صلو عالنبي بحالة ❤

اللهم صل وسلم وبارك يا سيدنا محمد

Enzymology- An overview-1

Substrate → المادة اللي بتتفعل عليه التفاعل
to be converted into a product
بتتحول له

اكتر اشي مهم بدراسة التفاعل هو استخداماته بالتشخيص وفوائده
يعني بالمستقبل اعرف التحاليل اللي لازم تنكتب للمريض
The different laboratory investigations to be written for the patient to facilitate the diagnosis of the disease (mothers)

enzyme are protein in natura exept one type ribozymes
↳ proccesing of RNA

Enzymes- An introduction

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

↳ acceleration for the reaction

Simple protein → just consisted of amino acid only

Conjugated protein → molcule part of protein and other part another thing ← carbohydrate, lipid ... and soon.

glycoprotein → carbohydrate + protein

lipoprotein → lipid + protein

nucleoprotein → nucleic acid + protein

we have molcule consist of two different portion

conjugative ← metaloenzymes

consist of protein + metal ies

in organic catalysts

catalysts → inorganic (metal ion) / organic

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

Globular, fibres

structural fibers, having dinamic function ← Globular protein

and they (Globular) have short life span

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

maturation the RNA

↳ pices of RNA can act for processing RNA

→ short sigbent of RNA about 90 – 200 ribonucleticles but they have catalitic activity

RNA ... to be converted from non functional form into the functional form

synthesising RNA from DNA (transcription)

never consumed, never altered, life span of the enzyme

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

number of substrate molcules to be converted into product molcule per unit time per enzyme unit

organic and inorganic enzyme

one unit of an enzyme

one unit of metal ion

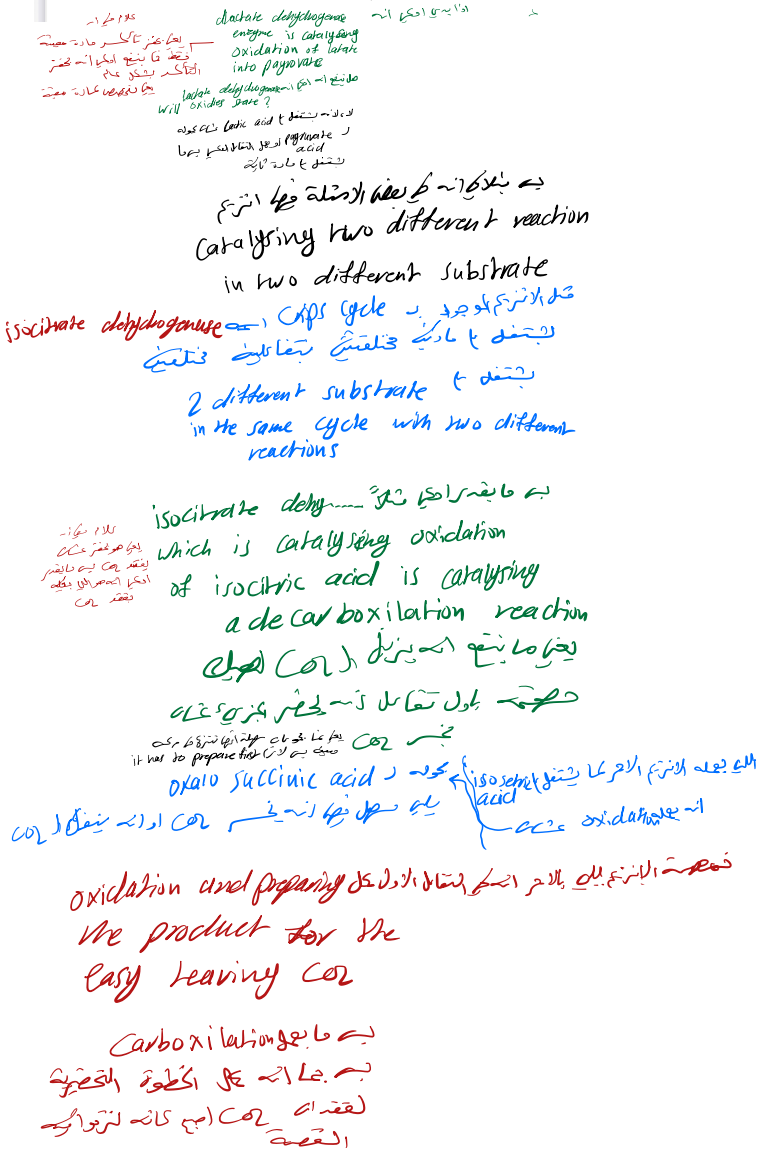
per unit time (1s)

in one second [enzyme can conver $10^6 - 10^{12}$ of the substrate molcules ; but metal ion can convert only 10^3]

