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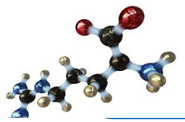

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture #13

Enzymes pt.3 (Kinetics)

Written by : Ali Alsharaeah
Nizar Alsabateen

Reviewed by :Muhanad Barhoom

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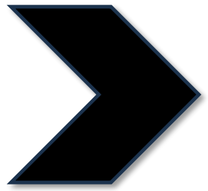
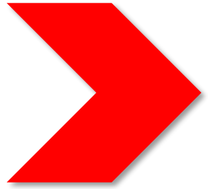


Black: the original slides

Blue: the doctor’s explanation/words

Gray: additional information and explanation



Red: important information

Enzymes II

Kinetics

Prof. Mamoun Ahram

Kinetics

Understand
Do not memorize

- Kinetics deals with the rates of chemical reactions.
- Enzyme kinetics is the study of the rates of enzymatic reactions (its not chemical reactions, doesn't change at a linear pace) .
- For the reaction (A $\rightarrow$ B), velocity (v) or rate of reaction is **the amount of B formed** (or **the amount of A consumed**) per unit time, t. That is,

The consumption of A or production of B should be (constant) at equal rates

$$\text{Rate of reaction (velocity or v)} = -\frac{\Delta [A]}{\Delta t} \quad or \quad \frac{\Delta [B]}{\Delta t} = -k[A] = k[B]$$

The rate law means that the rate of the reaction is proportional to the concentration

- This is known as the rate law, which describes how concentrations of reactants affect the rate of the reaction during a certain period.
- Note: the rate is proportional to the concentration of A, and k is the rate constant.
  - k has the units of $(\text{time})^{-1}$, usually sec^{-1}.

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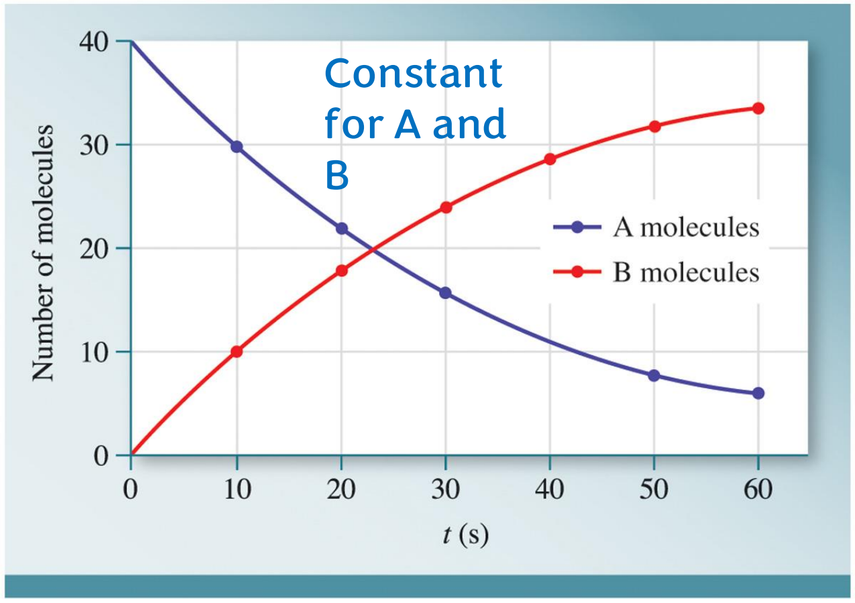


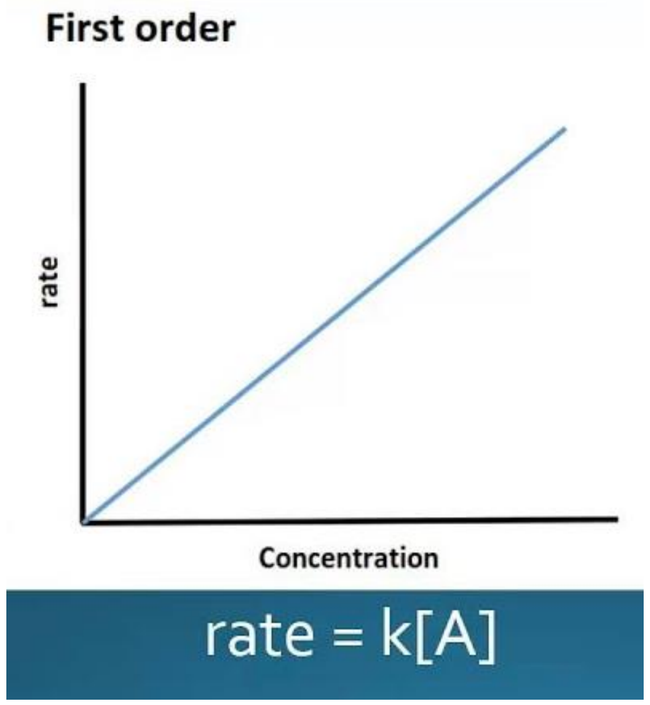
If (A → B) is (in chemical reactions)

Second order reaction : when u have
rate =[A][B]k so if u increase concentration of A and B it will affect greatly on the rate of the reaction

A first-order reaction

$$\text{rate} = k[A]$$

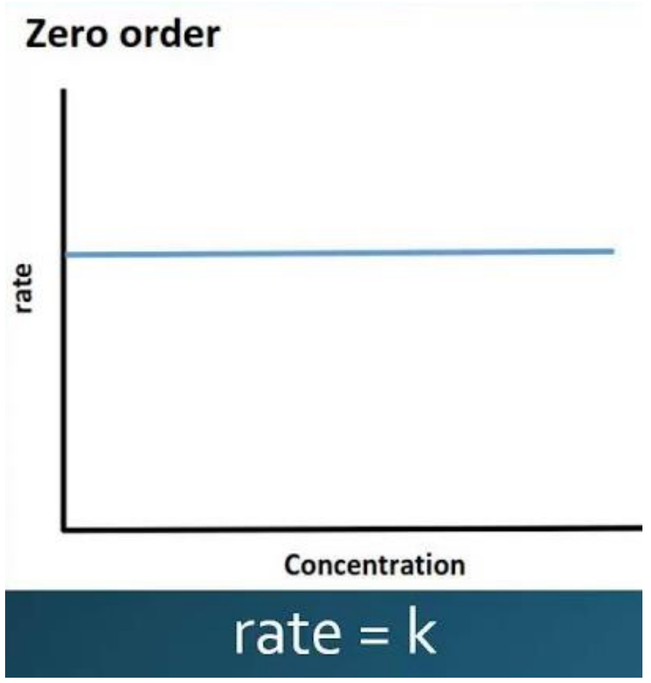
- The rate of a reaction increases **linearly** with increasing substrate concentration.



A zero-order reaction

$$\text{rate} = k[A]^0 = k$$

- The rate of the reaction is **independent** of substrates.



Rate of reaction (velocity) (in enzymatic reactions)

- Rate of reaction is calculated as : concentration of substrate disappearing (or concentration of product appearing) per unit time (mol L^{-1} . sec^{-1} or M . sec^{-1}).

