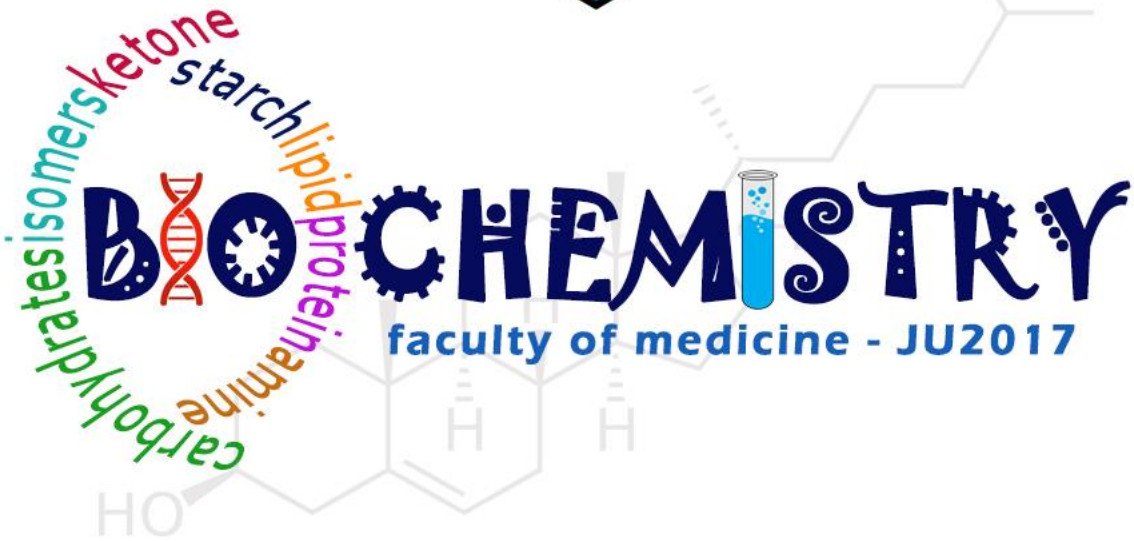
21



◉ Sheet ○ Slides

DONE BY

Ali Alabbadi

CONTRIBUTED IN THE SCIENTIFIC CORRECTION

Tamer Barakat

CONTRIBUTED IN THE GRAMMATICAL CORRECTION

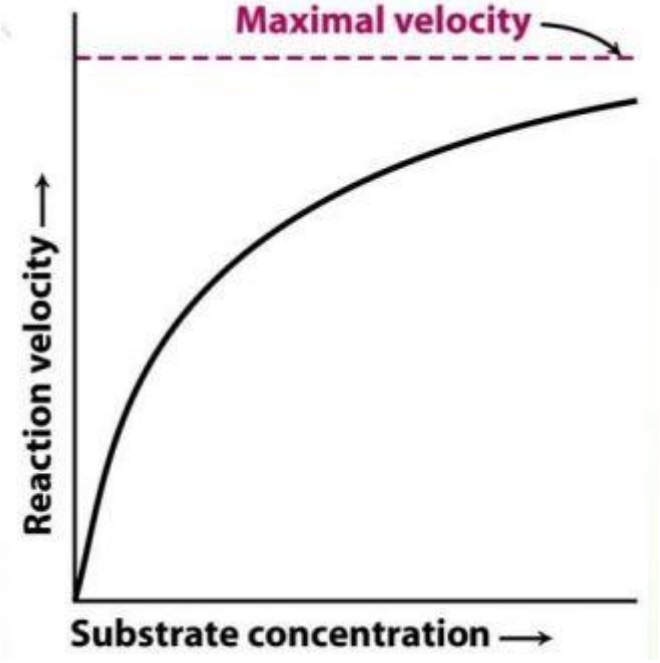
Farah H. Al-Bustanji

DOCTOR

Dr. Nafed

Review:

- Enzyme kinetics is the study of chemical reactions that are catalyzed by enzymes.
- The relationship between the reaction's velocity (rate) and concentration of reactant(s) is represented by rate law => **v = k[A]**
- k is a constant and its units depend on the reaction; it can have a unit of $time^{-1}$ when there is only one reactant, or it can have a unit of $time^{-1} \times concentration^{-1}$
- for any reaction involving enzymes a graph can be used to analyze their kinetics



