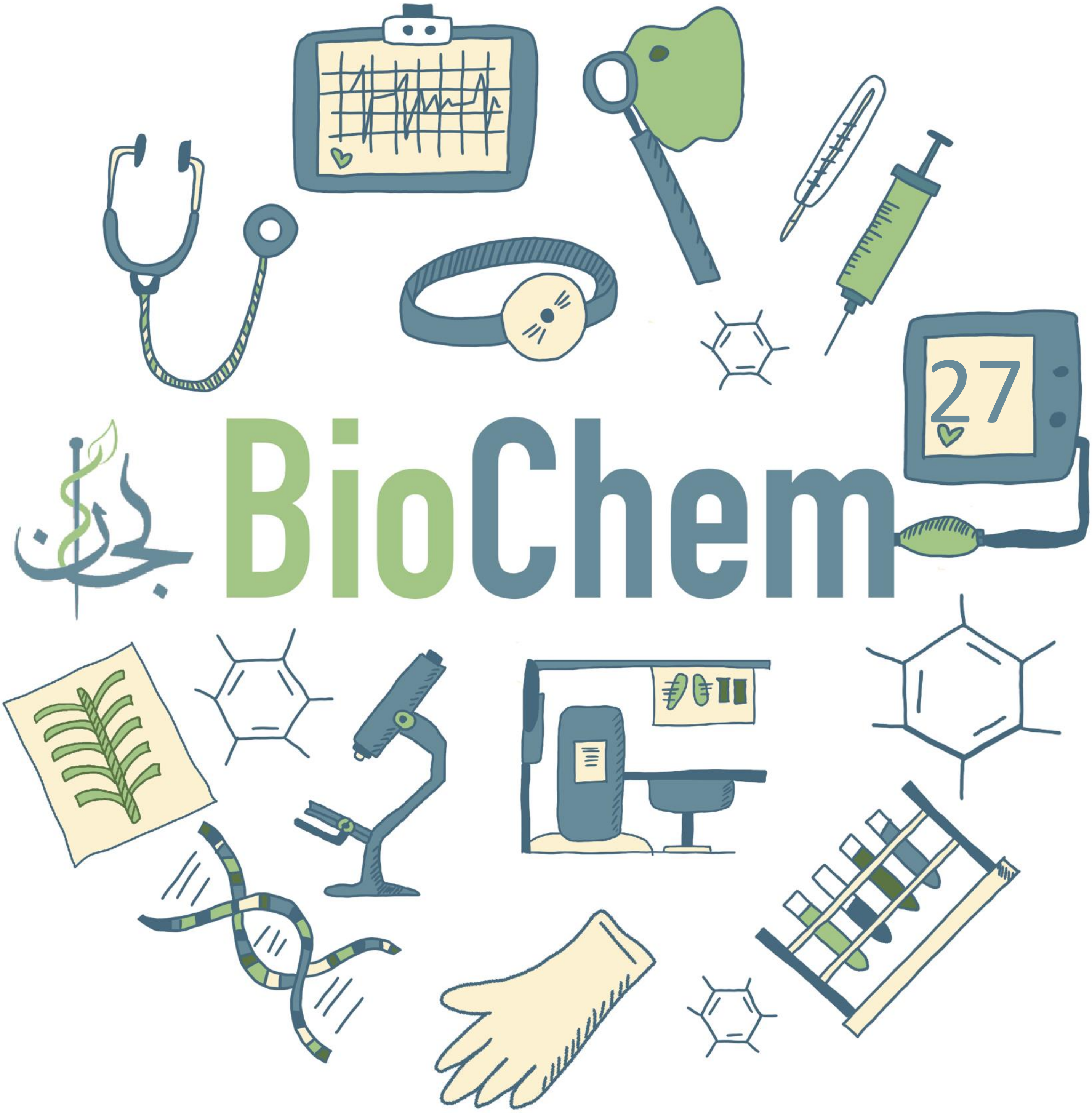
BioChem



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Topics discussed in this sheet (the 3rd lecture)

1. Oxidation-reduction coenzymes
2. General summary (table) about water-soluble vitamins and their corresponding coenzymes
3. Catalytic metals
   - Metalloenzymes
   - Metal-activated enzymes
4. Kinetics of enzymatic reactions
   - The rate of reactions
   - Michaelis-Menten Equation

In the last lecture …

Oxidoreductases are enzymes that catalyze oxidation-reduction reactions.

A Dehydrogenase is an enzyme belonging to the group of oxidoreductases that oxidize a substrate by reducing an electron acceptor, usually NAD^+/$NADP^+$ or a flavin coenzyme such as FAD or FMN.

