

صلواتنا عليك يا معلمنا  
الله صل وسلم وبارك يا سيدينا محمد

# Enzymology- An overview-1

Substrate →

المادة التي يتم تحويلها إلى منتج  
to be converted into a product  
مادة حيوية

التي يجب فهمها في راحة الاختراع هو الحد الأقصى للكفاءة وحواجزها  
لغرض الاستقبال الحركي في المختبر  
The different laboratory investigations  
to be written for the patient  
to facilitate the diagnosis  
of the disease (methods)

enzyme are protein in nature except one type ribozymes  
 ↳ processing of RNA

## Enzymes- An introduction

- Biologic (organic catalysts) polymers that catalyze the chemical reactions. ↳ acceleration of the reaction

Simple protein → just consist of amino acid only  
 conjugated protein → molecule part of protein and other part another thing + carbohydrate  
 glycoprotein → carbohydrate + protein  
 lipoprotein → lipid + protein  
 nucleoprotein → nucleic acid + protein  
 we have molecule consist of two different position  
 conjugative metalloenzymes consist of metal + metalloprotein

inorganic catalysts  
 metal ion  
 organic catalysts (enzymes)  
 hydrolysis reaction (as catalyst) H<sub>2</sub>O

- 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.

↳ pieces of RNA can act for processing RNA  
 transcription  
 Short segment of RNA about 90 ribonucleotides but they have catalytic activity  
 ↳ to be converted from non functional form into the functional form  
 near 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 molecules to be converted into product molecule per unit time per enzyme unit  
 one unit of an enzyme  
 one unit of metal catalyst  
 enzyme can convert 10<sup>5</sup> - 10<sup>12</sup> of the substrate molecules in one second but metal ion can convert only 10<sup>2</sup>  
 inorganic, organic catalyst enzyme  
 organic and inorganic enzyme

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

and specific

دالة في اذكي انه  
 lactate dehydrogenase  
 enzyme is catalyzing  
 oxidation of lactate  
 into pyruvate  
 ما يفتح اذكي انه  
 lactate dehydrogenase  
 oxidizes lactate  
 الى لاهت و يفتحه ل  
 pyruvate  
 و لاهت و يفتحه ل  
 pyruvate  
 و لاهت و يفتحه ل  
 pyruvate

به يفتحه ل  
 Catalyzing two different reaction  
 in two different substrate

isocitrate dehydrogenase  
 في دورة كريبس  
 2 different substrate  
 in the same cycle with two different  
 reactions

isocitrate dehydrogenase  
 which is catalyzing oxidation  
 of isocitric acid is catalyzing  
 a decarboxylation reaction  
 يفتحه ل  
 يفتحه ل  
 يفتحه ل

oxalo succinic acid  
 isocitrate dehydrogenase  
 يفتحه ل  
 يفتحه ل  
 يفتحه ل

oxidation and preparing the product for the  
 easy leaving CO<sub>2</sub>

Carboxylation  
 به ما يفتح ل  
 به ما يفتح ل  
 به ما يفتح ل

which is extracted from organism in the hot water called Thermus aquaticus  
 PCR يستخدم تيمت DNA polymerase enzyme  
 في درجة حرارة عالية  
 في درجة حرارة عالية  
 في درجة حرارة عالية

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

the enzyme activity in the cell membrane are not acting in other site of the cell. not just cell membrane in mitochondria or in nucleus --- not acting in one site ...

ممكن انه mitochondrial replication  
 it has its own DNA  
 Catalyzing replication of DNA?  
 it's DNA polymerase enzyme and it is nuclear enzyme  
 يمكن انه DNA polymerase  
 will be synthesis then it go to mitochondria  
 يحرك كل انتقاله الى الميتوكوندريا  
 بكونه الانزيم الذي يحفزها موجود بالميتوكوندريا

سؤال انكم زيادة ارتفاع الحرارة بالتاثير enzymatic activity  
 بيت انه بغيره denaturation  
 حرارة انتقاله الى 70 درجة  
 breaking down the bond that stabilize the tertiary and quaternary structure  
 So the protein molecule will be converted from tertiary or quaternary structure into primary structure.

يكون كجدة من انفسه الانتقال الى تertiary  
 لانه الاكبر يكون 3D-structure  
 او يمكن يكون quaternary اذا اكثر molecule  
 hydrogen bonding and quaternary structure  
 Salt bridge hydrophobic

peptide bond  
 disulfide bond  
 hydrogen bonding and quaternary structure  
 Salt bridge hydrophobic

Dr. Samir Saad, M. ...  
 10:44



No  
 Type a message

# Nomenclature of enzymes

اسم الـ substrate الى مشتق  
ase رابطة

- In most cases, enzyme names end in -ase

مكونة من  
المشتق لانها ما يتغير  
نوع التفاعل

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

انضمة التسمية صوابي بتسمى  
The name of the substrate to be active by the enzyme

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

oxidation الـ lactate  
removal of hydrogen

Pyruvate decarboxylase

removal of CO<sub>2</sub> الـ pyruvate

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

Salivary enzyme  
amylase

Catalase

Pepsin

in the stomach digestion

Chymotrypsin

amylase  
partial digestion of starch

Trypsin

+ aminopeptidase + carboxypeptidase  
+ dipeptidase + tripeptidase  
collagenase + elastase

انزيمات بتتفعل بالمخ البروتيني  
يكون انزيمات الـ ما يبدو بالمعدة يسير  
بالاسرار

