Biochemistry Important Notes

By, Girls Biochemistry Team

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Course/: 141 MBC
Topic: Enzymes (Lecture 1, 2, 3)

==== (Part 1) ===

Enzymes in General:

- Protein Catalysts.
- They are important because they increase the velocity of a reaction so that all the important metabolic happen.

Nomenclature:

- *Recommended Names :*

1. add the the suffix " -ase " to the Substrate. e.g. (Urea --> Ure**ase**)
2. add the suffix "-ase" to the Action that happen on the Substrate. e.g. (Lactate Dehydrogen**ase** <-- It is the action that happened.)
3. Old names they've got used to name it like this. e.g. (Pepsin, Trypsin)

- *Systematic Name:*

Divide the enzymes to 6 functional classes. We put the "-ase" after all the description.

The functional classes:

1. Oxidoreductase :

For example : "D-glyceraldehyde 3-phosphate: NAD+ oxidoreductase " The word underlined means that Oxidation and Reduction reaction happened here.

2. Transferases:

These enzymes catalyze the transfer of C, N, or P- containing groups from one molecule (donor) to acceptor.

Transferases is the class but Transaminases is one of the types of the class. These catalyze the transfer of an amino group to an α-Keto.

The α-Keto Acid -----> α-amino Acid.. Here the α-Amino Acid gives AA to the α-keto acid and come out -1 AA. e.g. : Glutamate-Asperatate Transmin**ase** (GOT) AST.

3.Hydrolases:

It is oxidation by H_2O. One of it's type is Lipase :

(3fatty Acid attached to glycride) --> Triglycerides + H_2O ---> 2Fatty Acid + Monoacid glyceride.

4.Lyases:

It break the bonds without Oxidation, Reduction, nor Hydrolases. and they are called desmolases.

5.Isomerases:

Change its appearance. It changes from D ----> L.

6.Ligases:

Attach or (أخيط) with need for ATP.

Properties of Enzymes:

- It is a biological catalyst.
- It support and increase the velocity of a reaction.
- Enzymes are formed from Protein. Except RNA which sometime act as an enzyme it called Ribozymes.
- Enzymes increase chemical reaction without being consumed.

Organic Enzymes	**Inorganic Enzymes**
High Efficiency and it made from protein.	It is not a protein.
Temperature + PH + Time, all have an influence on enzymes.	PH + Temperature + Time don't affect it.
More specific	Less specific.
A denaturation may happen to it after a period of time.	Denaturation doesn't happen to it.

- Active site: Are a pocket like structure that make a 3-D structure. A specific substrate attach to it. So that way it is a complementary to the substrate.
- Enzymes are highly specific. It catalyzes only one reaction.
- The catalytic efficiency: 100 -1000 substrate molecules.
- The Turnover number: is the number of substrate molecules converted to product/ enzymes molecules/ sec.

- Division of Enzymes according to substrate... :
    1. Simple: Made from Protein Only.

2. Complex: Made from a protein part and non-protein part. The Non-protein part is named as (Holoenzymes) and the protein part is named as (apoenzyme)

- Division of co-factore in non-protein part "holoenzyme".
  1. If it is Organic Co-factor, It is termed as Co-enzyme.
  2. Non-organic Co-factor.
- The Co-enzyme that is transiently associate with enzyme is called co-substrates.
- The Nucleotides: is a " Nitrogen Base --attach to --> Sugar --attach to--> Phosphate ".
- The Co-enzyme is often come from Vitamins.
- The Prosthetic Group: small organic molecule bond to apoenzyme by covalent bond.
- <u>The Regulation of Enzymes:</u>

Rate of product formation responds to the needs of cells.

- **<u>Enzymes Location Within Cell:</u>**
  - 1. Mitochondria: responsible of oxidation ..

TCA Cycle.

Fatty Acid Oxidation

Decarboxylation.

- 2. Cytoso:

Glyolysis

HMP.

Fatty Acid Synthesis.

- Lysosomes:

Degradation of macromolecules.

