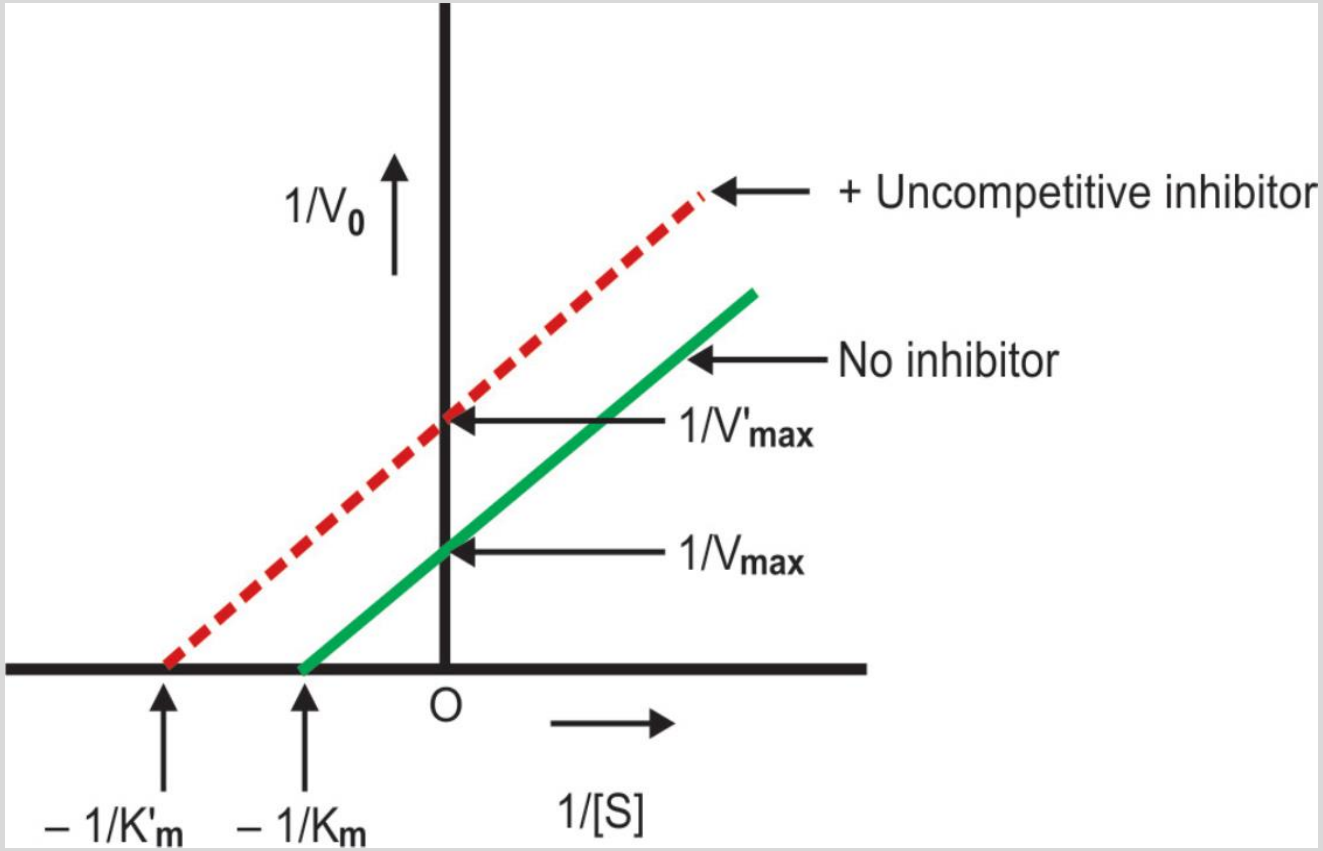
- There is no competition between inhibitor and substrate.
- Since inhibitor and substrate may bind at different sites; formation of both EI and EIS-complexes is possible.
- Km value=unchanged
- Vmax = lowered

