ENZYME KINETICS

Enzymology 2

إعداد
محمود بركات

Enzyme Kinetics

Definition: It is the field of biochemistry concerned with the quantitative measurement of the rates of enzyme-catalyzed reactions and the study of the factors affecting these rates.

The rate of a chemical reaction: is described by the number of molecules of reactant(s) to be converted into product(s) in a specified time period which is dependent on the concentration of the chemicals involved in the process and on rate constants that are characteristic of the reaction.

Before getting into enzyme kinetics let's view the factors affecting these rates

Factors affecting Enzyme activity:

I. **Factors affecting protein structure (3D structure)**
   A. Temperature
   B. pH
   Cause denaturation of the enzyme (protein)

II. **Changing concentrations**
   A. Enzyme concentration
   B. Substrate concentration

Note1: when studding one of these factors all of the other factors must be **constant**
Note2: These factors are studied inside test tubes rather than biological media
Note3: The active site the most affected domain by denaturation

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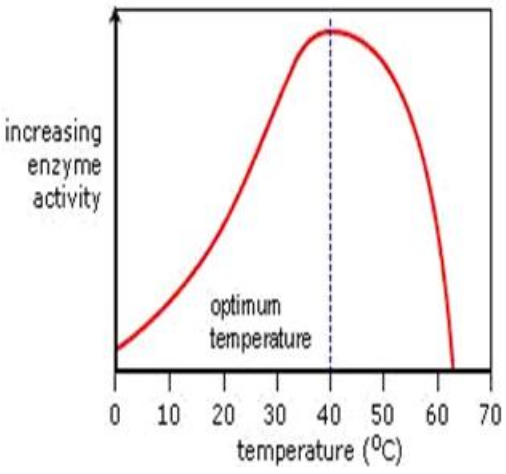
1. Temperature

- The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature
- Most animal enzymes rapidly become denatured at temperatures above 40°C



- The optimal temperatures of the enzymes in higher organisms rarely exceed 50 °C (such as plants)
- The Q_{10}, or temperature coefficient, is the factor by which the rate of a biologic process increases for a 10 °C increase in temperature.

Effect of Temperature

- For mammals and other homoeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.

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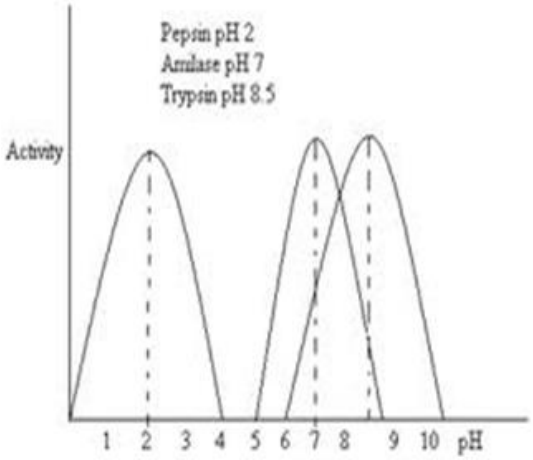
2. **<u>Effect of pH on enzyme activity</u>**

- The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration
- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9. Except for Pepsin, acid phosphatase and alkaline phosphatase
- The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation at high or low pH and effects on the charged state of the enzyme, the substrates, or both.



- If the pH is around the optimum value, amino acid side chains in the active site is ionized thus enhance the interactions between the substrate and the enzyme.
- As the pH decrease or increase away from the optimum pH the enzyme starts to denature

