20

Faculty of medicine – JU2018

◉ Sheet ○ Slides

DONE BY

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In the last lecture we said that we have two definition of km:

1- KM is the concentration of substrate at which the reaction velocity (V_0) is half of **V_{max}** – because half of the enzymes active sites are filled with substrates.

2- $V_0 = V_{max} \frac{[S]}{[S]+KM}$

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

NOTE: **k_{-1}**, **k_2** represent that the enzyme wants to **dissociate** from the substrate.

K1 represent that the enzyme wants to **bind** to the substrate.

⛩ km it is not really an accurate measure of affinity, because we have k2 which is very small in value relative to k-1 and k1.

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⛩ If you look at this equation again we have 3 cases:

$$V0 = Vmax \frac{[S]}{[S]+KM}$$

*When [S] = KM, then V0 = Vmax/2

* [S]<<<KM, which means that a very little number of Active sites are filled with substrates>> we increase **[S]** to increase the reaction rate **(dependent on substrate concentration)**

*[S]>>>KM, which means that almost all Active sites are filled with substrates- if we increase [S] there will be no free Active sites to bind with them. **(independent on substrate concentration) (V0=Vmax)**

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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

NOTES about the table above:

- The KM values of enzymes range widely (mostly, 10^{-1} and 10^{-7} M).

- The affinity of the same substrate doesn't change but for different substrates it changes, so we have an affinity of **Hexokinase** for **Glucose** which is different than its affinity for **ATP** and for **Fructose.** so we have phosphorylation of **Glucose-**by **Hexokinase**-with different affinity than phosphorylation of fructose by the same **Enzyme** -**Hexokinase**-.

- The same enzyme can bind to different substrate with different affinities. Also, with the same substrate at the same affinity.

- **V_{max}** will be the same for the same reaction of more than one substrate.

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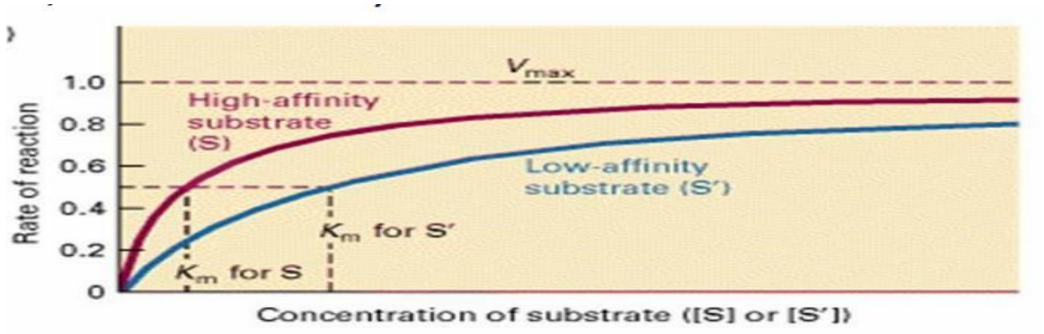
Example: Hexokinase – enzyme that phophorylates glucose

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



- For such reaction Vmax will be exactly the same because it is related to enzyme itself although the affinity of the first substrate is different than the second substrate, but Vmax will be the same for this particular reaction.

But if we have 2 reaction, for example hexokinase phosphorylates glucose and fructose at different Vmax values.

Fructose+ ATP ******* Glucose + ATP

The Vmax here of each reaction is different because each reaction generates different products.

Rates of phosphorylation of glucose and fructose in the brain

Sugar	Properties of brain hexokinase Vmax	K_M	Sugar concn in brain cell	Calculated rate of phosphorylation In vivo
Glucose	17	10^{-5}	10^{-5}	8.5
Fructose	25	10^{-3}	10^{-6}	10^{-2}

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A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately:

1- Vmax?
   a- 5000
   b- 699
   c- 621
   d- 94
   e- 700

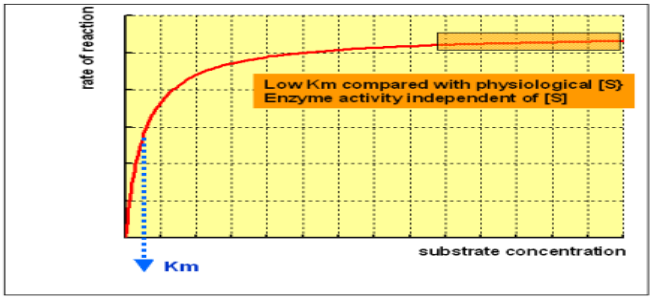
Substrate Concentration (μM)	Initial velocity (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

