Enzymes

Luc. 1

By

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Objective

- Definition
- Nomenclature
- Classification of enzymes
- Factors affecting enzyme activity.
- Application of enzyme inhibition.
- Isoenzymes.
- Enzyme in the Diagnosis of Pathology

- ***Definition***

Enzyme : It is a protein, catalyst, synthesized in all living cells that regulate a biochemical reaction without being changed.

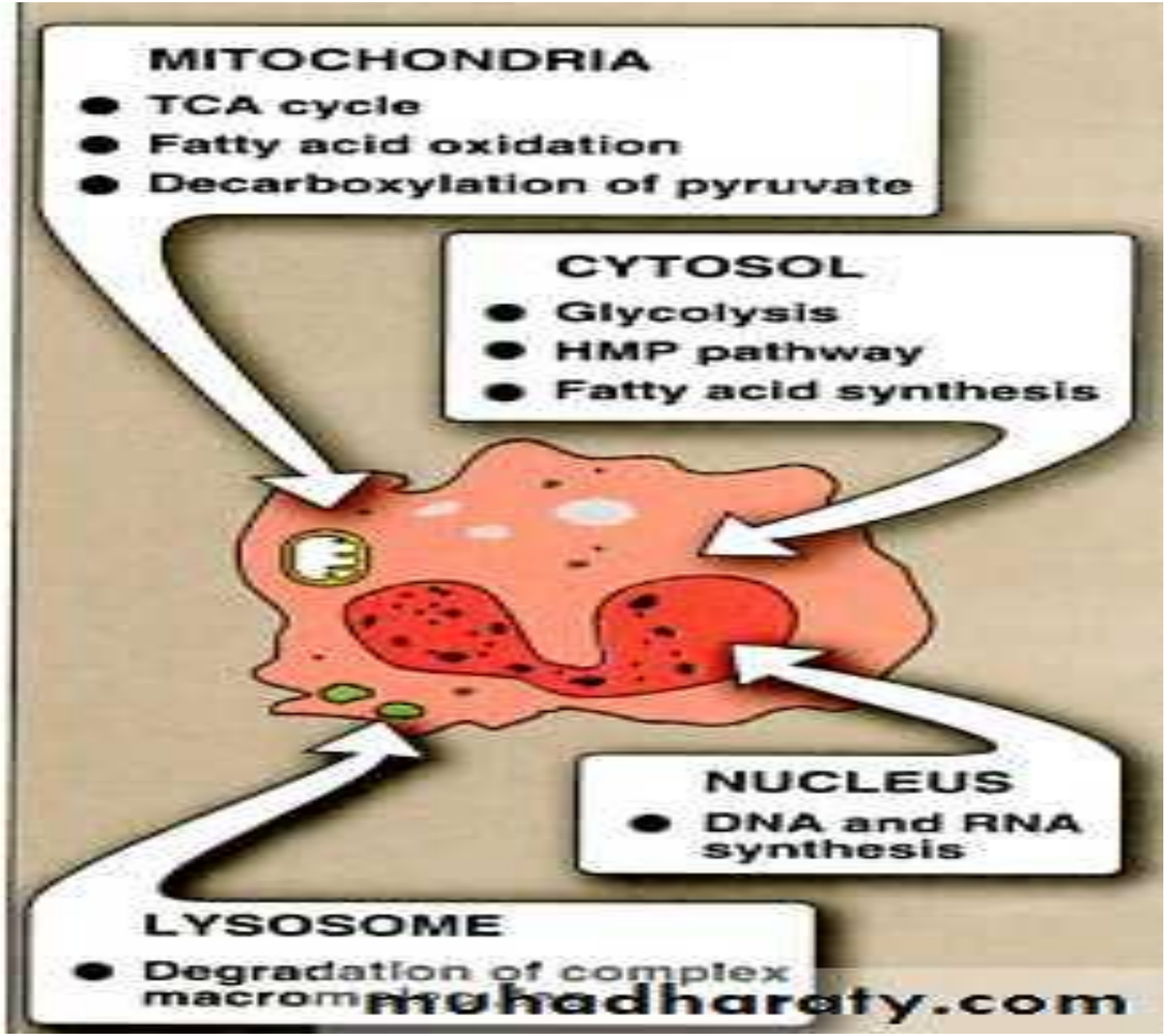
- ***Characteristics***

They are high catalytic rate.

They catalyze reaction without being changed.

They are very specific .