Remember: Concentration (M) = mol / vol

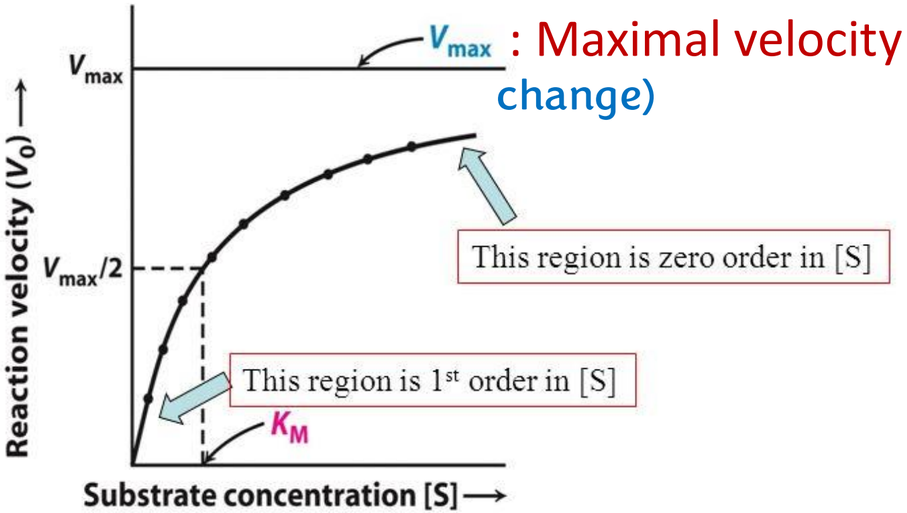
Enzyme kinetics

- Enzyme-catalyzed reactions have hyperbolic plots.
- Initial velocity (V_0) varies with the substrate concentration [S] where the **rate of catalysis rises** linearly as the **substrate concentration increases** and then levels off and approaches a constant, maximal velocity (Vmax) **at very high substrate concentrations**.

- When the concentration of the substrate is low it is considered a first order reaction meaning with the increase of [S] the rate will increase (dependent on substrate concentration)

- And if the concentration is very high it is considered zero order reaction meaning increase in concentration doesn't alter the rate that much.
- Finally, it will reach a level where the rate will stop being affected by the change in [S],, when reaching Vmax(not dependent on substrate concentration, but on the enzyme's efficiency itself)

A commonly observed behavior for enzyme catalyzed reactions showing the change in V_o as [S] is changed



This behavior can be described mathematically by the Michaelis-Menten equation

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

Why?

- At low concentrations of the substrate , the rate of the reaction depends on the concentration of the substrate. But why is that??

The reaction depends on the collision between the substrate and the enzyme.So when you increase the substrate concentration , you increase the probability of the collision making the reaction faster.

- At high concentrations of the substrate, the rate of the reaction depends on the enzyme itself.

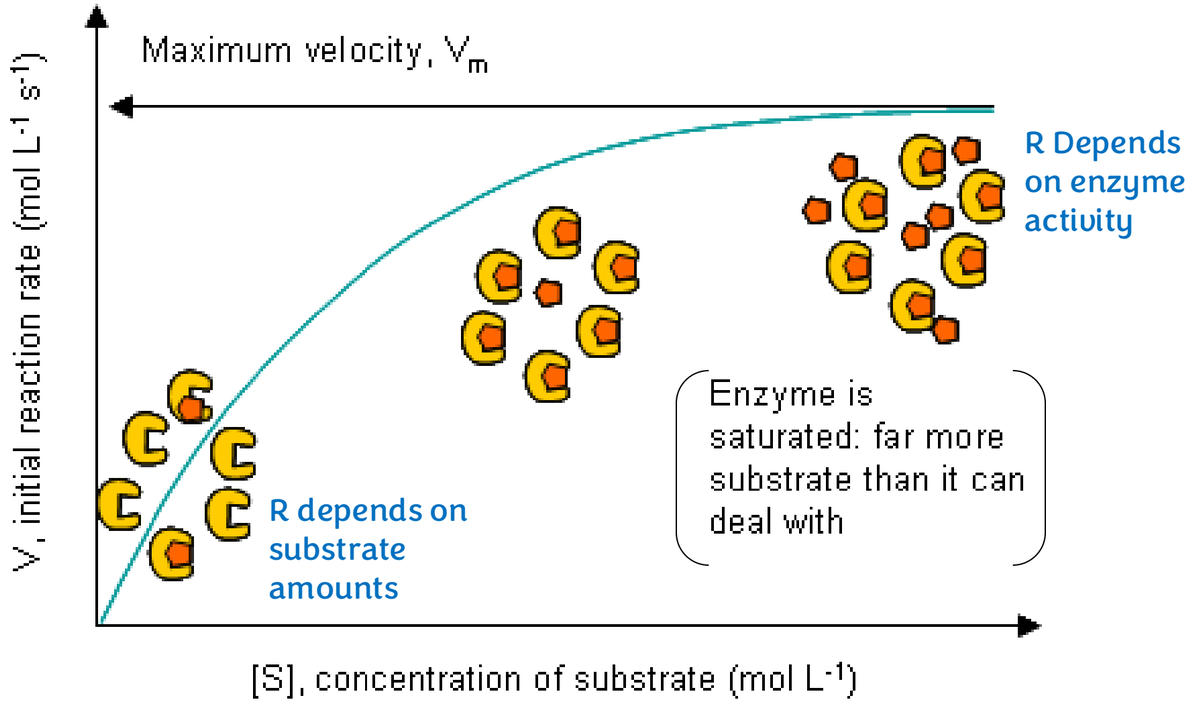
When the concentration of the substrate is at high level, the active sites will be saturated. So if you have increased the concentration even more, the rate of the reaction will not be altered. So now reaction rate depends on the enzyme itself and it's efficiency , Not at the random collision anymore.

- The hyperbolic plot is known as a saturation plot because the enzyme becomes "saturated" with the substrate, i.e., each enzyme molecule has a substrate molecule associated with it.
- Increasing substrate concentration more wont differ, because it depends on the enzyme catalic activity

What is the limiting factor of the speed of a car? The car or the road?

