Enzymes : (Organic catalysts)

Slide 1

* Function: catalyze (accelerate) of reactions
* Nature : Protein in nature exept ribozymes
  * Ribozymes : short segment of RNA molecules (about 90-300 nucleotides) which act as enzymes in processing RNA molecules
* Characteristics: 1. neither consumed nor permanently altered
  2. highly efficient
  3. high turnover number
  4. highly selective (specific)
  5. Thermolabile
  6. Site specific
* Notes: 1. enzymes neither consumed nor permanently altered as a consequence of their participation in a reaction throughout their life span
  2. Turnover number: the number of substrate molecules that can be converted by one molecule of enzyme into product molecules in a unit time.
  3. Affect of Temperature : if protein is exposed to high temperature it will be Denatured (breaking of bonds that stabilize the 3D structure (tertiary & secondary structure) turning the enzyme back to the primary structure). and by that the enzyme lose it activity
  4. Site specific: enzymes found in cell membrane are diffrent from those found inside the cytoplasm, nucleus, .. etc.
* Nucleic Acids :

DNA	RNA
1) Nuclear DNA 3.5×10^9	1) mRNA 4) micro RNA 7) Ribozymes
2) Metochondrian DNA 1.6×10^4	2) rRNA 5) small interfering RNA
	3) tRNA 6) heterogeneous nuclear RNA

* Ribozymes: transforming RNA from immature (inactive) to mature (active) form and this process in called RNA processing
* Inorganic catalysts such as : Ni, Vitamines
* Inorganic catalysts are thermostable
* Regarding turnover number : enzymes cat. $10^6 - 10^{12}$ molecule
  inorganic cat. 10^3 molecule

Slides 3-5

Nomenclature of enzymes : (-ase = enzyme usually)

[1] Hydrolases: common names: derived from the substrate

[2] Other enzymes : names: derived from the substrate and the reaction catalyze

[3] Hydrolases : historical names : no direct relationship to substrate or reaction type

[4] EC - according to IUBMB : each enzyme was given 4 digit numbers

E.g. [1] urease , lactase

[2] Lactate dehydrogenase , Pyruvate decarboxylase

[3] Amylase, Catalase, Pepsin, Chymotrypsin, Trypsine (digestive enzymes)

[4] [EC 1.1.1.1] , [EC 2.7.1.2]

Classification of Enzymes : [EC 1.2.3.4]

1st : 6 major classes / 2nd : subclass / 3rd : sub-subclass / 4th : serial number

Major Classes :

(EC.1) Oxidoreductase :

* catalyze redox reactions
* Reductases , Oxidases
* In biological system whenever oxidation reaction happen a reduction reaction must occur because leaving H^+ alone will change the pH thus its need a carrier
* need H carrier such as : NAD^+ (in oxidation reactions), NADH (in reduction)

(EC.2) Transferase :

* Transfer a group from one molecule to another (from donar to accepter)
* Transaminases , Kinases
* Transaminases → amine group // Kinases → phosphate group from ATP

PNMT → methyl group from a donar (SAM) will be converted to SAH

* (Sam) (s-adinosine methionine) is the most common methyl donar and form SAH (s-adinosine Homocysteine) as it lose it methyl group

(EC.3) Hydrolases :

* Cleaves bonds by adding water
* Phosphates , Peptides , Lipases
* Digestive enzymes

(EC.4) Lyases

Slide 5+6

* Catalyze forming double bonds or the reverse break double bonds
* Decarboxylases, Synthases, Fumarase
* Fumarase catalyze the addition of H_2O to Fumarate and converting it to malate
* Adding of H_2O is for converting the status of the bond (double → single) without cleavage of the molecule

(EC.5) Isomerases

* Catalyze intramolecular rearrangements change the position of some groups intramoleclarly
* Epimerases, Mutases, Racemases

(EC.6) Ligases

* catalyze C-C, C-O, C-S, C-N bond making and breaking
* Most important example is T4 DNA ligase enzyme which link the fragments of the DNA
* Result in linking of two molecules with each other
* Require energy for catalyzing the reaction – the only enzyme that require energy ↑

Active Site (Catalytic Site)