Enzyme distribution


MITOCHONDRIA
TCA cycle
Fatty acid oxidation
Decarboxylation of pyruvate
CYTOSOL
Glycolysis
HMP pathway
Fatty acid synthesis
NUCLEUS
DNA and RNA synthesis
LYSOSOME
Degradation of complex
macrom
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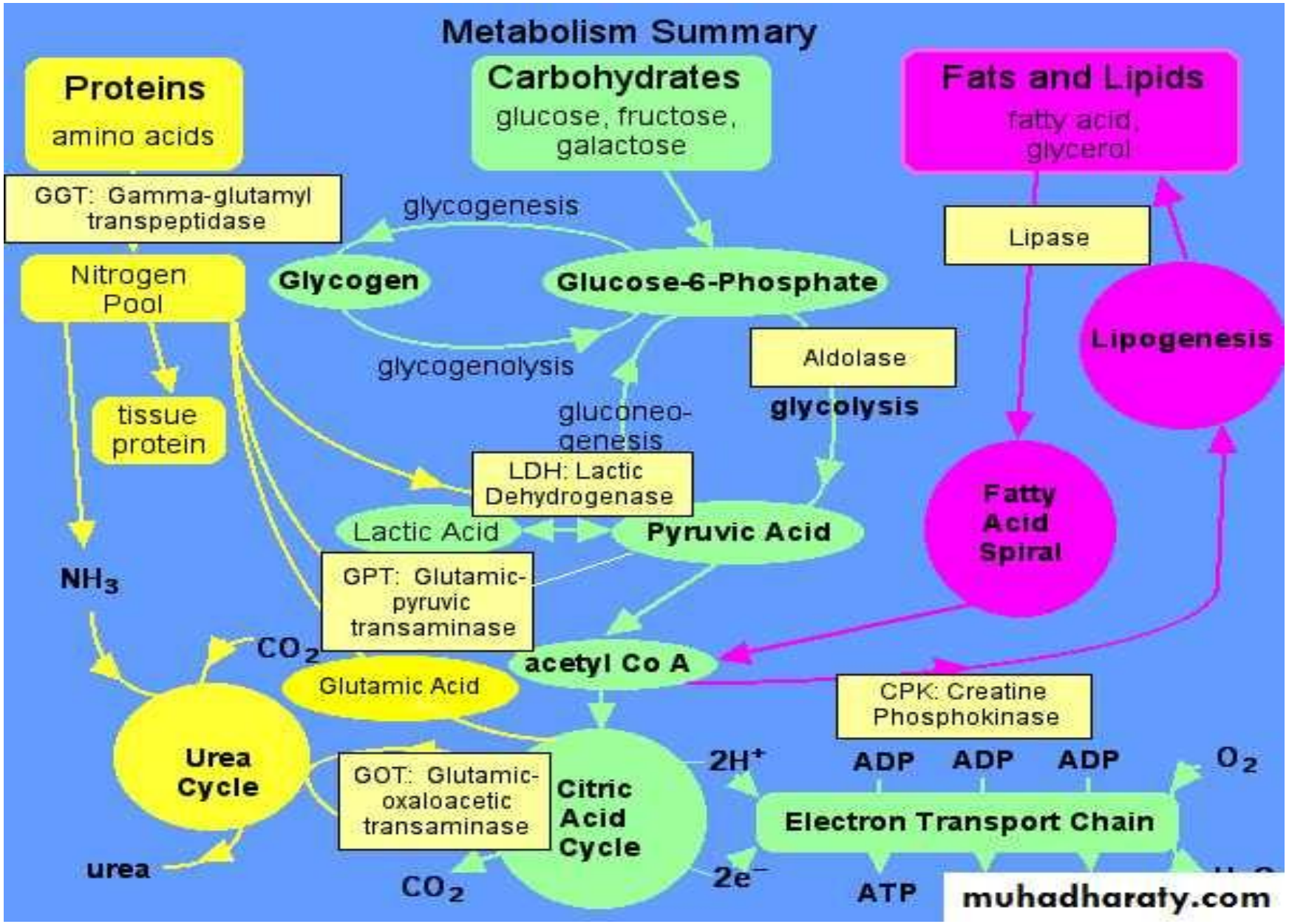
Metabolism Summary
Proteins
amino acids
Carbohydrates
glucose, fructose, galactose
Fats and Lipids
fatty acid, glycerol
GGT: Gamma-glutamyl transpeptidase
glycogenesis
Lipase
Nitrogen Pool
Glycogen
Glucose-6-Phosphate
Lipogenesis
glycogenolysis
Aldolase
tissue protein
gluconeo-genesis
glycolysis
LDH: Lactic Dehydrogenase
Fatty Acid Spiral
Lactic Acid
Pyruvic Acid
NH3
GPT: Glutamic-pyruvic transaminase
CO2
acetyl Co A
Glutamic Acid
CPK: Creatine Phosphokinase
Urea Cycle
2H+
ADP ADP ADP
O2
GOT: Glutamic-oxaloacetic transaminase
Citric Acid Cycle
Electron Transport Chain
urea
CO2
2e−
ATP
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Cofactor

Definition: A non-protein unit ,its presence is important in many enzymes.

Types:

1-Inorganic metals: Mn ,Zn ,Fe ,Cu.

2-Organic Complex (Coenzyme) .

Cofactors

Metal-activated enzymes:

- active in the presence of metal ions as K+, Mg+ or Ca++.

- Example: Kinase uses Mg++ , ATP.

Metalloenzyme:

- Firmly bound metal ion in the active site as Iron , copper , Zn & Co.

Examples:

1-Carbonic Anhydrase Zn.

2- Cytochrome oxidaseFe2+.

COENZYMES

Many enzymes require for their action on substrate, specific ,heat stable ,low M. wt.

and organic substance called ***coenzymes***

Enzyme which requires a coenzyme for its catalytic action is called ***apoenzyme*** and complete catalytic unit which

contain enzyme and its coenzyme is called ***holoenzyme.***

Catalytic unit (Apoenzyme + Coenzyme ==== Holoenzyme)

Apoenzyme: *inactive protein part.*

Cofactor: *Non protein part.*

Holoenzyme: *Active enzyme .*

Coenzyme itself may covalently or non covalently bound to enzyme and when coenzyme is covalent linked to its enzyme it will be then called ***PROSTHETIC GROUP.***

Majority of enzyme in the body required coenzyme in their action

(Nomenclature)

Unsystematic nomenclature:

1- Enzyme is named by adding (ase) to the name of the substrate e.g. (Urease).

2-Some other enzymes as (Trypsin ,pepsin) are known by their historic names.

one enzyme has one name or many enzymes have the same name.

Systematic Nomenclature

Adopted by **(IUB)** ; According to the type of reaction which is catalyzed.