اول انزيم  
بتتفعل  
بالاسرار  
المعدة  
بتتفعل  
بالاسرار  
المعدة  
بتتفعل  
بالاسرار  
المعدة

# 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** one of the 6 major classes of enzyme activity

الترتيب بعبرته الحجومه يك يتبعها الترتيب

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

هو نوع substrate الذي يستعمل بالانزيم او هو الرابطة التي يكسرها

**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

oxidation and reduction  
 تفاعل أكسدة واختزال

(Alcohol dehydrogenase [EC 1.1.1.1])

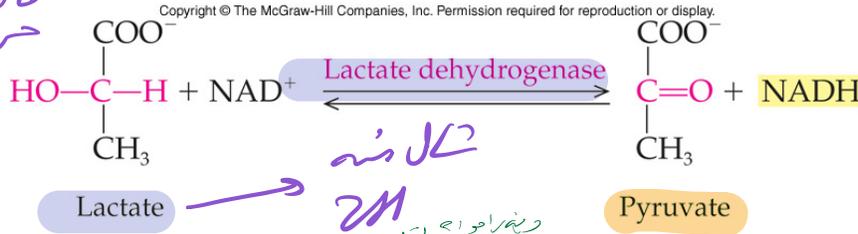
- Reductases

- Oxidases

in the biological system the oxidation never happens without reduction

loss of H or loss of e-  
 فقدان إلكترون أو فقدان هيدروجين

Oxygenation  
 أكسدة



ما يبرر دافعة التفاعل يكون عندها حرارة غير كافية  
 (بغيره) دافعة التفاعل تكون كافية  
 نشأ عن الاتزان

تفاعل  
 2H

reduction reaction the acceptor of the electron  
 تفاعل اختزال المتقبل للإلكترون

# 2- Transferases (EC.2) transfer a group from one molecule to another, such as

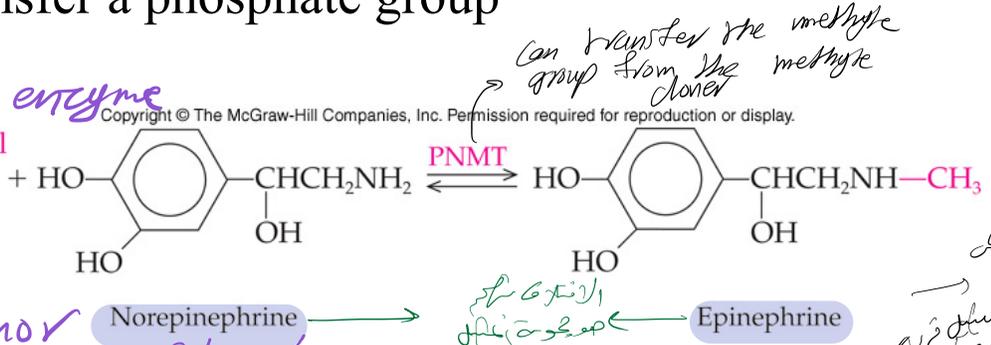
تحويل

(Hexokinase [EC 2.7.1.2])

- Transaminases catalyze transfer of an amino group

- Kinases transfer a phosphate group

methyl transfer enzyme  
 الإنزيم الذي ينقل الميثيل  
 methyl group from the donor to acceptor  
 مجموعة الميثيل تنتقل من المتبرع إلى المتقبل



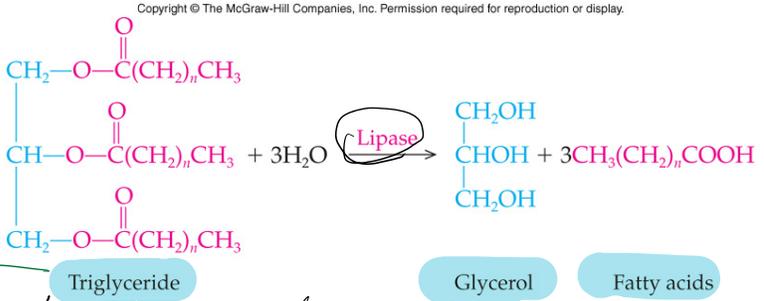
can transfer the methyl group from the donor

لا يتم نقل الميثيل من المتبرع إلى المتقبل  
 الإنزيم الذي ينقل الميثيل  
 مجموعة الميثيل تنتقل من المتبرع إلى المتقبل  
 (acceptor)

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

- Phosphatases
- Peptidases
- Lipases

*lipase enzyme*  
*3 molecules of water*  
*3 fatty acid*



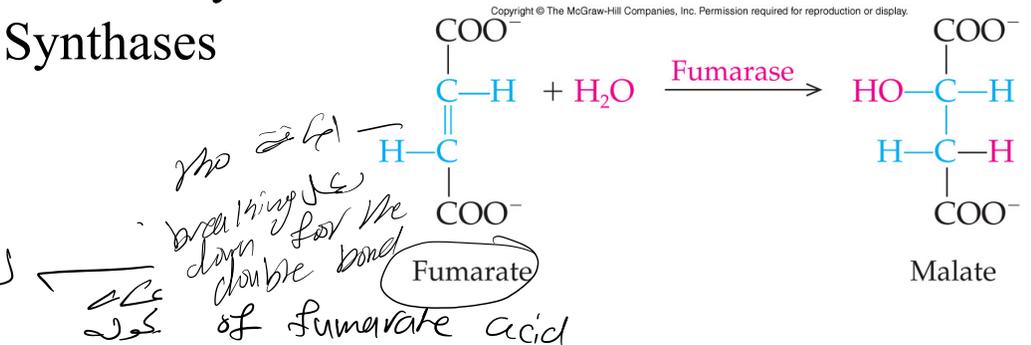
*Can create a double bond or breaking down a double bond*

