20

BIOCHEMISTRY
faculty of medicine - JU2017

ketone
starch lipid protein
carbohydrates isomers amine

◉ Sheet   ○ Slides

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Quick recap

- **The rate of Chemical Reactions** → Rises **linearly** as the substrate concentration [S] **increases**.

  The rate of Enzymatic Reactions → Rises **rapidly** with the substrate concentration [S] initially and then begins to **level off** *(when the enzyme is saturated)* and approaches a **maximum** *(V_{max})* at higher substrate concentrations where the enzyme is over saturated *(Plateau)*.
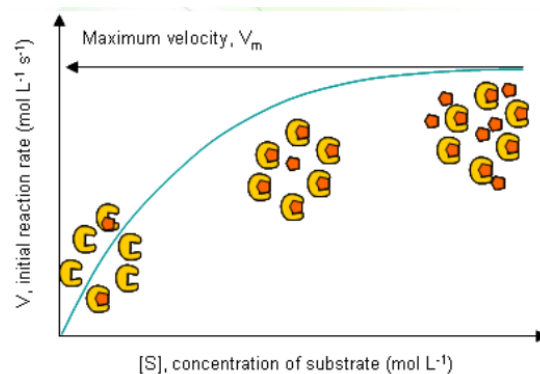
- **When the substrates' concentration is very high:** The rate of the reaction depends on the **catalytic activity** of the enzyme, not on the concentration of the substrate. *The only way to change the rate at this point is to change the [E].*

  When the substrates' concentration is low: The rate of the reaction is almost linearly proportional to the **substrates' concentration**.

- **Turnover Number:** is the **number of substrate molecules** converted *(Turned over)* into **product** by a **single enzyme** molecule in a **unit time** when the enzyme is fully saturated with substrate.

The Michaelis-Menten Equation

V_0 → Enzyme Reaction Rate

$[S]$ → Substrate concentration

K_M → Rate Constant

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

1) **What happens if the [S] is very low relative to K_m?**
The enzymatic reaction rate *(V_o)* would be **low**.

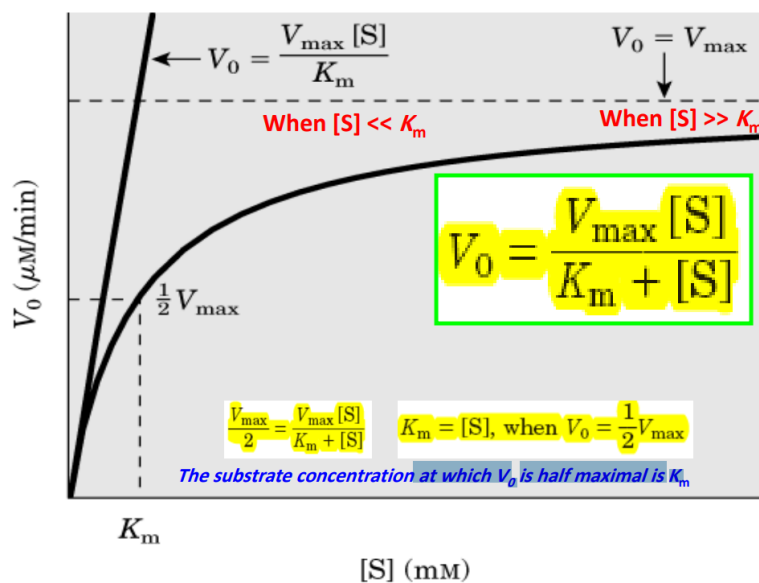2) **What happens if the [S] is very high relative to K_m?**
K_m would almost be **negligible** → $Vo = V_{max}$

3) **What happens if [S] = K_m?**
$Vo = \frac{1}{2} V_{max}$ *Quick mafs ;)*

> **Note:** Very low values are only **negligible** in **addition** and **subtraction**. However, it is not in **multiplication** and **division** since even low values can have a **huge** impact.

From the previous 3rd question, we notice that the **K$_M$** is actually the **substrate concentration** [S] at which V$_o$ is **half maximal** (V$_{max}$).

$$V_0 = \frac{V_{max}\,[S]}{K_m}$$

When [S] << K_m

$$V_0 = V_{max}$$

When [S] >> K_m

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

$$\frac{V_{max}}{2} = \frac{V_{max}\,[S]}{K_m + [S]} \qquad K_m = [S],\ when\ V_0 = \frac{1}{2}V_{max}$$

The substrate concentration at which V$_o$ is half maximal is K$_m$

$V_0\ (\mu M/min)$ — $\frac{1}{2}V_{max}$ — K_m — [S] (mM)

The Michaelis constant (K$_M$)

Steady State Approximation, is a chemical equation derived by Michaelis and Menten. It included that the **substrate enzyme complex** (ES) is **used up** as soon as it has **formed**, thus the concentration of the ES does **not change**.