There are 2 types of dehydrogenase

- **Alcohol dehydrogenases** catalyze the interconversion between **primary and secondary alcohols** to the corresponding aldehyde or **ketone**
- **Lactate Dehydrogenase** catalyzes the the interconversion between lactate and pyruvate

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In this lecture, we will focus on oxidation-reduction coenzymes that may assist dehydrogenases in redox reactions.

Oxidation-Reduction Coenzymes : are molecules which have the ability of binding to and carrying electrons from one place to another.

- Unlike activation-transfer coenzymes , they don't form a covalent bond with substrates ; they abstract electrons from them.

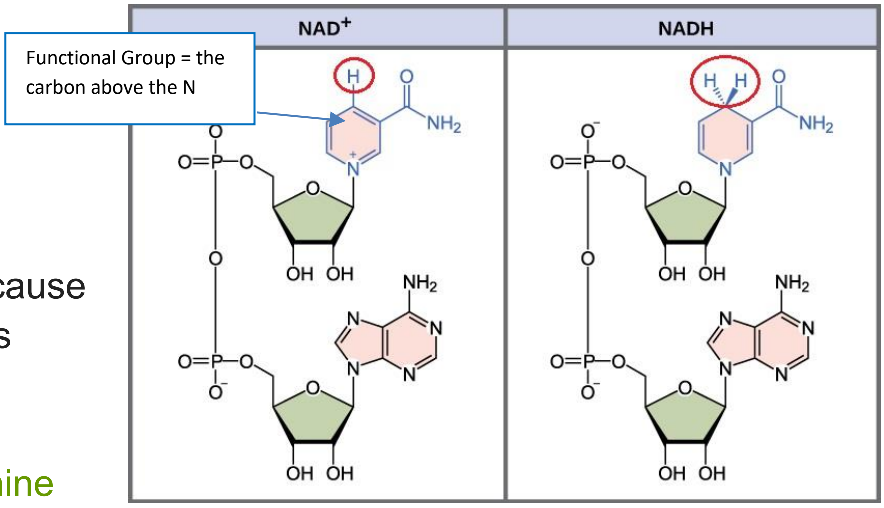
NAD^+ is the most common electron recipient in dehydrogenases
Unless noted , the coenzyme is NAD^+

Types of oxidation-reduction coenzymes

- **NAD^+ & $NADP^+$**
  Source : vitamin B3 (niacin)
- **FAD & FMN**
  Source : vitamin B2 (riboflavin)
- **Others** work with metals to transfer single electrons to oxygen
  Source :vitamin C & E
  (They are antioxidant)

1. NAD+

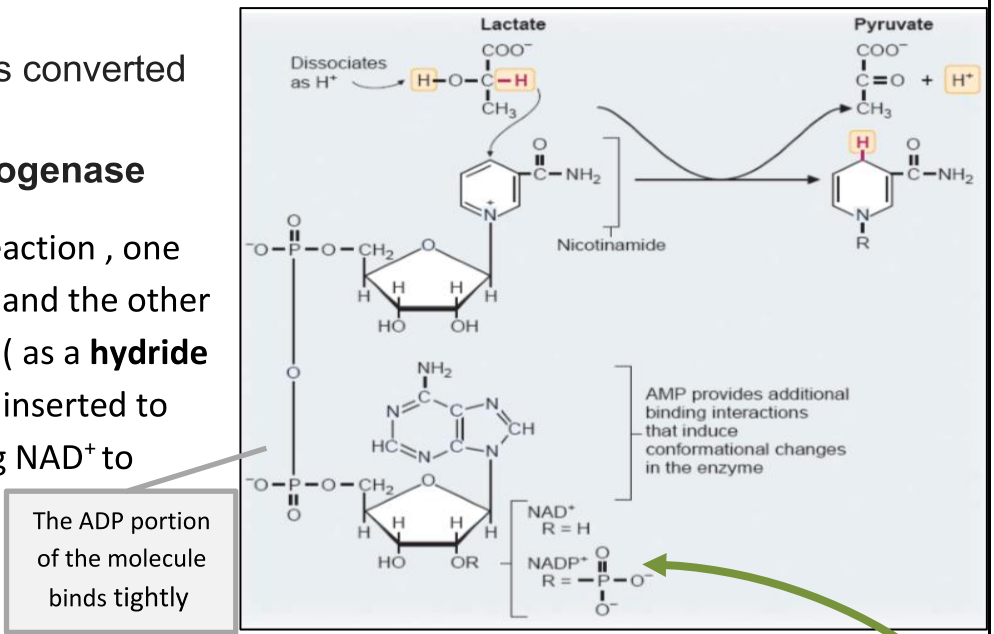
NAD is an abbreviation for **Nicotinamide Adenine Dinucleotide**



It's called a dinucleotide because it consists of two nucleotides joined through their phosphate **groups**. One nucleotide contains an adenine nucleobase and the other nicotinamide.