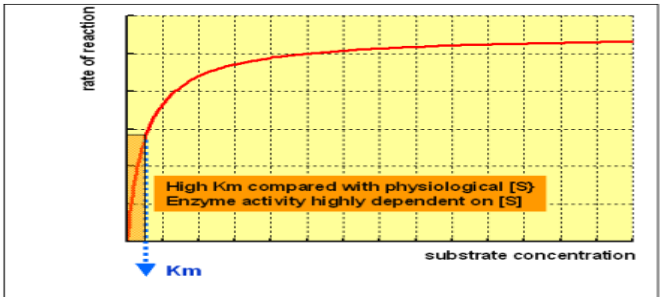
As you see in the table the velocity increases and you see a plateau and the Vmax is around 700 μmole/min.

2- Km? (you can estimate)
   a- 699
   b- 5000
   c- 50
   d- 1
   e- 8

Answer is 8 ,Why? Because we have the Vmax=700 … half of it349 … so the km=8

Remember that the km is [S] when the Vmax has half of its value.





We have 2 enzymatic reaction right here:

1- In the first one, Km is much lower than the [S] , so in this case when the [S] is higher than km physiologically any variation in [S] doesn't really affect the rate of the reaction.(The enzyme is normally saturated with substrate).
2- In the second case, the [S] much lower than km, so any variation in [S] will really make huge different in the rate of the reaction. (The enzyme is not saturated with substrate).

Let's say that we have hexokinase that catalyzes 2 reactions:

Glucose + ATP ⇨ Glucose-6-phosphate

Fructose + ATP ⇨ Fructose-6-phosphate

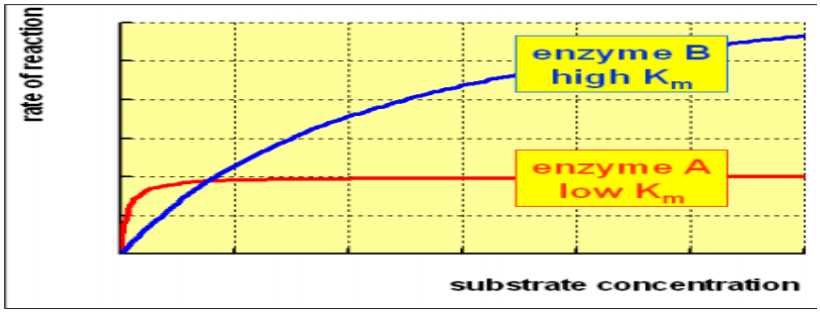
I said that the Km &Vmax are different in the 2 reactions.

NOTE: the Vmax is the same for one reaction only not for more than one reaction with same enzyme.

10:46

Let's say that we have a substrate like Glucose-6-phosphate which is can go through different pathways; it can go through formation of glycogen, it can go through glycolysis forming pyruvate at the end, it can go through another pathway known as pentose phosphate pathway produces 5 carbon sugar, it can backward into glucose, it can be released from lever cells outside peripheral tissues, AND each one of these pathways are catalyzed by a certain enzyme and each enzyme has different Vmax & different Km value. And each one has a different plot, ok

all of them are hyperbolic but the values are totally different, look at the picture below for understanding:



The blue one ⇨ (high km value ⇨ low affinity) & (Vmax is really high ⇨ high efficiency).

The red one ⇨ (low km value ⇨ high affinity) & (Vmax is really low ⇨ not efficient as the first enzyme).

NOTE: the higher the efficiency of the enzyme, the higher the Vmax.

⛫ I said that the Km is the reflexion of affinity, but it is not really an accurate measure of affinity because we have k2 as we said before.

KD= $\frac{k_{-1}}{k1}$ we neglected **k2** as it has a very low value.