*plots with simple enzymes follow a hyperbolic path, with the upper limit being the maximum velocity of the reaction (V_{max})

- the Michaelis-Menten equation describes the graph of the enzyme kinetics

$$Vo = \frac{V_{max}[S]}{Km + [S]}$$

V0 = Initial velocity (Molar /times)
[S] = substrate concentration (molar)
V_{max}= maximum velocity
K_m = substrate concentration at half V_{max}

K_M:

For a reaction:

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

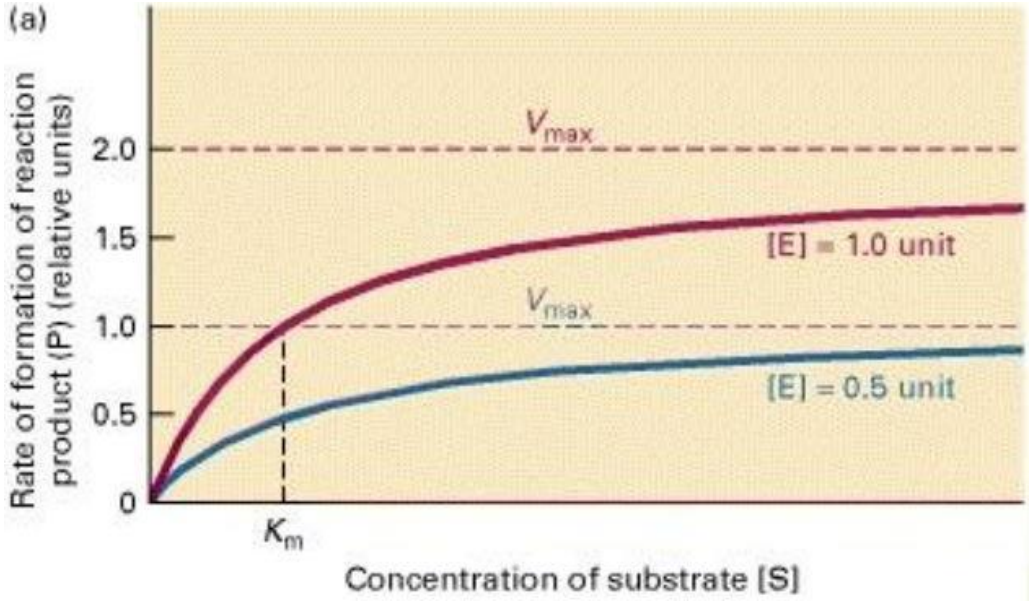
$$K_M = \frac{k_{-1} + k_2}{k_1}$$

K_M describes the affinity of an enzyme to its substrate

- this is because it sums the rate of dissociation of the enzyme substrate complex (k_{-1} and k_2) divided by the rate of formation of the enzyme substrate complex (k_1)
    - therefore, the smaller a K_M value is, the higher the affinity of the enzyme.
- remember: K_M is equal to the amount of substrate concentration needed to reach $V_{1/2}$, so the smaller concentration needed to reach $V_{1/2}$, the higher the enzyme's affinity.
- despite this, a better way of describing the affinity is by ignoring k_2, and only using k_1 and k_{-1}: this value is called K_D (dissociation constant)

V_{max}:

- the V_{max} is not affected by the substrate concentration, and the only way to affect it is to change the enzyme concentration.



- think of it like this, a car has a maximum speed, the only way to increase its maximum speed is to increase the power of the engine.
    - in relation to K_M, it is <u>not</u> affected by the enzyme concentration, because it is an inherent property of the enzyme; in order to reach half of the maximum speed in a slow car or a fast car, you need to press the gas pedal halfway down.
- K_{cat} (turnover number) is a value that describes how quickly one enzyme can act
    - It is the concentration of substrate molecules converted to product per unit time when the enzyme is fully saturated.
    - $K_{cat} = V_{max} / [Enzyme]_T$
    - K_{cat} is a constant for any given enzyme, meaning changing the enzyme concentration will affect V_{max}, but not K_{cat}.
    - The unit for K_{cat} is seconds^{-1} and it tells you how many reactions an enzyme can catalyze per unit time, which is usually seconds.

