

*The Behavior of proteins : Enzymes

Lecture 18

- Enzymes study in term of kinetics
- 2 Catalytic mechanism

Affected by
the presence
or absence of
catalysts, con. of
substrate & effector
of life molecules

study of reactions
rates, we look at
the changes in con. of
products & reactants within
a time

as it is very difficult to
velocity of the reaction, how
fast the reaction is happening

→ Some reactions they happen quickly.
Some slowly, but in Biochem. describing
reactions as slow & quickly isn't enough
we need to provide quantitative description
of the rate of reaction, to be able to
compare different reaction, different enzymes,
substrates

* In Biochem. we try to provide quantitative description of biochemical reactions & most of these reactions are catalyzed by enzymes

- All these reactions require substrates, enzymes. & typically enzyme concn. is very low in our cells compared to the substrate con. Why?

- Because enzymes they aren't consumed in the reaction

(As, products left, if 1 molecule of a substrate is dislodged it'll other substrate molecule & it's a large quantity of enzyme)

* The quick appearance of product means we have a very fast reaction

- we are monitoring the velocity of the reaction by looking at the appearance of the product with respect to time

Note → Velocity of reaction differ at different sub. Con.

~~we~~* we have 3 curves because we are looking at product accumulation at 3 different substrate concn. with respect to time

- ~~size~~ Enzyme initial tube → ~~size~~ - enzyme ↑ initial ↓ substrate

Some of the substrate will be converted to product & with more time, more product will be accumulated but also with more time, more products might be converted back to substrate

* In the early stages of reaction we will have mainly the substrate & some products (or maybe no products)

With more time, more products will be produced. With more time some products will go back to substrate & we reach the state of equilibrium

* Initial velocity → When we have a linear relationship between accumulation of product with time

* we typically in enzyme catalyze reaction we are interested in initial velocity of reaction why?

velocity of the reaction will not be the same throughout the reaction, it will keep changing & in enzyme catalyze reaction we are interested in the initial velocity because

we want to measure velocity when there is no, or very little product because if there is large quantity of product being made, this means that we aren't measuring the actual velocity of reaction

because some product go back to substrate & with time some products might inhibit the enzyme activity

Note

product goes to equilibrium
substrate

* We can calculate initial velocity from these lines but also the velocity of reaction could be calculated by determining the change in product conc. over time ($\frac{\Delta P}{\Delta t}$)

reaction \rightarrow velocity is ~~is~~ ~~in~~ ~~le~~ ~~H's~~ ~~*~~
 by either monitoring disappearance
 of the substrate with time or
 appearance of product with time

$$\text{rate} \rightarrow -\frac{\Delta [A]}{\Delta t} = -\frac{\Delta [B]}{\Delta t} = \frac{\Delta [P]}{\Delta t}$$