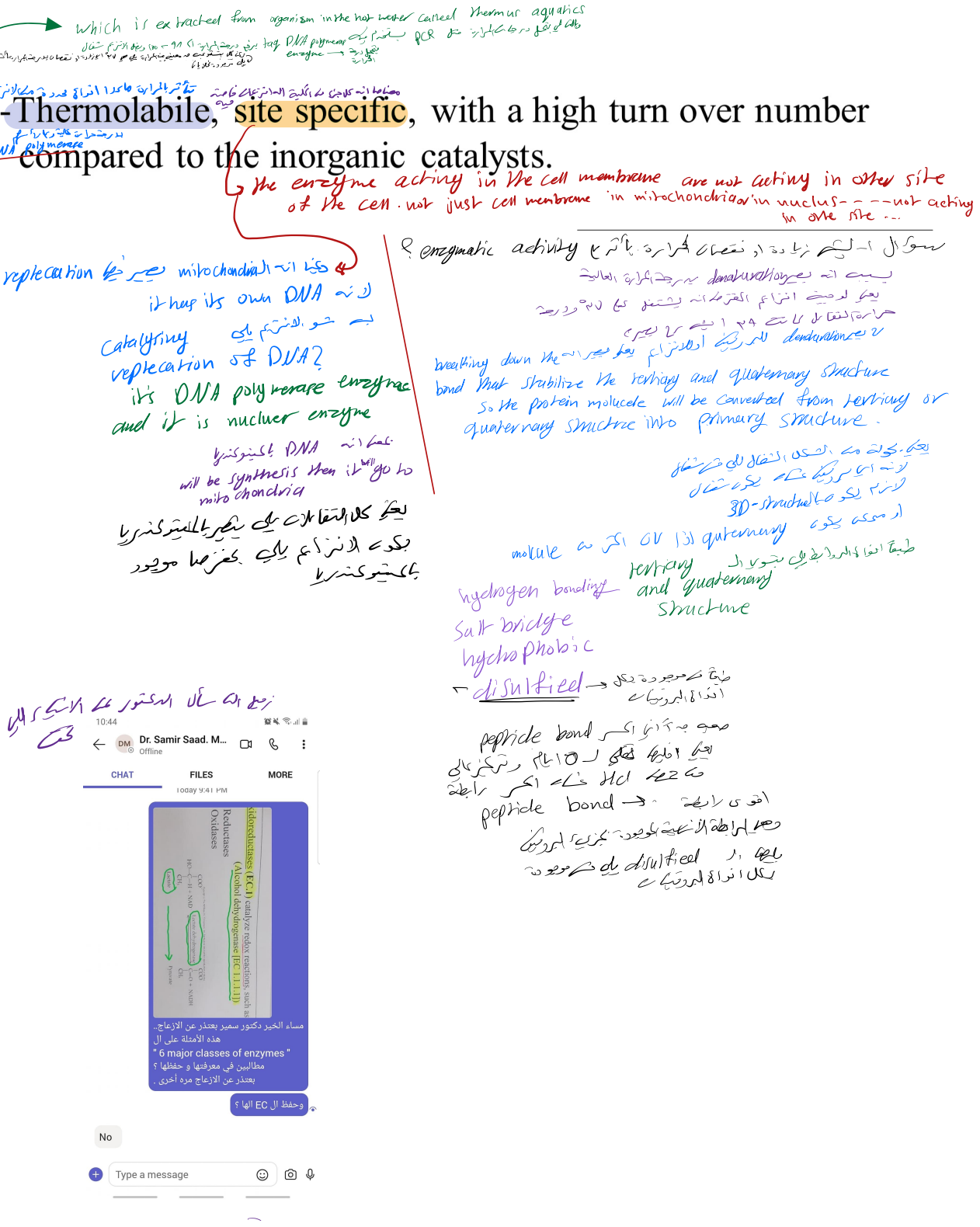
inorganic, organic catalyst enzyme

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

and specific



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



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

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

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

Lactate dehydrogenase

Pyruvate decarboxylase

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

Catalase

Pepsin

Chymotrypsin

Trypsin

Classification of Enzymes

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

- Each enzyme was given 4 digit numbes [1.2.3.4]