It divided the enzymes into **6 classes.**

Classification of enzymes

Class no I Oxidoredoctase

Class no II Transferase

Class no III Hydrolases

Class no IV Lyases

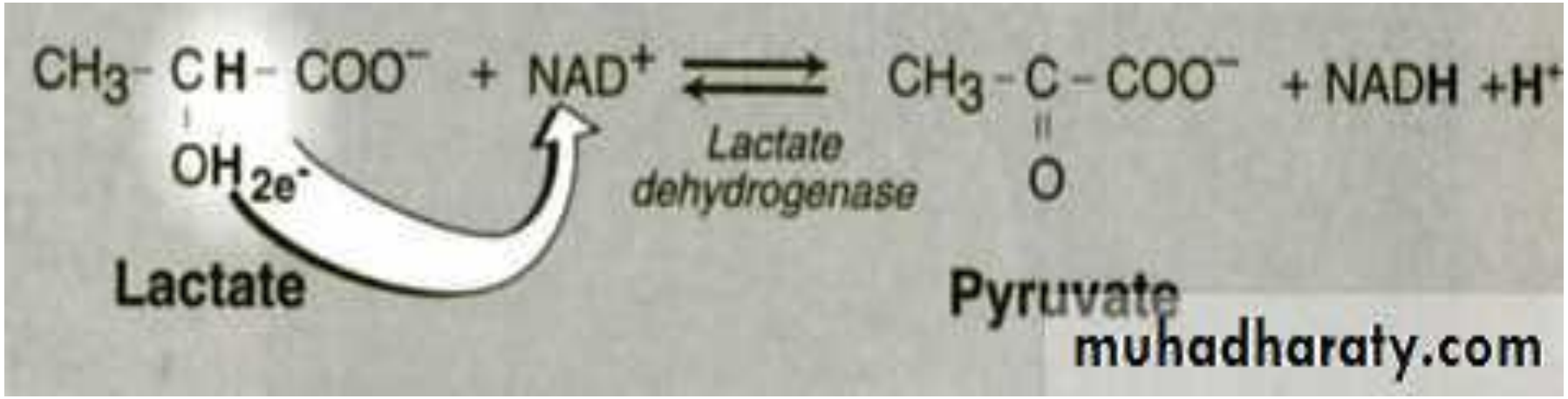
Class no V Isomerases cis and Trans

Class no VI Ligases

Class 1: Oxido-Reductase:

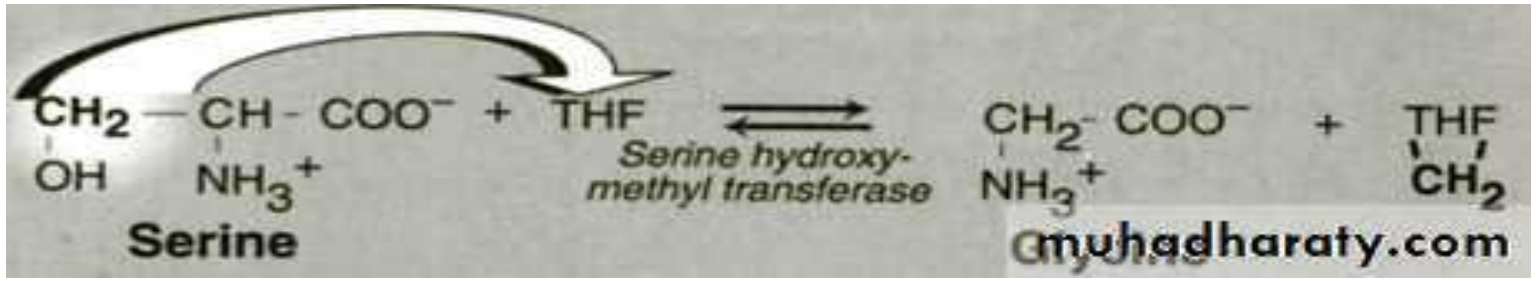
Catalyses Oxidation ,reduction reactions as : Dehydrogenase ,Oxidase ,Hydroxylase ,Peroxidase.

Usually they require coenzymes as : (NAD+,NADP+,FAD,FMN).



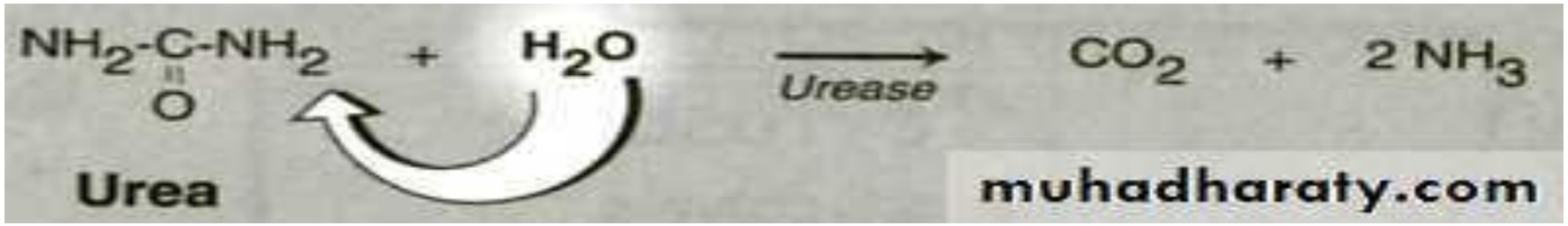
Class 2: Transferase

Catalyze transfer of functional group between donor & acceptor molecule as methyl , formyl ,carboxyl ,nitrogenous, phosphorus & sulfur containing groups