⛫ USES OF KM:

1- **Determine the substrate preferences of an enzyme**.
   If more than one endogenous compound can act as a substrate for an enzyme, the substrate with the lowest Km is probably the preferred physiological substrate. (low km means higher affinity).
2- **Distinguishes isozymes, which are different enzymes catalyzing the same reaction.**
   Isozymes often have different affinities for the same substrate.
3- **Check for abnormalities in an enzyme**

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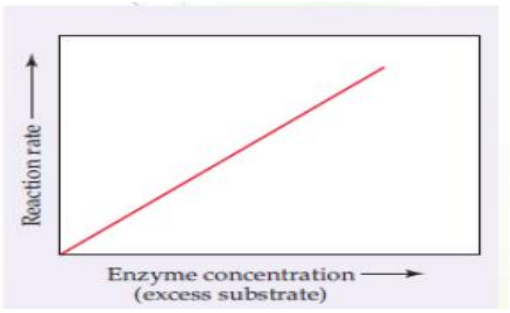
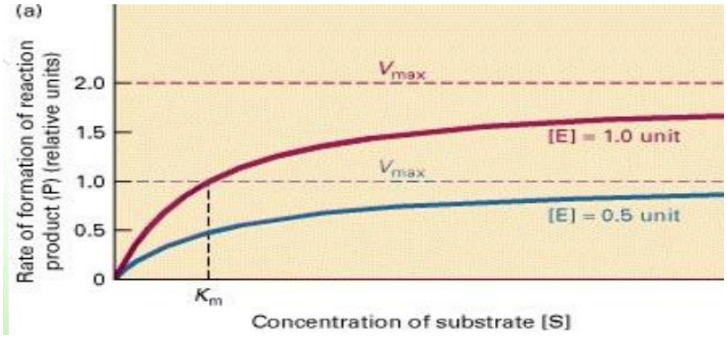
⛫The question is now, what is the effect of increasing of the enzyme concentration in any enzymatic reaction?

ANSWER: logically we said that Km is not different because the affinity of each enzyme exactly still the same.

*What about Vmax??

If you increase the enzyme conc. the rate of the reaction will be increased because the enzyme will find the substrate much easier.





So, as you see. Doubling the concentration of enzyme causes a proportional increase in the reaction rate but km exactly the same.

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(A measure of enzyme efficiency)

Vmax is not a measure of an enzyme efficiency because it varies according enzyme concentration!

actually, k2 is the measure of enzyme efficiency, and we can calculate K2 which is known kcat (catalytic constant) using this formula:

$$V_{max} = k_2 [E]_T$$ (Vmax depends on efficiency& enzyme conc)

If you double the [E], Vmax will be doubled but the K2 is constant!

Because the efficiency of the enzyme should not change at the same affinity (substrate).

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

ABOUT THE TABLE ABLOVE: (OF COURSE DON'T MEMORIZE)

Let's see what these numbers mean:

Kcat of Catalase= 40,000,000, which means that it can catalyze 40,000,000 reactions per second (by a single enzyme).

Kcat of RecA protein=0.4 , (it is not efficient), which means that it can catalyze around half reaction per second (by a single enzyme).

kcat, the turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated. kcat = Vmax/ [E]T

It describes how quickly an enzyme acts, i.e. how fast the ES complex proceeds to E + P.(EFFICIENCY)

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EXAMPLES

1-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. The turnover number (kcat) for the enzyme is:

NOTES: At best means Vmax.

M.W=50,000 g/mol mass of the enzyme= 10µg Vmax= 9.6/60 = 0.16 µmol/sec

Kcat = Vmax/ [E] = 0.16/ (10/50000) = 800 s^{-1}

3- A 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H2CO3 per second when it is fully saturated with substrate.

ANSWER: Kcat = Vmax/[E] = 0.6 /10^{-6} = 6 x 10^{5} sec^{-1}

6 x 105 x 60 sec/min = 3.6 x 107 min^{-1}

1 / 3.6 x 107 = 2.7 x 10^{-6} min per reaction

NOTES:

1- Each catalyzed reaction takes place in a time equal to 1/k2, which equals to 2.7 μs for carbonic anhydrase.
2- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second.

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Kcat vs. KM

- **Kcat** is **related** of the efficiency.
- **Km** is the **reflexion** of affinity.