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

*Malate acid*



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

تغيره isomer لايه  
 ان isomer كالم  
 ان isomer كالم  
 ان isomer كالم

(Alanine racemase [EC 5.1.1.1])

## Epimerases

### Mutases



3-Phosphoglycerate

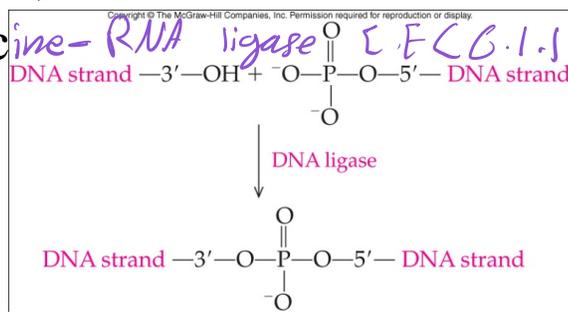
2-Phosphoglycerate

جلوكوز الى الفودوس وضرير يكتوز سير  
 لصيله يكره اسم الاتزام  
 aldoheto isomerase enzyme  
 او الاتزام يغيره جلوكوز  
 جلوكوز جلاكتور  
 Epimerase

# 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

فارة نشغل برصو  
 ATP  
 معظم الايمان يتعمل  
 Adenosin triphosphat

(Isoleucine-RNA ligase [EC 6.1.1.5])



Ty DNA ligase enzyme  
 برصو جلاكتور  
 to give continue of  
 the molecule DNA

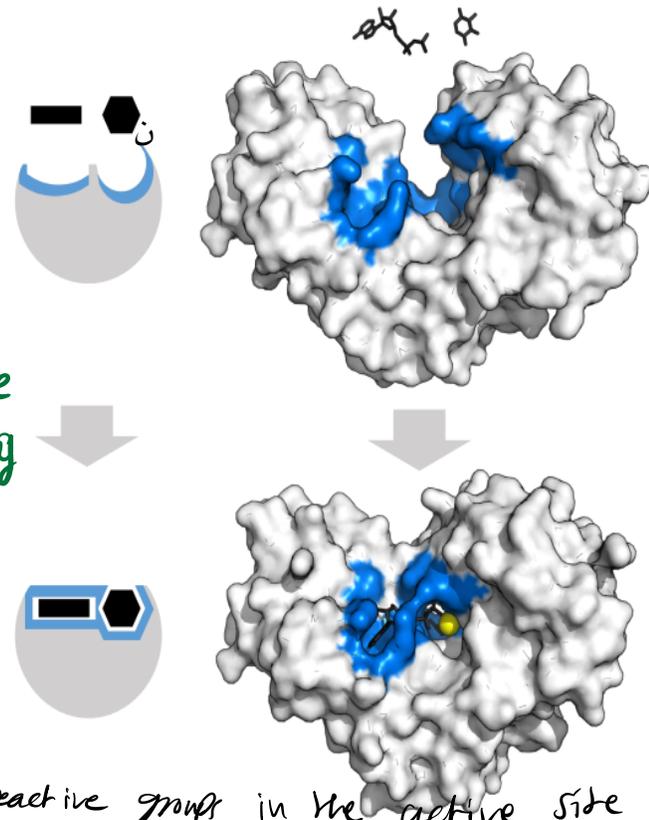
**Active site** → The site of the enzyme to which the substrate is binding for catalysing the reaction  
 صفة كل الإنزيم (قطعة رصدة من الإنزيم) لكه الخا صفة كده

- 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 character of the active site to allow the binding of the substrate to the enzyme?

1- It have 3D-Configuration active sites by complementarity *reses* and the substrate  
 يكواح 3D صفة  
 (الصفة)

complementarity *reses*, they should be specific reactive groups in the active site of the enzyme



The substrate should be stabilized to the active site

active site of substrate → product

لأنه في الموقع النشط للإنزيم يجب أن تكون المجموعات الوظيفية قادرة على تثبيت الركيزة

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

functional groups

When they are ionized they will give a negative charge

glutamate, aspartate } acidic amino acid

lysine, histidine, arginine } basic amino acid

تكونت من مجموعتين من الأحماض الأمينية  
 highly reactive groups } reactive groups } functional groups  
 to be stabilized active site of enzyme & binding site of substrate  
 Stabilized active site of enzyme & binding site of substrate  
 Consisted of more than one protein molecule  
 Consisted of two protein molecules  
 in the center face between the two polymer (two protein molecules)  
 located in the center face between the substrate and functional groups  
 this is very good → functional groups from the both protein molecule and it will form functional group from the second protein molecule and this will facilitate the binding and the stabilization of substrate to the active site.