- NAD+ is found in all living cells

- How does NAD+ work?



In this example , **lactate** is converted to **pyruvate** by the enzyme **lactate dehydrogenase**

In this dehydrogenation reaction , one hydrogen will leave as **H+** , and the other will leave with 2 electrons (as a **hydride ion H-**) ,which will then be inserted to nicotinamide ring reducing NAD+ to NADH.

So NAD is involved in :

- The interconversion between **alcohols** to the corresponding **aldehyde** or **ketone**
- The interconversion between **lactate** and **pyruvate**
- Role of enzymes' **histidine** >> if we have histidine inside the active site , it helps taking H+ out and release it to the solution preparing substrate to be activated and for the hydride ion to bind to NAD+ converting it to NADH

NADP+

is simply **NAD+** with a third phosphate group attached as shown at the bottom of the figure.

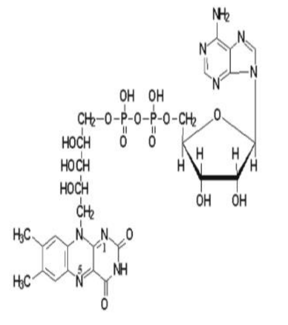
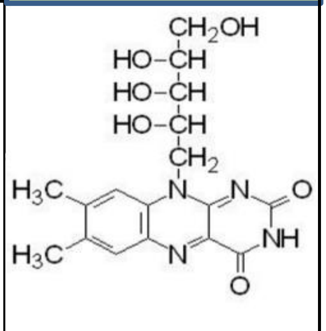
☒ Both NAD^+ and $NADP^+$ behave exactly the same because the insertion of the hydride ion is to the nicotinamide ring in both molecules

Why would we have 2 different coenzymes which behave the same ?
For regulatory reasons ; they are present in different places. For example, **NAD^+** is involved in energy metabolism , and **$NADP^+$** is involved lipid biosynthesis

- NAD^+ doesn't go into the one electron state_**free radical state**, which might be cancerous, at all ; it either receives the 2 electrons in one step or lose them ... so we find it in solutions swimming freely e.g. mitichondrial matrix , it's not dangerous ... the same thing apply to $NADP^+$

2. FAD & FMN

FAD is an abbreviation for **Flavin Adenine Dinucleotide** while
FMN is **Flavin Mononucleotide** (no adenine)

FAD	FMN
	

- Despite the difference in composition they both behave the same because the two electrons and two protons are added to the 2 nitrogen in flavin ring.

  So Why would we have 2 different coenzymes which behave the same ?
  for regulatory reasons too (like NAD and NADP) and because they differ in space (size)

- Note: **the addition of 1 H^+ and 1 e^- occur sequentially** in both

  FAD → FADH → $FADH_2$
  FMN → FMNH → $FMNH_2$

  So, it goes into the **free-radical state**, which is dangerous! Because of that, we will never find FAD or FMN in solutions swimming freely; they are hidden inside proteins (like succinate dehydrogenase) ... these enzymes have strategies to deal with these radicals to prevent damage to cell (specially to prevent DNA mutations)

Antioxidants are substances that may protect your cells against free radicals, which may play a role in **heart disease, cancer and other diseases**

- FAD and FMN are **prosthetic groups** (tightly bound) to enzymes such as
  a. **succinate dehydrogenase**
  b. **pyruvate dehydrogenase complex**

After we discussed all the vitamins and their corresponding coenzymes we willl go into them generallay as summarized in this table (as a recap)

Helps in decarboxylation rxn

Water-Soluble Vitamins

Vitamin	Name	Coenzyme or Active Form	Primary biochemical function
B1	Thiamin	Thiamine pyrophosphate (TPP)	Aldehyde-group transfer
B2	Riboflavin	Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer
B3	Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer
B5	Pantothenic Acid	Coenzyme A (CoA)	Acyl-group transfer
B6	Pyridoxine	Pyridoxal Phosphate	Amino-group transfer
B7	Biotin	Biocytin	Carboxyl transfer
B9	Folate	Tetrahydrofolate	One-Carbon group transfer
B12	Vitamin B_{12}	Coenzyme B_{12}	1,2 shift hydrogen atoms
	Lipoic Acid	Lipoyllysine	Hydrogen-Atom and Acyl-group transfer
C	Ascorbic Acid	Ascorbic acid, dehydroascorbic acid	Cofactor in hydroxylation

