Lecture : 20 (5th After Mid)

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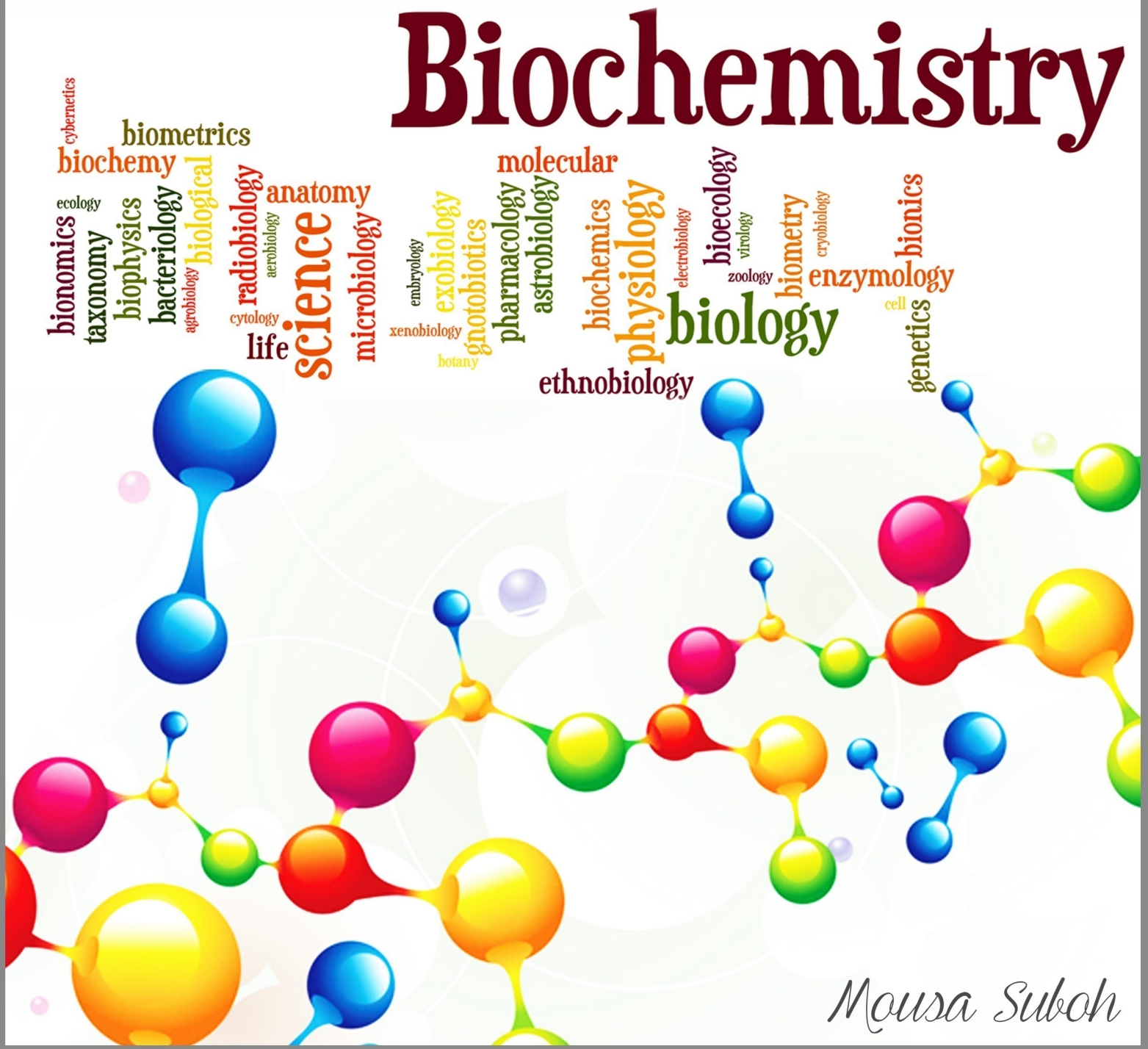
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

Biochemistry



Mousa Suboh

Kinetics of enzymatic reactions

My brilliant colleagues : hope you find this lecture easy and this sheet helpful. You should be focused while you studying this topic .