When the road (substrate) is smooth, the speed of the car (enzyme) **depends on the car, not the road.**

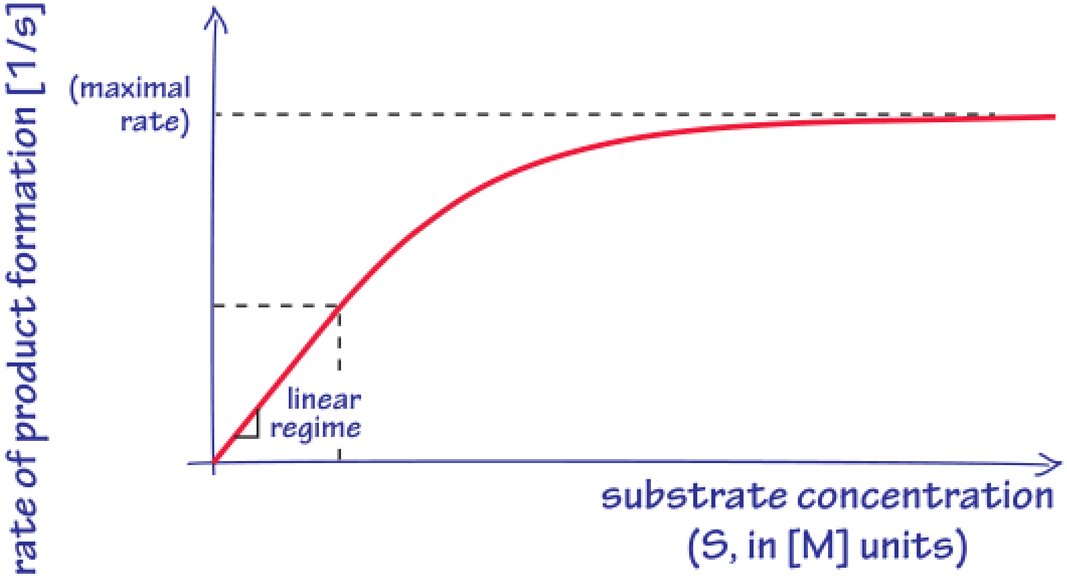
When the road (substrate) is rocky, the speed of the car (enzyme) depends on the road, not the car.



More explanation

- At a fixed concentration of enzyme, V_o is almost linearly proportional to [S] when [S] is small.
- However, V_o is nearly independent of [S] when [S] is large.
- The maximal rate, Vmax, is achieved when the catalytic sites on the enzyme are saturated with substrate.

- Vmax reveals the turnover number of an enzyme:
  - The number of substrate molecules converted into products by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.



The Michaelis-Menten equation

- The Michaelis-Menten equation is a quantitative description of the relationship between **the rate** of an enzyme catalyzed reaction (V_o), substrate concentration [S], a rate constant (K_M) and maximal velocity (Vmax).

Memorize:

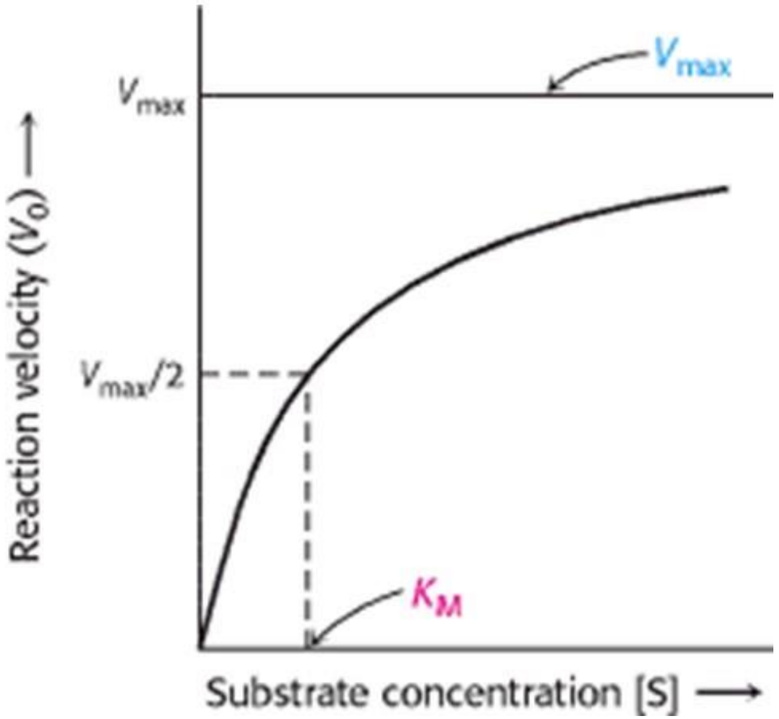
$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

The Michaelis constant (K_M)

- K_M is the **concentration of substrate** at which half the active sites are filled or half the maximal velocity is reached, its constant.
- When [S] = K_M, then V_o = Vmax/2
- Therefore, it provides a measure of enzyme affinity towards a substrate.
  - **It is not a true measure of affinity, though.**
- The lower the K_M of an enzyme towards a substrate is, the higher its affinity to the same substrate is. (it doesn't explain affinity but gives us the idea)

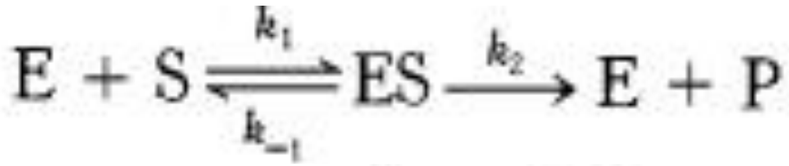
- When substrate equals Km the velocity is half of Vmax.

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



The Michaelis constant (K_M)

- For a reaction:



Rate of dissociation
(the enzyme being free)

Rate of association
(enzyme being linked to the substrate)

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

Assume $k_{-1} >> k_2$), $K_M = k_{-1}/k_1$

K2 is usually neglected because it's very small compared to the other constants, so the affinity of an enzyme could be described by K1 and K-1.

- K_M is related to the rate of dissociation of a substrate from the enzyme to the rate of enzyme-substrate association.
- K_M ***describes*** the affinity of an enzyme for its substrate but is ***NOT an accurate*** measure of affinity.

- When [ES] is formed, it has two options either it goes back to E+S or it can go forward and become E+P:
- Going back to E+S(by breaking the non-covalent interactions) has its own constant (k-1).
- Going forward has its own constant (k2).

K1: the rate of binding the substrate and enzyme.
K-1 :the rate of unbinding the enzyme and substrate without the product being formed. (the substrate becomes free).
K2 :rate of reaction to change the substrate into a product and unbinding from the enzyme.

