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THE KINETICS OF ENZYMATIC REACTIONS:

If you want to study any biochemical reaction you can study it from two points of view:

thermodynamically: (where you can study ΔG of the reaction, the starting and ending points of the reaction, and deal with potential energies of the reaction).

kinetically: (where you can study the reaction within the ending and starting points or within a specific period of time. Also you can study the path of the reaction, the steps of the reaction and the velocity [rate] of the reaction), all of the above-mentioned definitions are working effectively to change ΔG of the reaction.

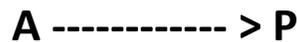
Biochemical kinetics: the science that studies rates of chemical reactions.

- If you want to define the rate or the velocity of something, obviously you will look for the change of that thing divided by the time >>> so in case of the rate [velocity] of the reaction we are looking for the change in the concentrations of the reactants or products divided by time.

➤ Biochemical Kinetics: the science that studies rates of chemical reactions
➤ An example is the reaction ($A \rightarrow P$), The velocity, v , or rate, of the reaction $A \rightarrow P$ is the amount of P formed or the amount of A consumed per unit time, t . That is,

in following chemical reaction:

$$v = \frac{d[P]}{dt} \quad \text{or} \quad v = -\frac{d[A]}{dt}$$



The rate can be calculated by $v = d[P]/dt$ or $v = -d[A]/dt$

- Applying arithmetic operations to this equation is not a simple matter because of the presence of integration, and in this case the result must be found by integrating the area under the curve, In order to facilitate these steps, the scientists noticed that this curve, which contains the required area under it, increases by a fixed amount. Therefore, they replaced the integration with a fixed value (K), which led to a change in the reaction rate law to the following:

$$\text{Rate} = k * [A]$$

In general:

the reaction rate law = $K[A]^{n1}[B]^{n2}[C]^{n3}$

K : the rate constant (the higher the activation energy -energy barrier- the smaller the value of K .)

$(n1+n2+n3)$: the overall order of the reaction. I.e: $n1$ the order of reactant A , $n2$ the order of reactant B , $n3$ the order of reactant C .

- The rate is a term of change over time
- The rate will be proportional to the conc. of the reactants
- It is the mathematical relationship between reaction rate and concentration of reactant(s)
- For the reaction ($A + B \rightarrow P$), the rate law is

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

- From this expression, the rate is proportional to the concentration of A , and k is the rate constant

THE ORDER OF THE REACTION:

- When any reaction occurs, it is logical that the concentrations of all the reactants contribute to an increase or decrease in the rate of the reaction. However this case may not be necessary and there may be exceptions in which not all reactants participate in contributing to the rate of the reaction. -These exceptions will be mentioned and explained later-So if you have one reactant ($A \rightarrow C$) $\text{Rate} = k[A]^{n1}$ // // // // 2 reactants ($A+B \rightarrow C$) $\text{Rate} = k[A]^{n1} * [B]^{n2}$.

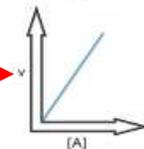
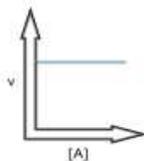
And if we have 3 or 4 , or 5 ... reactants the rate law will be written in the same way because the rate of the reaction depending on every single reactant which make a participation in the reaction.

- A multistep reaction can go no faster than the slowest step

$$v = k(A)^{n1}(B)^{n2}(C)^{n3}$$

- k is the rate constant: the higher the activation energy (energy barrier), the smaller the value of k
- $(n1+n2+n3)$ is the overall order of the reaction
- Dimensions of k

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



How do we derive the unit of constant K ?

When the overall order is 0:

$$V=K \gg M/S = K \gg K = M * S^{-1}$$

WHERE M(MOLARITY) S(SECONDS)

when the overall order is 1:

$$V=K*[A] \gg M/S = K*M \gg K=1/S \gg K=S^{-1}$$

- So when we say that the overall order for the rate of the reaction is zero that's mean the concentration of the reactants doesn't effect the rate of the reaction so $V=K$ (constant value) mathematically :

$$V = K * [A]^0$$

$$V = K * 1$$

$$V = K$$

- When we say that the overall reaction order is equal to one, this means that the rate of the reaction depends on the concentration of one reactant, and that does not mean that the reaction contains only one reactant. It is possible that the reaction contains more than one reactant, but the rate of that reaction depends on the concentration of only one of them. Mathematically :



$$V = k[A]^0 * [B]^1 * [C]^0$$

$$V = k * 1 * [B] * 1$$

$$V = k[B]$$

- What is used to determine the reactants with the required concentrations that affect the rate of the reaction? First when the overall reaction order is zero :

When you increase the concentration of reactants in this case you are increasing the velocity of the reaction but there is a limit for this increasing process this limit becomes when we have saturation or over saturation for the whole active sites of the enzymes.

- recall: In order for the enzymatic reactions to take place correctly reactants must be close to each other and putting them in the exact orientation for the active site.