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}

- Let's say that the enzyme has really high **affinity(low km)** for substrate but not really **efficient(low Kcat)**, so it binds to substrate with a high affinity but it needs more time to convert the substrate into products.

- But if it has a really low affinity (**high km value**) and high efficiency (**high Kcat value**) it converts substrates into products with high efficiency(**very little time**).

- so, there are **2 factors** that plays a major role in how good an enzyme is; It has to have high affinity to substrate and has to be really efficient in converting substrate into product.

- Simply by taking a value of Kcat/km that gives a really a good indication of how good the enzyme is. So, these values are really important.

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⛩ There are number of **enzymatic terminologies** you need to know in terms of calculations:

1- Rate of reaction (velocity)

Rate of reaction is calculated as (concentration of substrate disappearing OR concentration of product appearing) per unit time within a certain volume. (mol L^{-1}. sec^{-1} OR M. sec^{-1}).

2- Enzyme activity

In order to measure enzymatic activity, we measure (the number of moles of substrate disappearing OR products appearing) per unit time. (mol. sec^{-1}).

IN OTHER WORDS

enzyme activity = rate of reaction × reaction volume

3- Specific activity

Specific activity is usually a measure of enzyme purity and quality in a sample. It should be the same in any enzyme.

It is described as moles of substrate converted per unit time per unit mass of enzyme (mol. sec^{-1}. G^{-1}).

Specific activity =enzyme activity / mass of enzyme (grams)

This is useful in determining enzyme purity after purification. It is also used when the molar concentration of the enzyme is unknown.

(If the moles of the enzyme present are unknown, it is impossible to calculate kcat)

EXAMPLE ON THIS TOPIC

Let's say that you give me a powder and you tell me that I have an enzyme in this powder, then I asked you how pure is it?

Maybe 50% of powder is enzyme

NOW I know the specific activity of the enzyme, so by considering the mass of the enzyme in this powder I can tell you how pure this enzyme is.

- If I get the same specific activity it means that this powder is really pure (all of it is an enzyme).
- If I get half of the specific activity then I know that the powder is not really pure (it contains things other than enzyme).

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4- Turnover number (Kcat)

*Turnover number (kcat) is related to the specific activity of the enzyme where it is.

*Turnover number = specific activity × molecular weight of enzyme

* It is expressed as moles of substrate converted into product per unit time (usually per second)/moles of enzyme (min^{-1} or sec^{-1})

Remember: kcat = Vmax/ $[E]_T$

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Sample calculations:

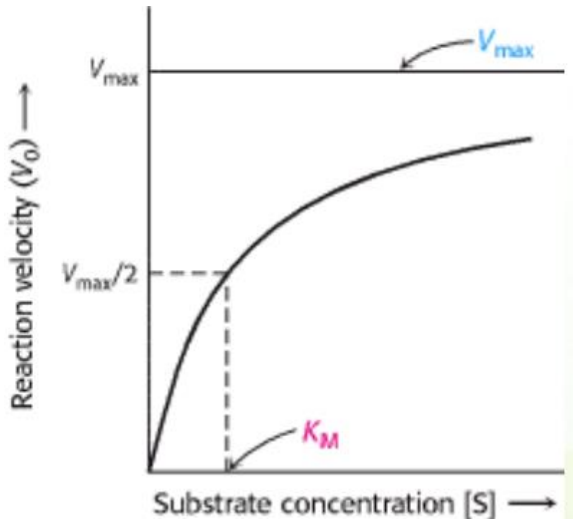
A solution contains initially **25.0 × 10^{-4} mol L^{-1}** of peptide substrate and **1.50 µg chymotrypsin**, in **2.5 mL** volume. **After 10 minutes, 18.6 × 10^{-4} mol L^{-1}** of peptide substrate remain. Molar mass of chymotrypsin is **25,000 g mol^{-1}**.

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

THE DOCTOR SAID that in the exam I will give you a simple mathematics

Disadvantage of Michaelis-Menten equation

There is a problem in **Michaelis-Menten equation;** in reality you cannot reach Vmax, in order to reach Vmax you need a very high concentration of substrate which is **impossible** for a such reaction to be done in laboratories so Km from hyperbolic plots is not accurate, so in this **case you cannot measure Vmax!**



ʃYou can calculate Km then you know how much Vmax.