Substrate containing molecules  
 cysteine

enzyme binding site and the binding site of the substrate

Substrate enzyme complex it should be stabilized

Transition state  
 Product

specific groups in the active site of the enzyme

3-specific arrangement of these functional groups which is responsible for the specificity of enzyme  
 3D configuration

Complementarity between the substrate binding site and the active site of the enzyme  
 3-specific arrangement of

# 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.

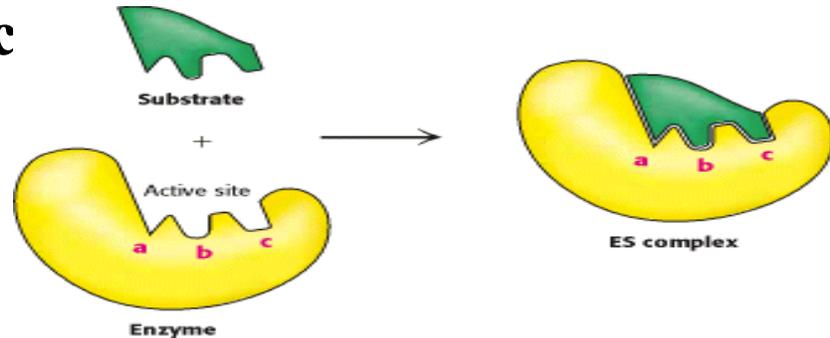
*مستقر*  
*Two models of binding the substrate to the active site*

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

*rigid & not flexible*  
*non changeable model*  
*if there is no complementarity between the substrate binding site and the active site of the enzyme there will be no reaction*



substrate for active site binding  
البروتين للركائز

# Induced-Fit Model of Enzyme-Substrate Binding

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

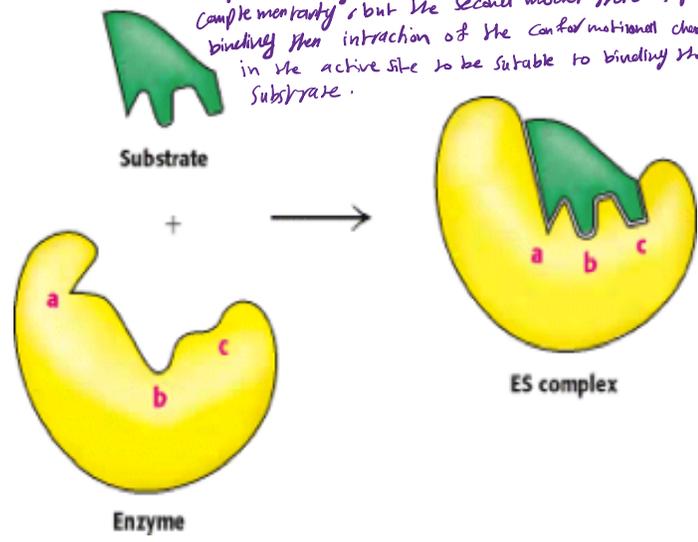
binding of the substrate to the original form of the active site is not good because there is no complementarity between the two, but once there is partial binding of the substrate to the active site to the enzyme

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

fit for binding the substrate = binding the substrate suitable for 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).

The first model → rigid and the second is flexible  
The first → unchangeable but the second → changeable  
The first model will binding if it is (there is) complementarity and no binding if there is no complementarity, but the second model there is partial binding then interaction of the conformational change in the active site to be suitable to binding the substrate.







# Processes at the active site

Where the substrate is binding to the enzyme and it is small cleft active site  
حيث يوجد الإنزيم ويجتمع فيه جزيء الركيزة في حفرة صغيرة

## 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.

(proximity) of the active groups in the active site of the enzyme and in the binding site of the substrate

اقتراب (proximity) of the active groups in the active site of the enzyme and in the binding site of the substrate

اقتراب (proximity) of the substrate (to the active site)

They pushing each other

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

will react in we call bond forming distance

stabilization of the substrate molecule in the active site of the enzyme because if there is no stabilization the reaction will not be correct

in what we called bond-forming distance

crowdedness of the substrate molecules around the active site of enzyme

orientation of these active groups in the active site of substrate

كثافة الجزيئات القريبة من الموقع النشط للإنزيم  
توجه الجزيئات النشطة في الموقع النشط للركيزة  
توجيه المجموعات الوظيفية النشطة في الموقع النشط للركيزة

to the bond forming distance

reaction

will react

in we call bond forming distance

in the active site of the enzyme because if there is no stabilization the reaction will not be correct

reactive groups in the binding site of the substrate

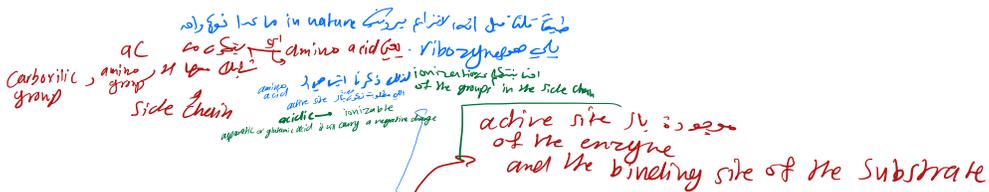
reactive groups in the active site

crowdedness of the substrate molecules around the active site of enzyme

orientation of these active groups in the active site of substrate

specific reactive group in the active site  
cysteine, sulfur, hydroxyl, carboxyl, amino acid, imidazole ring, hydroxyl, amino acid

stabilization of the substrate molecule in the active site of the enzyme because if there is no stabilization the reaction will not be correct



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.