Slide 7

[1] The site to which the substrates is binding to the enzyme

[2] found at any region of the enzyme and takes up a relatively small part of the total volume of an enzyme

[3] Shape → Take the form of a cleft or pocket

[4] * Active site specific characters:

1. Should have 3D configuration
2. should be complementary to the bind site of the substrate
3. should contain specific groups → highly reactive groups

The most highly reactive groups of amino acids:

- Hydroxycontaining amino acids: serine, threonine, tyrosine
- Modified hydroxycontaining amino acids: hydroxy proline
- Acidic amino acids: glutamate, Aspartate
- Imidazole containing amino acids: Histidine
- Sulfhidral containing amino acids: cysteine

Note: special arrangement of reactive groups in the active site of the enzyme will defined the special 3 D arrangement of the active site

Q: What is the hydroxy containing amino acid that participate the most in the active site ? (serine, threonine, tyrosine, hydroxyproline)

A: Tyrosine: participate in the production of hormones, participate in the production of melanin → doesn't participate the most

Threonine: It's an essential amino acid thus it's amount is not that much in our cells → doesn't participate the most

Hydroxyproline: It's a modified amino acid thus it's amount is not sufficient → doesn't participate the most

Serine: Non essential, non modified, sufficient amount → The most

[4] In order for the enzyme to work probably the substrate should be stabilized in the active site of the enzyme, and this stabilization require functional group in the binding site of the substrate and reactive groups at the active site of the enzyme because they are going to make the bonding together - multiple weak attractions

Types of bonds (attractions):

1. Hydrogen bonding 2. Electrostatic (Ionic) interaction
3. Hydrophobic reaction 4. Disulfide bonds (strongest attraction)

Note: These attractions are important for stabilizing the substrate

[5] The arrangment of the functional groups (reactive groups) in the active site are responsible of the specificity

[6] Regarding "multimeric enzymes" (consist of more than one polypeptide chain (quaternary structure)) ⇒ the active site will be located at the interface between subunits thus recruit (يستدعي/يجذب) residus (functional group) from more than one monomer (polypeptide chain) ⇒ The substrate will be more stabilized because the subtrate will form bonds with the first monomer and bonds with second monomer... thus stabilizing the substrate

How will the substrate bind to the enzyme ?

There are two models of enzymes :

1. lock and key model 2. Induced-fit model

* Lock-and-key model:

- The active site is Rigid, not flexible, must be complementary in the shape to the substrate.
- If there is a diffrence or an alteration in the active site of the enzyme or in the binding site of the substrate (they become incomplementary to each other) no reaction will occur because the active site of the enzyme is not able to undergo any conformational changes in its shape thus no bonds will form → no reaction
- This model accounted for the exquisite specificity of enzyme-substrate interactions ⇄ هذا النموذج يصف المثال على الدقة والاتقان في نوعية وتمييز الرابط بالهوية:

* Induced-Fit model:

- Active site shape is not suitable for binding of substrate but with proximity of substrate to the active site, the active site will form a complementary shape to the shape of substrate (enzyme a conformational change to fit the shape of the substrate)
- After the enzyme undergo (catalyze) the reaction it goes back to it original shape.
- There are some factors which induce this conformational changes such as : hormones
- This model is not rigid and can undergo some dynamic changes

Mechanism of Action of Enzymes

$$S + E \underset{-K_1}{\overset{+K_1}{\rightleftharpoons}} SE \underset{-K_2}{\overset{+K_2}{\rightleftharpoons}} P + E \xrightarrow{\text{go to catalyze other reactions}}$$

substrate — enzyme — enzyme-substrate complex — Product — Enzyme

(association constant)

+K 1 : constant for the forword reaction: (allow binding of substrate to enzyme)

-K 1 : constant For the reverse reaction: (Dissociation constant)

+K 2 : constant for the forword reaction: (constant of the production of the product + enzyme)

-K 2 : constant for the reverse reaction: (dissociation of product to substrate)