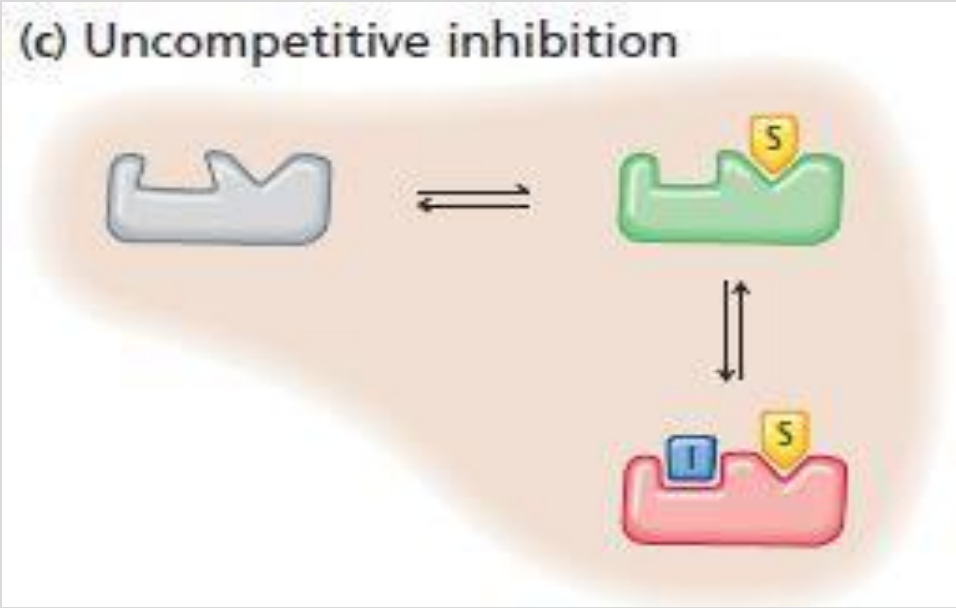




Reversible inhibitors Uncompetitive Inhibitor

- Uncompetitive inhibitor can bind only to the enzyme-substrate (ES) complex.
- It does not have affinity for free enzyme.
- decreases both Vmax Km





Irreversible Inhibitor

- An irreversible inhibitor binds with an enzyme tightly **covalently** and forms a stable complex.

➢ Decreased in V_{max} , no effect on the K_m

Examples

i. Iodoacetamide and heavy metals like, Pb^{2+}, Ag^{+}, Hg^{2+}

ii. 3-Bromoacetol phosphate (BAP).

iii. Drugs : *Penicillin* irreversibly inactivates an essential bacterial enzyme glycopeptidyl transpeptidase