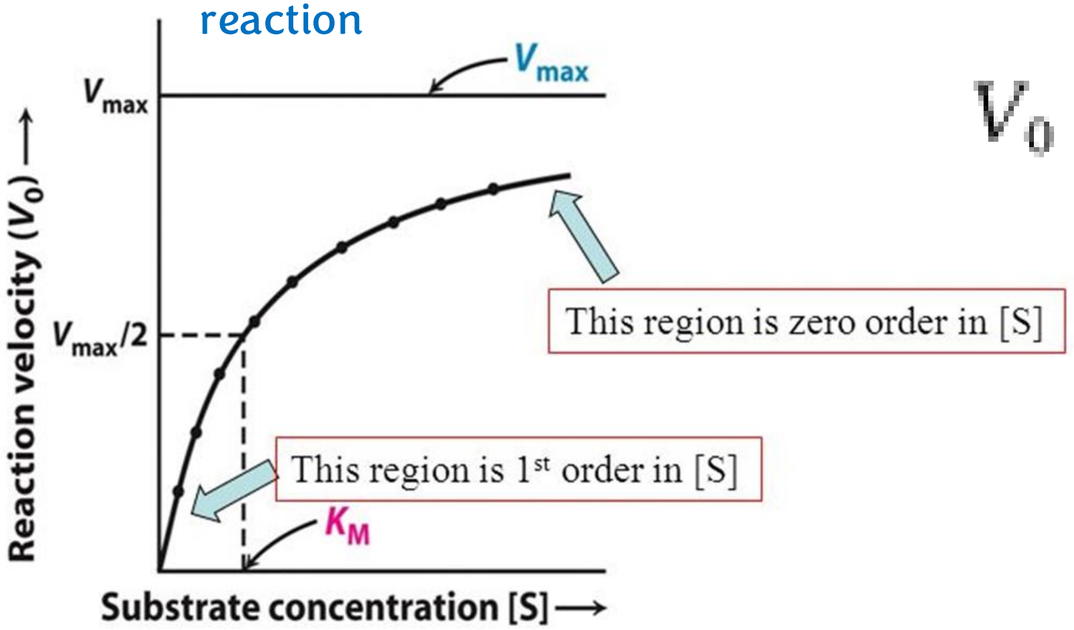
Reaction order in relation to K_M

- At very low substrate concentration, when [S] is much less than K_M, V_0 = Vmax.[S]/(K_M + [S]); that is, the rate is directly proportional to the substrate concentration and is affected by how well a substrate binds to an enzyme.
- At a high substrate concentration, when [S] is much greater than K_M, V_0 = Vmax; that is, the rate is maximal, independent of substrate concentration or how well an enzyme binds to the substrate.

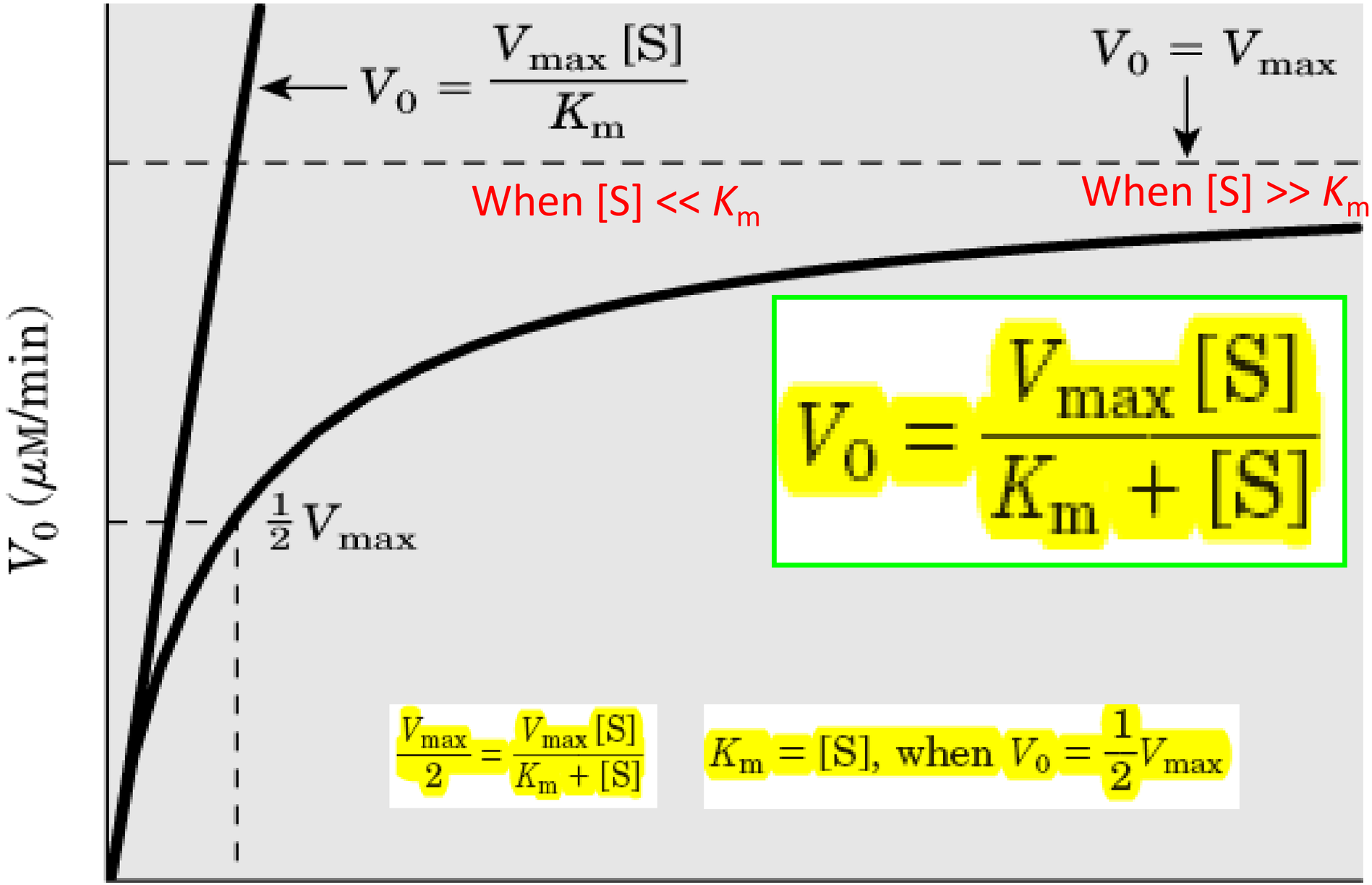
- You can apply in the equation when you have high A very, very high [S] comparing with the KM you would notice that the rate of the reaction equals Vmax.

- While if the concentration was much less than km, you would notice that any slight change in the substrate concentration makes considerable difference in the Rate of reaction

Lower Km results in higher affinity and vice versa.



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



The substrate concentration at which V_0 is half maximal is K_m

Note

Understand
Do not memorize

- The K_M values of enzymes range widely (mostly, 10^{-7} to 10^{-1}).
- Each substrate has a unique K_M for a given enzyme, but Vmax is related to the enzyme and is the same for the same reaction of more than one substrate.

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

Example: Hexokinase – enzyme that phophorylates glucose

Glucose + ATP ⟶ Glucose – 6-P + ADP + H^+

Glucose and ATP both have different Km values when binding to Hexokinase as they are different molecules.

- Hexokinase usually works on hexoses like fructose and glucose and you still can notice that there is a difference in the Km values.