- Nucleus: DNA Synthesis.

==== (Part 2) ===

<u>Factor Effecting Reaction velocity,</u>

- Reactant must be converted to a product; it will take a long time to happen if the enzymes weren't there.
- To convert the reactant to products, the reactant must first go to an intermediate level before it converted; the problem here is that the intermediate energy is higher than the product's.
- Different enzymes show different response to the factor's affecting them such as (Substrate concentration, Temperature, PH).

- Substrate Concentration. (S.C.)

Every time you increase the S.C. the Rate of Reaction increase.
Because every time S.C. increased the Active site will increase.
Until the Active site is finished.
This mean " The Enzyme Substrate Complex " is the one controlling the velocity. The Relationship between the " Enzyme " + " Substrate " is shown in the Hyperbolic Curve (Fig. 5.6 P.57)

- Temperature

1. It will give energy to the molecules so it can pass the energy barrier.
2. It will increase the collision which will give more energy.
3. It has a limit, after this limit there will be a denaturation of the enzymes because it is made of protein.
4. The optimum human temperature between 35 - 40. above that a denaturation will happen. But Thermophilic bacteria have optimum temperature of 70 degree.

- PH.

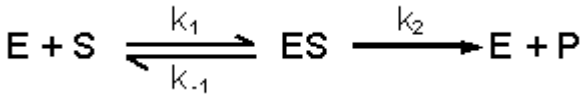
Enzymes are affected by changes in pH. The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH.
Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.
Because active site has functional group that has charges, the active site and the substrate must have the same charge.
The PH optimum varies from different enzymes.
Pepsin PH= 2
Amylase PH = 7
Alkaline phosphatase PH= 9t
Extremes of pH can lead to denaturation of the enzyme.

- Enzyme Concentration

==== (Part 3) ===

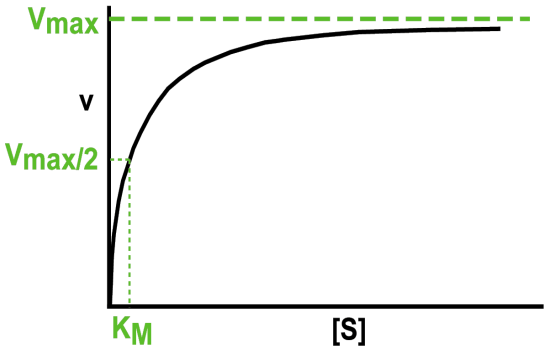
Michaelis-Menten Equation

It is a relationship between the enzyme and it's substrate.



E= Enzyme , S= Substrate, ES= Enzyme Substrate Complex, K1, K-1, K2 = rate constant.

On this graph we can label the V_{max}, and
the $V_{max/2}$.
By using the $V_{max/2}$ we are able to determine
the value of Km.



Michaelis-Menten Equation:

$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$

It represents the effect of substrate concentration on the reaction rate of the enzyme.

V_o= Initial reaction velocity.
Vmax= Maximal Velocity.
Km= Michaelis Constant = (K_{-1} + K_2) / K_1
[S]= Substrate concentration.

The Steady State assumplation:

The Rate of synthesis of ES complex = The rate of it's degradation.
(يتكون -يتكسر-يتكون-يتكسر .. وهكذا)

The Initial Velocity :

It is used in the analysis of enzyme reactions. We can measure the rate of reaction as soon as the enzyme is mixed with its substrate. In this state the concentration of the product is very small so it can be ignored.

Km :

It is the substrate concentration at which the velocity of reaction =half of Vmax. Which mean half of Active sites are saturated.

If ...

Km (Low) = The Affinity between the S and E (Increase).

Km (High) = The Affinity between S and E (Decrease).