Kinetics:

Kinetics science is the science that studies **the rate** of chemical reactions.

- We don't talk about thermodynamics but kinetics here.

Rate: ((for anything in this world))

Rate is velocity

- Velocity of anything in this world: It's a measure of change of any material over a period time (distance or anything else)

- You have a RXN with a substrate A and a product P, what is the velocity of RXN???

Either the change in product formation over a time, ***or*** the change of substrate consumption over the time.

Is the change in product formation equal in value with the consumption of substrate?

- yes, why?

You have: $A + B \longrightarrow P$

Two materials form **one** product.

Is the change in A = change in B = change in P,in value or not?

The idea behind enzymatic RXNs is that when you have multistep RXN, when you have more than one material in RXN, **The reaction can't go faster than the slowest step.**

*** Illustrative example:**

If you have multiple cars each one have a capacity to go to a certain speed (one car has speed of 250 km/hour and other is 150 km/hour ..etc) and they are linked with each other by rope>

Let them go , their speed can't go faster than the slowest car (because they are linked togother) same story for the enzymatic RXN

<u>Any RXN rate can't go faster than the slowest step in that RXN.</u>

*** Change in product formation = Change in substrate consumption**
Multiple reactants are **equal** to each other

Change is not simple mathematics. It needs integrals and derivatives. The change is the area under the curve.

- **For the reaction (A + B → P), the rate law is**

$$\text{Rate} = \frac{-\Delta[\text{A}]}{\Delta t} = \frac{-\Delta[\text{B}]}{\Delta t} = \frac{\Delta[\text{P}]}{\Delta t} \qquad v = \frac{-d[\text{A}]}{dt} = k[\text{A}]$$

In enzymatic RXN : we can replace the change by a rate constant.

- So far any mathematical calculations of enzymatic RXNs:

((V = [A]*K))

V :- The velocity or the rate of enzymatic catalyzed RXN

[A] :- concentration of the substrate been consumed

K :- The rate constant for that material. (<u>K never changes for a certain RXN</u>)

- There are some enzyme catalyzed reactions that don't depend on any substrate concentration, we call this reactions : **Zero order reactions**

The order: means if the velocity of RXNs depends on the substrate or not ? and if it does, in which order? It's multiplied in which magnitude?

- ***There's a RXN:-*** 1 mole of reactant is converted to 1 mole of product & the velocity of the RXN is dependent on the reactant ,we call it : ***first order reaction.***
- If 2 moles of the substrate are needed to make 1 mole of product then this RXN is a ***2nd order reaction***.

If there's 2 materials each of them in 1 mole :

1 mole of A + 1 mole of B ⟶ 1 mole of product.

- ***So it's 2nd order RXN***.

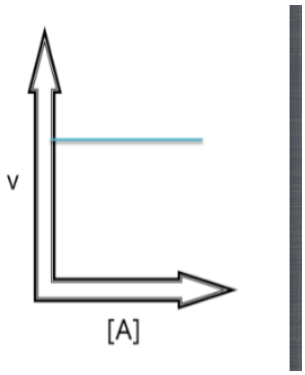
- If there's 3 materials or 3 moles, the cycle continues we called it 3rd or 4th & so on.

- Note: Zero order reactions are reactions don't depend on any substrate concentrations. There are **no** RXNs in nature don't depend on substrate concentration (unless special case we well talk about later)

If you want plot a velocity against substrate concentration:-

- ((Zero order kinetics))

- So the plot is horizontal line in certain velocity which doesn't change over substrate concentration.

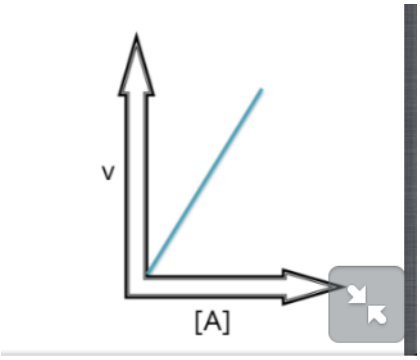


- **1st order RXN**

If you plot the 1st order RXN where velocity depends on single substrate then the velocity will be linear relationship

The linear relationship is represented like the:

V= k * [A] …… A: the material



A is the x axis . The **V** is y axis & the slope of the line is the **K**

Doctor said that:

He mentioned the zero order & the 1st order to let us understand how enzymes work.