Note: adding the four constants togther give us Km constant

In other words : +K1 : association between the enzyme and substrate
+K2 : conversion of the substrate to product
-K1 : dissociation
-K 2 : breaking down or inability of enzyme to form product
Note : K 2 is the least constant to occur because the enzyme have the tendency to complete the reaction
The mechanism of action of enzymes can be explained by two perspective:
1. Thermodynamic changes 2. Processes at the active site
* Thermodynamic changes
- uncatalyzed reaction → need higher activation energy
- catalyzed reaction → need lower activation energy
- Activation energy (ΔGF) : energy needed to be supplied inorder to reach the transition state
- Enzyme function : decrease ΔGF thus more molecules have the required energy to reach the transition state
⇒ decreasing the time of reaction
* Processes at the active site
1. Catalysis by proximity :
- for the molecules to react they must come within bond forming distance of one another
- Enzyme (Active site) provides microenvironment that attract the substrate creating a region of high local substrate concentration (crowdness of substrate molecules) this crowdness will result in pushing the substrates into the active site
- Enzyme - substrate interactions orient reactive groups and bring them into proximity with one another
2. Acid-Base catalysis :
- Depends on the ionizable functional groups of aminoacyl side chains of prosthetic groups Glutamate, Aspartate, imidazol ring of histidine
- Acidic or basic functional groups ⇒ Ionizable (will carry charges) ⇒ these charges will facilitate chemical interactions ⇒ stabilize the substrate ⇒ Catalyze the reaction

3. Catalysis by strain: (لوي)

- It's a special property of the enzymes that catalyze the lytic reactions (involve breaking a covalent bond)
- By binding of the enzymes to their substrates in a configuration slightly unfavorable for the bond they are forced to undergo cleavage

4. Covalent catalysis:

- formation of covalent bond between the enzyme and one or more substrate which introduce a new reaction pathways whose activation energy is lower. Enzyme + Substrates $\xrightarrow{\text{covalent bond linkage}}$ pathways with lowe $\Delta G^{\ddagger}$
- By this process the enzyme can undergo an alternative reaction (alternative pathway) with lower energy of activation

Enzyme SPecificity

[1] Absolute specificity: working only on substrate with a particular bond

[2] Group specificity: will act only on specific functional groups such as: methylase which transfer methyl group from donar to any acceptor

[3] Linkage specificity: act on a particular type of chemical bond regardless of the rest of the molecular structure such as: Lipase which work on the ester bond of the TAG regardless fatty acids binded

[4] Stereo chemical specificity (optical specificity): the enzyme will act on a particular steric or optical isomer. Example: in our body enzymes only act on L-amino acids, and it can't catabolize D-amino acids

[5] Dual specificity of enzyme: (which is not specificity) enzymes which can act on two diffrent substrates catalyzing the same reaction or on two diffrent substrates catalyzing two different reactions

e^-

Look wide, beyond your immediate surroundings and limits, and you see things in their right proportion. Look above the level of things around you and see a higher aim and possibilty to your work. ♡

Cofactors:

* Regarding cofactors we can classify enzyme in to 2 types:
  1) Enzyme active without a cofactor (Protein portion of this enzyme is active)
  2) Enzyme which need a cofactor to get activated (Protein portion is inactive) Apoenzyme $\xrightarrow{\text{cofactor}}$ Holoenzyme

* We can classify cofactor acording to 2 factors:
  1) According to cofactor origin 2) According to bond strengh

1- According to it's origin cofactor could be:
  A) small organic molecules: are often vitamins or made from vitamins
    - if they are bonded losely: coenzymes
    - but if they are bonded tightly: prosthetic group
  B) Metal ions: they are always considered as prosthetic group

2- According to bond strengh:
  A) Tightly bounded to the enzyme: (Prosthetic group)
    - Attached tightly to the enzyme by a covalent or non-covalent bond thus (Not easily removed)
    - It's important (essential) for enzymatic activity (if they were removed from the enzyme there will be no reaction)
  B) losely (weakly) bonded to the enzyme: (Coenzymes)
    - Coenzyme act in a reaction on an enzyme then it leave this reaction to catalyze another one and so on.
    - Catalyse the same type of reaction. E.g. NAD is used in redox reaction
    - Not all losely bonded substances or molecules are considered coenzyme thus coenzymes have some special characteristics:
      1) They are small molecules - small organic molecules
      2) They act as recyclable (shuttles) - group transfer agents
      3) Bind with weak bonds in transient, dissociable manner either to the enzyme or to a substrate because it's not specified for a particular reaction.