$$* \text{rate} = k[A]^x[B]^y \rightarrow \text{exponents}$$

rate constant
no. of molecules

temperature dependent

Describe

the number of
molecules involved

in the detailed

steps that constituted

the mechanism

? ~~is~~ means ~~a~~

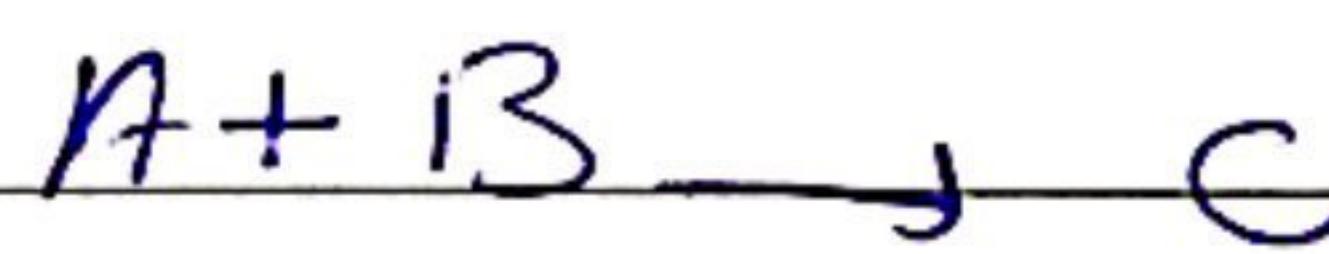
means that exponents

will determine how many

molecules of $[A]$ must collide with
how molecules of $[B]$ to produce C

Note \rightarrow enzymes
no H^+

activation Energy

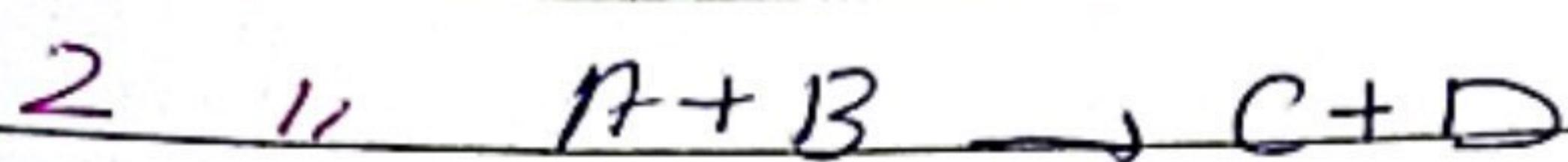
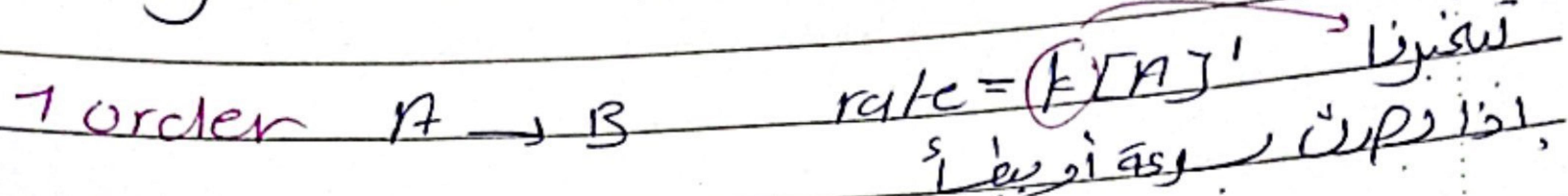


$$\text{rate} = k[n]^x[B]^y$$

L'vov's rule
all \downarrow slip.

* $2A + B \rightarrow 1C$ ~~simple~~

- order of the reaction is Determined by sumation of F & G



- Most of enzyme catalyze reaction are either 2, 1 order, or zero order reactions
Mainly \downarrow
(k , new)
Con. of reactants

Catalyst \rightarrow (k , new) Rate

lower activation Energy w/ catalyst \downarrow
& activation Energy is related to (k),
which mean the catalyst might have
affect on (k)

* Typically when we have zero order reaction in the presence of Enzyme we basically reach a point were the enzymes are saturated with the substrate

? - Some reactions proceeds as 1 ordered reaction but with time they become at higher con. of substrate, the same reaction might be zero order if the reaction is catalyzed by Enzyme

\uparrow Substrate \uparrow velocity
Con.

~~as \uparrow Con. of substrate \rightarrow \uparrow Enzyme~~

all the enzymes are saturated so
we have the Maximum velocity

- Many reactions will require 2
substrate binding to the enzyme

~~as \rightarrow One step reaction
both first order~~

Certain order to happen

~~simple step order \rightarrow one order~~

~~big 2 substrate \rightarrow two step order~~

* Scientist thought that enzymes
proceed according to this simple equation



but it turned out that most
enzymes catalyzed reaction they proceed
through $E + S \rightleftharpoons ES \rightarrow E + P$

* Enzymes can bind the substrate in a
region called active site, typically it's small,
allows the binding of 1 or more substrate
~~as the shape of it is somehow complementary~~
to the substrate shape