HELPS IN REDOX RXNS

TO BE DISCUSSED LATER

- NOTE: There are ***8 B Vitamins***(1,2,3,5,6,7,9,12) … No vitamins B4,B8,B10,B11

Not only coenzymes can help enzymes also some metals can

Catalytic Metals

Metals can be tightly bound to enzymes like prosthetic groups (**metalloenzymes**) or loosely bound (**metal-activated enzymes)** acting as electrophiles

- In **Metal-activated Enzymes**, the metal is either required or enhances activity (Mg2+, Mn2+, Ca2+, & K+)
- In **Metalloenzymes**, metal ions may contribute either to the structure or the catalytic mechanism. Metal ions are usually incorporated during synthesis, and removal of the metal causes denaturation

- If we extracted the metal from a metalloenzyme it will be denatured.

However, in the case of metal-activated enzymes, the structure is preserved even if the metal is dissociated .If the metal is present, the enzyme is active; if it's not, the enzyme is inactive.

Examples of metals and their associated enzymes

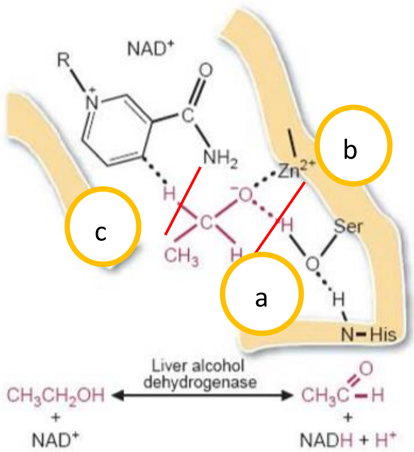
Metal	**Enzyme**
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	Hexokinase
Se^{2+}	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase

Examples of Metalloenzymes (zinc is the catalytic metal)

a. **Liver alcohol dehydrogenase** **(dimer)** is a metalloenzyme which have 2 Zn^{+2} in each monomer; one for structural maintenance (joins the two subunits), the other is catalytic.
b. **Carbonic anhydrase**; zinc atom is essentially always bound to four or more groups

Metals can benefit enzymes by <u>stabilizing the oxyanion</u> which is formed during the catalytic process.

e.g. **Zn^{2+} in ADH (liver alcohol dehydrogenase)**
In the process of converting ethanol to acetaldehyde by ADH as shown in the figure



Not Required

a. When ethanol comes to the active site, serine is activated (because of the change in PH) .. Because serine is unstable it will take the H from ethanol
Therefore, the negative charge in the oxygen is now more pronounced (an oxyanion is formed), and this cause instability for the molecule
b. So, zinc will stabilize the molecule with its positive charges.
c. And the binding from the outside will weaken to the bonds inside(the bonds around the carbon) the molecule so the H will be lost for NAD^{+} and double bonds will form ,so acetaldehyde is formed and thus the affinity will be lost and the molecule will dissociate

Kinetics of enzymatic reactions

Biochemical Kinetics: the science that studies rates of chemical reactions what happens throughout the reaction the intermediates, activation energy ,etc.

- e.g. In the reaction (A → P)
  The **Velocity**, **V**, or **rate, of the reaction A → P** = the amount of P formed or the amount of A consumed per unit time, t.

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

- *Rate of consuming reactants = Rate of forming the products*; because **matter** is conserved, it can change form through physical and chemical changes but it can't be created or destroyed.
- **The rate** is a term of change over time and will be proportional to the conc. of the reactants

For the reaction (A + B →P), the **rate law** is

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

The negative sign means the substance is consumed

The derivative is replaced by a constant

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

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

$$\mathbf{V = k(A)^{n1}\,(B)^{n2}\,(C)^{n3}}$$

- **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
  - n1 is the order of A, n2 is the order of B, and so on
  - The **order number** is close to the number of moles in a balanced equation (it's not the same thing but we can assume it is); the order number helps us determine how many moles of the material is participating in the reaction

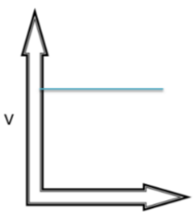
- For example, in the reaction **A + B →H**, If the velocity is not affected by the change of B's concentration then <u>the order number of B=0</u>

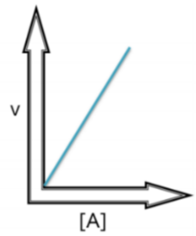
How is it possible that B's change in concentration doesn't affect the overall velocity?