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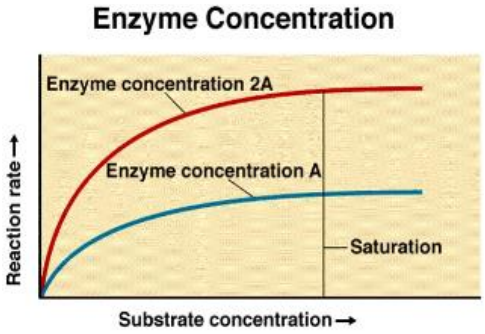
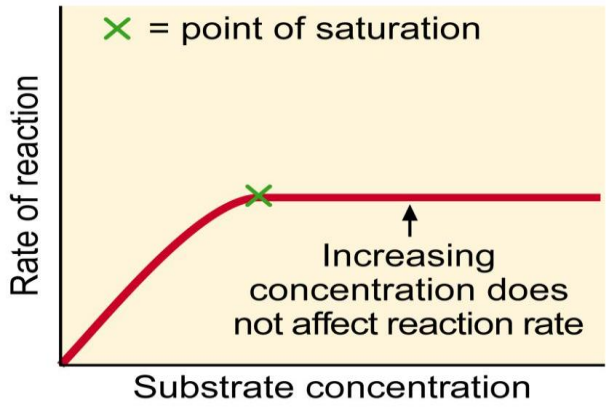
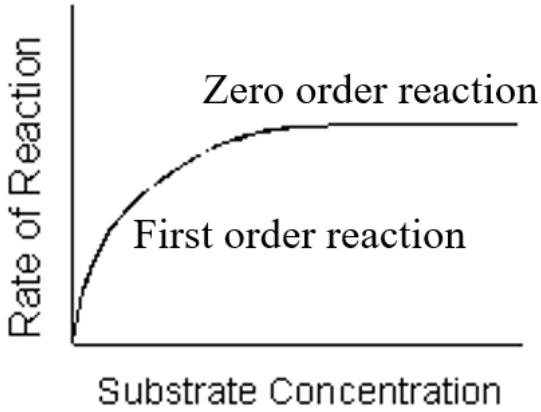
3. **<u>Effect of enzyme concentration</u>**

- As the amount of enzyme is increased, the rate of reaction increases.
- If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate.
- Reaction rate therefore increases then it levels off.

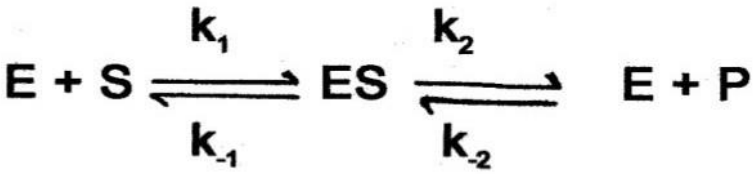


4. **Effect of substrate concentration**
   - At lower concentrations, the active sites on most of the enzyme molecules are not filled because there is not much substrate.
   - Higher concentrations cause more collisions between the molecules.
   - The rate of reaction increases (First order reaction).
   - The maximum velocity of a reaction is reached when the active sites are almost continuously filled.
   - Increased substrate concentration after this point will not increase the rate.
   - Reaction rate therefore increases as substrate concentration is increased but it levels off (Zero order reaction). (V_{max})





Enzyme Kinetics



K_1: association between the enzyme and the substrate

K_2: conversion of the substrate to product

K_{-1}: dissociation between the enzyme and the substrate

K_{-2}: breaking down or inability to form product

Note: K_{-2} is the least constant to occur because the enzyme has the tendency to complete the reaction. Thus, can be neglected when calculating kinetics:

E + S ⇄ ES → E + P

$$E + S \underset{-1}{\overset{1}{\rightleftarrows}} ES \underset{-2}{\overset{2}{\rightleftarrows}} E + P$$

$RATE_1 = K_1 [E] [S]$ $RATE_2 = K_2 [ES]$

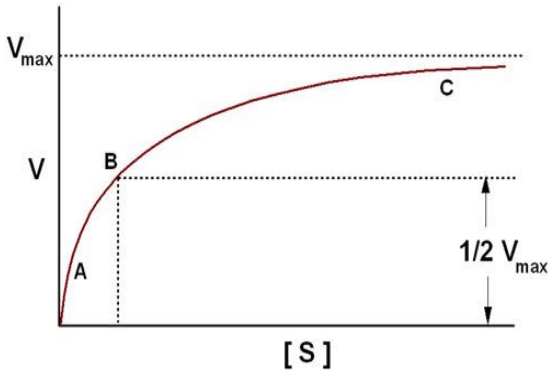
$RATE_{-1} = K_{-1} [ES]$ $RATE_{-2} = K_{-2} [E] [P]$