Order Of Reaction :

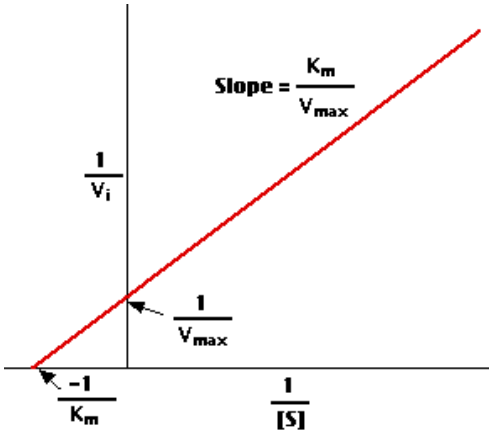
The First Order = When [S] is less than Km.

The Zero Order = When [S] is greater than Km.

==== (Part 4) ===

Lineweaver-Burk Plot:

In the Michaelis-Menten Equation the Diagram is a Hyperbolic Curve, and because of this you can never tell if you reached the Vmax or not. To be able to know when we reach the Vmax we can change the diagram to a straight line.



So the equation become :

$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max}[S]} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}$$

Here ..

1/ Vmax= كل ما يطلع على فوق يقل لانه مقلوب وكل ما نزل على تحت يزيد

1-/Km = كل ما أقترب من الصفر يزيد وكل ما بعد يقل

==== (Part 5) ===

Inhibition of Enzyme Activity

It is a substance that will change the action of Enzymes.

- If we increase the bond between the Inhibitor and the enzyme it becomes a Covalent Bond. The substrate wont be able to breakdown this bond so it wont bind on the enzyme (it wont work) ... Here we call it "Irreversible "
- If we decrease the bond between the Inhibitor and the enzyme the bond here is not Covalent. The substrate will be able to breakdown the bond and then work with the enzyme normally... Here we call it " Reversible "

Competitive Inhibitor :

Here there is a competition between the Substrate and the Inhibitor on the active site.. Why??

Because the Inhibitor has a Structure Analog that make the active site unable to determine if this is an Inhibitor or a Substrate.

The Stain Drugs as examples of Competitive Inhibitors:

The Reaction of Cholesterol Synthesis is catalyzed by *hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase).*

The Stain drug atrovastatin (Lipitor), Simvastain (Zocor) are structure analogs to HMG-CoA reductase.

They inhibit de novo (anew) cholesterol synthesis. Thats why they are used to lower the cholesterol level.

Non-Competitive Inhibitor :

Here there isn't any competition between the Substrate and the Inhibitor. Both of them attach to the active site.

It is either :

Enzyme (E) + Inhibitor (I) ----> EI ... and then the S come and attach with the Inhibitor on the active site.

Or

E + S ----> ES and then I will come and attach with the S on the active site.

Competitive	**Non Competitive**
Here Vmax is constant.	Vmax Decrease
Km Increase because S increased to breakdown the bond between I and E.	Km Constant.
The reaction Reversible	Some of them Irreversible

==== (Part 6) ===

Regulation of Enzymes Activity

By:

1. Substrate Availability
2. Allosteric Binding Sites.
3. Regulation of enzymes by covalent modification.
4. Induction and repression of enzyme synthesis.

The regulation maybe :

Short Term Or Long Term ..

Short Term:

Allosteric Binding Sites + Covalent modification

Long Term:

Synthesis of enzymes.

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1. Allosteric Binding Sites :

Occupy another space, other than the substrate.

They are enzymes that it's metabolic change by Allosteric modifiers.

Usually contains multiple sub-units.

It is usually found in metabolic pathway.

It is regulated by molecules called effectors or modifiers. And they are the substrate that binds on the Allosteric. They attached to it with non-covalently which mean

Reversible.

It's function:

1. Change the affinity of the enzyme for its substrate.
2. Modify the maximal catalytic activity of enzyme.

Or do both of them in the same time.