1st one of the 6 major classes of enzyme activity

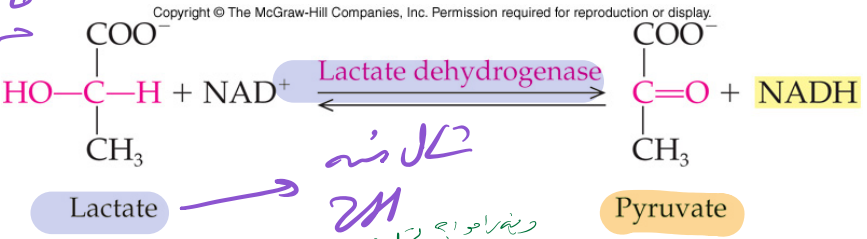
2nd the subclass (type of substrate or bond cleaved)

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

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

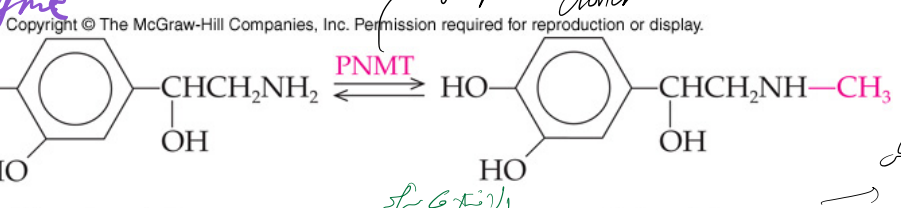
1- Oxidoreductases (**EC.1**) catalyze redox reactions, such as (Alcohol dehydrogenase [EC 1.1.1.1])

- Reductases
- Oxidases



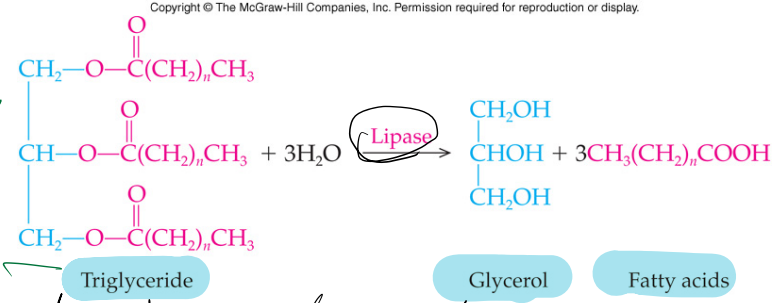
2- Transferases (**EC.2**) transfer a group from one molecule to another, such as (Hexokinase [EC 2.7.1.2])

- -Transaminases catalyze transfer of an amino group
- Kinases transfer a phosphate group



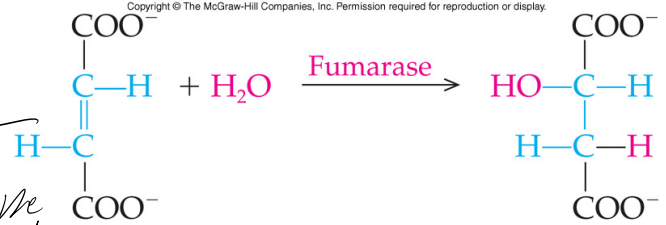
3- Hydrolases (**EC.3**) cleave bonds by adding water, such as (Alkaline phosphatase [EC 3.1.3.1])

- 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])

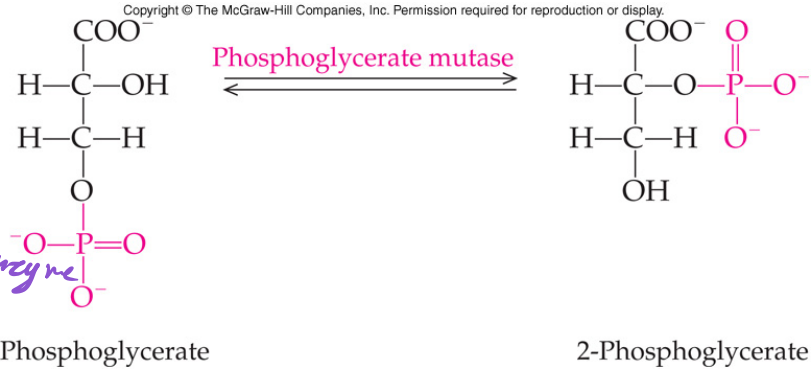
- Decarboxylases
- Synthases



5- Isomerases (**EC.5**) catalyze intramolecular rearrangements, such as

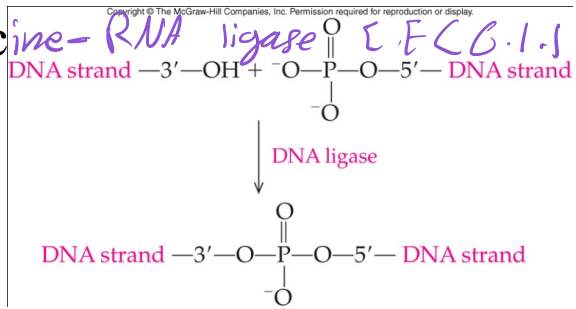
(Alanine racemase [EC 5.1.1.1])