RATE = V = speed of the reaction

↑RATE → ↑[S], ↑[E], K is constant

Some Assumptions:

1) Our solutions are behaving ideally
2) Our constants are indeed constants
   - The total [E] is constant → V_{max} is constant because at high [S] the enzymes will be saturated. Even if ↑↑[S] there will still be V_{max}
   - K is constant → environmental factors (temperature, pH) are constant
3) S → P without enzyme is negligible
4) The steady-state assumption → [ES] is constant → Formation of [ES] = Loss of [ES]



Michaelis-Menten Kinetics

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

اشتقاق المعادلة أخر صفحة مهمة لفهم العلاقات بين المتغيرات والثوابت

- The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme-catalyzed reaction [V_i], the concentration of substrate [S] and two constants, V max and km (which are set by the particular equation).
- The symbols used in the Michaelis-Menten equation refer to the reaction rate [V_i], maximum reaction rate (V max), substrate concentration [S] and the Michaelis-Menten constant (km).

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

In this equation we have 2 variables (V_I, [S]) and 2 constants (V_{max}, K_m):

V_i = is the speed (rate) of reaction

[S] = is the concentration of the substrate in the reaction

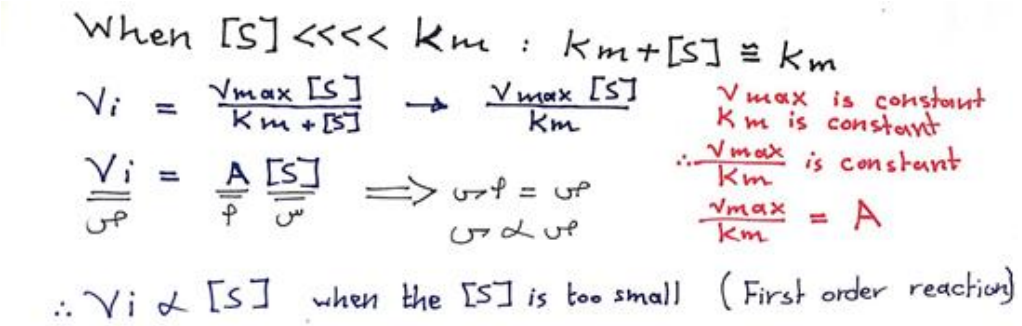
V_{maax} = is the maximum speed (rate) that this reaction can occur at (because the total amount of enzymes is constant)

K_m = equal to the [S] when the rate of the reaction (V_i) is equal to the maximum rate (V_{max})

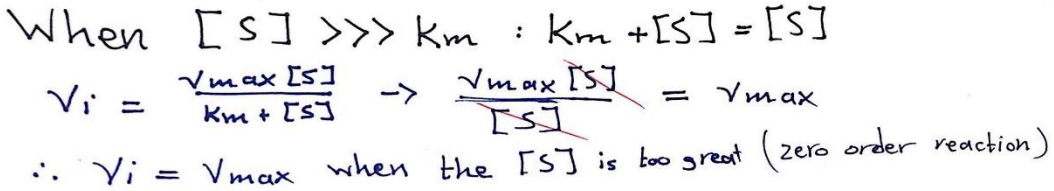
K_m = [S] when V_I = ½ V_{max}

The dependence of initial reaction velocity on [S] and Km may be illustrated by evaluating the Michaelis-Menten equation under three conditions.

1) When [S] is much less than km, the term km + [S] is essentially equal to km. Since V max and km are both constants, their ratio is a constant (k).
   In other words, when [S] is considerably below km, V max is proportionate to k[S].
   The initial reaction velocity therefore is directly proportionate to [S].



2) When [S] is much greater than km, the term km + [S] is essentially equal to [S].
   Replacing km + [S] with [S] reduces equation to Vi = Vmax
   Thus, when [S] greatly exceeds km, the reaction velocity is maximal (V max) and unaffected by further increases in substrate concentration.