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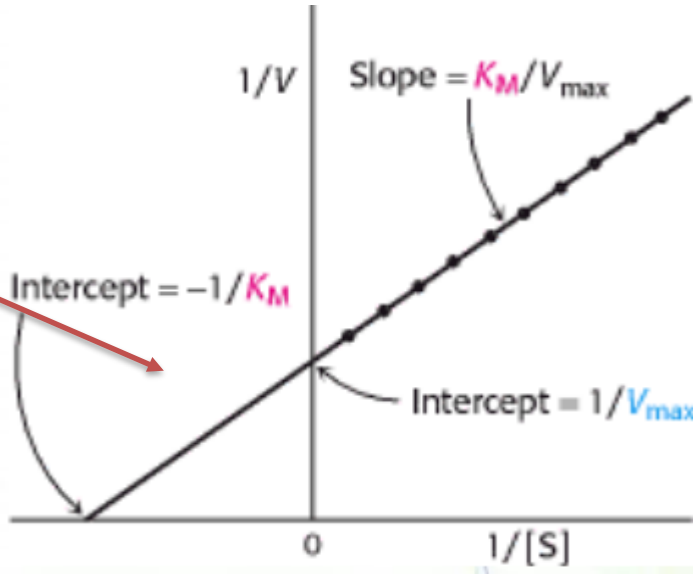
There are 2 scientists **The Lineweaver-Burk** said that the simplest thing that we can do is to draw a straight line like this



- The intercept on the x-axis is -1/KM (minus because you cannot get 1/[S] negative)
- The intercept on the y-axis is 1/Vmax
- And then we convert the **Michaelis-Menten equation** into this equation:

$$\frac{1}{V0} = \frac{1}{Vmax} + \frac{Km}{Vmax} * \frac{1}{[S]}$$

This formula right here is similar to: y=b+mx

y is y-axis = 1/ V0

x is x-axis = 1/[S]

m is slope = KM/ Vmax

B is 1/Vmax

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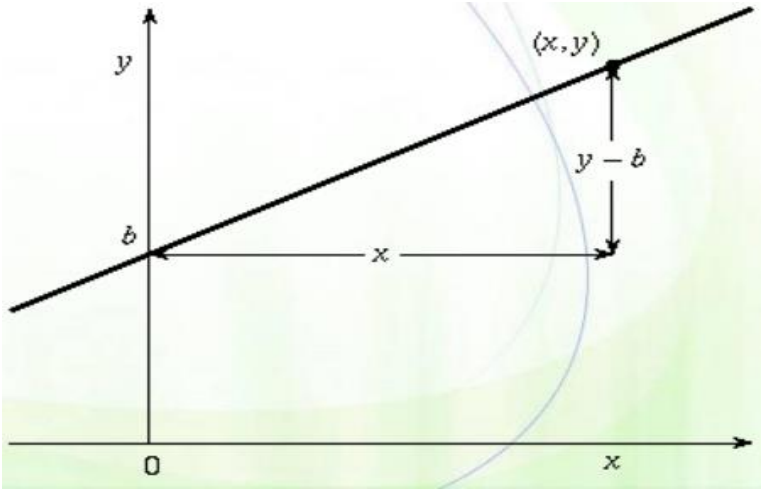
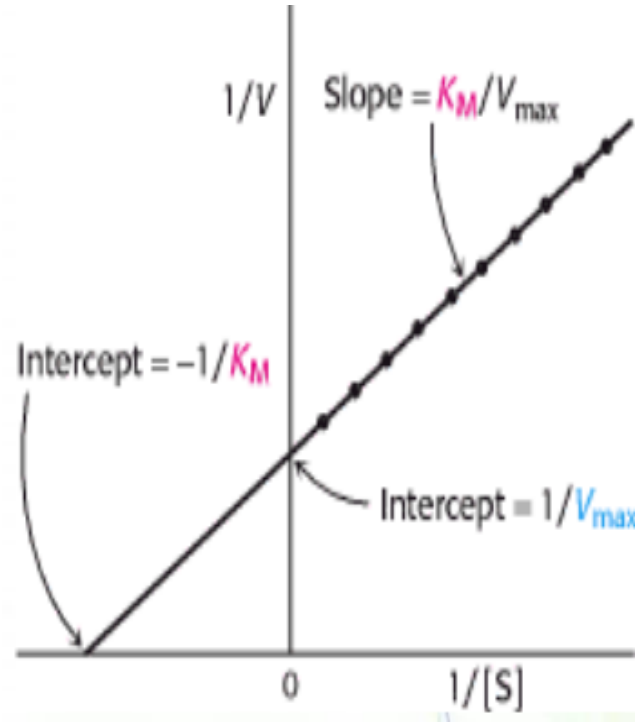