It's because B is oversaturated (مادة فائضة) and A is limited (مادة محددة)
B is excess (high concentration of B is added)

Important !

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





For the same example of A and B

We can represent the velocity of the reaction as constant (horizontal line) with respect to B which is zero order

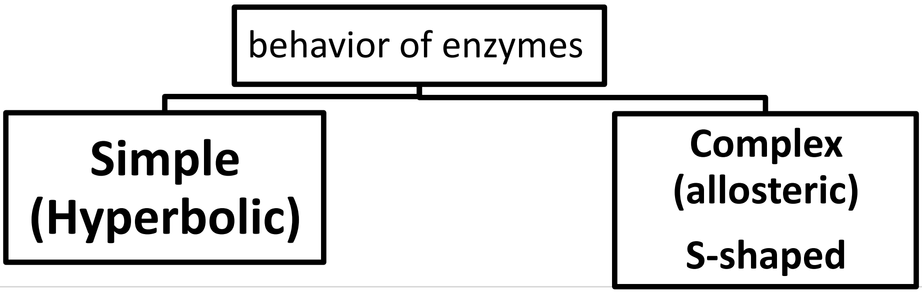
And linear with respect to A which is first order

Overall, linear at the beginning, constant in the end forming a plateau, and there is a curve in between

- ***We can use this concept in the lab to simplify the experiment***

For example, if the velocity is dependent on 4 reactants (that means that there will be a first, second, third and fourth order number each with fluctuating concentrations. To simplify, we can add 3 of these reactants with very high concentrations (excess) this reaction is called **pseudo-first order reaction**

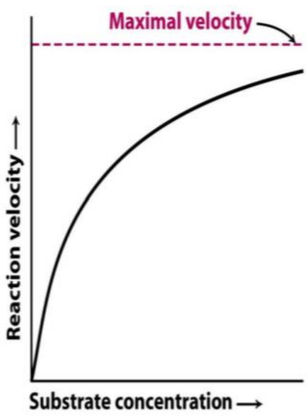
When Michaelis and Menten studied enzymes behaviors in all conditions regardless the substrate, environment it's put in), they concluded that enzymes can be divided into two types to according to their behaviors

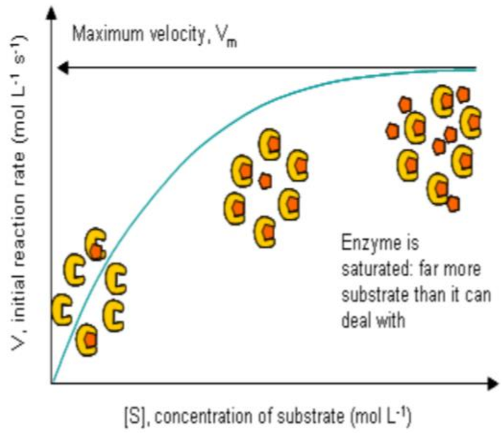


Simple behavior of enzymes

- as the concentration of the substrate rises, the velocity rises until it reaches a limit ...

¬ Thus; enzyme-catalyzed reactions have hyperbolic (saturation) plots**;** it starts sharp where small change in the x-axis (substrate concentration) cause big changes in the y-axis(velocity)





The **maximal rate, Vmax**

- is achieved when the catalytic sites on the enzyme are **saturated** with substrate
- Vmax reveals the **turnover number** of an enzyme
- It is the number of substrate molecules converted into product by an enzyme molecule in a unit of time when the enzyme is fully saturated by the substrate
- At Vmax ,the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction
- **Each enzyme has a specific V_{max} with respect with substrate it deals with. (certain active site with certain geometry and deals with a certain substrate with an exact chemical structure)**

In this case because the attitude (idea) is repetitive we can derive a mathematical equation ..