In other words, the rate of ES formation = the rate of its dissociation.

The derivation of the Michaelis-Menten Equation is not required.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\longrightarrow} E + P$$
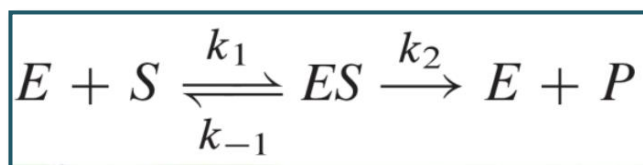
K$_1$ → Rate constant of the **formation** reaction of **ES**

K$_{-1}$ → Rate constant of the **degradation** reaction of **ES**

K$_2$ → Rate constant of the **formation** of **E + P**

Taking these constants into consideration, Michaelis and Menten came up with **K$_M$**.

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

→ *Dissociation Constants*

→ *Association Constants*

KM up until now can indicate the following:

1) The **substrate concentration** [S] at which V_o is **half maximal** (V_{max}).
2) The **affinity** of an enzyme to a substrate *"discussed below"*.
3) The **substrate concentration** [S] when **50%** of the enzymes' active sites are bound with the substrate.
4) Related to the **rate of dissociation** of the substrate from the enzyme, to the formation of the enzyme-substrate complex.

> **Rule:** The **lower** the K_M value of an enzyme towards a substrate is, the **higher** the **affinity** to the same substrate is. It's only an **indication** for affinity but not an **accurate measure** of it.

For further explanation, imagine these two situations:

1) 200 molecules of a substrate + 10 molecules of an enzyme
*After a while, we notice that **5 out of the 10** enzyme molecules are bound to the substrate (which is 50% of enzymes' active sites)* → $K_{M\,1} = 200$

2) 50 molecules of a substrate + 10 molecules of an enzyme
*Here we used the same enzyme but a **different substrate** (which has a different affinity).*
*After a while, we notice that **5 out of the 10** enzyme molecules are bound to the substrate* → $K_{M\,2} = 50$

Conclusion: *The 2nd substrate had **more affinity** because the number of molecules required to fill half of the active sites on the enzyme was **fewer**.*
Notice that $K_{M\,2} < K_{M\,1}$

→ *K_M is a measure of affinity and it is **inversely proportional** to it.*
It is the same concept when we mentioned that myoglobin has more affinity than hemoglobin, since myoglobin requires 2 Torr only of O_2 to be 50% bound while hemoglobin requires 25 Torr.

The K_M values of enzymes range widely mostly between 10^{-1} and 10^{-7} M

Each **substrate/enzyme** combination has a unique K_M value. If the enzyme binds to **another substrate** generating **different products**, then K_M *(V_{max} too)* will be **different**.

Examine the table in the next page.

- **Catalases' KM = 25 mMol**

It means that **25 mMol H_2O_2** is needed to fill **50%** of the enzyme active sites.

Also it means that **25 mMol** is required to reach **1/2 V_{max}.**

- **Hexokinase**, *which phosphorylates hexoses sugars* has **different** K_M values for each different **substrate** *(each give different products).*

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

- **Which substrate has the highest affinity to Hexokinase?**

Glucose, because it has the **lowest** K_M value, so you need only 0.05mM of Glucose to reach half V_{max}.

- **Which substrate has the lowest affinity to Hexokinase?**

Fructose, because it has the **highest** K_M value so you need lots of it to reach half V_{max}.

Please note that the enzymes' concentration [E] in the previous comparisons is constant.

The binding ability differs betweent different substrates, but does the V_{max} differ when it's the same enzyme?

No, V_{max} only reflects **the catalytic activity of the enzyme**, it does **not** depend on the **substrate**. However, **K_M differs** since it **depends** on the substrate.
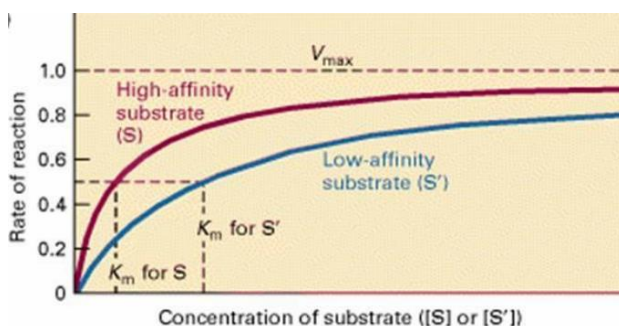
A reaction is catalyzed by an **enzyme** with **2 different** substrates, **S** *(high affinity)* and **S'** *(low affinity).*

Vmax is the same *(depends on the enzyme),* but **K_M** *(depends on the substrate)* **differs.**

S' has a **higher** K_M because it has a **lower** affinity.