According to this equation:

$$\frac{1}{V0} = \frac{1}{Vmax} + \frac{Km}{Vmax} * \frac{1}{[S]}$$

If we assume that x=0 so 1/[s]=0 then km/Vmax=0

Finally 1/V0=1/Vmax (y=b)

According to this equation:

$$\frac{1}{V0} = \frac{1}{Vmax} + \frac{Km}{Vmax} * \frac{1}{[S]}$$

If we assume that y=0 then

mx = -b

0 = 1/Vmax + (KM/Vax) . (1/[S])

-1/Vmax = (KM/Vax) . (1/[S])

-1 = KM . (1/[S])

you need to know how you can calculate Km, Vmax from this equation of this plot and again it will be simple calculations.

Regulation

Let's talk about regulation, this is a really important topic, you need to know a different mechanism of regulation whichs help you in metabolism.

There are different strategies that the body use to regulate the enzyme activity:

1-Expression of isoenzymes.

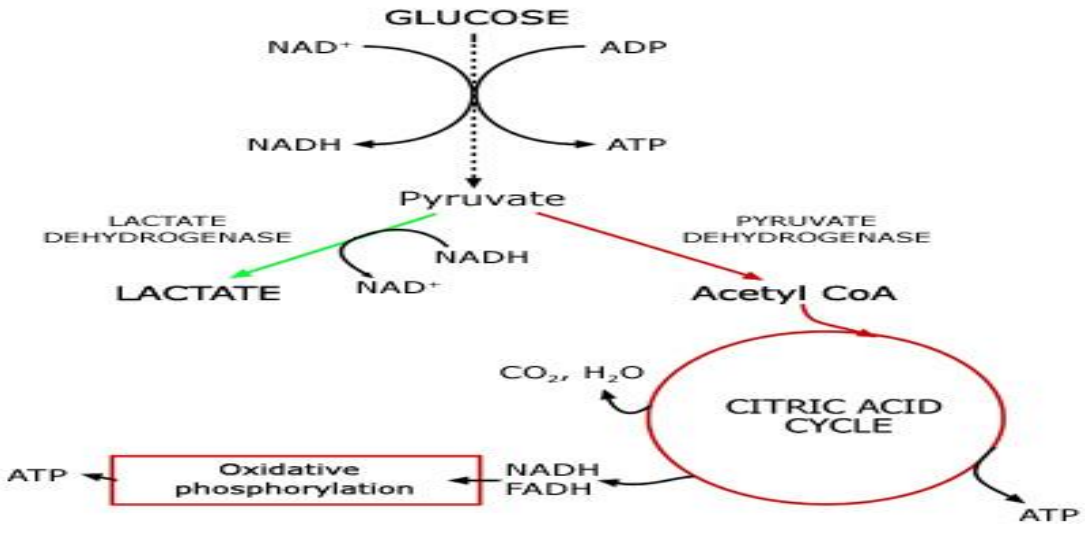
2-Regulation of enzymatic activity:

a- Inhibitors
b- Conformational changes (changing the structure of enzyme).
c- Allostery (like hemoglobin even it is not an enzyme but it is really nice protein to study how allosteric enzymes work).
d- Modulators
e- Reversible covalent modification
f- Irreversible covalent modification

3-Regulation of enzyme amount

4-Location (Compartmentalization and complexing of enzymes) (location of enzyme in such organelle)

5-Non-specific regulation



- Glycolysis: which is actually conversion of glucose into pyruvate.