because they can be ionized ← active site of the enzyme amino acid

carrying a charge to allow the reaction between another reactive groups also ionized in the binding site

ionization of the reactive group in the active site of the enzyme and ionization of another reactive group in the binding site of the substrate

general base catalysis partial proton abstraction from the acceptor also involve the ionization of particular group to allow the bonding between the reactive groups in the active site of the enzyme and the reactive group in the binding site of the substrate of the energy of activation and to be stabilize in a transition state

in the formation of bonds between enzyme and substrate and stabilization of the substrate in the active site transition state enzyme substrate complex formation or transition state

lowering partial transfer of proton from a donor general acid catalysis

يقدر اطيبي صانها بشكل تام كل انزيم  
الانزيم اعلى الجاهز للتحلل لانها تملك

Salivary amylase enzyme - starch  
and pancreatic amylase enzyme

starch highly branched  
منه تفرعها الى  
مجموعات فرعية  
مختلفة

### 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.

الانزيمات التي تحلل  
الارتباطات التساهمية  
تربط  
لل Substrate  
بشكل غير ملائم  
للتفاعل  
بشكل  
يعد ميل ما يكون  
يرتبط به  
بشكل غير ملائم  
للتفاعل

والمكانة غير ملائمة لتهيئة  
الارتباط التساهمي بين  
الانزيم والركيزة  
تسهل على  
الانزيم كسر  
الارتباط التساهمي

unfavorable slightly configuration of the binding substrate to the active site to the enzyme bond under strain

لأنه يسهل على  
الانزيم كسر  
الارتباط التساهمي  
بين الركيزة  
والانزيم

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

The reaction will be carried out by forming covalent bond between substrate and the enzyme for more stabilization → means that energy of activation needed to be in the transition state

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)**

Covalent bond done

يكون  
الارتباط التساهمي  
مستقر  
في  
موقع  
الانزيم  
والركيزة  
في  
موقع  
الانزيم  
والركيزة  
في  
موقع  
الانزيم  
والركيزة

كيفية  
تكوين  
الارتباط  
التساهمي  
بين  
الانزيم  
والركيزة

removal of electrons of substrate and the active groups in ionized state → binding between substrate and active site of the enzyme to be converted into product → transition state of reaction

# 5- Metal Ion catalysis

تقسيم الانزيمات المعدنية

Two classes of metal ion dependent enzymes:

تقسيم الانزيمات التي تعتمد على metal ions  
 1- metal ions  
 2- metal ion dependent enzymes

1- Metalloenzymes contain tightly bound transition metal ions

(Fe<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>)

if the metal ion is removed from this type of enzyme there will be no reaction

2- Metal-activated enzymes loosely bind metal ions (alkali or

alkaline metal including Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>)

في الحالة التي يكون فيها المعدن موجودا بالتقاليد  
 the reaction will proceed but not efficiency  
 but not efficiency

بعدها ان المعدن موجود  
 will take place but not efficiency  
 metal ion is not an essential part of the enzyme

- Metal ions enhance catalysis in three major ways:

1- Binding to and orienting substrates for reaction as Mg<sup>2+</sup>

binding to ATP

الانزيم الذي يرتبط بالمركب المدخل في التفاعل  
 metal ion substrate  
 attracting the substrate toward the active site of the enzyme

2- Mediating redox reaction through changes in oxidation state

such as reduction of O<sub>2</sub> to H<sub>2</sub>O through electron transfer

3- Electrostatic stabilization or shielding of negative charges as

Mg<sup>2+</sup> binding to ATP

facilitated the interaction between the reactive groups in the active site of enzyme and the binding site of the substrate  
 specific  
 as an acceptor of electrons  
 reactive... ionization/oxidation in the active site of the enzyme and the substrate there is no need for more ionization  
 Shielding the negative charges in the active site of the enzyme



enzyme metal substrate bridge

active site of the enzyme  
 Substrate is pushing towards the active site of the enzyme

# 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

دفع الانتزاع لل  
distribution and arrangement of the charges in the active site of the enzyme  
catalysis هو ترتيب توزيع الشحنات في الموقع النشط من الإنزيم  
orientation = 2  
the reactive group in both the enzyme and the binding site of the substrate  
Catalysis هو ترتيب توزيع الشحنات في الموقع النشط من الإنزيم  
reactive group  
to stabilize the transition state  
removal of the water from the active site and lead to generate what we called dielectric constant in the active site

لجعل توزيع الشحنات في الموقع النشط من الإنزيم والتمسك بالموقع النشط من الركيزة  
active site of the enzyme and the binding site of the substrate  
guiding polar substrate to their active site  
لتنجذب الركيزة القطبية نحو الموقع النشط من الإنزيم  
active site of the polar substrate  
their particular enzymes



# Cofactors

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

*Coenzymes*

*derivative from vitamin  
VB-complex*

*VB<sub>1</sub> → Co factor → thiamine pyrophosphate  
VB<sub>2</sub> → Flavin adenine dinucleotide and flavin adenine mononucleotide  
VB<sub>3</sub> → nicotinamide adenine dinucleotide*

*B-complex member  
of vitamins are providing us with different  
Coenzymes*

- Cofactors that are small **organic molecules** are called **coenzymes**.

- Most common cofactor are also metal ions.