Please see the last page for further explanation.

Example: A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, what is the V_{max} and K_M of this enzyme?

V_{max} is the value when the **velocity** changes are **very minimal**.

V_{max} is approximately **700**.

K_M is the substrates' concentration at which V_o **is half maximal**.

➔ $V_o = 700$
   $1/2\ V_o \approx 350$
   $K_M = [S] = 8$

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

Dissociation constant (K_D)

K_M describes the **affinity of enzyme** for the substrate, but it's not an **accurate** measure of affinity. K_D *(dissociation constant)* is the **actual** measure of the affinity.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$
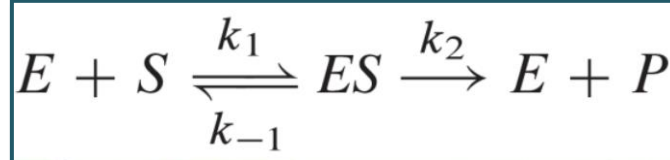
Which constants do you think are related to the affinity?

*K_2 is related to the **kinetic activity** of the enzyme to form products.*

*However, **K_1 and K_{-1}** are related to the **formation** and **dissociation** of [ES].*

➔ $K_D = \dfrac{K_{-1}}{K_1}$

Vmax and enzyme concentration

The **reaction's rate** depends on the ability of the substrate to find an active site on the enzyme. So by **increasing** the **enzymes' concentration**, the **rate** of the reaction is **increased**.

K_M, **affinity** and the **catalytic power** of the enzyme are **NOT** altered because it is the **same** substrate.

(a)
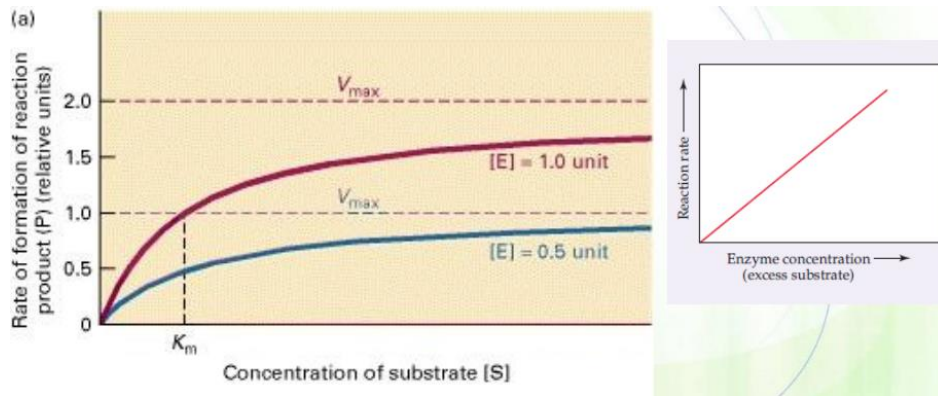
As you can see in the graph above, the concentration of the enzyme was **doubled** which caused a **proportional increase** in the **reaction rate** *(V$_{max}$ is doubled)*. The **K$_M$**, however, is **unchanged**.

K$_{cat}$

- **K$_{cat}$** *(also known as K2)* is the **turnover number**, which is the number of substrate molecules converted into product per unit time of a fully saturated enzyme.

- It describes how **quickly an enzyme acts** *(how fast the ES complex proceeds to E + P, check the equation).*

- In other words, the maximal rate, **V$_{max}$**, reveals the K$_{cat}$ of an enzyme if the total concentration of active sites **[E]T** is known, using the following equation:

$$Kcat = \frac{Vmax}{[E]total}$$

- K$_{cat}$ means, the number of reactions that can happen per second per one catalytic molecule.

- **Each enzyme has its own Kcat. Example:** Catalase's K$_{cat}$ = 40 *10^6

➔ 40 *10^6 molecules of H$_2$O$_2$ are **converted** to H$_2$O and O$_2$ by **one catalase** molecule within **one second**.

Turnover Numbers (k_{cat}) of Some Enzymes		
Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H$_2$O$_2$	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

Note that K$_{cat}$ is directly proportional to the efficiency of the enzyme.