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}

For example, according to this graph lysozyme can do 0.5 reactions per second, while catalase is able to do 40 million reactions per second.

<u>Enzyme Activity:</u>

- Remember: rate of reaction is the change in concentration per unit time
- In order to measure enzyme activity, we measure the number of <u>moles</u> (not molars) of substrate disappearing or product forming per unit time.
    - Therefore, enzyme activity has a unit of moles/time
- In order to get rid of the volume in the concentration, we can multiply the rate of the reaction by the volume in order to get the enzyme activity.
    - This is some dimensional analysis for you to understand what happens to the units

Enzyme activity = rate of reaction × reaction volume

$$\frac{Moles}{time} = \frac{moles}{\cancel{volume} \times time} \times \cancel{volume}$$

Moles/time = moles/time

Enzyme Specificity:

- It is a measure of enzyme purity and quality
- It is calculated as moles of substrate converted per unit time per unit mass of enzyme (moles / (time × mass)
    - $Specific\ activity = \frac{enzyme\ activity}{mass\ of\ enzyme(grams)}$

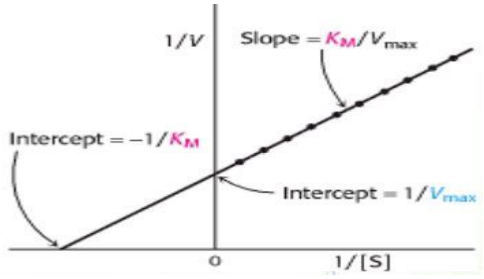
Turnover Number:

- K_{cat} is related to specific activity
    - K_{cat} = specific activity × molecular weight of the enzyme

Lineweaver-Burk Equation:

Realistically there are disadvantages in using a hyperbolic plot in real world situations, in our case it is because the enzyme velocity that is determined experimentally will never reach V_{max} and this is due to the properties of hyperbolic plots. Because we can never determine V_{max} experimentally, we cannot determine $V_{1/2}$ and therefore K_M. In order to start having more accurate values, two scientists named Lineweaver and Burk started plotting the reciprocal of the Michaelis-Menten equation, and this resulted in a linear graph.

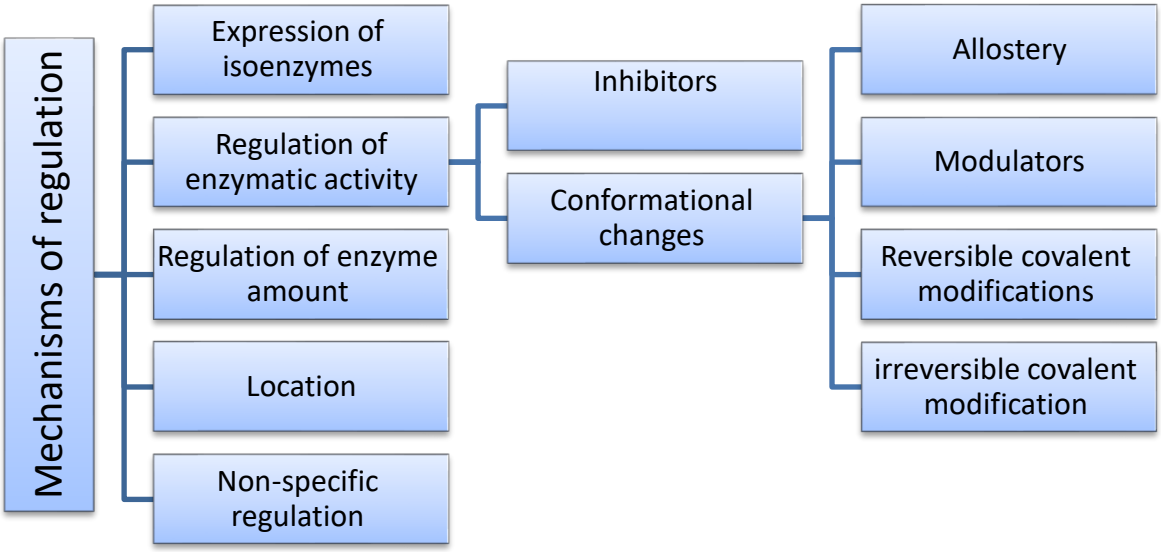
Note: Even if we want to approximate Vmax using Michaelis-Menten equation, we would need to repeat the experiment a lot of times to get an accurate value (by approximation), which would need a lot of money for one enzyme.