Michaelis-Menten equation

- Is a quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_0) & substrate concentration [S], the rate constant (K_m) and maximal velocity (V_{max})

- This equation is derived from the **Steady State Assumption**

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Not Required

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

$$v = k_2\, ES$$

$$\frac{d\,ES}{dt} = k_1\ E\ .\ S - k_{-1}\ ES - k_2\ ES$$

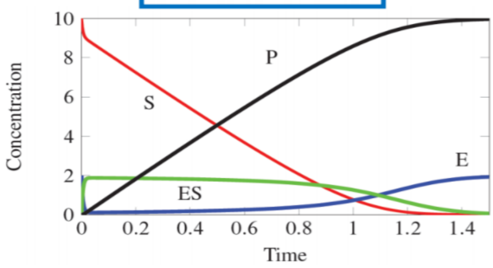
$$0 = k_1\ E\ .\ S - k_{-1}\ ES - k_2\ ES$$

$$E_t = E + ES$$

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

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

$$v = \frac{Vmax\ S}{K_m + S}$$



K_m (Michaelis-Menten constant)

- *how they derived it …*

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

E : the enzyme **, S:** the substrate **, p:** the product

Not Required

STEADY STATE APPROXIMATION

$$\frac{d[ES]}{dt} = k_1[E][S] - K_{-1}[ES] - K_2[ES] = 0\ \text{(approx.)}$$

$$\frac{[E][S]}{[ES]} = \frac{K_{-1} + K_2}{k_1} = K_M \qquad \text{Equation 1}$$

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

$$K_m = \frac{\text{rates of degredation of ES comlex}}{\textit{rates of formation of ES complex}}$$

- **K_m** describes the affinity of enzyme for the substrate; the smaller the Km, the greater the affinity and vice versa.

Starts at 1:22:26

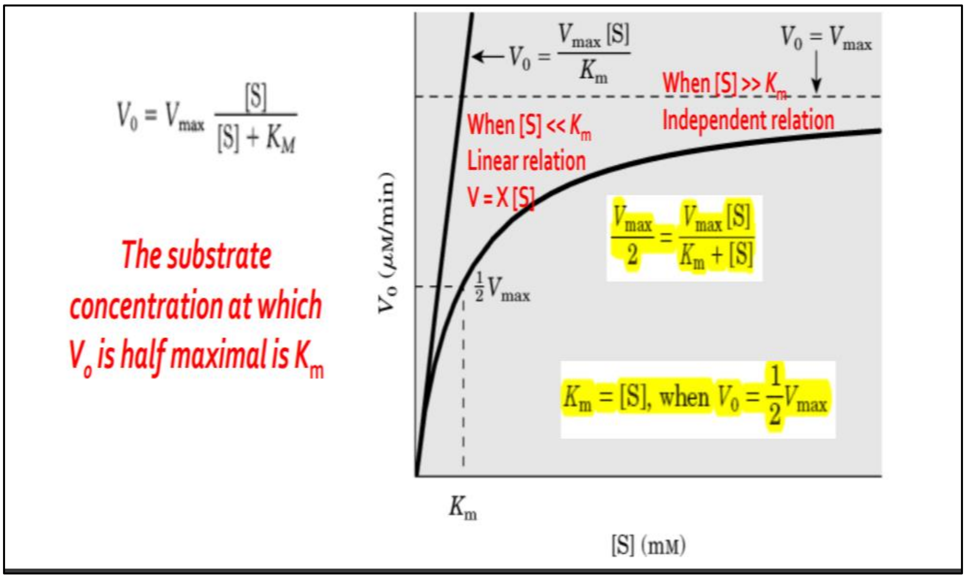
K_D: dissociation constant, The **actual** measure of the affinity

¬ K_D = (k-1 /k1)

الفرق حسب كلام الدكتور انه **K_D** أدق من **K_m**

The explanation starts at 1:26:18

We can use Michaelis-Menten equation to describe the simple (hyperbolic) behavior of enzymes



1. At the beginning of the reaction [s] will be very small

[S]<<< $\mathbf{K_m}$

So [S] can be neglected accordingly the graph becomes linear

هذا يؤكد انه في بداية التفاعل يكون التفاعل

first order (linear)

In mathematics, very small numbers can be neglected in addition and subtraction **but not** in multiplication and division

2. At the end of the reaction, [S]>>> $\mathbf{K_m}$ so $\mathbf{K_m}$ can be neglected
   V=Vmax
   So at the end the reaction is zero order (constant)
3. What happens if the [S]= $\mathbf{K_m}$

$\mathbf{V_0}=1/2\mathbf{V_{max}}$

In order to conclude :

- $\mathbf{K_m}$ is the Concentration of substrate needed to reach the $V_0=1/2\ V_{MAX}$ …
- Why do we need $\mathbf{K_m}$? It's a concentration unit used to compare enzymes
- $\mathbf{K_m}$ is dissociation constant over association constants
  $\mathbf{K_m} = \frac{\text{rates of degredation of ES comlex}}{rates\ of\ formation\ of\ ES\ complex}$
- $\mathbf{K_m}$ determines the affinity of the enzyme to its substrate