Class 3: Hydrolases

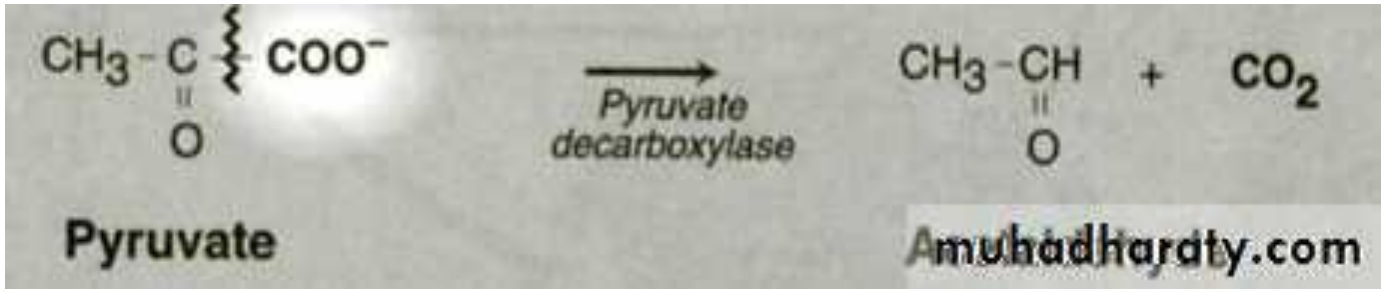
Catalyze hydrolytic reaction by adding H2Ocleavage of bond between C & others as : C-O , C-N & C-S.



Class 4 : Lyases

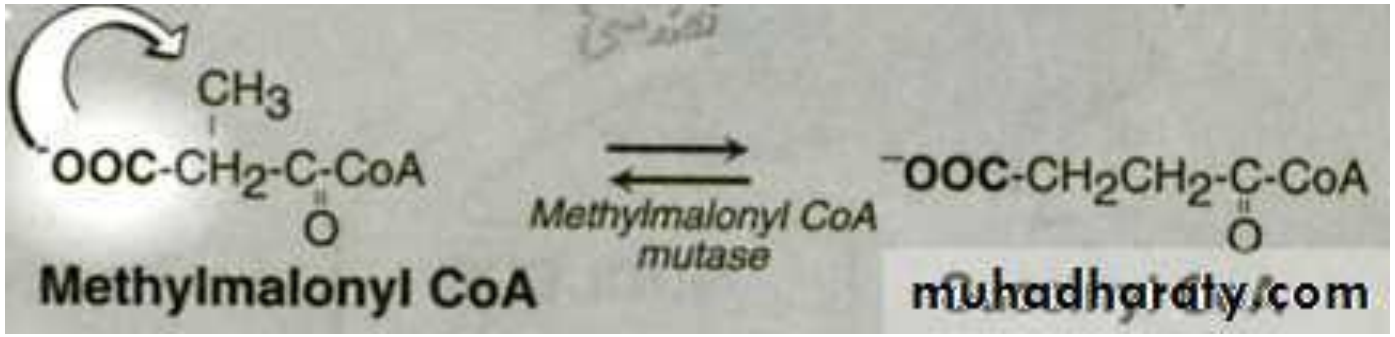
Catalyze non-hydrolytic reaction

Examples: Decarboxylase .



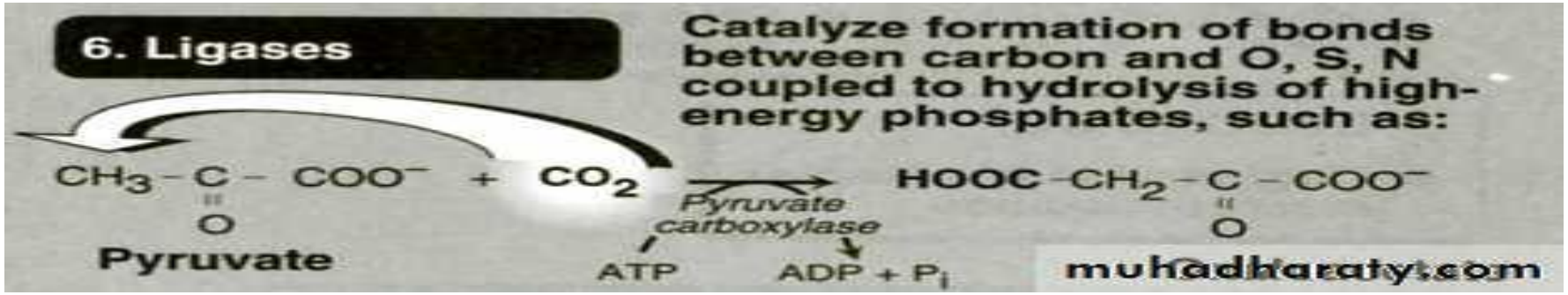
Class 5 : Isomerase

Catalyze transfer of groups within a molecule (rearrange).



Class 6:Ligase

Catalyze bond formation coupled to ATP-hydrolysis joining 2 molecules.



Substrate

The molecule being utilized and/or modified by a particular enzyme at its active site

Enzyme Specificity

The most significant properties in the enzyme catalytic reaction is the ability of the enzyme in catalyze one specific reaction and no other that is a characteristic of enzyme and when these enzyme is absent the respective reaction will not occur and this behavior is called ***specificity*** of enzyme and this behavior is usually appear in the following **TWO** properties:

I-optical specificity

II- Selective group

I-optical specificity

The enzyme has an absolute specificity in particular optical region of the substrate. Almost all human enzyme are being specific for an optical part of substrate . ex: enzyme acting on CHO. Metabolism (sugar breakdown)are usually specific for D-sugar not act on L-sugar or other enzyme acting on amino acid metabolism are usually acting on L- amino acid (not D-amino acid) with exception of D- amino acid oxidase in the kidney .

