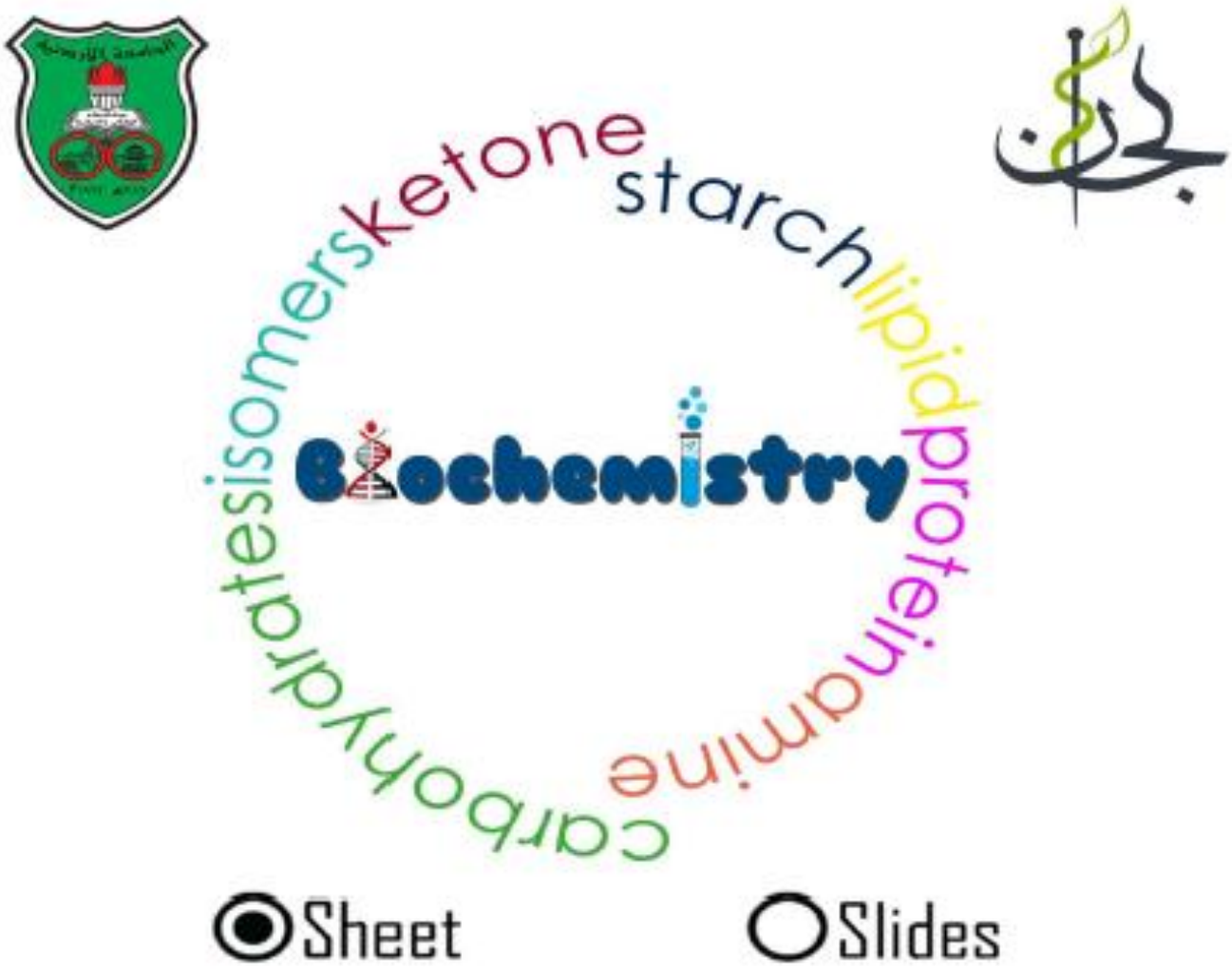

Subject :	**Kinetics**
Done By :	**Hanin Saleh**
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Number :	15

We are going to continue with what we talked about in the previous lecture which is kinetics of enzyme catalytic reactions.