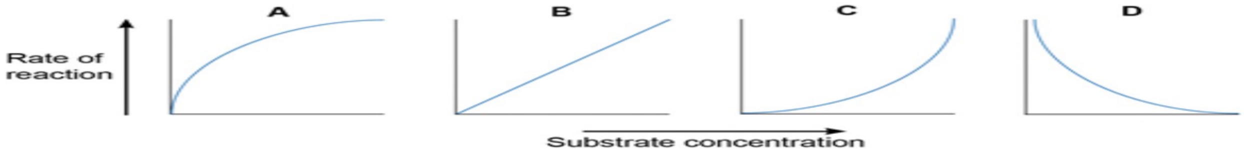
the smaller the Km, the greater the affinity.

FIN

"You can't connect the dots looking forward; you can only connect them looking backwards. So you have to trust that the dots will somehow connect in your future. You have to trust in something — your gut, destiny, life, karma, whatever. This approach has never let me down, and it has made all the difference in my life."

~ Steve Jobs

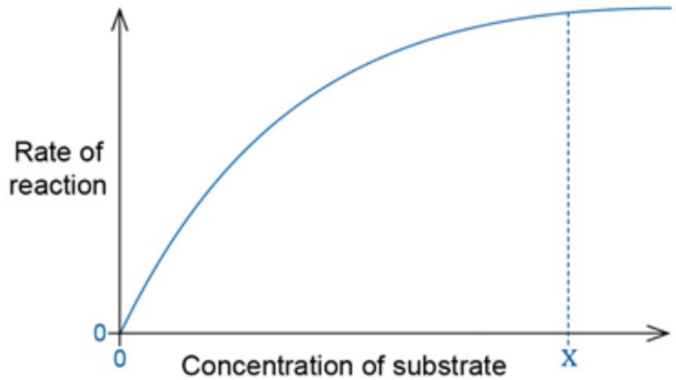
1. Which of the diagrams illustrates the way in which the rate of an enzyme-controlled reaction depends on substrate concentration?



2. Which of the following enzyme groups can catalyse oxidation reactions?

A)phosphorylases
B)isomerases
C)hydrolases
d) dehydrogenases

3. At concentrations of substrate greater than X, which of the following statements is true?



A -The rate of reaction is limited by enzyme concentration.
B -The rate of reaction tends towards zero.
C-The substrate has an inhibitory effect.
d-The products have an inhibitory effect

4. Succinic acid dehydrogenase is the enzyme which catalyses the oxidation of succinic acid during cell respiration. If malonic acid is added to the system, the rate of reaction is reduced. An increase in the substrate concentration, succinic acid, increases the rate of reaction again.
Using this information what might be deduced about the action of malonic acid?

A-It decreases the pH of the system.
B-It forms a permanent attachment to the active site of the enzyme.
C-It has a similar molecular configuration to that of succinic acid.
d- It acts as a coenzyme.

5. Which type of enzyme catalyses the conversion of a dipeptide into two separate amino acids?

A-decarboxylase
B-dehydrogenase
C-hydrolase
d- oxidoreductase

6. The concept of “induced fit” refers to the fact that:

A) enzyme specificity is induced by enzyme-substrate binding.
B) enzyme-substrate binding induces an increase in the reaction entropy,thereby catalyzing the reaction.
C) enzyme-substrate binding induces movement along the reaction coordinate to the transition state.
D) substrate binding may induce a conformational change in the enzyme, which then brings catalytic groups into proper orientation.
E) when a substrate binds to an enzyme, the enzyme induces a loss of water (desolvation) from the substrate.

7. Which of the following statements about a plot of V0 vs. [S] for an enzyme that follows MichaelisMenten kinetics is false?

A) As [S] increases, the initial velocity of reaction V0 also increases.
B) At very high [S], the velocity curve becomes a horizontal line that intersects the y-axis at Km.
C) Km is the [S] at which V0 = 1/2 Vmax.
D) The shape of the curve is a hyperbola.
E) The y-axis is a rate term with units of mm/min

8. Which of these statements about enzyme-catalyzed reactions is false?

A) At saturating levels of substrate, the rate of an enzyme-catalyzed reaction is proportional to the enzyme concentration.
B) If enough substrate is added, the normal Vmax of a reaction can be attained even in the presence of a competitive inhibitor.
C) The rate of a reaction decreases steadily with time as substrate is depleted.
D) The activation energy for the catalyzed reaction is the same as for the uncatalyzed reaction, but the equilibrium constant is more favorable in the enzyme-catalyzed reaction.
E) The Michaelis-Menten constant Km equals the [S] at which V = 1/2 Vmax

9. Vmax for an enzyme-catalyzed reaction:

A) generally increases when pH increases.
B) increases in the presence of a competitive inhibitor.
C) is limited only by the amount of substrate supplied.
D) is twice the rate observed when the concentration of substrate is equal to the Km.
E) is unchanged in the presence of a uncompetitive inhibitor.