II- Selective group:

In this properties enzyme is usually affective on specific chemical group that is present in the structure of substrate. ex: glycosidase, glycosidase catalyze hydrolysis of Glycosidic bond between sugar and alcohol are highly specific for sugar portion not specific for alcohol.

Trypsin and pepsin act on peptide bond.

Some enzymes have a higher degree of specificity ex: amino peptidase act on amino group , carboxypeptidase act on carboxy end of peptide bond .

Chymotrypsin will act on peptide bond on which carboxy terminal end of peptide bond is being contributed to an aromatic a.a. Which may be phenyl alanine , tyrosine and tryptophan split of a.a one at a time from the carboxy or amino terminal end of polypeptide chain respectively.

Tyrosine

Tyrosine

CH NH2COOH

CH NH2COOH

CH2

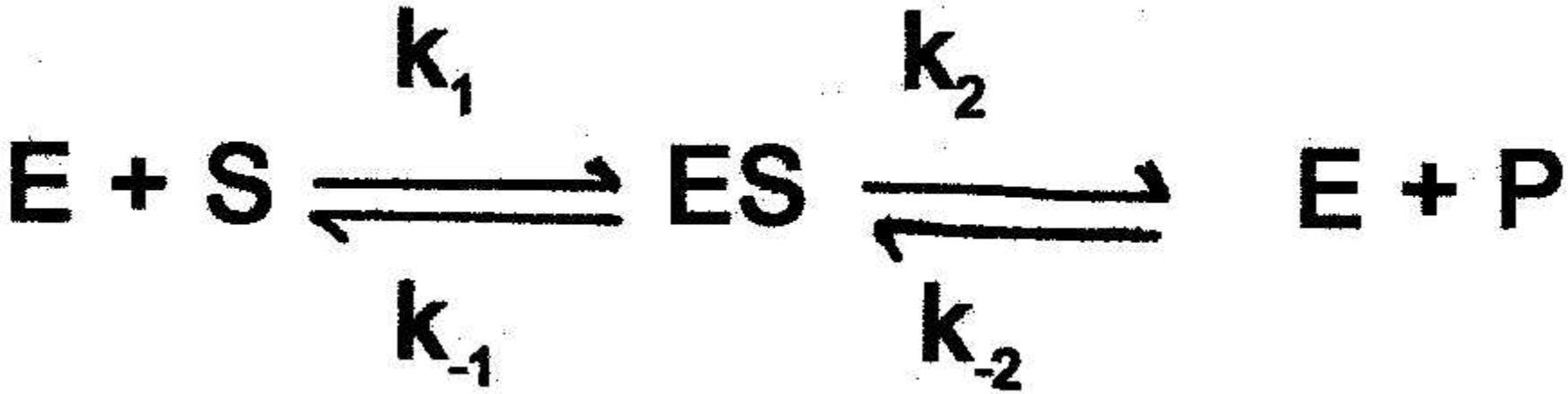
CH2

-HO

-HO

Enzyme velocity (V)

It is moles of product (P) appearing or substrate (S) disappearing per unit of time. (Mole / liter /sec.)



S = substrate P = product

E = enzyme

ES = enzyme-substrate complex

k_1, k_{-1}, k_2, k_{-2} are rate constants

Enzyme units

International unit (IU): a mount of enzyme that converts one micromole (µmol) of substrate per minute at 25°C under the optimal conditions of the measurement.

Katal: amount of enzyme that converts one mole of substrate to product/sec

(Active site)

Active site: is an important structural feature to recognize and to bind substrates.

It is very specific.

Catalytic Site:

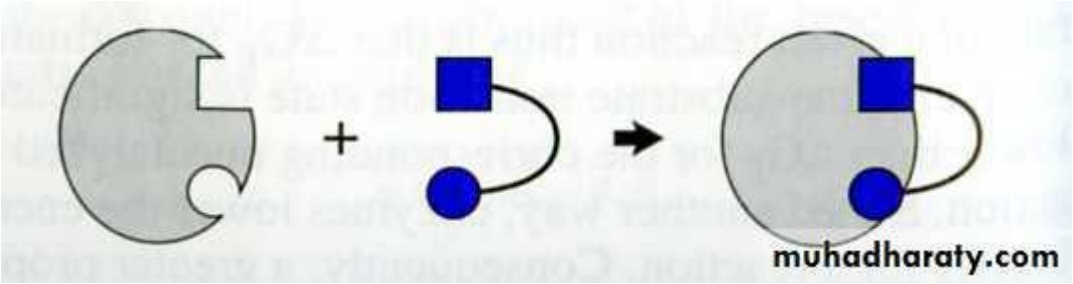
The large size of the enzyme molecule in comparison with substrate size that a small part or limited number of amino acids in the enzyme molecule is being responsible for the catalytic reaction these size is called ***CATALYTIC SITE*** or ***ACTIVE SITE*** or ***ACTIVE CENTER*** of the enzyme.

There are two theory or mode or type to explain the interaction between the substrate and enzyme.

Type I

The lock & key model:

- Enzyme fits substrate as a lock & key .
- Its rigid type.