Enzymes that have the 2nd , 3rd & 4th order RXN are enzymes that depend on more than one materials for their RXN(for their rate) how to solve them?

- The same with of mathematics equations:-

How to solve equation with more than one variable ?

You should write more than one equations

2 variables → 2 equations

3 variables → 3 equations

Then solve it by addition and subtraction equations till you end up with one variable, so you can solve the equation and find the other variables>

Same way here.

If the RXN velocity depends on more than one material, you have more than 1 variable that controls RXN speed, So how to solve these equations?

You can only deal with one variable and if you remove one of the reactants, then the RXN won't go

To know how much the velocity: you have to increase the concentration of one of the reactants in excess then you will neglect its effect & you will deal only with one concentration.

WHAT is the dimension of K?

Overall order	V=	Dimentions of k
Zero	k	$(conc.)(time)^{-1}$
First	$k(A)$	$(time)^{-1}$

- In zero order RXNs → $V= k[A]^o$ …. because it's independent on the material concentration so → V= k …. & the dimension of k will be the same of velocity which is → ((conc. * $time^{-1}$))

- 1st order reaction → $V= k[S]^1$…… & Dimension of k will be → $(time)^{-1}$

time either by minute, per second or whatever you want.

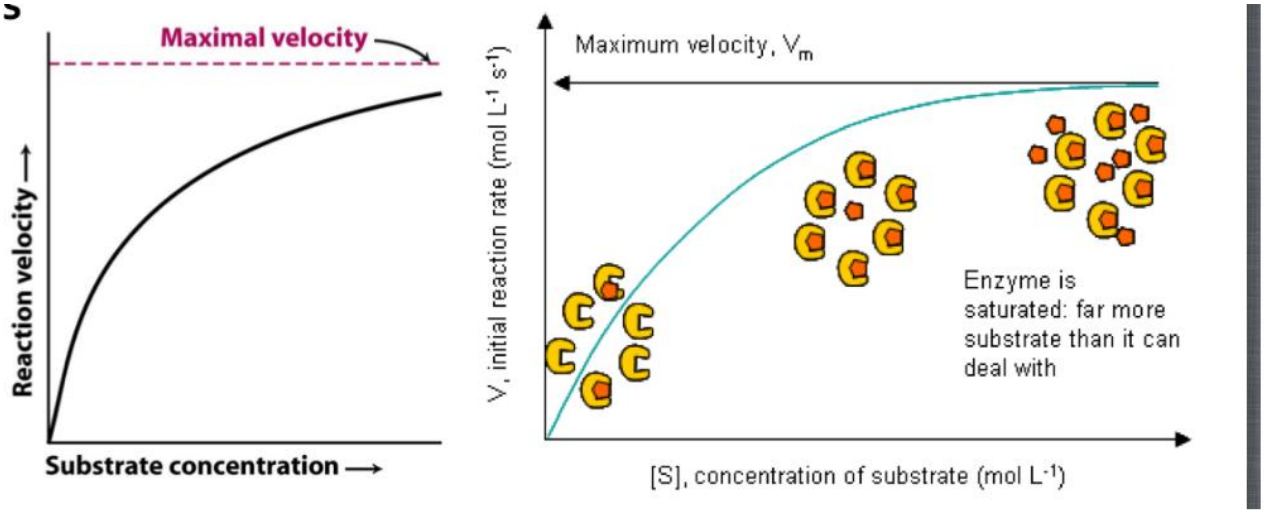
How do enzymes work?

When you have substrate concentration & enzymes with their active sites, you start increasing the substrate concentration. What happen? How enzyme will behave?

-:enzymes according to their behavior, can be classified into 2 types -

*(It can be classified as simple and complex (allosteric)

remember that we have classified the enzymes before as simple and :complex*(conjugated) according to the structure ***Simple enzymes***



What does this plot mean?

When we increase the substrate concentration the velocity will be increased in first order manner. (so the relation will be linear)

However that doesn't continue to happen or to occur, why? What will change?