بالعربي : لنفترض أنو عنا 10 مواقع نشطة و عدد كبير من المواد المتفاعلة و كل موقع نشط لإنزيم يعمل على تحويل مادة متفاعلة واحدة إلى نواتج خلال ثانية واحدة , عند ارتباط المادة الأولى بموقعها كم تكون السرعة ؟ أكيد 1 ث و عند ارتباط المادة الثانية بموقعها كم بنكون حولنا خلال ثانية واحدة ؟ مادتين متفاعلتين بمعنى آخر زدنا من سرعة التفاعل , و هكذا حتى نصل إلى المادة المتفاعلة رقم 10 في هذه اللحظة يكون قد تم نقل 10 مواد متفاعلة خلال ثانية و واحدة و بعدها يصبح تقريبا عدد المواقع النشطة و عدد المواد المتفاعلة تقريبا متساوية في هذه اللحظة تزداد سرعة التفاعل و لكن بمعدل أقل من السابق و عند حدوث الاشباع او فوق الاشباع للمواقع النشطة تدخل سرعة التفاعل في مرحلة الثبات.

Second when the overall reaction order is one:

Here, in this case, it was discovered by certain experiments in the laboratory, where the properties of each reactant and its impact on the reaction are known. First, the reaction takes place in the presence of enzymes, then we put an excess of a specific reactant and exclude a substance and surround the reaction with all the factors that guarantee its success, as the substance that was made its exception is the substance that we want to study. When the excluded substance is placed in the reaction, the reaction occurs efficiently, and from here we know the impact of this substance on the reaction. These reactions, which are controlled to make them first order reactions, while they aren't are called pseudo first order reaction. So we do a lot of these pseudo first order reactions so we can be able to study them.

Enzyme kinetics:

The two experts, Michaelis and Menten, began studying and observing the behavior of enzymes in their solutions and reactions, and they conducted several experiments in which they were able to reach an important mathematical relationship to calculate the maximum reaction rate.

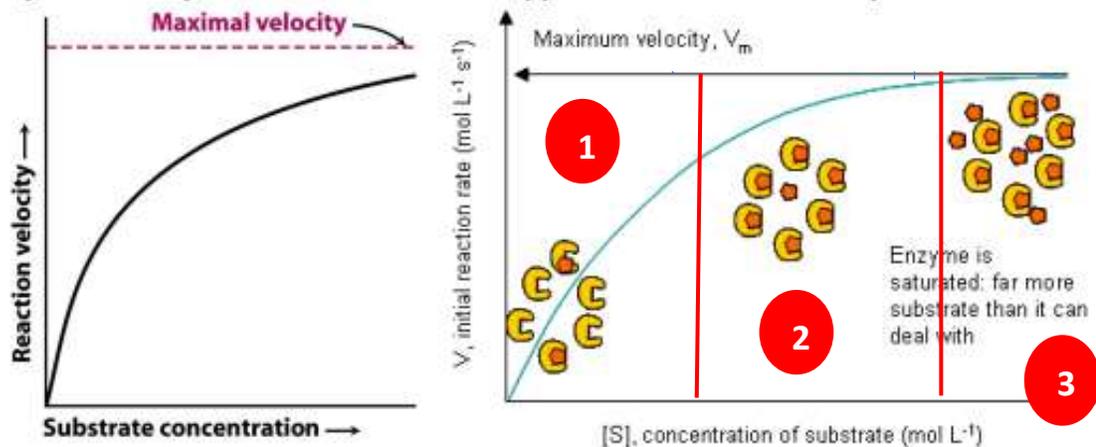
They first began to bring large quantities of enzymes, regardless of their nature, structure, function and even their reactions, and they put their substrates. they started with several experiments so that the concentration of the substrates is increased in each experiment and they started taking notes and studying the

effect of this increase on the rate of the reaction. They found that there are many similar behaviors between these enzymes that are completely different from each other. As they drew a curve representing the increase of the reactants and their impact on the reaction rate. They concluded that part of these enzymes represented their curve in the form of **hyperbolic** plot which are called (*simple enzymes*) and the other part are presented in a **sigmoidal** plot they called (*allosteric enzymes*). -we will focus on the simple enzymes and their curve-

WHY DO WE MEAN BY **HYPERBOLIC PLOT OR HYPERPOLIC ACTIVITY ?**

Any small change in the concentration of the reactants (the x-axis) is offset by a large change in the reaction rate (the y-axis) until both the concentration and the rate reach the stability state.

- **Enzymatic reactions: either have a simple or complex (allosteric) behavior**
- **Simple: as the concentration of the substrate rises, the velocity rises until it reaches a limit**
- **Thus; enzyme-catalyzed reactions have hyperbolic (saturation) plots**



- 1) At the beginning we have a straight increasing line (first order reaction) that's due to the abundance of active sites compared to the small amount of reactants. In this case, when two reactants bind to two active sites, the reaction rate increases to double, and if 3 reactants are bound to 3 active sites, the reaction speed increases to 3 times and so on.
- 2) Here we have a number of reactants almost equal to the number of active sites for enzymes, and in this case the reaction rate increases, but at a lower rate than before, and the stability phase begins

3) At the end we have almost horizontal line(zero order reaction) that's due to the over saturation for the active sites by the substrates in this case we have a "plateau" what do we mean by plateau ? The stage in which no matter how much the concentration of the reactants increases, the reaction rate is not affected at all. In addition the reaction reaches the maximum reaction rate (V_{max}) in this stage.