10. Enzyme X exhibits maximum activity at pH = 6.9. X shows a fairly sharp decrease in its activity,when the pH goes much lower than 6.4. One likely interpretation of this pH activity is that:

A) a Glu residue on the enzyme is involved in the reaction.
B) a His residue on the enzyme is involved in the reaction.
C) the enzyme has a metallic cofactor.
D) the enzyme is found in gastric secretions.
E) the reaction relies on specific acid-base catalysis.

11. Define the terms “cofactor” and “coenzyme.”

12. Which aspects of its reaction will be changed by Enzyme Y?

a) the activation energy of the reaction and the energy of the product
b) the rate of the reaction and the energy of the transition state
c) the equilibrium position of the reaction and the energy of the substrate
d) the reversibility of the reaction and the energy of the active site

****** Enzyme X and Enzyme Y are both involved in monosaccharide metabolism. Enzyme X uses glucose as a substrate while Enzyme Y uses fructose as a substrate. At pH=7.0, Enzyme X has a Vmax of 10 μM/s while Enzyme Y has a Vmax of 20 μM/s. Both enzymes have a KM of 3.0 mM for their respective substrates. (Questions 12-16)**

13._____ When its reaction is carried out at pH = 2.0, the Vmax of Enzyme X is 1.0 μM/s because

a) the enzyme is inhibited by its product at low pH.
b) the enzyme is saturated with substrate at low pH.
c) the enzyme is able to stabilize the transition state at low pH.
d) the enzyme is partially denatured as R-groups protonate at low pH.

14._____ When the reaction is carried out at pH = 7.0 and the substrate concentration is equal to the KM value

a) X will produce more product than Y.
b) Y will produce more product than X.
c) X and Y will produce the same amount of product.
d) X and Y will both work at their Vmax value.

15._____ Enzyme Y can also use the monosaccharide galactose as a substrate with a KM of 8.0 mM. Which will be a characteristic of Y as it binds galactose compared to its binding to fructose?

a) Y will form more non-covalent bonds with galactose.
b) Y will form more covalent bonds with galactose.
c) Y will have an active site that is less complementary to galactose.
d) Y will undergo a greater conformational change as it binds galactose.

16. Which kinetic property would Enzyme X display as it binds its normal substrate and catalyzes its reaction?

a) It could have an initial velocity independent of [S] when [S] < KM.
b) It could have a KM value that decreases as [S] decreases from 3.0 mM to 0.3 mM.
c) It could double the rate of its reaction as [S] increases from 3.0 mM to 30 mM.
d) It could have a Vmax value that is dependent on [S] when [S] < KM.

17. Coenzyme is

(A) Often a vitamin
(B) Always an inorganic compound
(C) Always a protein
(D) Often a meta

18. Coenzymes FMN and FAD are derived from vitamin

(A) C
(B) B6
(C) B1
(D) B2

19) An enzyme catalyzing the reaction E+A->EA->E+P was mixed with 4 mM substrate (compound A). The initial rate of product formation was 25% of V max The Km for the enzyme is which one of the following?

(A) 2mM
(B) 4mM
(C) 9mM
(D) 12mM
(E) 25mM

20) The liver enzyme glucokinase catalyzes the phosphorylation of glucose to glucose 6-phosphate. The value of K,,, for glucose is about 7 mM. Blood glucose is 5 mM under fasting conditions and can rise in the liver to 20 mM after a high-carbohydrate meal. Therefore, if a person who is fasting eats a high-carbohydrate meal, the velocity of the glucokinase reaction will change i which one of the following ways?

(A) Remain at less than 50% V mn
(B) Remain above 80% Vmu:
(C) Increase from less than 50% V mn to greater than50% vmu
(D) Decrease from greater than 50% V mu: to less than 50% vmu
(E) Remain at V mu

Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
A	D	A	C	C	D	B	D	D	B

Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18	Q19	Q20
	B	D	B	C	C	A	D	D	C

A cofactor is any chemical component required for enzyme activity; it includes both organic molecules, called “coenzymes,” and inorganic ions.