* Active site → Made of α-α, some made of nucleotides

We have functional Groups that are the R Group of certain α-α, some Groups are imp. for making interaction (weak H-bond - ionic interactions - hydrophobic interactions) with substrate, & some functional Groups are imp. for the conversion of sub. → product

* Enzymes are considered highly specific which mean it bind specific type of substrate because of the way of active site designed (active site where the reaction happen)

* In term of the binding of substrate to the active site, 2 Major Models

(1) Lock & key Model : we have an active site that have a complementary shape to the shape of sub. (100% perfect match), Active site / sub. have rigid structure

(2) Induced fit model : we don't have 100% match ~~highly~~, Dynamic active site

* The shape of active site is more perfectly match with the transition state of the substrate

* Major charact. of Transition state

higher energy, very short local structure between S & P, Non-stable (bond making at the same time broken)

* If you Give the sub. some Energy, the sub. will become in the transitional state & once we have transitional state we will have equal chances to Go to P or Go back to S.

- In order to convert S to S^+ we need energy called Activation Energy
It's a matter of chance \rightarrow

Energy needed to convert sub. to transitional state.

& chance is \propto Activation Energy \propto $e^{-\frac{E_a}{RT}}$

* One way by which the enzyme lower activation Energy is by stabilizing transition state because its active site will be perfectly match with transition state

Remember the sub. binds to the active site because there is weak interactions happening between the sub. & the active site. In the lock & key model we expect more interactions happening here.

* In induced-fit model, the sub. bind to active site, but because a-s isn't perfectly match to the shape of molecule there might be few molecular interactions & ~~so~~ lock ~~will~~ ^{has} 4 sites

* ES complex have a lower energy compare to the E+S

Whenever E+S bind together it will form interaction, & whenever interactions are formed energy is released

* If the substrate binding goes through lock & key model, the ΔG of this step will be much greater because there will be more interactions

- The more interactions you have between the sub & active site, means we have lower energy of ES complex (More energy will be released)

* Most enzymes they follow induced fit model

T_{sub} con. we reach the maximum velocity

V_{max}

we won't have any further change in the rate of the reaction because there is a lot of substrate & all enzymes are saturated (all enzymes bind to sub.)

Add more Enzyme \rightarrow V_{max} is i.e.

- Some enzymes are allosteric Enzymes, their velocity against Con. generate a Sigmoidal curve

* Adding more sub. won't increase the reaction which mean the rate of reaction doesn't depend on Con. of s.

Note Binding step happen much faster than the catalit. step

Remember Many reactions happen through intermediate step, so SP reaction might be $S \xrightarrow{k_1} I \xrightarrow{k_2} P \xrightarrow{k_3} P \xrightarrow{k_4} P$ (each step will have a rate constant, but the overall rate of this reaction will be always determined by the slowest step (rate limiting step))

Lecture 19

- Many Enzymes follows a type of kinetics called Michaelis-Menten

- Reaction rate (velocity) could be calculated by $\frac{\Delta P}{\Delta t}$ or $k \cdot [con. obs]$

v_0 initial velocity - No product accumulate
 v_{max} highest velocity

* Velocity of reaction isn't constant, it's affected during what happens

$E_P \rightarrow E + P$ Most enzymes

in reversible eq.

Note It take milliseconds to E & S to form ES complex

Focus on ES comp. Rate of reaction goes *

- Con. of ES doesn't change, it remain constant (milliseconds go to)
(Steady state reaction)
equili. (is not eq)

but con. of Es for majority of reaction is constant, which mean Es being formed & Es being converted to product (reactions produce ES & reactions breakdown Es)

* The net reactions doesn't change the con. of ES

↳ net ES con. is ~~in jis jis curc swiss tie gel -~~
proportionally is well, tied,
~~in direk~~
increase in con.
constant.