*مرتبة بالانترام اربطها قويا  
metal ions  
and vitamin derivatives*

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

*↳ covalent or not covalent bond but tightly to the enzyme*

**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

*metal ions  
or vitamin derivatives  
الانترام اربطها قويا  
الانترام اربطها قويا  
الانترام اربطها قويا*

*coenzyme*

*metal ions  
or vitamin derivatives  
الانترام اربطها قويا  
الانترام اربطها قويا* prosthetic group

*cofactors*

*metal ions  
or vitamin derivatives  
الانترام اربطها قويا  
الانترام اربطها قويا* loosely bound to the enzyme

## 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

metalloenzymes *معدنية الإنزيمات* metal ion *أيون المعدن*  
and metal activated enzyme *إنزيم نشط بالمعدن*  
binding and orientation of the substrate *ربط وتوجيه الركيزة* metal ion *أيون المعدن*  
essential in the structure of the enzyme or not *أساسية في البنية الإنزيمية أم لا*  
oxidation states in the active site of enzyme *حالات الأكسدة في الموقع النشط للإنزيم*  
electrostatic shielding to prevent of the extra negative charge *حجب كهروستاتيكي لمنع الشحنة السالبة الإضافية*

- 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 →

derived from vitamins  
مشتقة من الفيتامينات

usually bound to the enzyme  
يعتقد كجزء من تركيب الإنزيم  
it can do more than one reaction  
in the same time

لا يمكنه العمل مع كل الإنزيمات  
NAD  
it doesn't mean that it will act at a particular enzyme  
لا يمكنه العمل مع كل الإنزيمات  
Lactate dehydrogenase  
لا يمكنه العمل مع كل الإنزيمات

- They serve as **recyclable shuttles**—or group transfer agents—that transport many substrates from their point of generation to their point of utilization.

for the neutralization ← acceptor

- 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

بستقیمہ کے تشخیصی  
اعتماد یہ کہ ان مراح

chemical components  
that can help in estimation

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

نہایت لائقہ لوانہ فعل  
استعمال کیلئے عام صاف  
الستادہ کے محدود مستویا  
of glucose oxidation reaction in which we are using an enzyme for the oxidation of glucose

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

بعض کے لئے استعمال  
علاج کے لئے  
الاستادہ لوانہ استعمال  
of other components

## Enzymes as diagnostic markers

دیباہ کے لئے نسبتاً آخری الاستادہ کے استعمال کے لئے

1- Functional plasma enzymes ( Plasma derived enzymes):

وجودیہ ہائیم سے موجودہ بالکل

- 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.

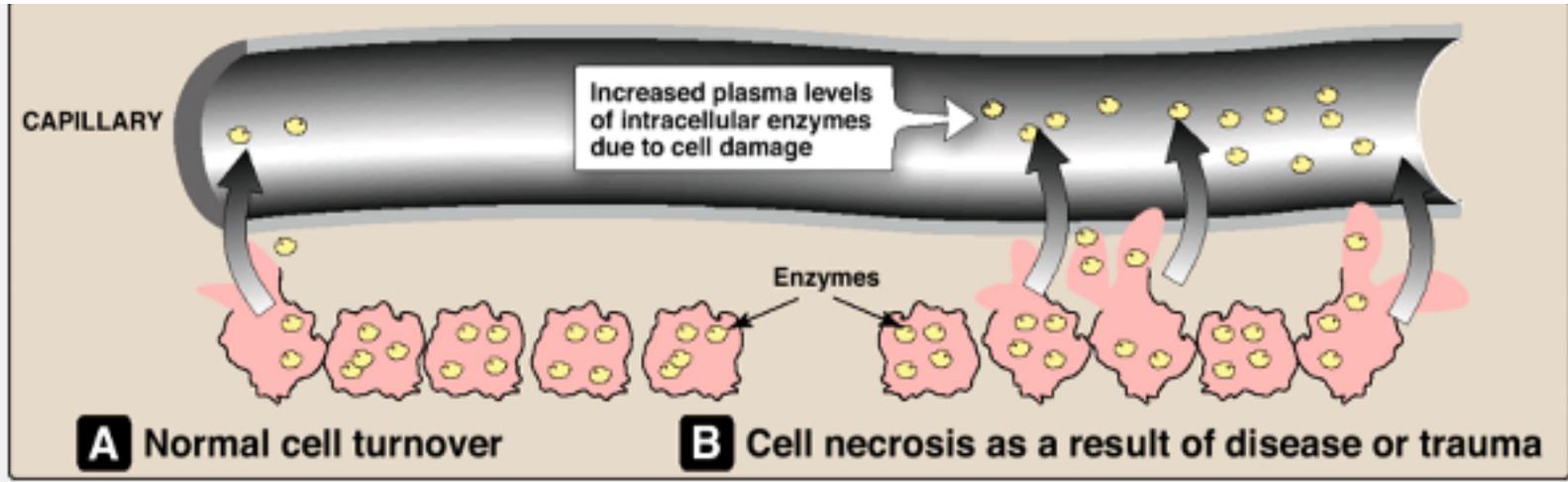
لو (فیکٹا) علیہا کلہا صلاہ انہ کے change in the membrane of the cell and the enzyme is existing in the

abnormal state  
Concentration  
high concentration of these enzymes in the blood

abnormal state  
Concentration  
high concentration of these enzymes in the blood

## 2- Nonfunctional plasma enzymes (Cell derived enzymes):

- Plasma also contains numerous other enzymes that perform no known physiologic function in blood. *مجموعة متنوعة من الإنزيمات*
- These apparently nonfunctional plasma enzymes arise from the routine normal destruction of erythrocytes, leukocytes, and other cells. *تسبب destruction of the cells containing the enzyme severe illness can sometimes damage breathing system*
- Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes. *concentration of enzyme cell turn over*



# Isoenzymes (Isoenzymes)

particularly cell derived enzymes  
بعض الإنزيمات المشتقة من الخلايا  
particular cell derived enzymes  
بعض الإنزيمات المشتقة من الخلايا

- 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**, → origins  
 They are coding in the same substrate. They are coding in the same reaction and they are coding in the same product.  
 في نفس الركيزة، في نفس التفاعل، في نفس المنتج.  
 They have different molecular weight.  
 لديهم أوزان جزيئية مختلفة.  
 They have different origins.  
 لديهم أصول مختلفة.