3) When [S] = km

Equation states that when [S] equals km, the initial velocity is half-maximal. Equation also reveals that km is a constant and may be determined experimentally from—the substrate concentration at which the initial velocity is half-maximal.

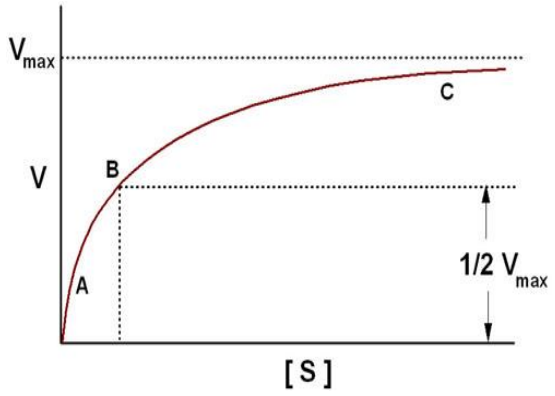
When $[S] = Km$: $Km + [S] = 2[S]$

$V_i = \frac{V_{max}[S]}{Km + [S]} \rightarrow \frac{V_{max}[S]}{2[S]} = \frac{1}{2} V_{max}$

$\therefore Km = [S]$ when $V_i = \frac{1}{2} V_{max}$

Km is the substrate concentration when the rate of the reaction equal half the maximum rate

And now as we combine these three conditions together, we get this plot



الرسمة البيانية التالية تمثل العلاقة بين تركيز المواد المتفاعلة وسرعة التفاعل الابتدائية حيث انه بزيادة تركيز المواد المتفاعلة في التجربة سيزيد من سرعة التفاعل الابتدائية إلى أن تصل سرعة التفاعل الابتدائية إلى أقصى سرعة ممكنة وحينها أي زيادة في كمية المواد المتفاعلة لن تحد أي تغير.
ملاحظة: هذا الرسم مبني على فرض ان كل إنزيم يعمل على مادة متفاعلة واحدة فقط (يعبر عن بداية التفاعل - السرعة الابتدائية - فقط)

Km and its significance

- **The Michaelis constant K_m** is the substrate concentration at which V_i is half the maximal velocity (Vmax/2) attainable at a particular concentration of enzyme
- It is specific and constant for a given enzyme under defined conditions of time, temperature and pH
- K_m determines the affinity of an enzyme for its substrate, lesser the Km higher is the affinity and vice versa, it is inversely proportionate to the affinity
- K_m value helps in determining the true substrate for the enzyme.

Note1: km unit is molar or mole per liter

Note2: The lower the km the better the enzyme is at working when substrate concentrations are small

Note3: Catalytic efficient = K_{cat}/K_m ; ↑Catalytic efficient = ↑K_{cat} , ↓K_m
$K_{cat} = V_{max}/[E]_T$; also known as Turnover number: How many substrates can 1 enzyme turn into products in one second at maximum speed

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Lineweaver-Burk Plot

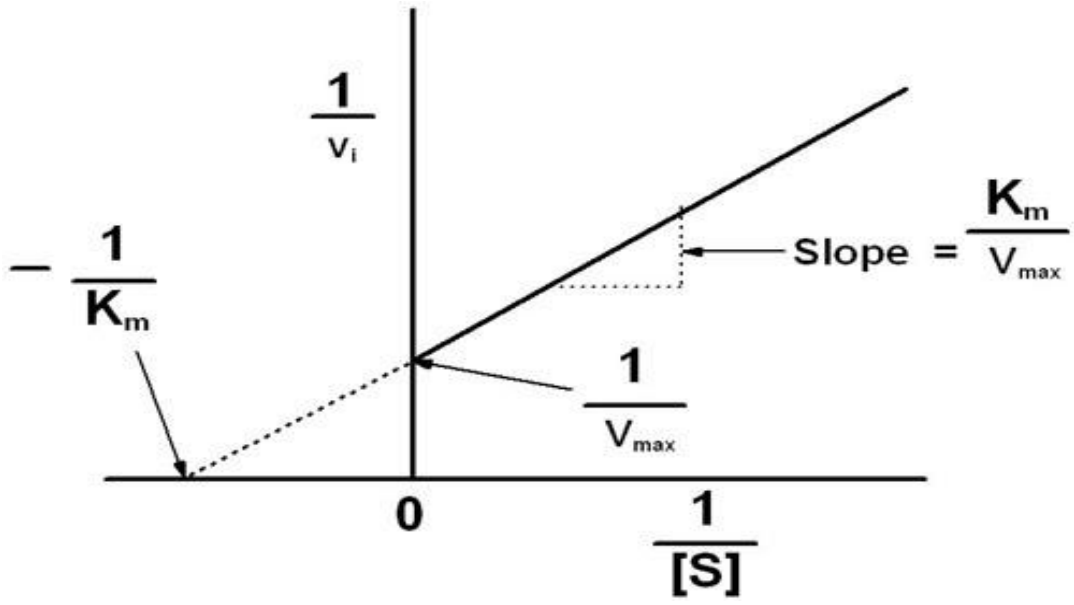
A Linear Form of the Michaelis-Menten Equation is used to determine km & V max.