Questions

1) An enzyme has a **molecular weight of 50,000 g/mol**. **10 µg** was used 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.
   The turnover number *(Kcat)* for the enzyme is:

MW = 50,000 g/mol

Weight = 10^{-5}g

➔ $[E]_T = \dfrac{10-5\ g}{50,000\ g/mol} = 2 * 10^{-10}$

V_o = 9.6 * 10^{-6} mol per min = 1.6 * 10^{-7} s^{-1}

$$K_{cat} = \frac{V_{max}}{[E]total} = \frac{1.6 * 10-7}{2*10-10} = 800/s$$

2) Calculate the **Kcat** for a **10^{-6} M** solution of carbonic anhydrase which catalyzes the formation of **0.6 M H_2CO_3 per second** when it is **fully saturated** with substrate.

$$K_{cat} = \frac{V_{max}}{[E]total} = \frac{0.6}{10^{-6}} = 6 * 10^5\ s^{-1}$$

*K_{cat} calculates the **number of reactions** that occur **per unit of time**. So to know how long each reaction takes, we divide the rate by K_2.*

*__Example:__ From the previous calculation we know that $6 * 10^5$ reactions occur per second which is K_{cat} (equals K_2), so **each catalyzed reaction** takes place in a time equal to $\dfrac{1}{K2}$, which is **1.7 µs** for the carbonic anhydrase.*

*__Note:__ The **turnover numbers** of most enzymes with their **physiological substrates** fall in the range from 1 to 10^4 per second.*

Vmax **does not change**:

Same Enzyme, different substrates **(Glucose/ATP),** same products **(Glucose 6-phosphate).**
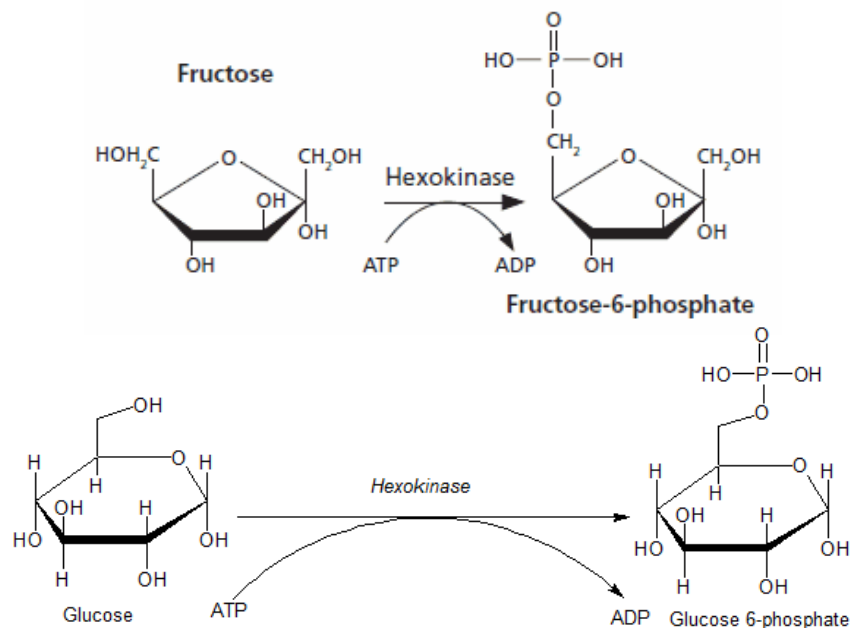
→ **Same chemical reaction**
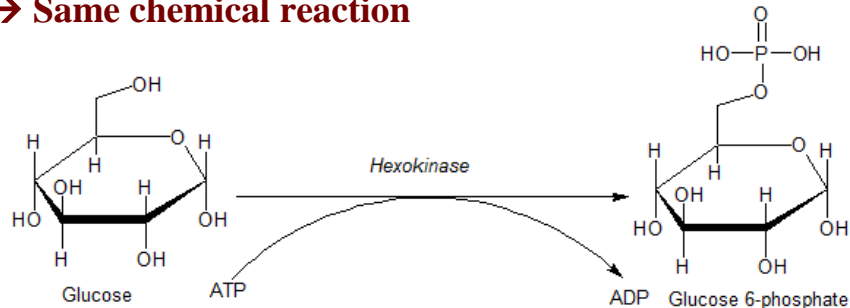
Vmax **changes**:

Same Enzyme, different substrates **(Glucose/Fructose**), different products **(Glucose 6- phosphate/ Fructose 6- phosphate).**

→ **Different chemical reaction**

Refer to Dr. Nafeths' lecture 20 record if you did not understand this point. 40:00

Good Luck ☺