- 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.

عضلة القلب H، عضلة الهيكل العظمي M

They have different origin and they have different physical and they differ in the effect of the activator and inhibitor.  
 لديهم أصول مختلفة، لديهم خصائص فيزيائية مختلفة، وهم يختلفون في تأثير المنشطات والمثبطات.  
 Lactate dehydrogenase one is inhibited by iron.  
 إنزيم اللاكتات ديهيدروجيناز أحدهم يثبطه الحديد.  
 lactate dehydrogenase is inhibited by iron.  
 إنزيم اللاكتات ديهيدروجيناز يثبطه الحديد.  
 - the reaction may catalyze  
 - the substrate may be acting on  
 - the product may be different  
 - origin  
 - chemical composition  
 - effect of activator

- There are 5 different isoenzymes. *Can be separated by electro phoresis and it also can be separated by ion exchange chromatography*
- The relative ratio of the isoenzymes depends on the location in the organism as well as the developmental stage.

Isoenzyme	Tissue origin
LDH1 (H4) <i>optimized to act under aerobic condition high O<sub>2</sub> supply cardiac muscle</i>	Cardiac and kidney
LDH2 (H3M) <i>4 polypeptide chains 3H - 1M</i>	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. *myocardial infarction*
- Measuring them is of value in the presence of elevated levels of CK or CPK to determine the source of the elevation. *dimeric molecule 2 polypeptide chains only*
- Each isoenzyme is a dimer composed of two protomers 'M' (for muscles) and 'B' (for Brain). *specific biomarker in the particular disease*
- These isoenzymes can be separated by, electrophoresis or by ion exchange chromatography. *in the diagnosis to give the final and different diagnosis*

Isoenzyme	Electrophoretic mobility	Tissue of origin	Mean % in blood
MM(CK3) <i>من نركه وادى قدام</i>	Least	Skeletal muscle Heart muscle	97-100%
MB(CK2)	Intermediate <i>يتضمنه في الكلى والكبد وعضلات القلب والبنكرياس</i>	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.

*very little concentration  
في الكلى والكبد وعضلات القلب والبنكرياس  
polypeptide  
Brain  
in high concentration in the brain*

# **Enzymology- An overview-2**

## Factors affecting Enzyme activity

we are talking about some factors studied in the test

- Numerous factors affect the reaction rate: *نوع*

### Temperature

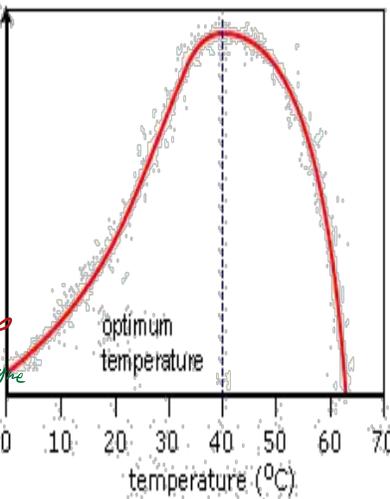
تأثير درجة الحرارة على سرعة التفاعل

- 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.

تبدیل به سرعت حرارتی همگرمها  
تدریجاً، البته در نقطه نقطه حیاتی  
و درجه حرارتی را  
maximum level  
as نشاط لا تترجم عند النسخة  
no more increase  
in enzymatic activity  
در حد حیاتی بحواله حرارتی  
optimum temperature

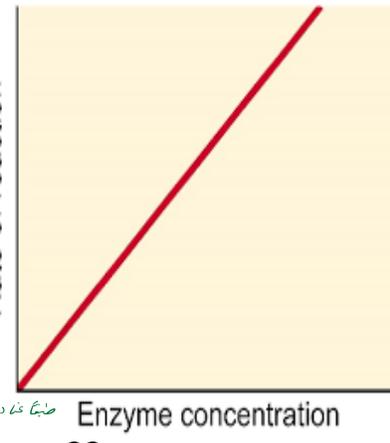


temperature at which the enzyme is acting maximally  
تبدیل حداکثری  
gradual decrease in the activity of the enzymes till zero  
تدریجاً درجه حرارتی  
activity of enzyme to zero why?  
denaturation  
انواع آهمیوتیک از عوامل مختلف الی انان که  
denaturation  
Quaternary structure  
درجه حرارتی

# 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.

Hydrophobic interaction  
electrostatic bond  
quaternary structure  
non functional form of the enzyme  
primary structure  
activity of enzyme  
Q10  
temperature coefficient  
what is the rate of decreasing activity of enzyme for each 10°C increase



شبهت کل العوائل ریکوه عتسماً متحرکاً فقط

عند كل الاحتمالات عند درجة الحرارة  
Enzyme concentration  
Enzyme activity

Substrate concentration  
Substrate  
enzyme activity  
Substrate  
enzyme activity  
Substrate  
enzyme activity

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# Effect of pH on enzyme activity