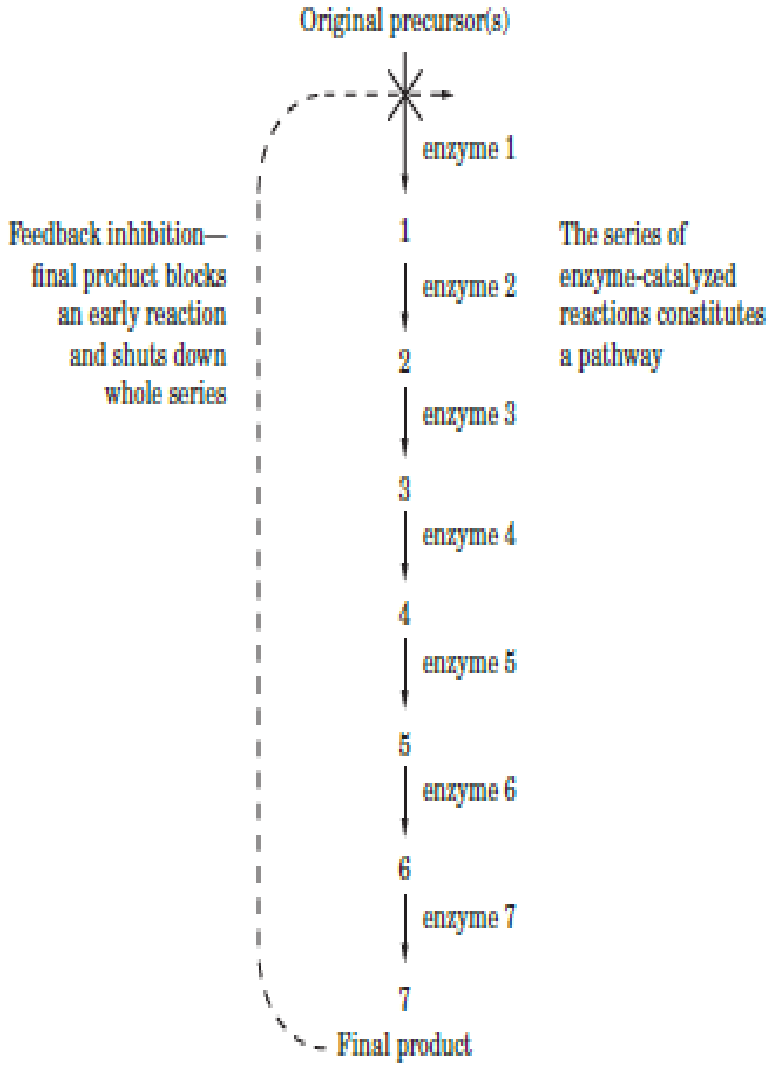
Regulation of enzyme activity

A. Feedback Control

is an enzyme regulation process in which formation of a product inhibits an earlier reaction in a complex system in which enzymes work cooperatively.

B. Zymogens

Some enzymes are manufactured by the body in an **inactive form (called proenzymes or zymogens)**. To make enzymes active, a small part of their polypeptide chain must be removed.

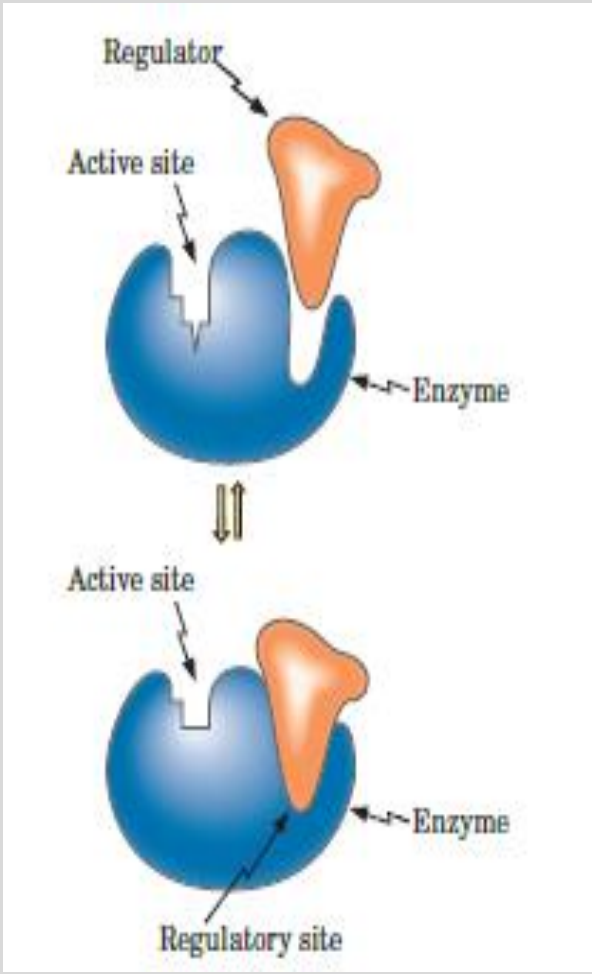


C. Allosterism

Allosteric enzyme An enzyme in which the binding of a **regulator** on one site on the enzyme modifies the enzyme's ability to bind the substrate in the active site.

If **regulator** binds to a site other than the active site:

- ❖regulator inhibit enzyme action (negative modulation تعديل سلبي)
- ❖ stimulate enzyme action (positive modulationتعديل إيجابي).