BUT doesn't change the enzyme specificity .. (يعني ما يشتغل اليوم على شي وبكره على شي ثاني)

fig. 5.16.. A) catalytic activity يشتغل على ال + Vmax التغير في ال

B) Affinity يشتغل على . +Km فيها تغير في ال

Division of Allosteric Binding Sites:

1. Homotropic effector: They are molecules that are the same type of substrate, act as the effector, but they are not the substrate itself. Usually it's (+).
2. Heterotropic effector: They are molecules different than the substrate. Sometime they are (+) and other time they are (-) depending of the substrate. Feedback Inhibition is an example for it. (look at fig. 5.17)

e.g. If the cell was synthesize fatty acid, if there was no inhibitor, he cell will continue the synthesize without a stop. But if there was inhibitor (feedback inhibitor) it will stop it from synthesing something that it isn't useful.

2. Regulation of enzymes by covalent modification:

Change in action of the enzyme by making the enzyme attach to a phosphate group.

Phosphorylation and dephosphorelation :

The enzyme either take the phosphate group from the ATP. Or reattach it to make the enzyme back.

The attachment or reattachment of a phosphate group depends on whether the enzyme is active or inactive.

The enzymes that help building are mostly in the dephosphorelation form.

The enzymes that break are mostly active in the phosphorelation form.

3. Induction and repression of enzyme synthesis.

Change in the amount of enzyme leads to change in the amount of active sites.

* Enzyme Repression= Decreasing the amount of enzyme.
* Enzyme Induction= Increase the amount of enzyme.

The enzyme in the plasma should be in the inactive condition until stimulation come along and activate it.

e.g. The digestion enzymes in the stomach are in the inactive condition until the food come and activate it. If it was active before the food comes it would digest the stomach membrane.

fig. 5.19 is not that clear for a better understanding of Regulation take a look at fig. 24.1 at P. 321.

==== (Part 7) ===

Enzymes In clinical Diagnosis:

Enzymes In Plasma :

* Functional = They are found in the plasma and their substrate also found in plasma. They are released into the plasma in an inactive condition until stimulation comes and activate it. Most of the functional are Zymogens.

*Nonfunctional= They are found inside the cells. But they leak to the plasma because of the cell turnover which mean .. (كل ما ماتت الخليه تطلع اللي بقلبها اللي هو الأنزيمز للبلازما) .. They don't have substrates in the plasma. and you can find a large amount of them in the plasma. Also, they have no physiologic use in plasma.

Plasma	Serum
Physiological fluid. It is inside the body. To differentiate Between them : 1. Put it in a test tube. 2. Add an anticoagulant. 3. Add other blood cells to it like RBC. 4. If they are mixed then it mean that it is plasma. 5. After this test you can find coagulation factor in the fluid.	It is prepared in the laboratory. It works in lab. To differentiate Between them : 1. Put in a test tube. 2. Don't add anticoagulant. 3. add other blood cells such as RBC. 4. They will not mix. and you can see the RBC separate from the serum. 5. After the test you will not find any coagulation factors n the fluid.

Alteration of plasma enzyme levels in diseases states:

Diseases are caused by increase release of intracellular enzymes into the plasma.

The diagnosis of disease for heart, liver, skeletal muscle and other tissues are determined by the activity of its enzymes.

Plasma enzymes as diagnostic tools:

Specific enzymes= If we knew each enzyme and what tissue it come from it help a lot in diagnosis. For example ALT enzyme is secreted from liver. So, if we found a high level of this enzyme in plasma, this is signal of a possible damage in hepatic tissue.

Non specific enzymes= Some enzyme are not specific for only one tissue some of them are secreted from more than one tissue. For example AST enzyme is secreted from liver, red blood cells, cardiac muscle, skeletal muscle, and Kidney. So if we see an increase level of this enzyme in the plasma it is hard to determine which tissue is the damaged one. So the lack of tissue specificity limits the diagnostic value of many plasma enzymes.

Isoenzymes and diseases of the hear:

They are enzymes that catalyze the same reaction. But they don't have the same physical properties because of the differences in the amino acid sequence.

Their level in the plasma may determine the presence of a disease of damage. For example, the plasma level of creatine kinase (CK) are commonly determine the myocardial infarction.

- Quaternary structure of isoenzymes,

fig. 5.21:

CK isoenzymes are:

CK1 = BB, CK2= MB, CK3= MM.