Because we are dealing with substrates and active sites, when all the active sites are saturated with their substrates then full saturation is there.

Any increase in substrates concentration won't affect the velocity. So we will start the RXN in simple enzyme like 1st order kinetics and end up by Zero

order kinetics (here where we have zero order RXN). Where any increase in substrate concentration doesn't affect the rate of the RXN.

- This plot is called **Hyperbolic** plot – hyperbolic means in Arabic يتسم بالغلو- this plot is called hyperbolic because at the beginning the increase will be very sharp then will start to plateau after a while.

When all the active sites are saturated by substances, can the enzyme increase its velocity? NO, so we maintain this velocity as the **Maximum velocity (Vmax).**

Maximum velocity (Vmax) : the amount of reactants consumed or products formed per unit of time at a **stable enzyme concentration**.

When increasing substrate concentration the rate will be increased until we reach a limit which is a full saturation, How to know a specific velocity for a specific substrate concentration?

If someone tells you that at the [S]=10mμ . how much will be the rate of RXN? Can you answer that? Is there a mathematical equation to solve this issue?

The Michaclis - Menten equation:-

At a specific substrate concentration there's specific velocity & the mathematical equation is :

$$V = (V_{max} * [S])/km+[S]$$

Km → Michaclis-Menten constant

Michaclis & Menten : they have proposed the constant for the RXN km

So Vmax is known & Km is known so if you were given the [S] you can solve the equation.

Michaclis & Menten study the **behavior of simple enzymes:**

For any enzymatic RXN an enzyme **E** will be combined to its substrate **S** will result in enzyme-substrate complex **ES** (transition state) then the product **P** will be released & the enzyme will back to its original state.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

The rate of formation of enzyme-substrate complex is K_1.

The rate of degradation of enzyme-substrate complex is K_{-1}.

The rate of formation of product is K_2.

The rate of degradation of product is K_{-2}.

Note: you find it in some text book as k_1, k_2, k_3 & k_4. Dr. Nayef also gives it like this way → It doesn't make a difference.

As the Dr said: the idea of have them as minus (K_{-1}, K_{-2}) is to show the reverse RXN.

The 1st assumption → Michaclis and Menten have assumed that this RXN is irreversible: once the product is formed it can't go back to form transitional state

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

However once the product form there's no reversible RXN. So we ended up by 3 terms: K_1,K_{-1} & K_2. The velocity of the RXN is determined **by K_2.**

- How much ES is giving you a product P!!

$V = K_2 * [ES]$

Simple as A gives P

V = [A] * K … that's the same situation where the velocity of the whole RXN will be:

$V = K_2 * [S]$

The 2nd assumption:-

The enzyme-substrate complex is in **steady state** that's why we called **Michaclis - Menten** equation steady state. **What does that mean?**

The complex formation (the transitional state formation) is in steady state which means → the rate of formation of transitional state **equal**s the rate of breakdown of transitional state ES

(It's in steady state, there's no change)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

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

$$v = k_2\, ES$$

$$\frac{d\,ES}{dt} = k_1\, E\,.\,S - k_{-1}\, ES - k_2\, ES$$

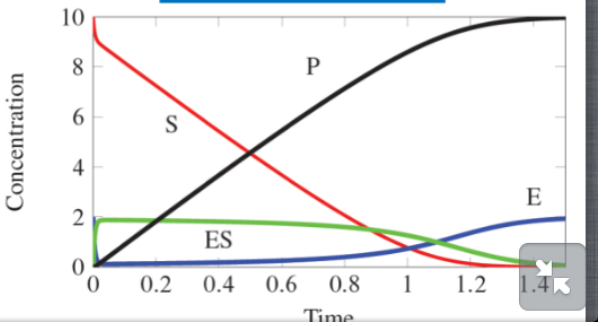
$$0 = k_1\, E\,.\,S - k_{-1}\, ES - k_2\, ES$$

$$E_t = E + ES$$

$$ES = \frac{E_t\,.\,S}{(k_{-1} + k_2)/k_1 + S}$$

$$v = \frac{E_t\, k_2\, S}{(k_{-1} + k_2)/k_1 + S}$$

$$v = \frac{Vmax\, S}{K_m + S}$$



Again: the rate of formation of transitional state **equal** the rate of breakdown of transitional state .

which means that the change in conc. Of transitional state over the time EQUALS zero (it's in steady state→ no change)

Δ[ES]/TIME = ZERO

the rate of formation of ES **equal** the rate of breakdown of ES.