*remember that the **rate** of anything is basically **change over time.**

So when we talk about rate in enzymatically catalyzed reactions the thing that is changing is the **concentration** **(**reactants are decreasing in concentration and products are increasing in concentration.)

So rates are changing according to concentration.

-The Rate of any enzymatically catalyzed reaction will be equal to the concentration of the product or reactant multiplied by a factor called rate constant (how the rate is changing)

We can replace the constant amount of change by a factor(K)

So when we want to measure the velocity of an enzymatic reaction it will be equal to the constant(K) multiplied by the concentration of that reaction.

But what concentration do we use to calculate (products or reactants)?

-Actually both of them can be used, you cannot have change in concentration on one side and lesser change on the other side (المادة لا تفنى ولا تستحدث) … so rate of change in products is **always** equal to rate of change in reactants

-the velocity is changing according to the concentration by a constant

In enzymatically catalyzed reactions there's something called ***order of reaction***

What is that?

-It is describing how the product is being released compared to the material that is being consumed.

If the production of one mole of a product needs one mole of a reactant we call that **first order reaction (**1 mole of A gives us 1 mole of product B**)**

But if we need for example one mole of A and one mole of B to produce one mole of C we call that **second order reaction**

1A+1B=1C or 2A=1B (both are second order reactions)

And if we need 3 moles we call it third order and so on ..

As we said in first order we need one reactant to make one product, So when we calculating the rate we have the concentration of one reactant so it is easy

but in the case of second order and third order, how can we calculate the rate of reaction ?

If we have more than one reactant what we do is we change the concentration of one reactant only and the others will be fixed . then mathematically we can know what is the rate .

So, if the product depends on many reactants , we increase the reactants concentration to very huge values except for one reactant .so, the last reactant will control the reaction . (although the reaction is second order , only one reactant controls the rate of reaction). by this way , all reactions become what we call "pseudo first order reactions"

(الدكتور ما حكا اشي زيادة عنها بس للي بده يفهم ع اليوتيوب بتكون مشروحة رياضيات بالتفاضل والتكامل وكيف بكون فعلا التأثير من مادة متفاعلة واحدة) للعلم فقط

NOW, as we know when we increase the concentration the velocity increases and that makes sense

But there's something we call **zero order reaction** where the production of products **does not** depend on the reactants .

That statement only makes sense in one state and that is when we have very high concentration of reactant.

When you put an enzyme and its reactant in a solution, there will be a movement of them and therefor binding . increasing the concentration of the reactants will increase the chances of binding, the velocity will increase, the product production will too ..the more you increase concentration the more you will increase the probabilities of collision until you reach a point where the increase will be negligible, it won't affect the product production and that's what's called **zero order reaction.**

So the conclusion : enzymes start as **first order reaction** where increase of reactants will increase products formation but end as **zero order reaction** where we reached a very high amount of concentration that the reactants do not affect products formation any more (saturation).

NOTE: this applies for all simple enzymes.

Question :

You have a reaction A+B……C and V=K [B], the true about this reaction is:

A. the reaction is independent on [C]

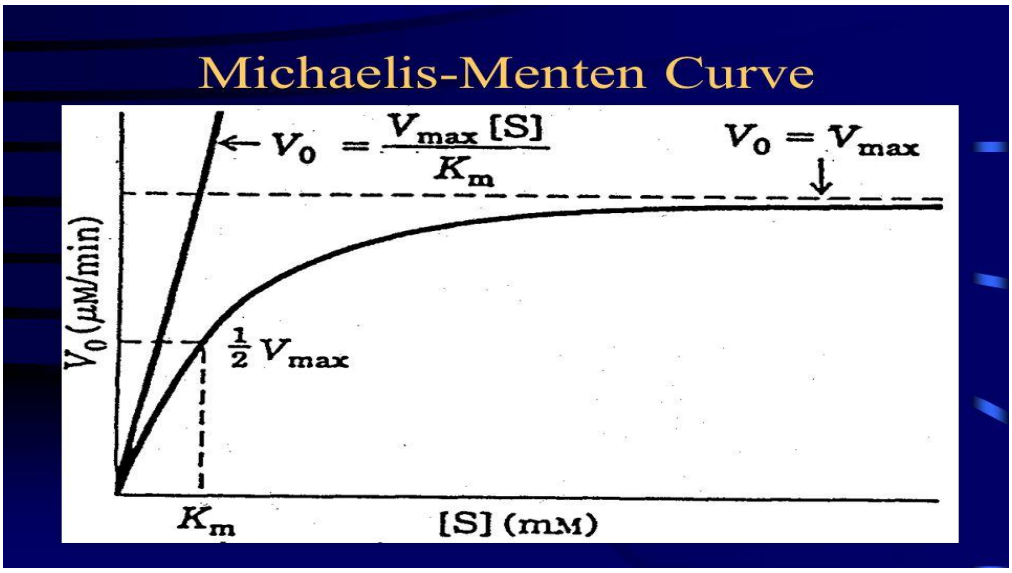
B. the unit of K is (time)-1

C. the reaction is independent on B

d. non of the above

(the answer is B)

If we have a certain concentration of enzymes that have a certain number of active sites and in one second they can take for example 10 substrates and change them into products ..that is the enzymes capacity, it cannot take any more than that and that is what we call maximum velocity, every enzyme has a **Vmax** that it cannot extend unless we increase the concentration of the enzyme.



Since we have a certain saturation point we will have a hyperbolic plot, a certain increase then a platue (Vmax).

And since all enzymes as we said have a Vmax this means we will always end with the same plot.

So two scientists called Michaelis and Menten derived a mathematical equation from the constant plot.

$$V_0 = \frac{V_{\max}[S]}{(K_M + [S])}$$

From this equation, the velocity of the enzyme depends on substrate concentration and Vmax.

-What does Km mean in the equation?

When an enzyme binds to a substrate it gives us a enzyme substrate complex then breaks down to product and the enzyme .

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

For this reaction , Km is :

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

In other words , km is govern the rate of degradation over the rate of formation.

Km describes the **affinity** (how they are binding and breaking)

Low km : high affinity

High km : low affinity

It gives us an indication But doesn't exactly give us a real measure of affinity because it also measures the product formation (K2) and in order to measure the **real** affinity we only need to measure the binding and separating of the enzyme and substrate.

That's why there's something else called **Kd** (dissociation constant**)** which is a real measure for affinity which equals rate of dissosciation from substrate/rate of formation .

-Kd >> measure for affinity

-Km >> indicator for affinity

Okay then since we have Kd which is more accurate why are we using Km ?

-Basically km easier to measure and use less effort and time.

Now at the beginning of the reaction the concentration is very small so the equation is **V=Vmax*S/km** and this is a ***linear*** equation which means and as we said before that at the

beginning of the equation the enzyme goes through **first order reaction**.

But at the end of the reaction the substrate concentration is much bigger compared to the value of Km so we can omit it from the equation so we are left with **V=Vmax** (zero order reaction).

So, michaelis menten equation fits with the hyperbolic plot

-let's talk about Km again a little

Now the unit of Km is **concentration** (of the substrate)

We talked about what happens when the substrate concentration is very low and when the substrate concentration is very high, but what happens if the substrate concentration equals Km?

Rearranging the equation we will find that when the concentration equals Km the velocity will be equal to half the Vmax

-when Km = S >>>>V=1/2Vmax

-So Another definition for Km is the substrate concentration needed to reach **half** the maximum velocity.

NOTE: please refer to the slides for further information (just this time)

What happens when Km value is high?

High Km Means I need high concentration of substrate to reach half the Vmax so>>rate of degradation is higher than the rate of formation therefore ***>>>low affinity***.

-High Km >>>>low affinity

-Low Km >>>>>high affinity

Question :

You have two enzymes, the first (1) acts on substrate A with Km =0.05 and Vmax= 100, the other (2) acts on substrate B with Km=0.1 and Vmax= 50, which of following is true:

A. enzyme 2 has catalytic activity higher than enzyme 1

B. substrate A binds faster than B

C. you can increase V max of enzyme 2 by increasing in [B]

D. affinity of B is higher than A E. None of the above

Answer...>b

Question :

In an experiment, a biochemist uses an enzyme with highly concentrated substrate (S= 10000 Km). After 5 minutes, 100 micromolar of the product were formed. In another experiment, he used 0.2 of the enzyme concentration that was used in the first experiment and the same substrate concentration. Expect the time needed to form the same amount of the product (100 micromolar).