      E.g. $NAD^+ \xrightarrow{\text{lactate dehydrogenase}} NADH$ and it can also participate in other redox reactions so it's not specified for this reaction only.

Prosthetic groups V.S Coenzymes (Summary and Examples)

1) Vitamin B complexes derivatives:

A) As a prosthetic groups if they are bonded tightly. Examples:

1) Pyridoxal phosphate → B6
2) FMN → B2
3) FAD → B2
4) TPP → B1
5) Folate → B9
6) Biotin → B7

} ← هما الفيتامينين وليس أحد مشتقاتهم

* Biotin (B7) only exist as prosthetic group

B) As a coenzyme if they are bonded losely. Examples:

1) NAD, FAD transport hydrogen atoms or hydride ions
2) Folates transport methyl group
3) Coenzyme A transport acyl group
4) Dolichol transport oligosaccharides

* Usually coenzymes are water-soluble B vitamins derivatives
* Coenzymes act as a temporary carriers of group from their point of generation to their point of utilization.

2) Metal ions - Co, Cu, Mg, Mn, Zn: (Most common prosthetic group)

They only act as prosthetic groups; they are either:

Metalloenzyme → tightly bounded metals → Essential for the reaction → (if they are removed no reaction will occur)

Metal-Activated Enzyme → losely bounded metals → Nonessential for the reaction but they are required for increasing the activity of the enzyme to the maximum level → (if they are removed the reaction will still take place but with slower rate)

The importance of metal ions:

1) Binding and orientation of substrate molecule:
   - The substrate must be oriented correctly to the active site to bing with it
   - If the substrate is going to bind to the enzyme incorrectly, metal ion will make some rotation in the substrate molecule to bind correctly

2) Interact with substrate to render them more electrophilic or nucleophilic:

اكثر حكي عن هاي النقطة الدكتور

By allowing substrate molecule to be more reactive by carrying a charge → Keep the substrate molecule in the reactive form → Stabilize the substrate in the active site → increase the rate of the reaction

3) Formation of covalent bonds with intermediate of the reaction

- Every metabolic pathway is consisting from more than one reaction, which begin with a substrates and end with a products and during the reactions of the pathway we get some unstable molecules or substances called intermediates
- Those intermediates can participate in other reactions (pathways)
- In order to keep the intermediates in the metabolic pathway and prevent them from participate in another pathway (reaction), Metal ions covalently bond with them

Uses of Enzymes in Medicine :

1. As reagents for biochemical estimations and detections. E.g. Glucoseoxidase enzyme (قياس نسبة السكر في الدم من طريق)
2. As bio markers → Diagnosis of certain diseases.

⇒ Regarding point 2, we classify into 2 groups :

A. Functional plasma enzymes (Plasma derived enzymes) :

- their substrates are normally located in blood plasma
- High concentration of these enzyme in blood is normal whereas Low concentration of these enzyme in blood is abnormal ← دليل على وجود مرض
- Majority of these enzymes are synthesized and secreted by liver

Examples : Lipoprotein lipase / pseudo cholinesterase / proenzymes of blood coagulating and blood clot dissolution. (pro = inactive)

Note: Any enzyme begin with pro or end with gen is biologically inactive

B. Nonfunctional plasma enzymes (Cell derived enzymes) :

- their substrates are found in high concentrations intracellularly
- High concentration of these enzymes in the blood is abnormal though we can find them with low concentration in the blood due to normal apoptosis of cells
- High concentration of these enzymes in the blood means damaging of the cells / tissues (E.g: erythrocytes, leukocytes, myocardial tissue, and other cells / tissues)
- Damage or necrosis of tissues is resulting from injury or disease leads to high concentration of these enzymes in the blood.