- rate of formation of ES = k_1 [E] [S].

- rate of breakdown of ES = K_{-1} [ES] $+K_2$[ES]

So this is how the equation:

- Rate of formation – rate of breakdown = 0
- K_1 [E] [S] – K_{-1}[ES] – K_2[ES]=0

While you are deriving the equation you will end up

- $(k_2 + k_{-1})/k1$= (E . S)/ES

All them are rate constant, Can you replace rate constants by one constant? YES

- **Michaclis - Menten** have proposed one rate constant to replac (K2+ K-1)/K1 And call it the **Mechalices constant ((Km))** which is included in the equation.

The enzyme concentration.

Here in eq. we have [E], they said the enzyme concentration is in 2 state: one is free as an enzyme & the other is bound to the substrate. So:-

Et = E + ES

Et → total enzyme conc. / E →free enzyme conc. / ES → bounded enzyme conc.

At the maximum velocity of the Enzyme, the total enzyme concentration will be [ES]. Because at the Vmax all active sites are occupied (There's no free enzyme) so:

Et = ES

As shown in the previous figure

That's why they replace the ES in eq. by Et

$V = K_2 [ES]$

$Vmax = K_2 [Et]$

-so at any point of the RXN if you want to know the velocity:

$V_o = (Vmax * [S]) / Km + [S]$
You will replace the [S] by what's given & you will result in the velocity that is required.

➢ **For a reaction:**

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

STEADY STATE APPROXIMATION

$$\frac{d[ES]}{dt} = k_1[E][S] - K_{-1}[ES] - K_2[ES] = 0 \text{ (approx.)}$$

$$\frac{[E][S]}{[ES]} = \frac{K_{-1} + K_2}{k_1} = K_M \quad \text{Equation 1}$$

Look at the figure above –try to study it well- :

$[E][S]/[ES] = (K_{-1}+K_2)/K_1 = K_m$

So what does K_m mean???

At any point there's a K_m for the enzyme. It represents the rate of breakdown (back to substrate) divided by by the formation rate

K_m describes **affinity** of that substrate toward the active site

- If the affinity is high so K_1 will be high and K_m will be low

Remember: K_1 is the rate of formation

So as K_m decrease the AFFINITY increase.

To understand that:
when increasing the energy value (activation energy)of any RXN the K will decrease (inverse relationship)

Again: K_m describes the affinity of the **substrate** toward the active site to some extent (This statement is not that precise)
To measure the affinity between the substrate and the active site (the rate of binding of the substrate S to the active site and the rate of breakdown of the complex (ES) to substrate again and the enzyme), It's not a logic in the term the rate of product formation K_2 (the product is a new state it has nothing to do with the substrate)

BUT $K_m = (K_{-1} + K_2)/K_1$
When the K_2 is very small compared to K_{-1} then we can neglect it