In electronic field,

BB = more (–) and attracted to the Anode (+)

MB= Midway between the + and the –

MM= more (+) and attracted to the Cathode (–) .

- Diagnosis of myocardial infarction,

The MB (CK2) it comes from skeletal muscle + heart muscle.. But in skeletal muscle it is less than 5% while in the heart muscle it is more than 15%. So, when you see a high level of this in the plasma that means it came from the heart muscle. Which mean that there is a myocardial infarction. It appears after 4-8 hrs. and reach the peak after 24 hrs. It returns to the baseline after 48-72 days.

- Newest markers for myocardial infarction.

Troponin I and Troponin T: (TnI) (TnT)

Are proteins involved in myocardial contractility. They are released after a damged of the cardiac muscle appears.

Cardiac Troponin I: (cTnl)

Is highly specific for damaged to cardiac tissue. It appears in plasma within 4 -6 hrs. after myocardial infarction. It reach the peak at 8 -28 hrs. and remain elevated for 3 - 10 days.
Elevated serum troponin are more predictive of adverse outcome in unstable angina or myocardial infarction than CK2.

Heart Angina	Myocardial Infarction
Blood flowing to the heart only decrease.	Blood flowing to the heart stop. Especially from coronary artery.
The muscles of the heart only suffer from the decrease flowing of the blood to it.	Here the muscles die because the blood flow stopped.

---- Here are extra notes, they are not mentioned in the book, but Dr. Mona has explained them in the lecture.

- LDH (Lactate dehydrogenase)

It can be used as a marker of myocardial infarction. Following a myocardial infarction, levels of LDH peak at 3-4 days and remain elevated for up to 10 days. In this way, elevated levels of LDH can be useful for determining if a patient has had a myocardial infarction if they come to doctors several days after an episode of chest pain.

Before the use of CK2 they used to use LDH as the main marker for myocardial infarction. But know they use CK2 or cTnI. and that's because LDH is not specific for myocardial infarction, so we only use it just to make sure that the patient is going through myocardial infarction. Because some patient come after few days of the beginning of the infarction, and by now the level of CK2 has dropped, so we use LDH.

- Sometime the symptoms of myocardial infarction is Atypical, Instead of a pain in the chest there is going to be pain in the abdominal. Here the patient won't know that it is dangerous, so he/she might come late to the hospital (after 2-3 days). By then if you did a CK2 you'll find it normal. But if you did LDH you will see it very high, and you can save the patient .

ملاحظات :

النوت لا تغني عن الكتاب بشيء

هذي نسخه محسنه من النوت .. الأولى أستعجلت فيها وكان فيها كم خطأ مطبعي بس .. بس بهذي النسخه عدلتها

. للبنات : بليز بنات أنتبهوا في كم معلومه بصححها في النوت القديم

أولا في نوت التتوريال في ثاني صفحه أول سؤال بدال Polypeptide Bond

أكتبوا Polypeptide Chain

للأولاد والبنات

في ملاحظات الأنزيم لأول 3 محاضرات في,Part 5 / Competitive and non competitive

الجدول في أخر فقره لل.Reversible and Irreversible

أولا كل النوعين من أنواع الReversible Inhibitors

بس ال Non competitive في منه أنواع Irreversible

... يعني موب كله بس بعضه

وفي كمان في هذي النوت الجديده أضفت كم شي وتوضيح لأول صفحه لـ

Transferases و Lyases

ياليت تنتبهون لها انا لونتها بأحمر عشان تنتبهوا اني غيرت

أخر شي هذي نوت مسونها طالبات يعني أحتمال فيها أخطاء .. فلو شكيتوا في شيء

أعتمدوا على اللي موجود في الكتاب والكتاب فقط ..

حاولنا قدر المستطاع ان نتجنب الاخطاء او المعلومات المتداخله والمختلفه

ان شاء الله تستفيدون

Good Luck in the Quiz.

Girls Biochemistry Team