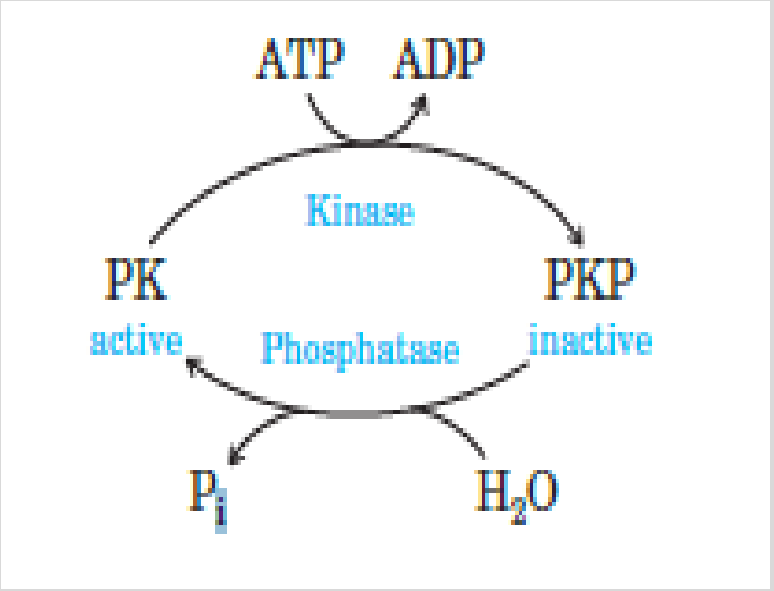
D. Protein Modification

Regulation of enzyme activity by covalent modification

The modification is usually a change in the **primary structure** (addition of a functional group **covalently** bound to the enzyme).

example of protein modification :- **phosphorylation.**

PK: pyruvate kinase



E. Isozymes

Isoenzymes or isozymes are multiple forms (isomers) of the same enzyme that catalyze the same biochemical reaction.

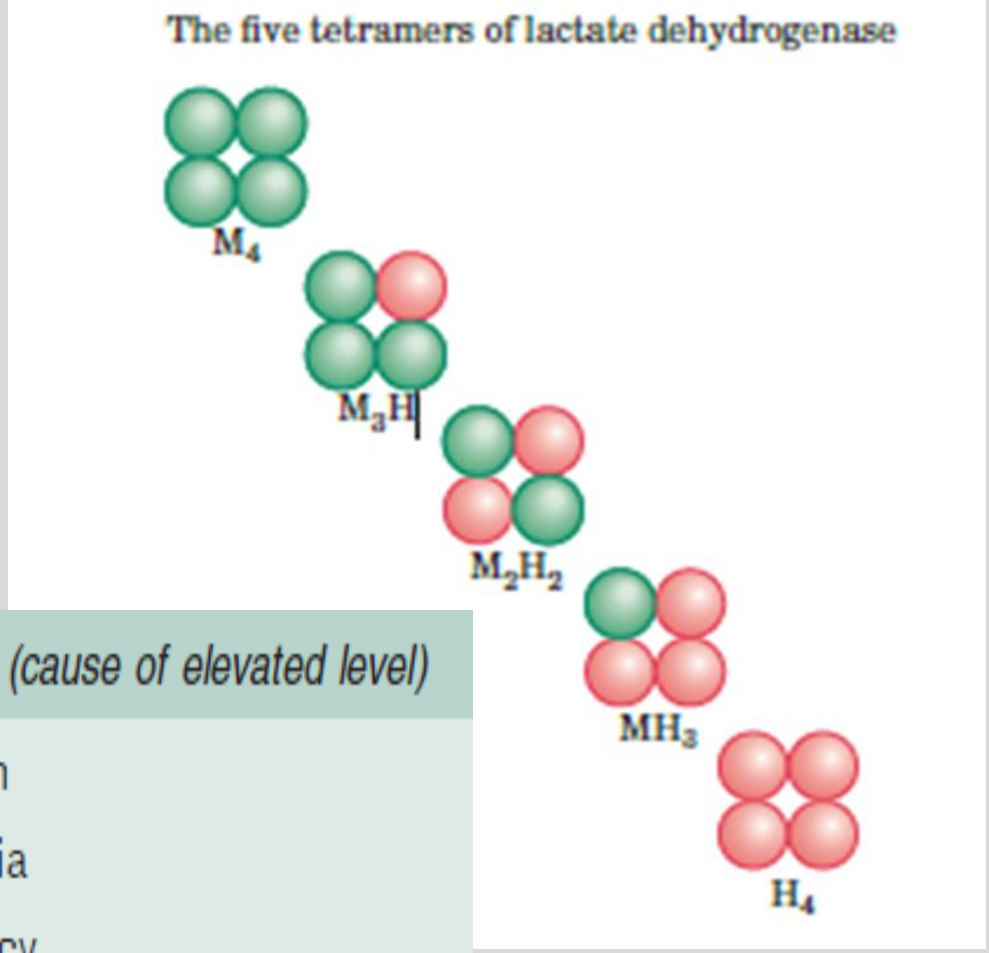
Examples:

- **Creatine kinase (CK)** : are **dimer** that are made up of two types of polypeptide chains, which may be either **M (muscle)** type or **B (brain)** type, three isoenzymes:

– CK1 (BB)
– CK2 (MB)
– CK3 (MM)

➢Lactate dehydrogenase (LDH)

catalyzes the oxidation of lactate to pyruvate
LDH is a **tetramer**, made up of two types of polypeptide **M (muscle)** type and **H (heart)**
has five isoenzymes:
LDH1, LDH2, LDH3, LDH4, LDH5



Type	*Composition*	*Location*	*Diagnostic importance (cause of elevated level)*
LDH_1	HHHH	Heart, RBC	Myocardial infarction
LDH_2	HHHM	Heart, RBC	Megaloblastic anemia
LDH_3	HHMM	Brain	Leukemia, malignancy
LDH_4	HMMM	Lung, spleen	Pulmonary infarction
LDH_5	MMMM	Liver, muscle	Liver diseases, Muscle damage/diseases
CK_1	BB	Brain	Neurological injury
CK_2	BM	Heart	Myocardial infarction
CK_3	MM	Skeletal muscle	Muscular dystrophies and myopathies

deficient enzymes

Phenylketonuria (PKU)

- The phenylketonuria is inherited in an autosomal recessive,
- PKU caused by deficiency of phenylalanine hydroxylase, associated with the **inability to convert phenylalanine to tyrosine.**
- there is an **accumulation of phenylalanine in tissues and blood and increased excretion in urine.**
- some minor pathway of phenylalanine occur
- accumulation of toxic metabolites of phenylalanine such as, phenylpyruvate, phenylacetate, phenyllactate and phenylacetyl glutamine.

The disease acquired its name (PKU) from the high levels of the keto acid, phenylpyruvate in urine.

Characteristics of PKU

• Increased level of: Phenylalanine, phenylacetate, phenyllactate, phenylpyruvate and phenylacetylglutamine, in tissues, plasma and urine.

(Phenylacetate gives the urine a mousy odor)

• Neurological symptoms

• Hypopigmentation: lighter skin color, fair hair and blue eyes due to deficiency of pigment melanin.

Diagnostic tests for PKU

- urine of newborns was assayed by the addition of $FeCl_3$ which gives an olive color in the presence of phenylpyruvate.
- The phenylalanine level in blood is detected by screening by using Guthrie test.
- The gene for human phenylalanine hydroxylase has been cloned.

Lactose Intolerance

- Intolerance to lactose (the sugar of milk)
- due to **deficiency of enzyme lactase**.
- In this condition, lactose accumulates in the gut which undergoes bacterial fermentation in the large intestine with the production of H_2 and CO_2 gases and acetic acid, propionic acid and butyric acid.
- Abdominal cramps and flatulence results from the accumulation of gases.
- diarrhea and dehydration.

- Treatment for this disorder is simply to remove lactose from the diet.

CLINICAL SIGNIFICANCE OF ENZYMES

- Diagnosis of the disease
- therapeutic agents
- analytical reagents.

References

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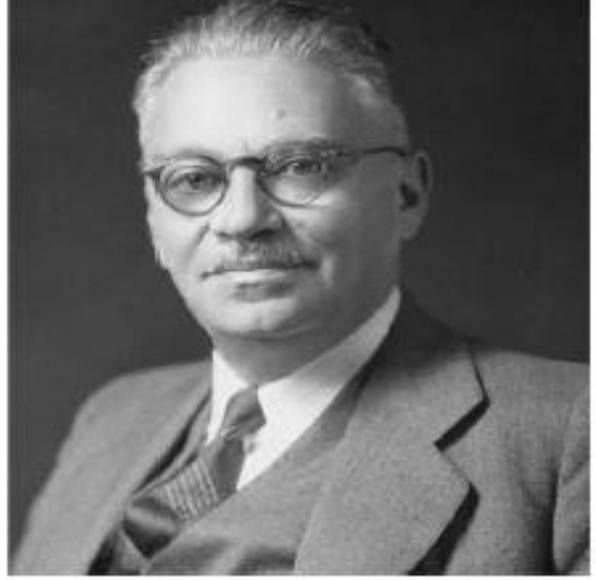
I wish you a happiness success and good luck

Dr. Rusul H. Hamza



Leonor Michaelis, 1875–1949
[Source: Rockefeller Archive Center.]

Maud Menten, 1879–1960