Then the cell makes a decision

1- **If there is an oxygen in the cell**, it will go through **Aerobic metabolism,** where is the pyruvate is converted into acetyl CoA which undergoes into **citric acid cycle**, and here we have production of some ATP as well as some electron carriers. these electron carriers go through oxidative phosphorylation & electron transport chain generating a lot of ATP.

2- **If there is not enough oxygen in the cell**, the pyruvate is converted into lactate **(anaerobic metabolism)**.
This happens in skeletal muscle cells that convert pyruvate into lactate.

- But there are some tissues like cardiac muscles that cannot function anaerobically.

It can only function aerobically. So, when lactate is released from muscles and goes through blood circulation to the heart, **PH** of the blood will be lowered.

- to pass through aerobic metabolism. lactate is converted into pyruvate using the same enzyme (lactate dehydrogenase), that is used for the reversible reaction, by heart muscles.

This is harmony between different organs

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Isoenzymes (isozymes)

- We will focus on **lactate dehydrogenase** which is a good example of isoenzymes.

1- Isoenzymes are enzymes that can act on the same substrate(s) producing the same product(s).

2- They are produced by different genes that vary only slightly (they are really different).
3- Often, various isozymes are present in different tissues of the body.
4- They can be regulated differently.
5- They can have different catalytic activities.

So, we have one isoenzyme work efficient than another, we have one isoenzyme regulated differently than another.

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Lactate dehydrogenases (LDH)

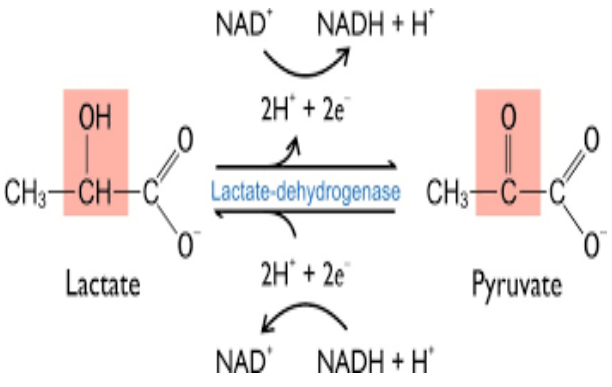
LDH is a tetrameric (4 polypeptides) enzyme composed of a combination of one or two protein subunits (FROM 2 DIFFERENT GENES) H (**Heart**) and M (**Skeletal muscle**).

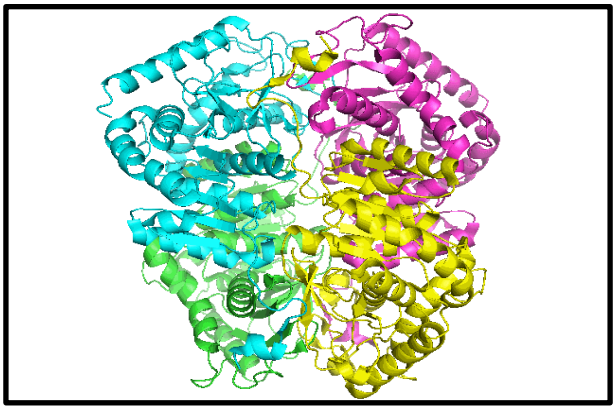
These subunits combine in various ways leading to leading to 5 distinct isozymes (LDH1-5) with different combinations of the M and H subunits. (1M3H, 3H1M 2M2H, ALL M, ALL H).

all **H isozyme** is characteristic of that from **heart** tissue, and all **M isozyme** is typically found in **skeletal muscle and liver**.

The question is what is the **action** that LDH catalyze?

Oxidase lactate to pyruvate producing NADH OR takes pyruvate and converts it to lactate.