Same enzyme, different substrates, **same** reaction

Different Km values, but Vmax is the same

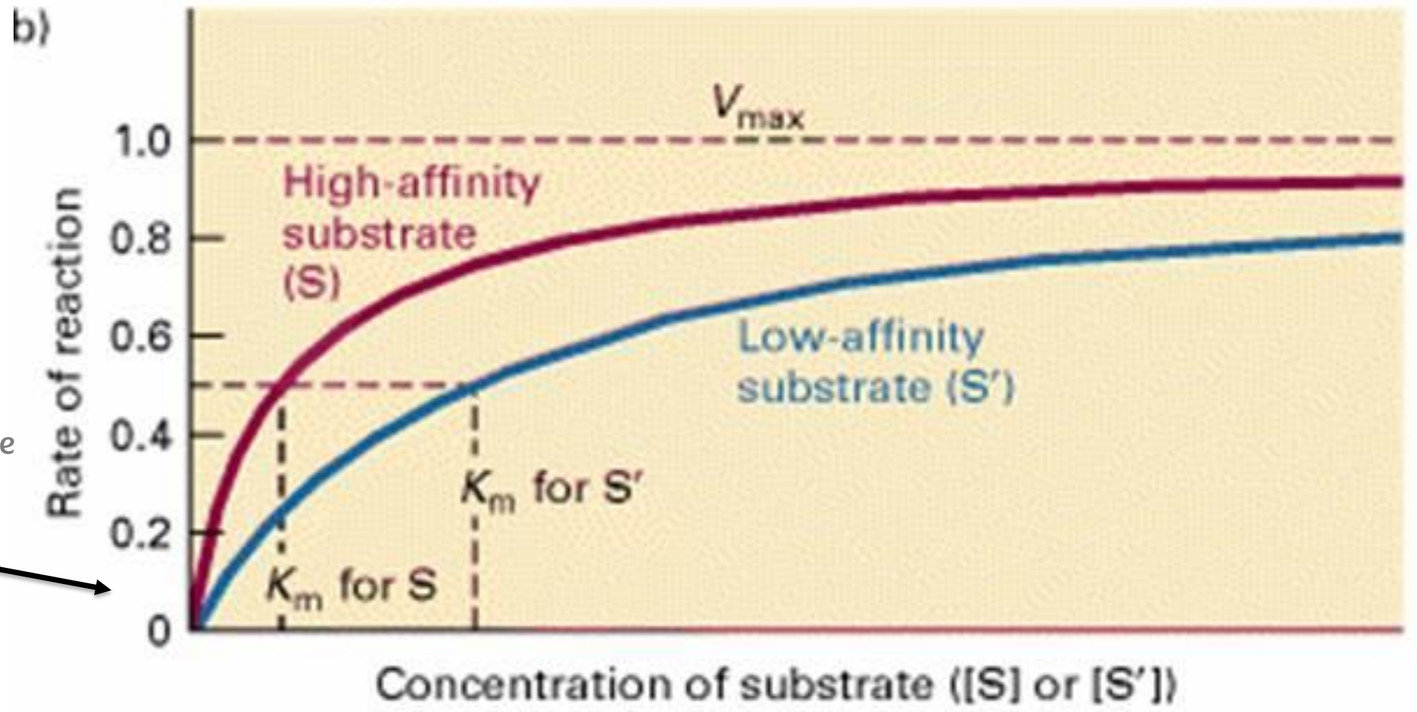
Example: Hexokinase – enzyme that phophorylates glucose

Glucose + ATP ⟶ Glucose – 6-P + ADP + H^+

- A reactions is catalyzed by an enzyme with substrate S (high affinity) and with substrate S′ (low affinity).
- Vmax is the same with **both substrates**, but K_M is higher for S′, the low-affinity substrate.

Vmax will be the same for different molecules if they're in the same reaction.

When you have a very high amount of glucose (non-limiting factor), the curve will be the same as the curve of ATP and vice versa. When both are non-limiting, we reach Vmax



Same enzyme, different substrates, different reactions

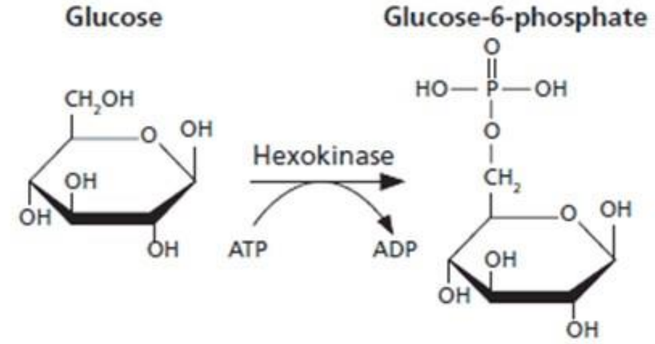
It does not necessarily mean that if the affinity is high , the υ max should be high.

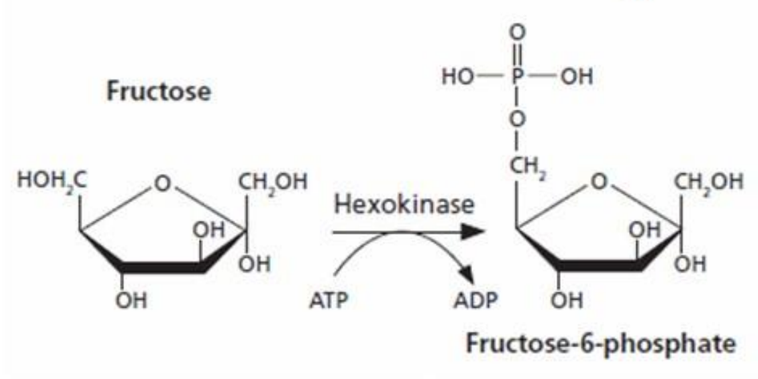
You can notice that the fructose has the lowest affinity , but it also has the highest Vmax, which means that once the fructose is bound to the enzyme , it will be converted swiftly.

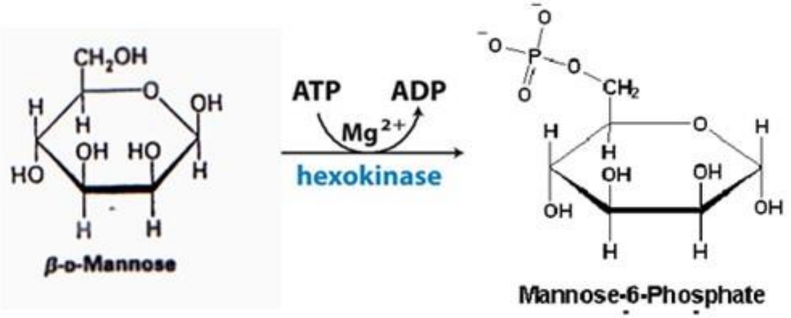
- If an enzyme binds to another substrate generating **different product(s)**, then **Vmax** will be **different**.
  - For example, hexokinase phosphorylates glucose, fructose, and mannose at different Vmax values.

Hexose	K_M (μM)	V_{max} $mol\ L^{-1}.sec^{-1}$
Glucose	59 ± 10	26 ± 2
Mannose	32 ± 2	13 ± 1
Fructose	4436 ± 2275	34 ± 5