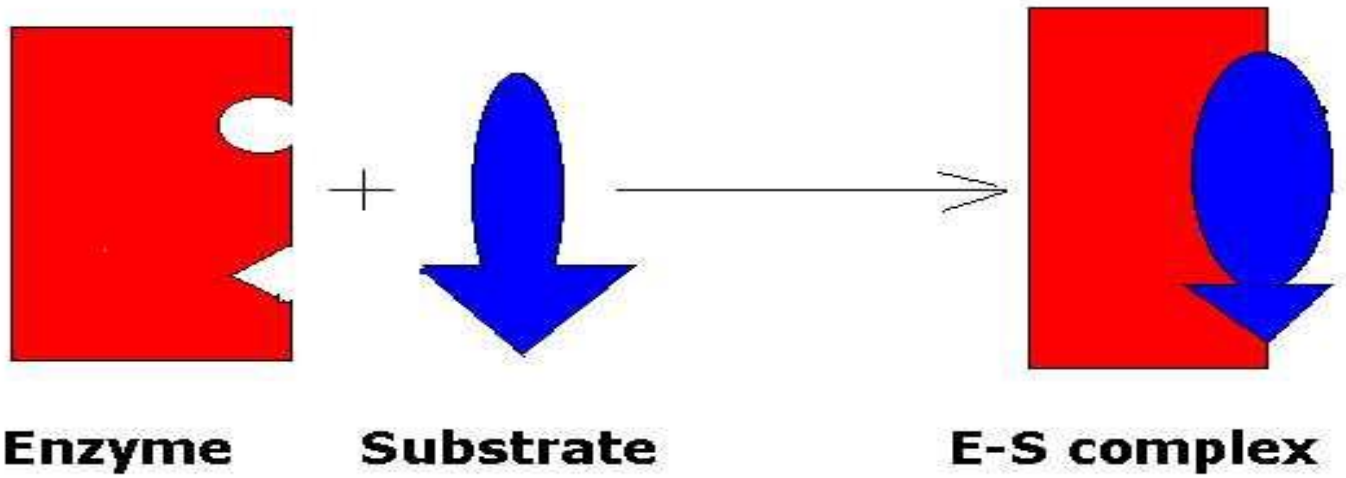


Type II Induced fit (Koshland model):

the substrate induces conformational changes in the active site rearrangement of the A.A Enzyme fits substrate exactly.

This type discovered by Koshland in which there is a source of flexibility in substrate – enzyme binding in which certain physical changes take place in the enzyme that include arrangement of certain (a.a)s both to the substrate binding site and at catalytic site.

These changes are called ***(conformational changes)*** and the site in which these changes take place are called ***Allosteric site*** being important for the enzyme catalytic reaction. This type is more flexible than the lock and key type and it has wide application in explainin



Catalytic efficiency

Most enzyme-catalyzed reactions are highly efficient, proceeding from 103 to 108 times faster than uncatalyzed reactions.

Factors affecting Enz. Activity

1.Enzyme concentration.

2.Temperature.

3.PH

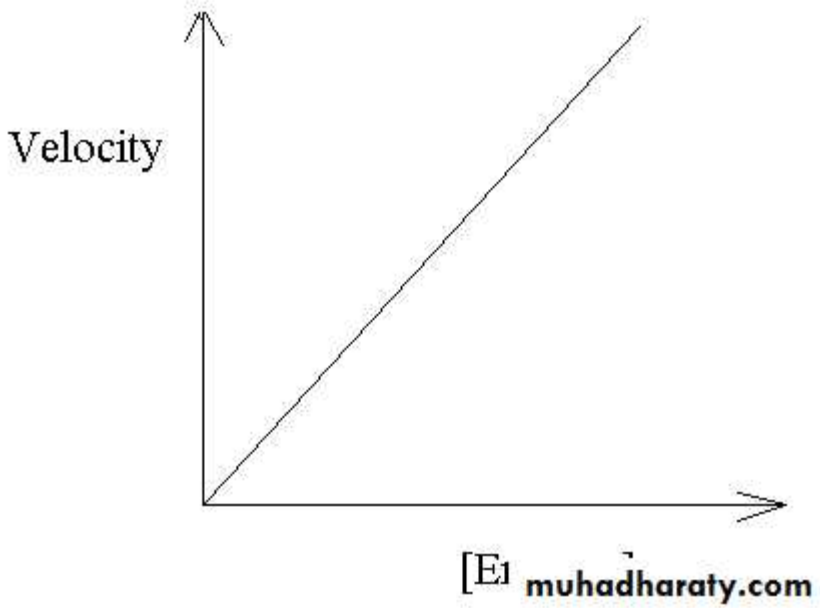
4.Substrate concentration.

5. Inhibiters

6. Activators

Enzyme concentration:

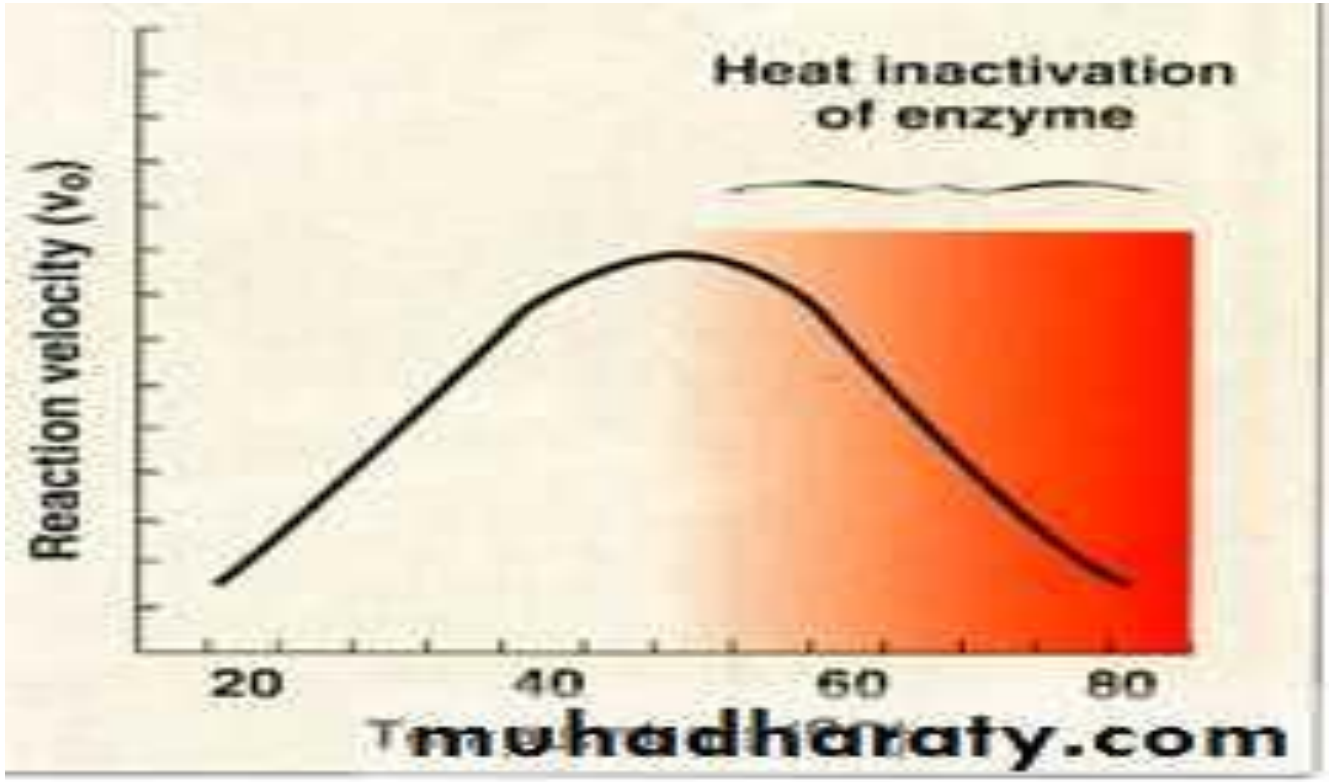
The rate of the reaction is directly proportional to [enzyme]



Temperature

The rate of the reaction increases with the temperature increasing until reaching the (Maximal velocity) at the (Optimal temperature) . Increasing of the temperature after the optimal temperature decreasing in the reaction velocity.

The velocity decreases due to (enzyme denaturation)

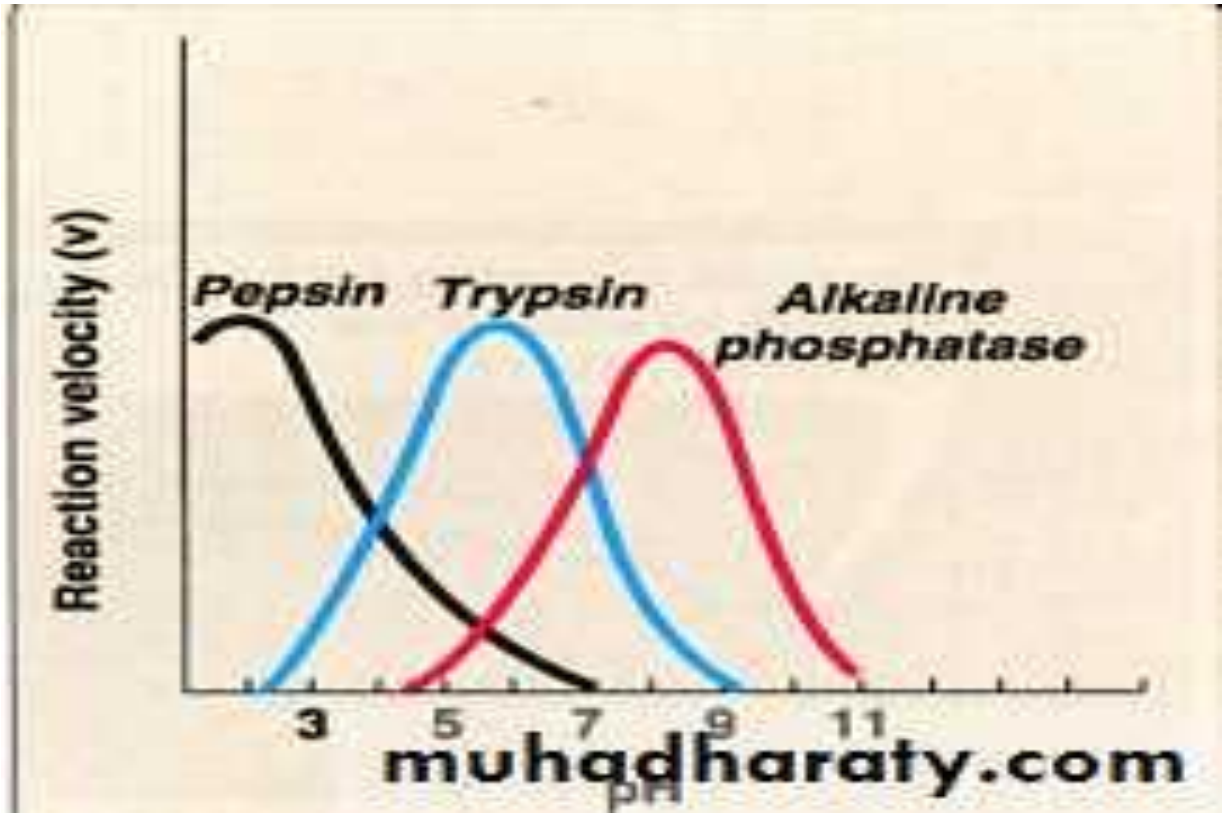


Effect of PH

Each enzyme has its own (Optimal PH).