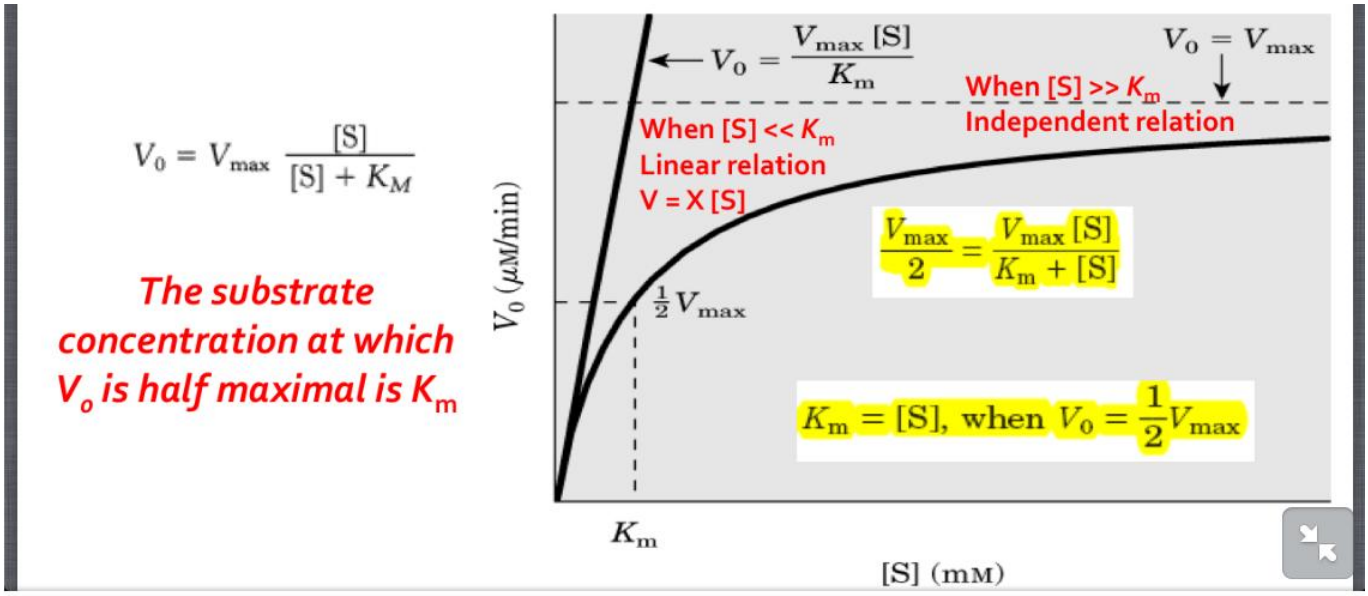
SO $K_m = K_{-1}/K_1 = K_d$

K_d → dissotiation constant

K_d → constant which measure the actual affinity of enzyme toward its substrate (we can calculate it

K_m → It infers affinity or measures it to some extent.(because it includes K_2 , it's not actual measure for affinity, (keep that in your mind))

So far we have talked about K_m and K_d



According to **Michaclis – Menten**, What we can understand from the figure above??

The **hyperbolic** plot of any enzyme indicates that :
when enzyme concentration increases, the velocity will be increase in a linear manner till it reaches a **limit** (this limit describes the full saturation of the active sites by the substrate)

When [s] > K_m(At very high substrate concentration we can neglect the K_m)

SO **V = Vmax**

As a result

K_m is the substrate concentration needed to reach 50% of Vmax

By calculating :

$V = ([S]. Vmax)/(K_m+[S])$

when $K_m = [S]$

$V = ([S]. Vmax)/(2[S])$

$V = (Vmax)/2$

IMPORTANT NOTES

- It's the [s] needed to achieve 0.5 (50%)Vmax
- K_m is a measure **of concentration** needed by the substrate to achieve the velocity. It's not a measure of velocity

SO: K_m is ameasure of concentration

- K_m is low → needs low amount of S to reach (0.5Vmax)→ means very high affinity of substrate toward the active sites.
  (with little [s], very tight binding, RXN will be fast)

- K_m is high → needs large amount of S to make the collisions very high because the affinity is not high

For reactions that involve more than one substrate:

We have talked in previous lectures about **Glucokinase** ((it's not simple enzyme but Dr. mentioned it as illustrative example)) In Induced theory when Glucokinase binds first glucose then will change the active site, one of the benefits of the active site change is to make ATP binding better.

So it first binds glucose then ATP. when the enzyme binds more than one substrate, Will both substrates have the same K_m or different K_m? Most of the time they're different ((K_m describe affinity)).

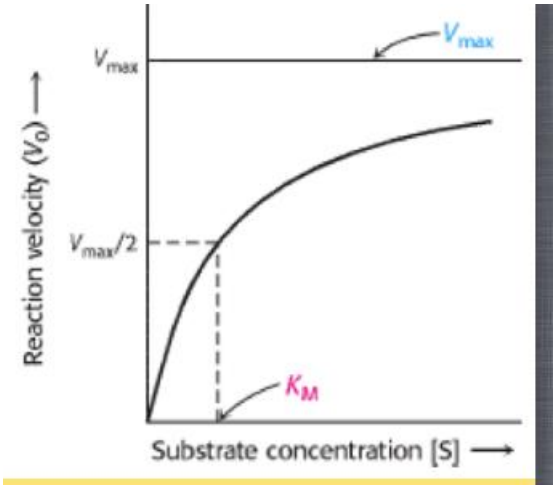
Each enzyme will have a certain **K_m** for each substrate. If they're providing you with same output (with same product) then they have the same **Vmax**