- Epimerases
- Mutases



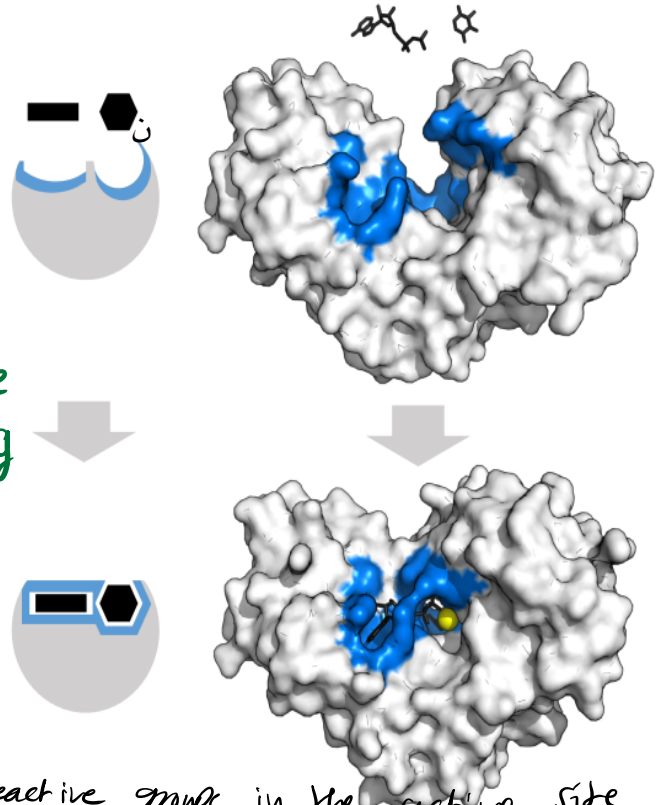
6- Ligases (**EC.6**) catalyze a reaction in which a C-C, C-S, C-O, or C-N bond is made or broken, such as

(Isoleucine–RNA ligase [EC 6.1.1.5])



Active site → the site of the enzyme to which the substrate is binding for catalysing the reaction

- Takes the form of a cleft or pocket
- 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



What are the characters of the active site to allow the binding of the substrate to the enzyme?

1- It have 3D -Configuration active sites complementarity and the substrate

2- they should be specific reactive groups in the active site of the enzyme

لسه

the substrate should be تكون جاهزة لكي
stabilize to the active site

يعني بيصير ال substrate لل active site, يرتبط بالكامل
ويحصل بعدين الازاي تحولها ل product

يعني لازم يكون في reactive في ال reaction groups
functional او groups

in the active site of the enzyme
and the binding site of the substrate

كلام عام لازم يكون في على الاقل مكانين
الاول بالانزيم وعلى
ال substrate وهو يتعلق
ويرتبط بالانزيم وبعدين تحوله
لل product

when they are ionized بيصير مشحونة
the will give a negtive charg

glutamate }
aspartate } acidic amino acid زي ال

lysen } basic amino acid
Histaden }
argenen

sulf hedral containing
molcules
cistein زي ال

لازم يتعرف القتال بين enzyme binding
site and the binding site of the substrate

تكملة للكلام الي تحت اضافة ان:

شي تسمى ال functional groups او ال reactive groups او ال highly reactive groups

في موجودة in the binding site of the substrate وفي كمان في functional groups في موجودة في ال active side of the enzyme to be stabilized

لازم stabilized

هل كل الانزيم بتتكون من one protein molcule, ولا موجودة انزيم consisted of more than one protein molcule

ال active site can be located وفي بيكون في انزيم consisted of two protein molcules

in the enter face between the two polymers (two protein molcules)

located in the enter face between the subunit and this is very good → functional ويحسب groups from the first protein molcule and it will يحتسب functional group from the second protein molcule and this will facilitate the binding and the stabilization of substrate to the active site.