Examples : Amylase enzyme → liver / pancreatic ~~diseases~~ diseases
Acid phosphatase → Prostate cancer
Alkaline phosphatase → Liver and bone cancer
Creatin phosphokinase → } Myocardial Infraction (MI)
lactate dehydrogenase → } Muscletal muscles disorder

نصيحة هامة مستقبلاً من دكتور سمير } Note: when diagnosis a disease never never never relay on one marker only but you should use more markers to emphasis the disease

Isoenzymes (Isozymes) : A type of intracellular enzymes

- they are different forms of the same enzyme
- they have the same charcteristics regarding the reaction they work on: متشابهين في كل شيء له علاقة بالتفاعل
  - A) Catalyze the same reaction
  - B) Act on the same substrate
  - C) Give the same product
- But they differ in some other charcteristics
  - A) Differ in their origins
  - B) Differ in the affect of activators and inhibitors
  - C) Differ in some of the physical properties such as: electrophoresis mobility and molecular weight

Examples of Isozymes : we have 2 examples

1. Lactate dehydrogenase isozyme (LDH) (Interconverts lactate and pyruvate)
   - Is tetrameric molecule → consist of 4 polypeptide chains → Classified into 2 different types of polypeptides:
     - M : found in muscles ; optimized to work under anaerobic conditions
     - H : found in heart ; optimized to works under aerobic conditions
   - There are 5 different isozymes:

Homotetramer	LDH_1 (H_4)	→ Cardiac + kidney
Heterotetramers	LDH_2 (H_3M)	→ Cardiac + kidney + Brain + RBCs
	LDH_3 (H_2M_2)	→ Brain + lung + WBCs → Used in the diagnosis of leukemia
	LDH_4 (HM_3)	→ lung + Skeletal muscles
Homotetrameric	LDH_5 (M_4)	→ liver + Skeletal muscles

2. Creatine enzyme (CK) / Creatine phosphokinase (CPK) Isozymes
   - Is a dimeric molecule → composed of 2 protomer
     - M : muscles B : Brain → M electrophorectic mobility < B electrophoretic mobility
   - There are 3 different isomers
     1) BB (CK_1) : Found in brain → Can't be used in diagnosis of deases
        - the lightest → maximum mobility
        - the lowest % in the blood ⇒ 0-1 %
     2) MB (CK_2) : found in heart muscles → is used to diagnose M.I
        - Medium size (weight) → intermediate electrophoretic mobility
        - low % in the blood ⇒ 0-3 %

3) MM (CK_3) : found in skelatal / Heart muscles
- the heaviest → least electrophoretic mobility
- the highest % in the blood ⇒ 97 - 100 %

- These isoenzymes can be separated by :
1) Electrophoresis 2) Ion exchange chromatography

Note : Biomarkers (Diagnostic markers) should be characterized by :

① High sensitivity : with least duration after occurance of disease the concentration of bio marker should increase four-five times the normal concentration.

② High specificity : Released from one organ

E.g. LH_5 : Highest specificity to diagnose liver diseases
CK-MB : Highest specificity to diagnose MI

* اخر سلايد (enzyme kinetics) ما حكاكنها الدكتور ولا شرحها و هي مش فيها شي ← قراءة فقط

Lecture 2 : Factors affecting enzyme activity

There are 4 factors affecting enzyme activity:

1. Temperature } both of these factors affect the protein structure (tertiary structure)
2. pH
3. Enzyme concentration } both depends on the ratio between enzyme molecules and substrate molecules (نسبة و تناسب)
4. Substrate concentration

Note : when we study one of the above factors we make sure that all other factors are Constant

Note 2 : we study these factors in test tube not under biological media

First : Temperature and pH (Hydrogen ion concentration)

- Every enzyme have an optimum tempreture and an optimum pH in which the enzyme is acting maximally
- Any change in the temp. or pH will reduce the activity of the enzyme thus if the change was much enough the enzyme will denaturate
- Denaturation: breaking the structure (3D struture) of the enzyme (protein) starting from the active site then the whole protein
- Denaturation: affect any protein portion of an enzyme
- From the graphs we can notice that increasing the temp. higher than the optimum destroy the enzyme faster than decreasing the temp. whereas in pH changing the pH in both ways result the same ~~destruction~~ decreasing