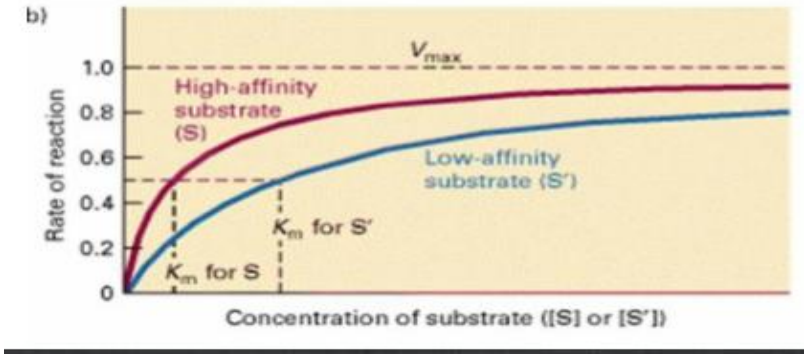
If they're 2 different RXNs like glucokinase it can use glucose or fructose & the results are different then each substrate will have certain K_m & Vmax for itself. (study the following table)

table 8–6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0





- What does this mean?

The affinity of glucose is higher which means if you have glucokinase, glucose & fructose, the hexokinase will bind glucose not fructose and this is why glucose is the preferred substance for glucokinase (hexokinase)

- for any substrate :

Km is low →affinity is high .

By knowing K_m value for the substrates, you can arrange them and determine which will bind first.

We have talked about Kd (the actual measure of affinity)

- best way to understand it :

If you have 5 molecules of substrates, 10 active sites and you increase the molecule of substrate. what will happen for the velocity of enzyme?

The velocity will increase till it reaches the Vmax

(Because you increase S conc you will achieve the Vmax , but if you increase S conc after reaching Vmax you won't achieve anything)

If you have 5 molecules of substrates, 10 active sites & you made it 20 active sites. what the velocity will be? What have changed is the chance of substrates to bind active sites (it is higher for sure) ((the chance is mathematical calculation)), and it will keep increasing in linear manner

- Enzyme concentration increases → rate will be higher → because the chance of binding between substrate and enzyme is higher.

The 5 molecules will bind the 5 active sites. The number is not changing what's changing is the time need to bind

- more active sites → less time to bind → more chance of collision.

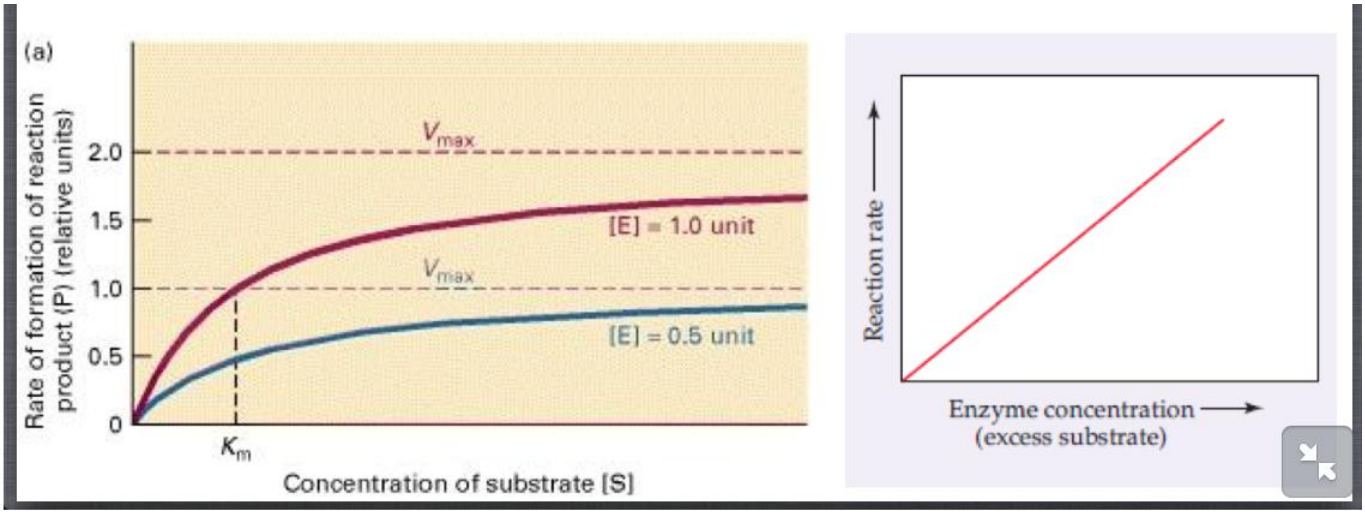
You're not changing the molecule concentration which are in the numerator, what we are changing is the time so when denominator is less the velocity will be higher