لما يصير ال substrate بتصير بدها ال ترتبط مع ال active site of the enzyme ما بمجرد ما تتعلق السبب انه ما يترابط
enzyme بيتحول الى substrate complex it should be stabilized
لازم
transition state
وبعدها تتحول ل
product

specific groups يعني لازم يكون ال في المكان in the active site of the enzyme

2- complentardy between the substrate binding site and the active site of the enzyme

3-specific arragement of the الازم

3-specefic arragement of these functional groups which is responsible for the specifeality of enzyme

يعني لازم يكون في 3D configuration

in the active side in a particular manner (arranged and distriputed) بطريقة معينة عشان ما التكلة فورا

يعني انه lactate dehydrogenase يعني acting only on lactate لانه الموجودات reactive الي موجودة بال active site مرتبة

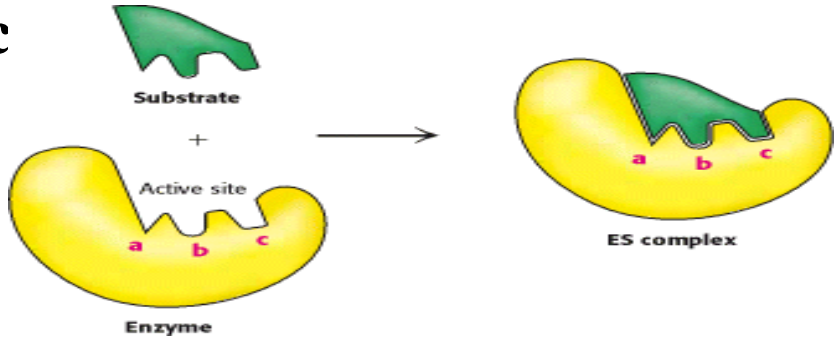
Enzyme substrate binding

-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.

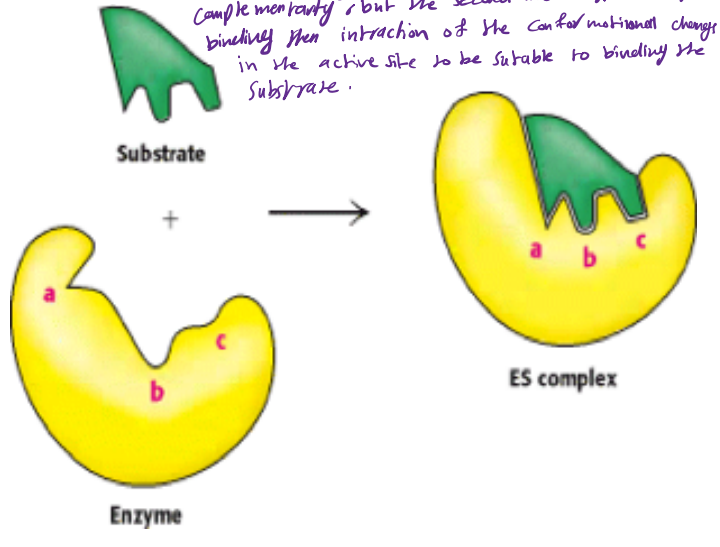
-"lock and key model" **accounted for the exquisite specificity of enzyme-substrate interac**

the implied rigidity of the enzyme's active site failed to account for the dynamic changes that accompany



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:

$$S + E \longrightarrow P + E$$

- 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:

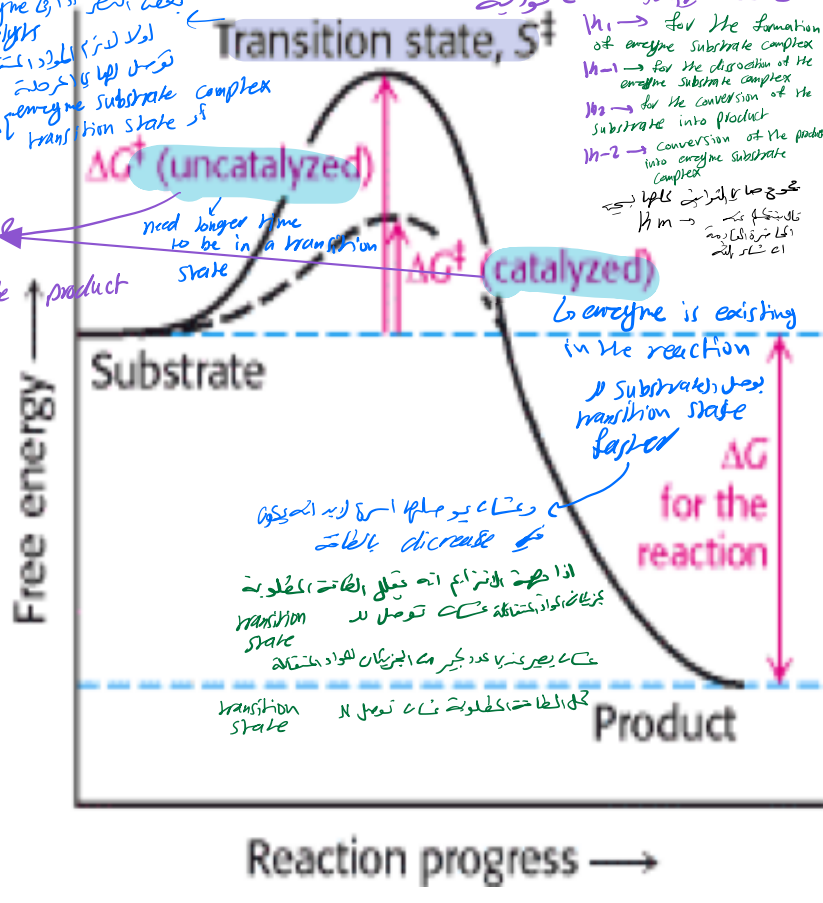
1- Thermodynamic changes

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.



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 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.

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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 withdrawl of electrons from substrate

3- 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 use charge distributions to guide polar substrates to their active sites

Enzyme Specificity

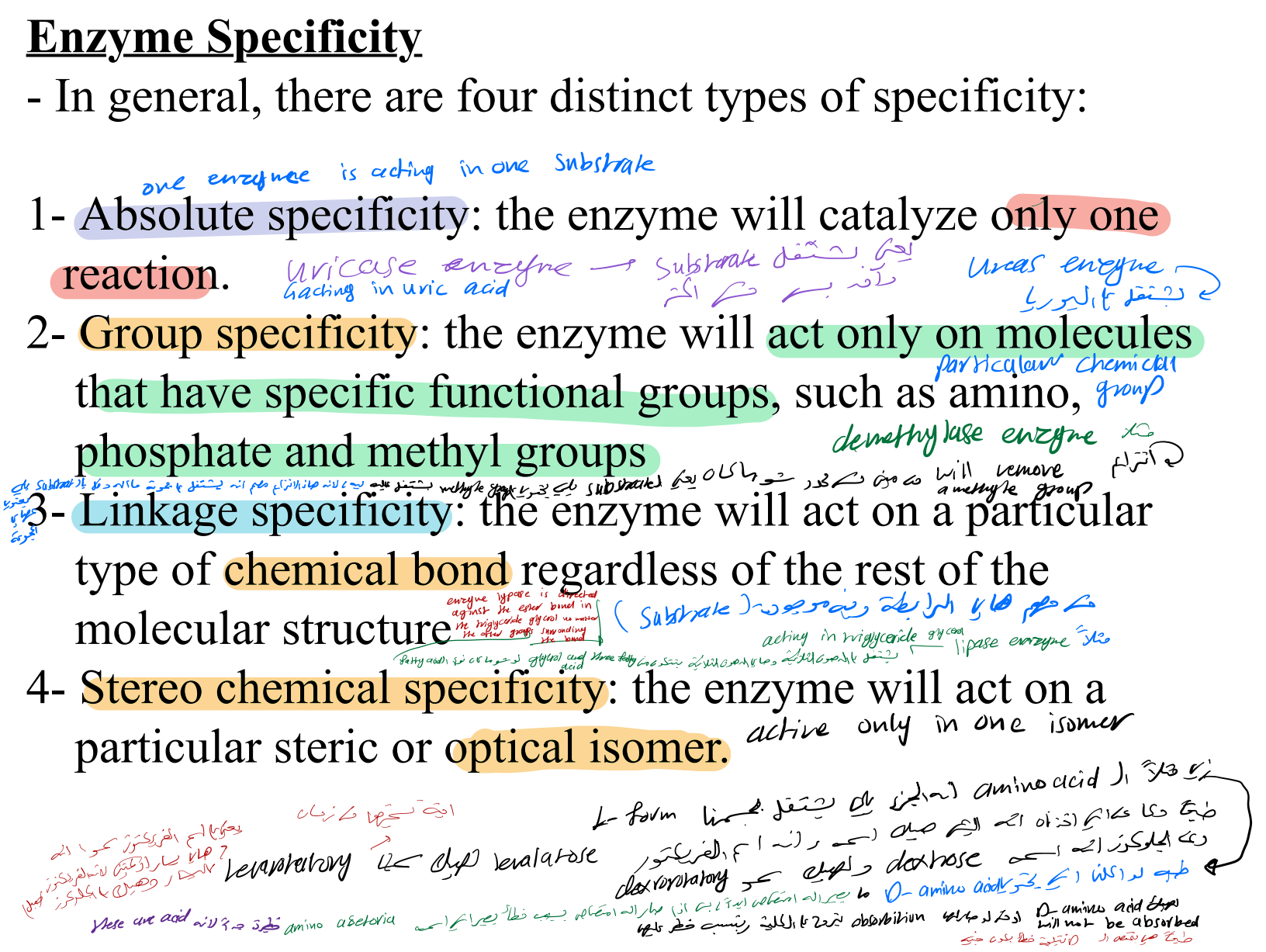
- In general, there are four distinct types of specificity:

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

- 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 VB6
Flavin mononucleotide(FMN) VB2
Flavin adenine dinucleotide(FAD) VB2
Thiamin pyrophosphate (TPP) VB1
Biotin VB7
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

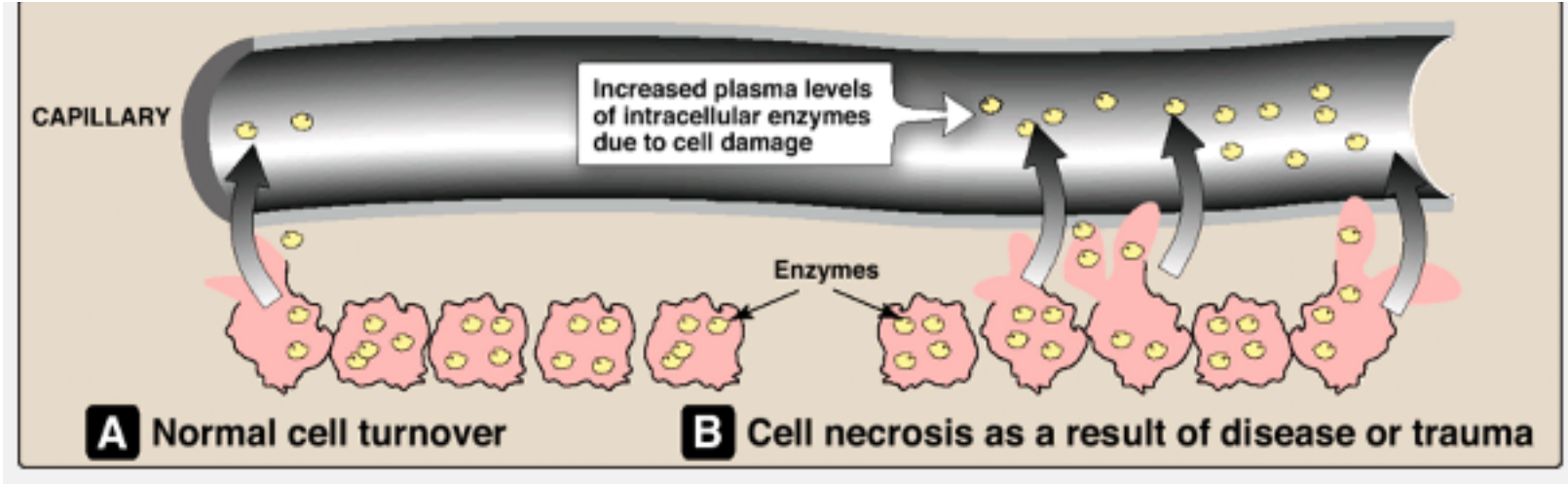
1- **Functional plasma enzymes** (Plasma derived enzymes):

- 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, (LDH)

- The enzyme interconverts lactate and pyruvate
- 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.

Enzymology- An overview-2

Factors affecting Enzyme activity

we are talking about some factors studied in the test tube

- Numerous factors affect the reaction rate:

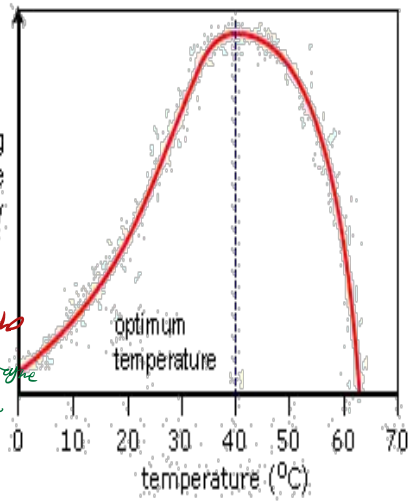
Temperature

هال ركزو ع السلايد كويس جداً

- The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature
- Most animal enzymes rapidly become denatured at temperatures above 40°C
- The optimal temperatures of the enzymes in higher organisms rarely exceed 50 °C
- The Q_{10}, or temperature coefficient, is the factor by which the rate of a biologic process increases for a 10 °C increase in temperature.