Understand
Do not memorize



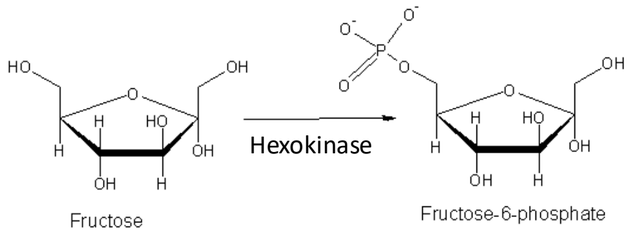


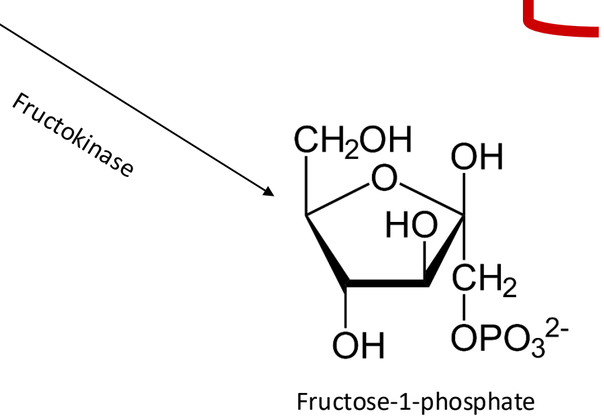


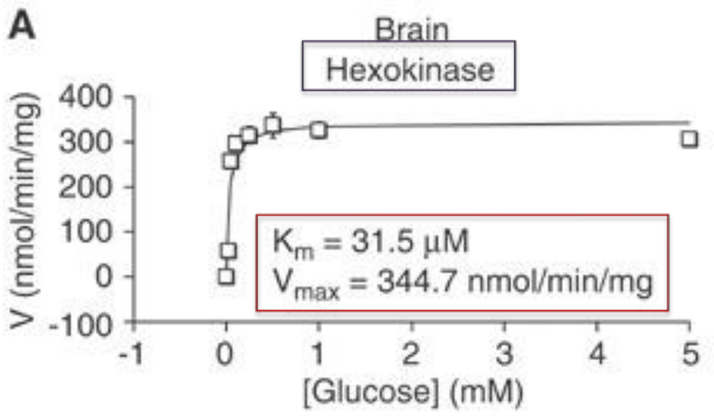
Different enzymes, same substrate, different reactions

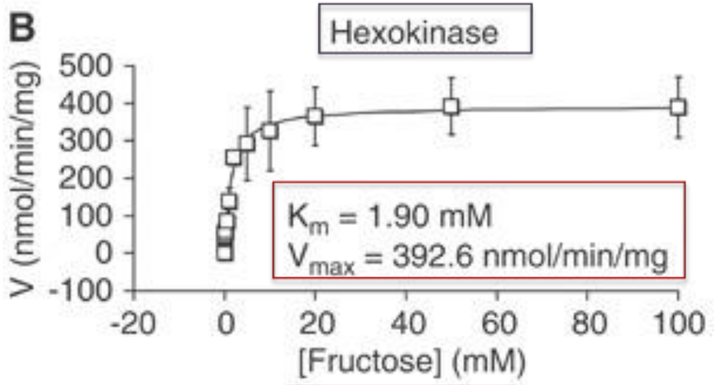
Understand
Do not memorize

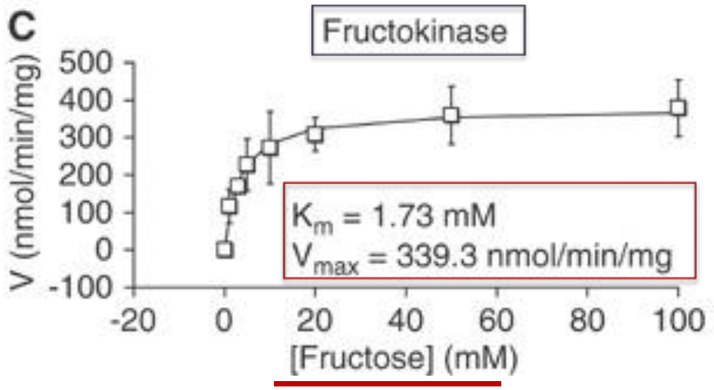
Same enzyme, different substrates

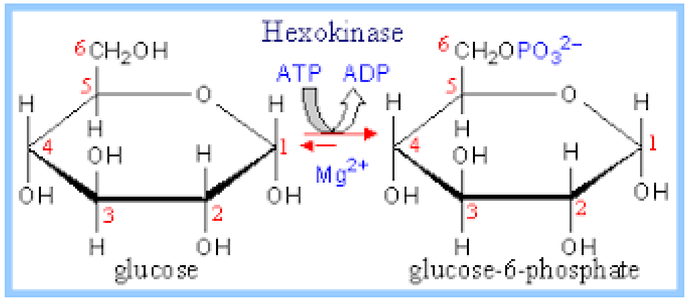












Different enzymes, same substrate

Example

- A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, Vmax of this enzyme is … & K_M is …?
  - A. 5000 & 699
  - B. 699 & 5000
  - C. 621 & 50
  - D. 94 & 1
  - E. 700 & 8

Substrate Concentration (μM)	Initial velocity (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

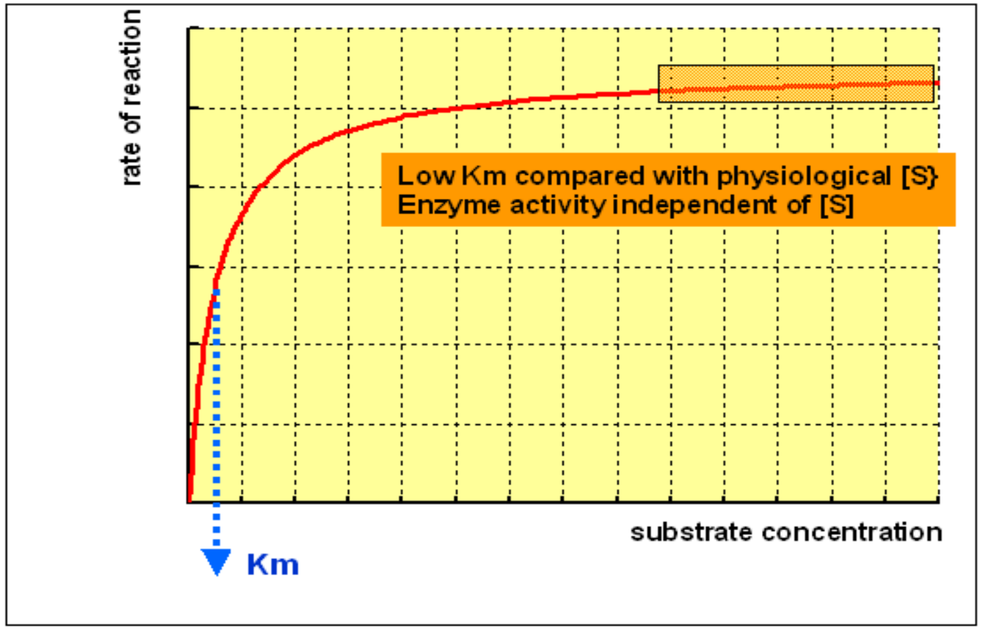
Explanation:
You can notice from the last few values that the increase in the initial velocity is a small comparing with the enormous increase of substrate concentration Which means that you are near to the Vmax .In this case, it is estimated as 700 and to find Km .You should find half of υ max , which is 350 and the concentration of the substrate at that rate , is 8