(the answer is 25 minutes)

km values differ in an enzyme but Vmax is always the same

-How?

For example the enzyme glucoKinase adds phosphate group to glucose (transfers a phosphate group from ATP to glucose) ..now glucoKinase has two active sites, one for binding of Glucose the other to ATP .. so both of them will bind to the same enzyme and both of them will give the same product (glucose 6 phosphate + ADP) .

But ATP and Glucose are two different chemical structures so they will bind to the enzyme at different affinities but they will give the same product.

-Km >> affinity

-Vmax = amount of products per unit of time.

Common question :

If you have an enzyme that gives you 10 molecules per second and another one gives you 20, which one is more efficient?

-actually we cannot know because as we said before the Velocity and Vmax changes with change of concentration of enzyme.

So to know the efficiency of enzymes we have something called **Kcat** .

-the highest speed an enzyme can reach divided by its concentration.

$$k_{cat} = V_{max} / [E]_T$$

Km, Kcat and Vmax are values used to compare between enzymes.

Example:

You are working on the enzyme “Medicine” which has a molecular weight of 50,000 g/mol. You have used 10 µg of the enzyme in an experiment and the results show that the enzyme at best converts 9.6 µmol of the substrate per min at 25˚C. calculate The turnover number (kcat) for the enzyme.

(the answer is 800)

But the problem is scientists noticed that values of Km vary in a very wide range between enzymes so the comparison is useless (it's like comparing 1 with a million)

So they came up with another solution which is dividing Km by Kcat (Kcat/Km) and used it as a **specificity constant**.

Specificity constant is indicative for :

1) **Enzyme's substrate specificity: the higher the ratio, the higher the specificity.**
2) **Enzyme's catalytic efficiency: the higher the ratio, the more efficient the enzyme.**

Enzyme activity :

If we want to calculate the **activity** of an enzyme we divide the number of moles by the volume.

enzyme activity = rate of reaction ×reaction volume

Another term used in enzymes is **specific activity** which is basically enzyme activity divided by the **quantity** of the enzyme (Grams) and it is useful to measure enzyme purity after purification.

Specific activity= enzyme activity/mass of enzyme

-Also another term is **turn over number (Kcat)** which expressed how much we can catalyze reactions per unit of time per enzyme concentration.

Kcat=specific activity x molecular weight of enzyme

Remember: $k_{cat} = V_{max} / [E]_T$

Sample calculations:

A solution contains initially 25.0×10^{-4} **mol** L^{-1} of peptide substrate and **1.50 µg chymotrypsin**, in **2.5 mL** volume. **After 10 minutes,** 18.6×10^{-4} **mol** L^{-1} of peptide substrate remain. Molar mass of chymotrypsin is **25,000 g** mol^{-1}.

peptide substrate consumed	$= 6.4 \times 10^{-4}$ mol L^{-1} in 10 minutes
Rate of reaction	$= 6.4 \times 10^{-5}$ **mol** L^{-1} **min**$^{-1}$
Enzyme activity (rate × volume)	$= 6.4 \times 10^{-5}$ mol L^{-1} $min^{-1} \times 2.5 \times 10^{-3}$ L $= 1.6 \times 10^{-7}$ **mol** min^{-1}
Specific activity (activity / mass)	$= 1.6 \times 10^{-7}$ mol min^{-1} / 1.50 µg $= 1.1 \times 10^{-7}$ **mol** μg^{-1} **min**$^{-1}$
Turnover number (sp. act. × molar mass)	$= 1.1 \times 10^{-7}$ mol μg^{-1} $min^{-1} \times 25{,}000$ g $mol^{-1} \times 10^6$ µg g^{-1} $= 2.7 \times 10^3$ min^{-1} = **45** s^{-1}

THIS IS THE END