With increasing enzyme concentration the Vmax of enzyme will keep increasing in a linear manner.

If you are increasing the enzyme concentration what will happen for the K_m of the enzyme?

Remember that K_m measures the affinity of substrates toward active site, and it doesn't change with increasing enzyme concentration. So the K_m stays the same.

<u>The maximum velocity of the enzyme</u> (**Vmax**) is the rate of how much substrates is converted to product per unit of a time when there's a full saturation.



There's a term called the **Catalytic activity** of the enzyme:

Mean → how much the enzyme is powerful, how much it can convert substrates into products per unit of a time.

So K_{cat} means K_2 ((convert complex to product))

For a RXN that has **K2 =10** which the catalytic activity of that enzyme it can convert 10 ES to 10Ps in ,for example, one second

If the enzyme concentration is increased. To 20, so ES = 20, P = 20, Rate is doubled however the K is constant.

So K_2 is not an actual measure of how much the enzyme is powerful.

For this reason they said that :

K_{cat} is the same of K_2 but when the [E] is normalizing (because enzymes can be different with different enzyme concentration → their rate will be different)

So **K_{cat} : ((the catalytic activity of the enzyme))** is how much substrates are converted to product per unit of time per enzyme concentration.

The question now is : Why we are dealing with (rates' constants) in any RXN?

- To tell us an information ,To help understanding what goes on and To be able to compare two materials together

If there're two RXNs one certain RXN with (K_2= 10) and the other RXN with (K_2= 10) but the P formed is doubled.(You can't compare both of them) however, when you divide them by the [E] being used then you're normalizing all the RXNs. Here, we talk about **The Catalytic Activity Of The Enzyme** if one unit of [E] is being used in each rxn. Now,we can compare between both RXNs (Which of them more catalytic or more reactive than the other because of using the same unit of [E]) .

K_{cat} = the maximum velocity of enzyme being used divided by the total enzyme concentration

K_{cat}= Vmax/[E]

This is a comparison between different enzymes:

Turnover Numbers (k_{cat}) of Some Enzymes		
Enzyme	**Substrate**	**k_{cat} (s^{-1})**
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Catalase: it can convert 40 millions of H_2O_2 to water H_2O molecules in one second. :O

Example: **K_{cat}= Vmax/[E]**

Example

- a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate
  - ✓ Hence, k_{cat} is 6×10^5 s^{-1}
  - ✓ 10^4 min^{-1}

REMEMBER in the EXAM to convert from Seconds to Minutes, okay? ☺

(you can use any time unit you want)

So **K_{cat}** is how much product formed per time unit divided by total enzyme concentration.

(you should be aware while dealing with different time units)

Q:- If you have $6*10^5$ RXNs/seconed, How much time needed for each reaction to take place???

- **Each catalyzed reaction takes place in a time equal to $1/k_2$, which is 1.7 µs for carbonic anhydrase**
- **The turnover numbers of most enzymes with their physiological substrates fall in the range from 1-10^4 (s^{-1})**

"SUCCESS IS NO ACCIDENT.

It is hard work, perseverance, learning, sacrifice

And most of all, **love** of what you are doing."

- PELE

BEST WISHES
Dyala Murad Al-Frijat ☺