E

Importance of K_M

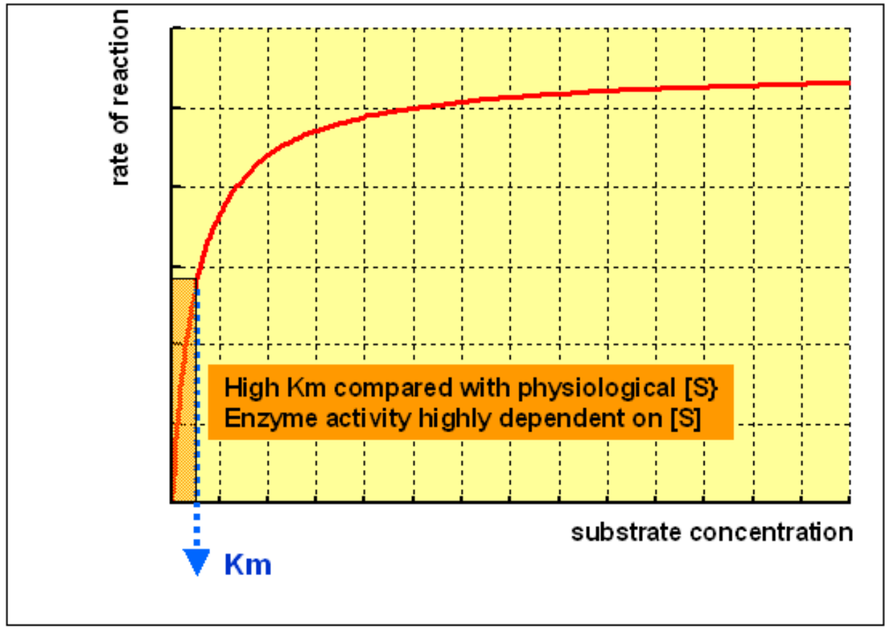
You can use Km to predict where the reaction is going.

If K_M is lower than physiological concentration of S



The enzyme is normally saturated with substrate and will act at a constant rate, regardless of variations in the concentration of substrate.

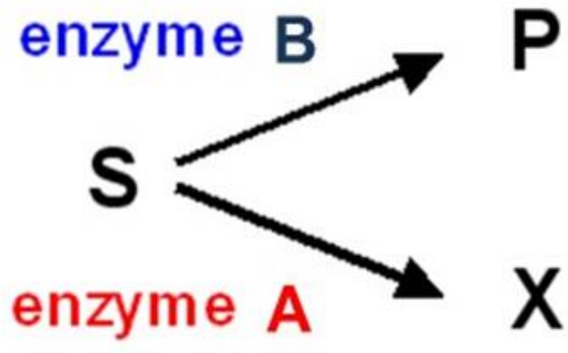
If K_M is higher than physiological concentration of S

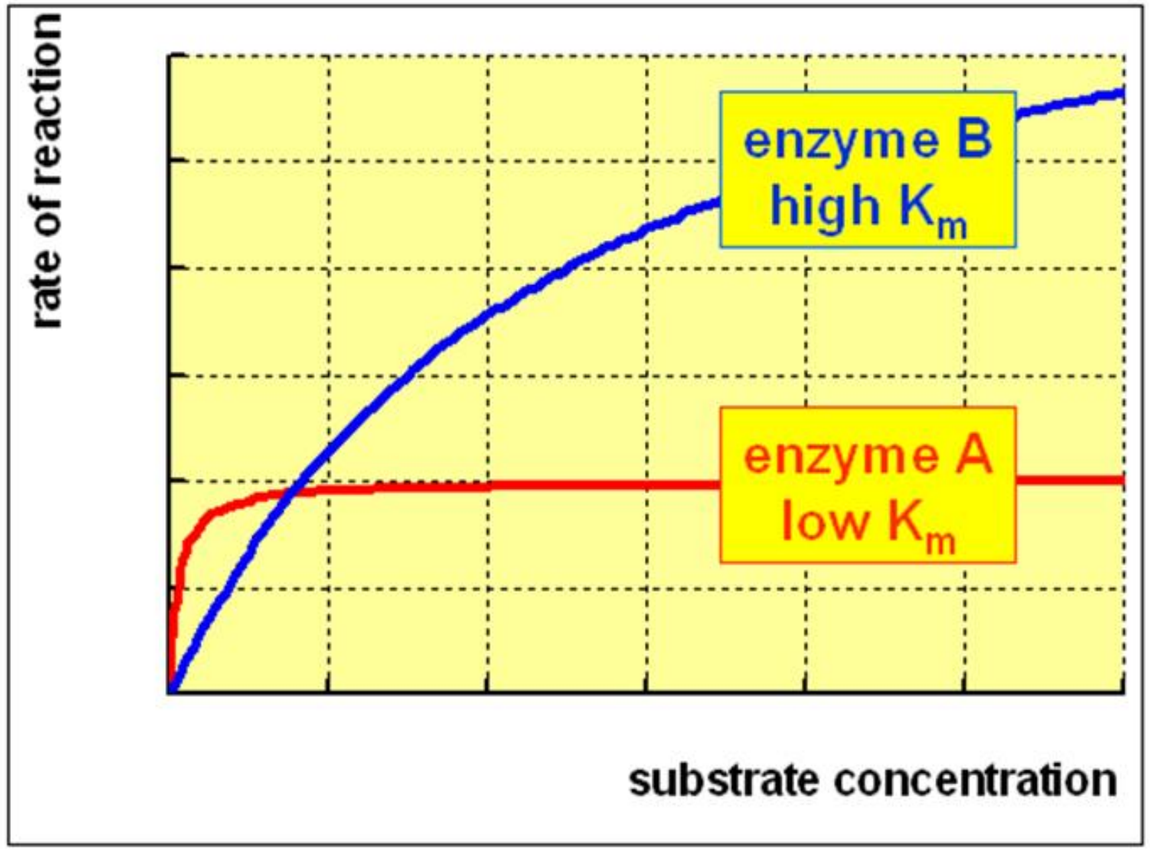


The enzyme is not saturated with substrate and its activity will vary as the concentration of substrate varies and the rate of formation of product will depend on the availability of substrate.

Metabolic pathways

- If two enzymes, in different pathways, compete for the same substrate, **then knowing the values of K_M and Vmax for both enzymes permits prediction of the metabolic fate of the substrate** and the relative amount that will flow through each pathway under various conditions.
- Which reaction is favorable when:
  - [S] is very low? **A**
  - [S] is very high? **B**





An enzyme in liver called the glucokinase and another enzyme in muscles called Hexokinase , both enzymes do the same function , which is phosphorylation of glucose.
In the liver, phosphorylation of glucose is needed to synthesise glycogen, while in muscles it is needed to break up the sugar molecules and make energy.

When someone's hungry enzymes in the liver (glucokinase) will work differently given it’s almost the same as the ones in the muscle (hexokinase) , hexokinase will function leading to breaking up the glucose to make the energy needed for the physiological activities.

Glucokinase in the liver stores energy while hexokinase in the muscles consumes energy, so when you're hungry liver's enzymes won't work as you need energy to use and not to store.