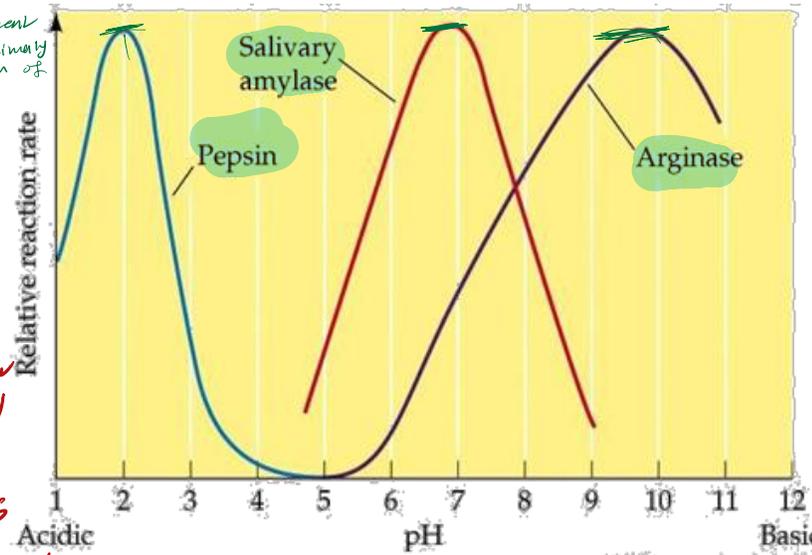
- The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration
- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.
- The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation 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 or 7.35 - 7.45 in the blood and the 6.9 intracellularly*

*acid base catalyst is ionization of the particular group of the active site of the enzyme and particular*

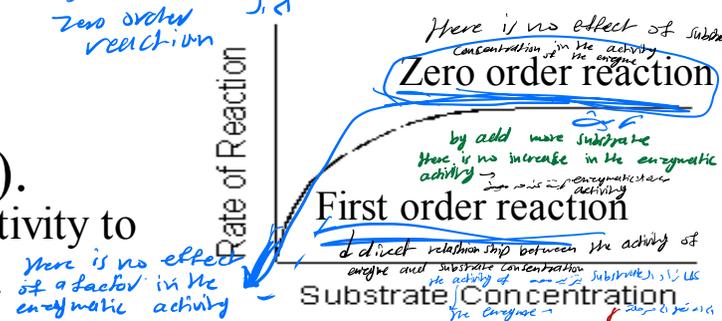
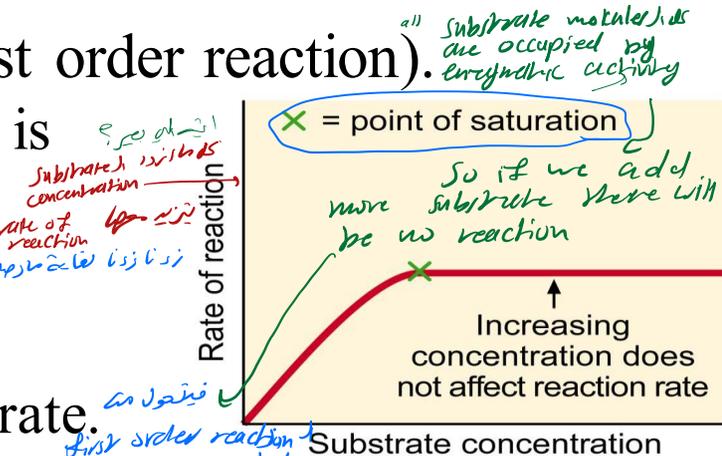


groups for the binding site of the substrate for the stabilization of the active state → breaking down of some of the inter action between the substrate and the active site of the enzyme

# Effect of substrate concentration

لبن قسمه ان کے تحت تمام عوامل دیکھئے کہ تیز کرنے سے کون سے عوامل متاثر ہوتے ہیں اور ان کے اثرات کیا ہیں۔

- 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.

صلى الله عليه وسلم

# Michaelis-Menten Kinetics

بے لپس ڈیٹا کے لیے لگاتار تجربے کی ضرورت ہے (تجربے کے نتائج)

in which there is no increase in the enzymic activity due to increase in the substrate concentration

point of saturation in which all enzyme active sites are occupied by substrate molecules

-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  $k_m$  (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 ( $k_m$ ).

# Enzymes- An introduction

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

يسرع  $\rightarrow$  (acceleration for the reaction)

Simple protein  $\rightarrow$  just consisted of amino acid only

we have molecule consist of two different portion  $\rightarrow$  conjugated protein  $\rightarrow$  molecule part of it protein and other part another thing - carbohydrate

enzymes are protein in nature except one type

ribozymes processing of RNA

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

organic catalysts  
inorganic catalysts

having dynamic function and they have structural function

carbo + protein  $\rightarrow$  glycoprotein  
lipid + protein  $\rightarrow$  lipoprotein  
nucleic acid + protein  $\rightarrow$  nucleoprotein

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

Short segment of RNA about 90-200 ribonucleotides but they have catalytic activity like control for the ribosomal RNA

pieces of RNA can act for processing RNA

consist of protein and conjugative + metal ions

metalloenzymes

synthesising RNA from DNA

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.

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

per unit of time  $\rightarrow$  inorganic, organic

one unit of metal ion  
one unit of enzyme  
turn over number

the enzyme acting in the cell mitochondria... one unit of metal ion in other site of the cell

DNA synthesis will be synthesis then it go to mitochondria

per unit of time  $\rightarrow$  inorganic catalyst  $\rightarrow$  metal ion  
organic catalyst  $\rightarrow$  enzyme  
inorganic catalyst  $\rightarrow$  inorganic