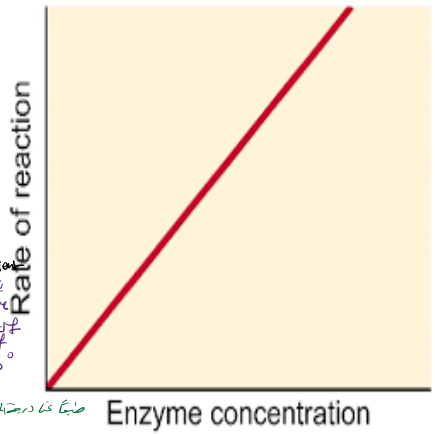
Effect of Temperature

- For mammals and other homoeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.



Effect of enzyme concentration

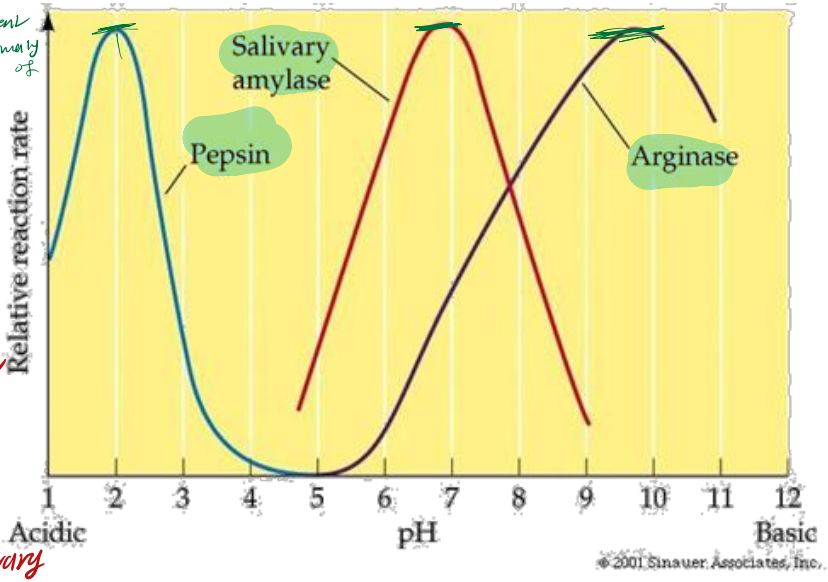
- As the amount of enzyme is increased, the rate of reaction increases.
- If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate.
- Reaction rate therefore increases then it levels off.



Effect of pH on enzyme activity

- The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration
- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.
- The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation at high or low pH and effects on the charged state of the enzyme, the substrates, or both.

we have different curves for different enzyme acting maximally at different optimum of pH



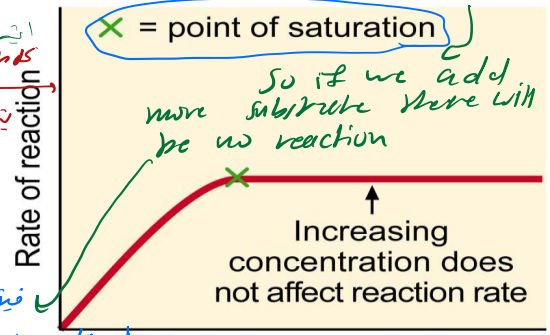
- Except for Pepsin, acid phosphatase and alkaline phosphatase, most enzyme have optimum pH between 5 to 9.

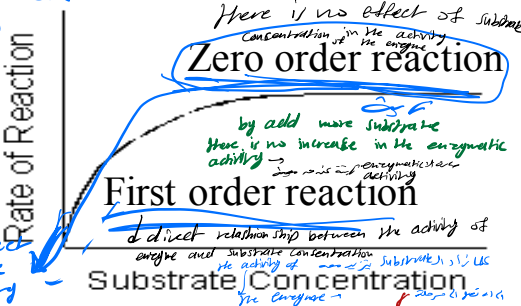
most of our enzymes acting maximally at the physiological pH at 7.35 – 7.45 in the blood and the 6.9 intra cellularly

Effect of substrate concentration

- At lower concentrations, the active sites on most of the enzyme molecules are not filled because there is not much substrate.
- Higher concentrations cause more collisions between the molecules.
- The rate of reaction increases (First order reaction).
- The maximum velocity of a reaction is reached when the active sites are almost continuously filled.
- Increased substrate concentration after this point will not increase the rate.
- Reaction rate therefore increases as substrate concentration is increased but it levels off (Zero order reaction).

The shape of the curve that relates activity to substrate concentration is hyperbolic.





Michaelis-Menten Kinetics

- The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme-catalyzed reaction [V_i], the concentration of substrate [S] and two constants, V max and km (which are set by the particular equation).

- The symbols used in the Michaelis-Menten equation refer to the reaction rate [V_i], maximum reaction rate (V max), substrate concentration [S] and the Michaelis-Menten constant (km).

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