* 3 rates that are involved directly in or affect con. of ES

- so basically in steady state scientists they assumed that for the majority of the reaction, the change in ES con. over time equals zero

↳ ~~sis jis jis b'con. tie~~
reactions allows the formation of ES reactions / breakdown of ES

ES II con. ~~is jis jis~~ ~~is jis jis~~
is the key to our quantitative measurement of the rate of the reaction but it's not easy to calculate con. of ES complex
~~is jis jis b'losup~~

Quantitative Description of the reaction without including ES con.

- & to simplify the way they derive the equation, they did some assumption

⇒ There is an equili. in the binding step

& also they made the steady state assumption which means for the majority of that reaction the ES complex doesn't change

based on 4 key assumption

$$1 \Delta ES = \text{zero}$$

2 assumed that con. of enzyme is very low compare to the con. of substrate.

3 assumed that

$k_2 \leftarrow k_{-1}$ → valid for many enzymes
but for other isn't

* Rate of the reaction depend on con. of ES, but con. of ES during reaction can't be determined. So they wanted to determine the Velo. without using ES

Note Every Enzyme has a specific Michaelis menten constant for a type of sub., some enzymes can operate on multiple sub. so they will have multiple K_m

K_m Enzyme N mol/L
 V_{max} reaction " "

Note → If we measure initial velocity at different sub. con.

hyperbolic curve ~~sigmoid~~ *

e. ~~sigmoid~~ to find *

Affinity between sub. & enzyme or ligand & protein

K_m could be used as a measure of the affinity of enzyme to sub., not entirely because we have k_2 , but for many enzymes k_2 is small, so it could be ignored, but for other, it can't be ignored

K_m → sub. con. at which reaction reaches half maximum velocity

V_{max} enzyme con. will be

* Enzyme activity increased / decreased by allosteric effectors, increased by making more enzyme, adding / removing phosphate group

JP, VP, JP
Hg, Ni, Co, Cr
congut

Note → Subs. con. = K_m

half maximum initial velocity

Notice that because of limits of solvability of sub. we can't actually experimentally determine V_{max} but scientist find a way to accurately find k_m & V_{max}

$$\text{linear equation } \frac{1}{v} = \frac{k_m + 1}{V_{max}[S]}$$

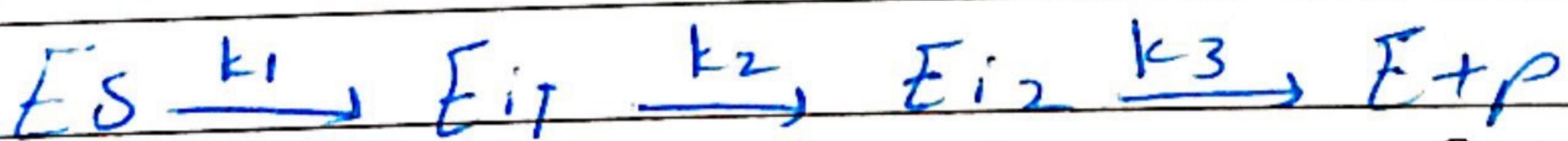
$$-\frac{1}{k_m} = \text{linear line} \rightarrow x\text{-intercept} *$$

$$\frac{1}{V_{max}} = y\text{-intercept} *$$

$$\text{slope} = \frac{k_m}{V_{max}} *$$

Remember V_{max} is reached when all the enzymes are occupied by sub.

Remember There might be intermediate step - Going from $E_S \rightarrow E_i$, $E_i \rightarrow E_{i2}$, $E_{i2} \rightarrow E + P$ each intermediate step will have its own k constant



If k_1 is very small, k_2 is small, k_3 is large \rightarrow k_{cat} will be small

k_{cat}

k_{cat} → - rate constant of the slowest step if there is multiple step

- Describe breaking down of Es into P

- Catalytic constant, called Turnover number

k_{cat} → Measure how many number of moles of sub. con. can be converted to product, per mol of Enzyme per second

ex → k_{cat} for catalase enzyme is 4×10^7
this means that this 1 mol of this enzyme
can convert 4×10^7 mol of sub. per 1 second

$\uparrow k$ value, the more faster/efficient the enzyme is converting sub. into a product

if, $\uparrow k$ is, $\downarrow K_m$ → -
enzyme bind to sub. higher affinity
 $\downarrow K_m \downarrow$ affinity

So some enzymes bind to sub.