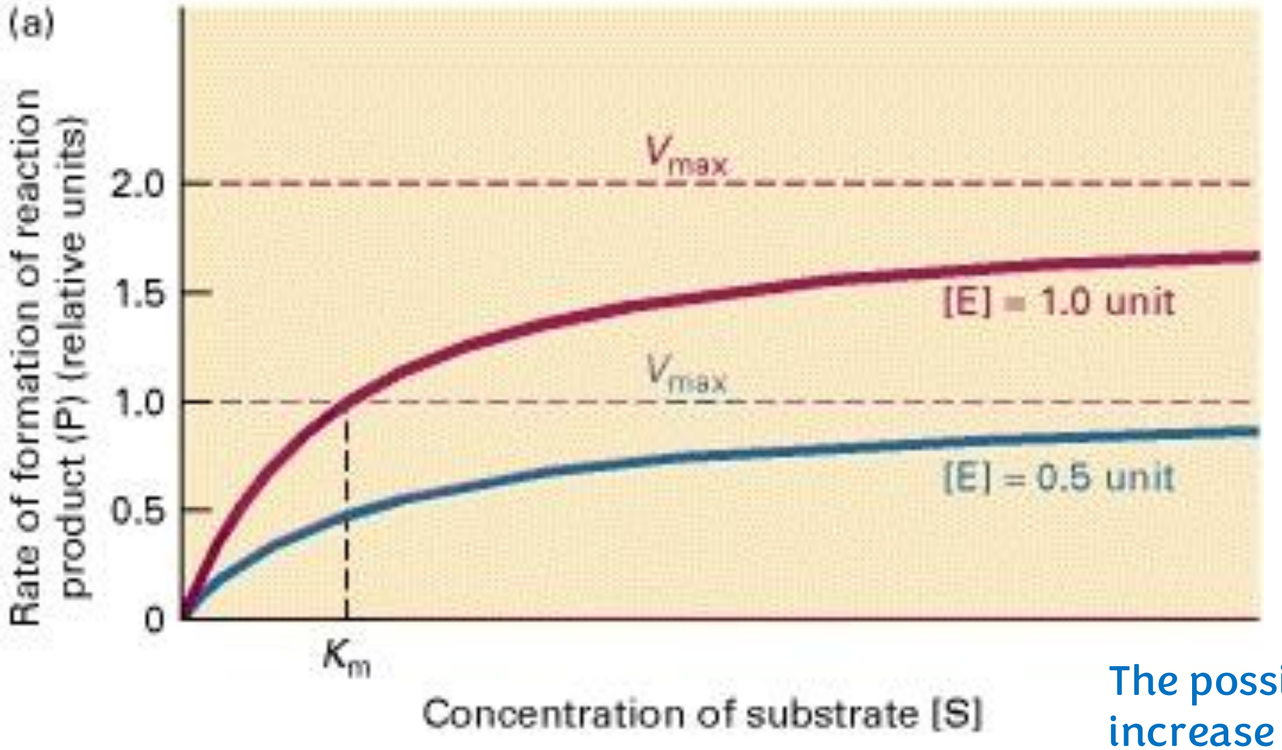
In normal conditions (you're not hungry) muscle enzymes will work just fine (there is no need to burn all sugar molecules), while liver enzymes will work efficiently.

Uses of K_M

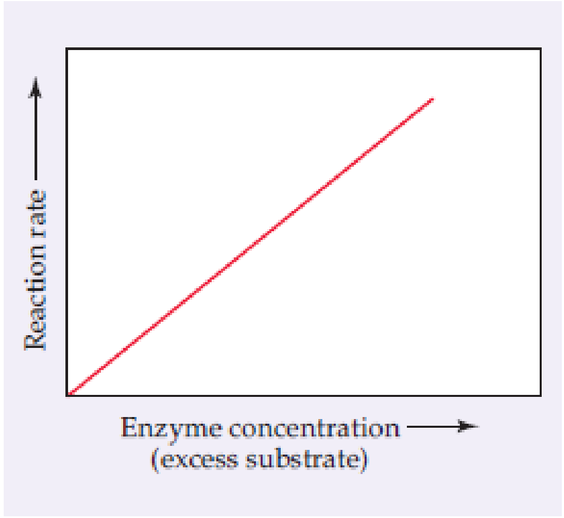
- Determine the substrate preferences of an enzyme.
  - If an enzyme has more than one substrate , the substrate with the lowest K_M is probably the preferred physiological substrate.
- Distinguish isozymes, which are different enzymes catalyzing the same reaction.
  - Isozymes often have different affinities for the same substrate.
- Check for abnormalities in an enzyme. **(change in affinity (*Km*) means there is a problem)**

Vmax and enzyme concentration

- Doubling the concentration of enzyme causes a proportional increase in the reaction rate, so that the maximal velocity V_{max} is doubled; the K_M , however, is unaltered.



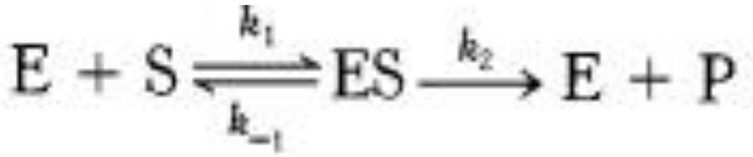
Linear relationship



The possibility of the enzyme finding the substrate is higher once you increase the concentration of the enzyme.(collision probability between the substrate and the enzyme increases).

Vmax & kcat

(a measure of enzyme efficiency)

- For the enzymatic reaction 

- The maximal rate, $\mathbf{V_{max}}$, is equal to the product of **k_2, also known as kcat**, and the total concentration of the enzyme.

$$V_{max} = k_2 [E]_T \; OR \; k_{cat} = V_{max} / [E]_T$$

Understand
Do not memorize

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

Once I increase the enzyme concentration Vmax increases, (**kcat stays the same).**

kcat is a constant for any given enzyme and a substrate.

Kcat

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

$V_{max} = k_2 [E]_T$ *OR* $k_{cat} = V_{max} / [E]_T$

- **kcat, turnover number**, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, **when fully saturated.**
- It describes how quickly an enzyme acts, i.e., how fast the ES complex proceeds to E + P.
- In other words, the maximal rate, Vmax, reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known.

- I have two factors that changes the efficiency of the enzyme , which are the affinity and the catalytic activity.

- The efficient enzymes are the ones who have high affinity and high efficiency.meaning who have low Km and high k2

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 <u>at best</u> converts 9.6 µmol of the substrate per min at 25˚C. The turnover number (kcat) for the enzyme is:

A. 9.6 s^{-1} B. 48 s^{-1} **C. 800 s^{-1}**

D. 960 s^{-1} E. 1920 s^{-1}

mol = g/MW

Explanation

- MW = 50,000 g/mol
- Weight = 10 µg
- Vmax = 9.6 µmol of the substrate per min

Kcat = (9.6/60)/(10 µg /50,000)

= **800 s^-1**

Number of reactions per second

Understand
Do not memorize

table 8–7

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

Catalytic efficiency (Kcat vs. K_M)

Catalytic efficiency depends on the **efficiency (Kcat)** and the **affinity (Km).**
Increasing kcat increases the catalytic efficiency while increasing Km decreases the catalytic efficiency.

Table 6.2

Turnover Numbers and Km for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M**
Catalase	Conversion of H_2O_2 to H_20 and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

Understand
Do not memorize

Catalytic efficiency of enzymes = k_{cat} / K_M

Quiz time

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks’ Basic Medical Biochemistry (6th edition)