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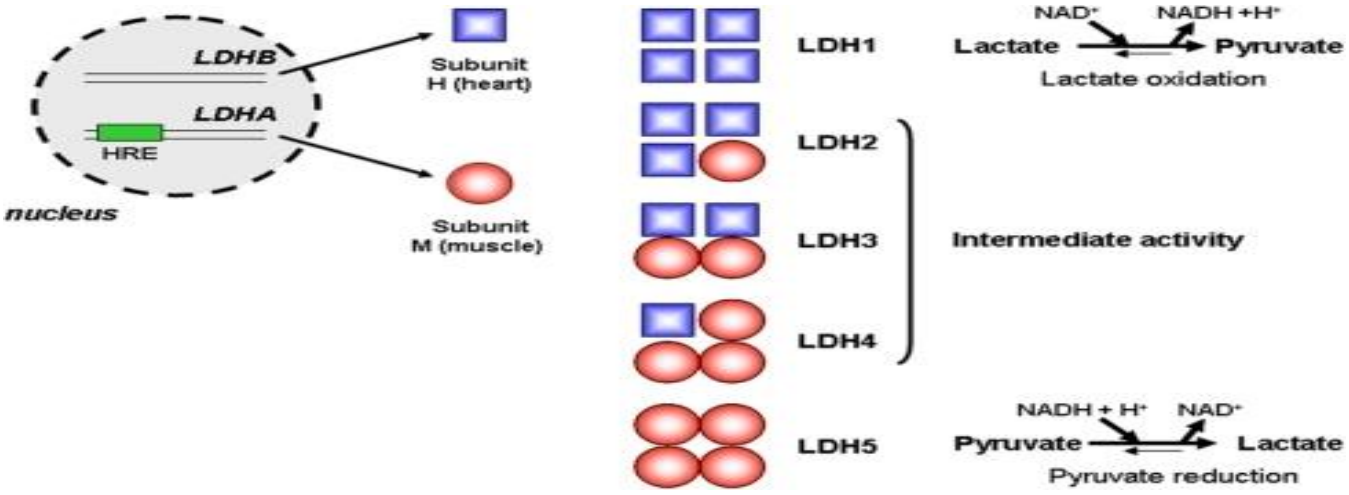

- Heart LDH can catalyzes the reaction of **lactate** to **pyruvate.**

- LDH take pyruvate to lactate and heart muscle can do the opposite but this is really rare.

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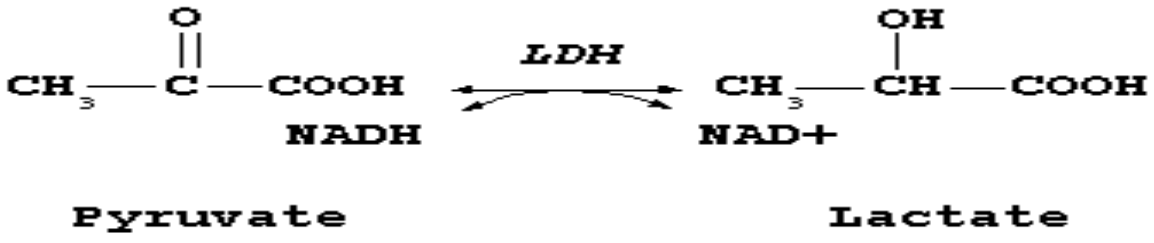
NOT for memorize

Isoenzyme	Structure	Present in	Elevated in
LDH1	(H_4)	Myocardium, RBC	myocardial infarction
LDH2	(H_3M_1)	Myocardium, RBC	
LDH3	(H_2M_2)	Kidney, Skeletal muscle	
LDH4	(H_1M_3)	Kidney, Skeletal muscle	
LDH5	(M_4)	Skeletal muscle, Liver	Skeletal muscle and liver diseases



- Muscles can function anaerobically, but heart tissues cannot.

⊓ Whereas the all M isozymes (M4) function anaerobically and catalyze the reduction of pyruvate into lactate, all H enzyme (H4) functions aerobically and catalyzes the reverse reaction.

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Regulation of LDH

⊓ H4 LDH has a low K_m (high affinity) for lactate and is inhibited by high levels of pyruvate..

- The H4 isoenzyme favors the oxidation of lactate.
- Heart muscle inhibited by high level of pyruvate (if it converts high lactate into pyruvate the cell says enough I don't need more pyruvate, get out or go to other tissue).

⊓The M4 LDH enzyme has a high K_m (low affinity) for pyruvate and is not inhibited by pyruvate.

⊓ It converts pyruvate into lactate only when there is a high level of it.

⊓ M4 LDH is always active even at high levels ensuring that pyruvate is always funneled to anaerobic metabolism.

Good luck my colleagues