- very quickly but they are slow
in converting sub. to a product

* scientist came up of joining k_{cat} & k_m in one equation

$$\frac{k_{cat}}{k_m}$$

The specificity constant / catalytic efficiency

- $k_{cat} = v_{max}$

$\therefore S_1, S_2$
are occupied

It can't exceed 10^{10} for most enzymes

appear limit

diffusion limit/rate
of diffusion

* Most of Biochemical reaction their are happening in an aqueous environment. for the reaction to happen the substrates must diffuse toward the enzyme, this diffusion process happen at a rate, the maximum rate of diffusion is called diffusion limit =

* The enzyme can't quicker convert sub. to product. Then hold quickly sub. diffuse to enzyme

* If the diffusion of sub. to enzyme happens at specific value that number can't be higher than this value.

- let's say the diffusion limit for most molecules in aqueous invi. is 10^{10} , k_{cat} for most enzymes can't be higher than 10^{10}

and it's 30

↓
when the diffusion limit can be higher than the upper limit, when there is forces bring the sub. to enzyme very quickly

Lecture 20

Enzyme inhibition

→ Many drugs are enzyme inhibitors, some diseases are caused by activity of some enzymes

For ex some enzymes become over activated & their activity isn't controlled like in certain type of cancer

* Enzyme inhibitors are sub. that can interfere with enzyme activity which mean they affect some chemical reactions (skip some biochemical reactions)

2 Major type of enzyme inhibitor

1 reversible

- can bind to enzyme & affect their activity in a negative way, but it could be released (interact with functional group of enzyme with weak interactions)

2 Irreversible

- react with the enzyme (interaction between sub. & some reactive groups in the enzyme)

& this reaction will result most of the time in formation of covalent bonds between sub. & the enzyme

Reversible → classify in the way they bind
— (1) the way they affect enzyme activity

\perp → competitive inhibitor

inhibitor, sub. → same shape

Note → binding of inhibitor to enzyme changes the K_m of enzyme to its sub.

Note inhibitor increase K_m , decrease affinity of sub.

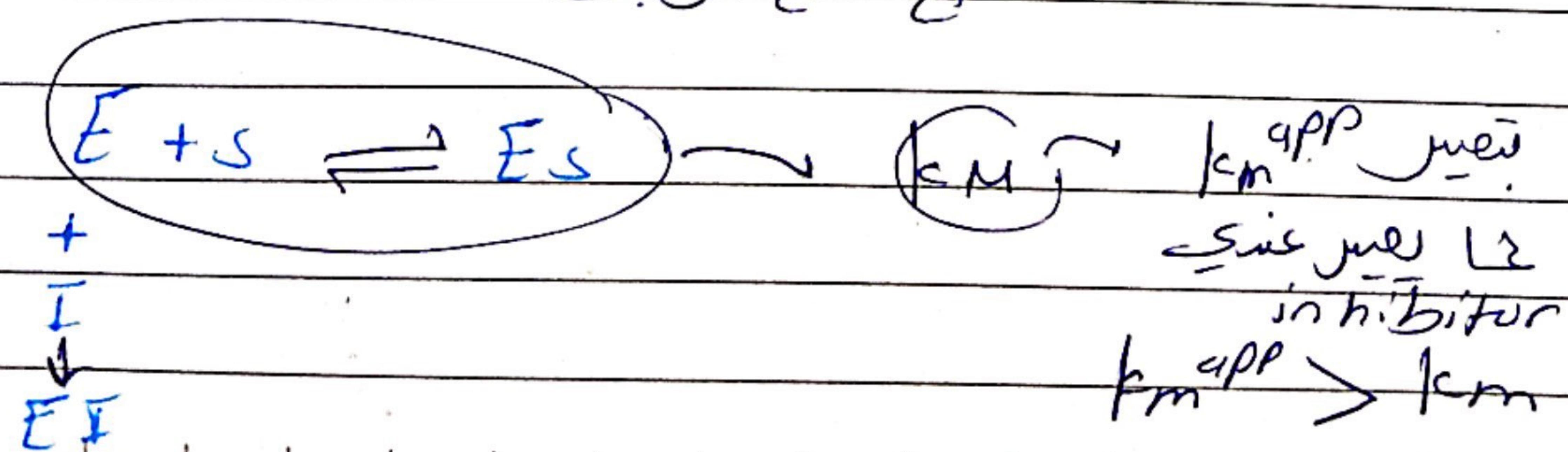
Note If the orientation is right, interactions between sub & functional group are right. sub. will bind