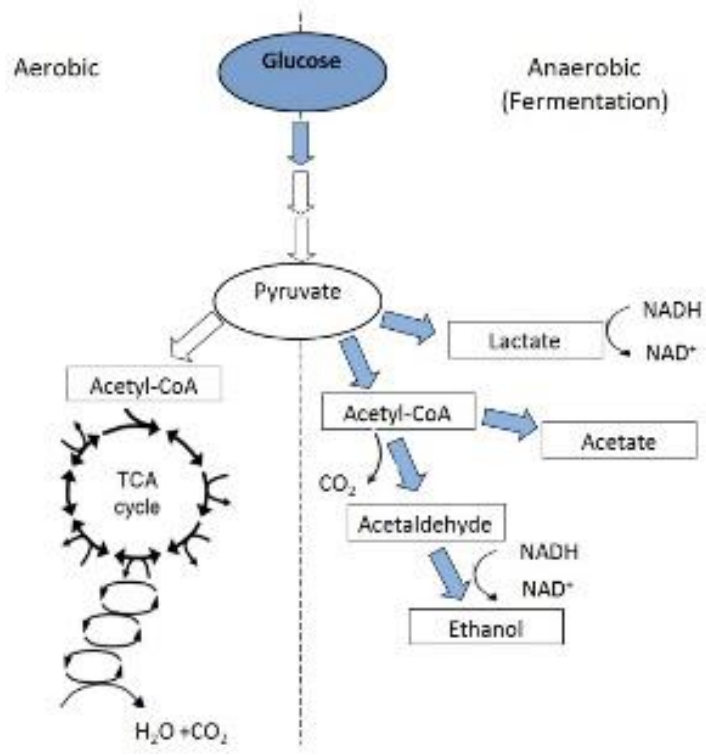
- This is the graph of $\frac{1}{V0} = \frac{1}{Vmax} + \frac{KM}{Vmax} \times \frac{1}{[S]}$
- This plot is also known as the double reciprocal plot.

- It follows the standard graph of linear equations y = mx + b, with the y-axis being 1/V, and the x-axis being 1/[S]
    - Because of this, we can say with confidence that the slope is equal to K_M/V_{max}, the Y-intercept is equal to $1/V_{max}$, and the X-intercept is equal to $-1/K_M$

Mechanisms of Regulation



Aerobic vs. Anaerobic Respiration:



- In the case of anaerobic metabolism, the enzyme that converts pyruvate to lactate and vice versa is called lactate dehydrogenase; during anaerobic respiration in the skeletal muscles, pyruvate is converted to lactate, but in the cardiac muscles, lactate is converted back to pyruvate by the same enzyme, why?
- They are the same enzyme, but are slightly different from each other, and they are defined as **isoenzymes**

Isoenzymes:

- Isoenzymes are enzymes that can act on the same substrate and produce the same product.
- They are produced by different genes that vary only slightly.
- They are distributed in different tissues (like in cardiac and skeletal muscles).
- Different methods of regulation, and different catalytic activity.

Example: Lactate Dehydrogenase (LDH):

- LDH is a tetramer, meaning it has 4 subunits in its quaternary structure
- They have two different types of subunits, M for skeletal muscle and H for heart muscle
- The subunits combine in different ways to form 5 different isoenzymes (LDH 1-5)
- 2 of the 5 enzymes are homotetramers (only H or only M subunits), the rest are heterotetramers
    - The H-subunit homotetramer does lactate oxidation, converting lactate to pyruvate, while the M-subunit homotetramer does pyruvate reduction, converting pyruvate to lactate, they are both able to do both reactions but their efficiency is more towards their respective reaction
        - The LDH found in the heart is much better at doing lactate oxidation, and the LDH found in the skeletal muscle is much better at doing pyruvate reduction

Have fun