$$v_i = \frac{V_{max}[S]}{K_m + [S]} \quad \text{Invert} \quad \frac{1}{v_i} = \frac{K_m + [S]}{V_{max}[S]} \quad \text{factor} \quad \frac{1}{v_i} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]} \quad \text{and simplify}$$

$$\frac{1}{v_i} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Lineweaver-Burk Plot

- A plot of $1/V_i$ as *y* as a function of 1/[S] as *x* therefore gives a straight line whose *y* intercept is 1/ V max and whose slope is km / V max.
- Such a plot is called a double reciprocal or Lineweaver-Burk plot.



Michaelis - Menten equation

The steady-state Assumption :

[ES] is constant :

Formation of [ES] = Loss of [ES]

$$Rate_1 + \cancel{Rate_{-2}} = Rate_2 + Rate_{-1}$$

$$K_1[E][S] = K_2[ES] + K_{-1}[ES]$$

$$\therefore [E]_T = [E] + [ES] \Rightarrow [E] = [E]_T - [ES]$$

عدد الإنزيمات الكلي = عدد الإنزيمات الحرة + عدد الإنزيمات المرتبطة بـ substrate

نستبدلها في الطرف الأول

$$K_1([E]_T - [ES])[S] = K_2[ES] + K_{-1}[ES]$$

فك أقواس — عامل مشترك

$$K_1[E]_T[S] - K_1[ES][S] = [ES](K_2 + K_{-1})$$

نقسم الطرفين على K_1

$$[E]_T[S] - [ES][S] = [ES]\left(\frac{K_2 + K_{-1}}{K_1}\right)$$

$$K_m = \frac{K_2 + K_{-1}}{K_1}$$

كل من k_2 و k_{-1} و k_1 نوابت

نستبدلهم بثابت واحد k_m

$$[E]_T[S] - [ES][S] = [ES]K_m$$

$$\cancel{+[ES][S]} \qquad \cancel{+[ES][S]}$$

$$[E]_T[S] = [ES]K_m + [ES][S]$$

$$\frac{[E]_T[S]}{K_m + [S]} = \frac{[ES](\cancel{K_m + [S]})}{\cancel{K_m + [S]}}$$

$$\frac{[E]_T[S]}{K_m + [S]} = [ES]$$

بضرب الطرفين بـ K_2

$$\frac{K_2[E]_T[S]}{K_m + [S]} = K_2[ES]$$

$$K_2[ES] = V_o \;,\; K_2[E]_T = V_{max}$$

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

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

- V_o : the speed of whole process
- $V_o = \frac{dP}{dt} = \frac{\Delta P}{\Delta t} = K_2[ES]$
- V_o = the rate of product formation

\# if $V_o = V_{max}$ ∴ all enzymes are saturated

- $\therefore [E]_T = \cancel{[E]} + [ES]$
- $[E]_T = [ES]$
- $\therefore V_o = K_2[ES]$
- $V_{max} = K_2[E]_T$