possible to bind w/ enzyme → can do - active site (inhibition)

- Sometimes inhibitor bind first

— sub orientation of \rightarrow

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- V_{max} → ~~comp. inhibitor, so V_{max} will decrease~~

2 Non competitive

- site \neq E_{active}

- Binding of another sub. will cause some structural changes that might slightly change structure of active site (affect catalysis) this means k_m won't be affected but V_{max} will decrease }

* Many enzymes in our cells their activity could be controlled in the presence of activators / inhibitors

↳ Might increase / decrease depend on the state of the cell

3 uncompetitive inhibitor

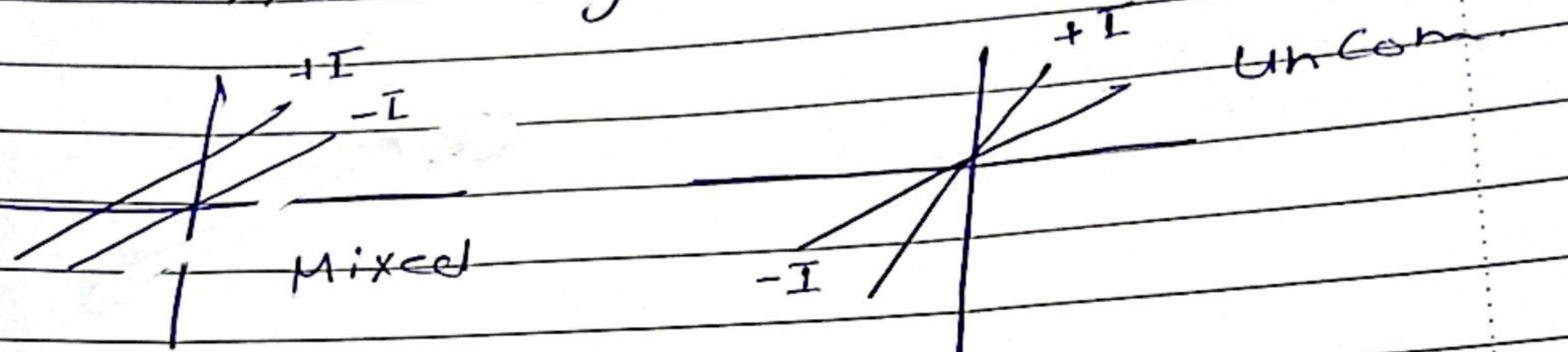
- bind E_S complex not free enzyme

Non Comp. $\xrightarrow{\text{bind}}$ free enzyme
 $\xrightarrow{\text{bind}}$ E_S complex

uncomp. → E_S Complex

4 Mixed inhibitor

- like noncom. in term of ability
to affect catalysis



Un comp. \rightarrow Decrease K_m

Non comp. \rightarrow K_m unif.