➤ **The maximal rate, V_{max} is achieved when the catalytic sites on the enzyme are saturated with substrate**

➤ **V_{max} reveals the turnover number of an enzyme**

➤ **The number of substrate molecules converted into product by an enzyme molecule in a unit of time when the enzyme is fully saturated with substrate**

➤ **At V_{max} the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction**

As we mentioned earlier, the main objective of these experiments carried out by Michaelis and Menten is to find a mathematical equation to calculate V_{max} . There are two ways to calculate this value. We start with the most difficult method, which is to do the previous experiment in its entirety. This method consumes a lot of money, energy and time. Therefore, scientists went to study the properties of enzymes and their similar repeated behaviors, which led to the conclusion of a mathematical equation to calculate this value without wasting any resources, which is The easiest and most accurate method.

Expression of enzyme kinetic reactions "Steady State Assumption"



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



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

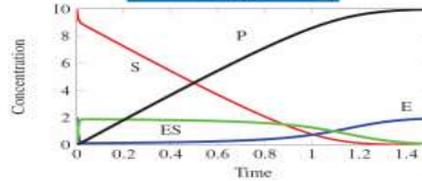
$$v = k_2 ES$$

$$v = \frac{V_{max} S}{K_m + S}$$

$$\frac{dES}{dt} = k_1 E \cdot S - k_{-1} ES - k_2 ES$$

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

$$E_t = E + ES$$



E: Enzyme / S: Substrates / ES: Enzyme substrate complex/ P: Product

K1: association constant between E & S which gives ES / k-1: degradation constant for ES which gives E&S

K2: degradation constant for ES which gives E&P / k-2: association constant between E&P which gives ES

$E_{total} = E + ES$ (the concentration of the free enzymes that are not bound to any substrates + the concentration for the enzymes that are bound to substrates).

The mathematical equation that was found by Menten and Michaelis was put on the basis of two scientific assumptions in order to find the maximum reaction rate, and these assumptions are:

- 1) Once you can see the product in a reaction that means the reaction is irreversible (when the product is produced it can not go back and form the enzyme substrate complex -ES- again) So there is no need for K-2.
- 2) "steady state assumptions" when the enzyme substrate complex in a steady state that means the concentration of ES is constant (not changing) Which leads to the conclusion that the rate of formation of ES and the rate of its degradation are equal, and therefore their subtraction is equal to zero.

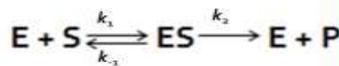
بالعربي: لازم سرعة تفكك و سرعة التكون للـ ES تكون متساوية حتى نحافظ على مستوى ثابت من تركيز ES كيف ؟ لانه بنفس مقدار و سرعة تكون ES من انزيم و مواد متفاعلة يجب أن يقابلها نفس المقدار و السرعة لتفكك ES الى انزيم و نواتج و هيك بيتم الحفاظ على تركيز ES ثابت . ففي حالة كانت سرعة تكون ES أكبر من سرعة تفككه حيصير over production for ES . و بناء على هذه الافتراضات تم وضع المعادلة الرياضية المطلوبة.

If you are interested in the derivation of the mathematical equation based on the previously mentioned assumptions watch this video

د نافع حكي انه اشتقاقها مش مطلوب منا المهم نفهم كيف اجت من الافتراضات و هاد الفيديو حيساعد في الفهم أكثر لكن فعليا مش مطلوب

https://youtu.be/7u2MkbsE_dw

➤ For a reaction:



STEADY STATE APPROXIMATION

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \text{ (approx.)}$$
$$\frac{[E][S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_M \quad \text{Equation 1}$$

➤ K_M , called the Michaelis constant is $K_M = \frac{k_{-1} + k_2}{k_1}$

➤ In other words, K_M is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex

➤ K_M describes the affinity of enzyme for the substrate

the chemical equation that is presented in the previous slide is the final equation according to Michaelis and Menten's assumption and through this chemical equation they arrived to :

$$V = V_{max} \cdot S / (K_M + S)$$

Since $K_M = k_{-1} + k_2 / k_1$

The importance of this constant lies in determining and giving an initial impression about the affinity between enzymes and substrates, as well as the ability of the ES complex to degrade to give the products.

And it is not considered a real measure that is adopted to calculate the attraction of enzymes with their substrates, but rather it calculates the approximate value, so we say that K_M gives an **INDICATION** for the affinity between enzymes and substrates.

ملاحظة بنهاية الشيت : مهم جدا جدا جدا تحضروا هاي المحاضرة من الدكتور لانه بيحكي تفاصيل جدا مهمة و اسلوبه في عرض المعلومة حيساعد في تثبيت و ترسيخ المعلومة .

GOOD LUCK 😊