Any change in the PH decreasing in the reaction velocity due to change in the ionization of the active site A.A.

This ionization inactivation of the active site decrease in enzyme activity.



Substrate concentration

Rate of the catalytic enzyme increases rapidly constant.

1-low [S] active sites are not saturated rapid reaction .

2-High [S] Saturated active sites slow reaction.

Substrate concentration

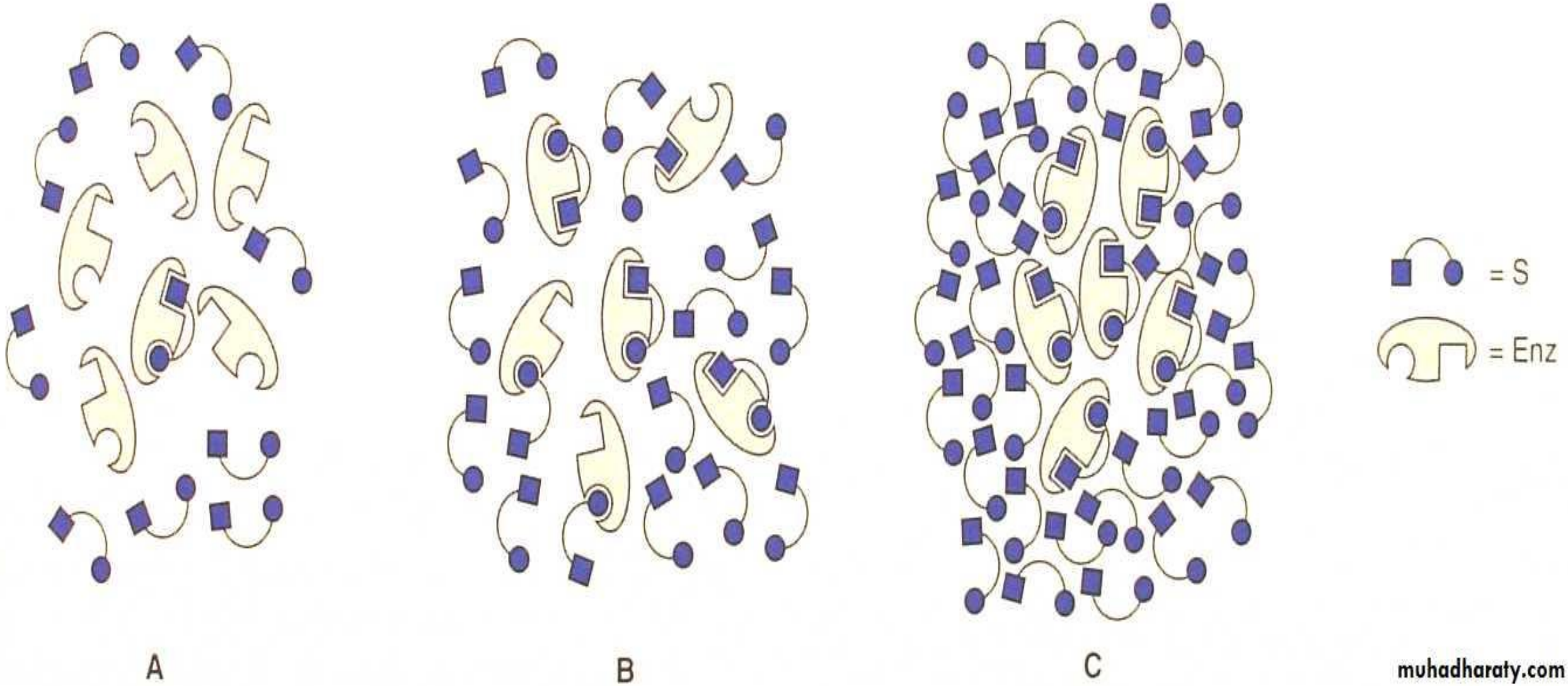
The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time and is usually expressed as µmoles product formed per minute.

The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached.

A. Low [S]

B. 50% [S] or Km

C. High, saturating [S]



The Michaela's- menten constant (Km).

The quantitative relationship between substrate concentration and Vmax. For different enzymes, it is defined as that substrate conc. at which a given enzyme give one – half it maximum velocity . In many cases the Km is an inverse measure of the affinity of the enzyme for its substrate : the lower the Km the higher the affinity .

$$v_o = \frac{[V_{max}][S]}{Km + [S]}$$

v_o = initial reaction velocity

V_{max} = maximal velocity

[S] = substrate concentration

Characteristics of Km

The Michaelis constant is characteristic of an enzyme and a particular substrate, and reflects the affinity of the enzyme for that substrate.

Km does not vary with the concentration of enzyme. A numerically small (low) Km reflects a high affinity of the enzyme for substrate because a low concentration of substrate is needed to half-saturate the enzyme.

Large Km:

A numerically large (high) Km reflects a low affinity of enzyme for substrate because a high concentration of, substrate is needed to half-saturate the enzyme.

The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations.

When [S] is much less than Km, the velocity of the reaction is proportional to the substrate concentration.

Uses of Km

Experimentally, Km is a useful parameter for characterizing the number and/or types of substrates that a particular enzyme will utilize. It is also useful for comparing similar enzymes from different tissues or different organisms. Also, it is the Km of the rate-limiting enzyme in many of the biochemical metabolic pathways that determines the amount of product and overall regulation of a given pathway. Clinically, Km comparisons are useful for evaluating the effects mutations have on protein function for some inherited genetic diseases