Enzymes

Biological Catalysts

Enzymes Have Specific Functions

- Enzymes are categorized into one of 6 biological activities according to the Enzyme Commission:
  - Oxidoreductases: catalyze redox reactions -- involve NAD and FAD
  - Transferases: catalyze group transfers
  - Hydrolases: use water to lyse bonds
  - Lyase: nonhydrolytic and non-oxidative group removal
  - Isomerases: catalyse isomerization reactions
  - Ligase: catalyzes reactions requiring ATP hydrolysis

Enzyme “Add-On’s”

- Cofactors ≡ a molecule or ion of a non-protein nature that is required by an enzyme for complete catalytic capacity, e.g., Mn^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, Mo^{2+}
- Coenzyme ≡ a carbon-based molecule required by an enzyme for complete catalytic capacity, e.g., NAD^{+}, FAD, vitamins – bound loosely to the apoenzyme
- Apoenzyme ≡ active enzyme minus the cofactor; catalytically inactive
- Prosthetic group ≡non-protein moiety tightly bound to apoenzyme
- Holoenzyme ≡ apoenzyme plus prosthetic group
- Zymogens ≡ immature enzymes that need “clipping” for activation – more later in course

- Enzymes are globular proteins
  - Exception: ribozymes
- Without enzymes, cellular reactions go too slowly to be conducive to life
- All enzyme names end in “ase”

Terminology

- Active site ≡ 3-dimensional cleft in the enzyme caused by/coded by the primary structure of the protein; complimentary to the shape (geometry of the substrate)
- Specificity characteristics ≡ due to the active site; crevice allows binding of 1) only one substrate or 2) 1 kind of R group
- Constitutive enzymes ≡ always in the cell without regard to the availability of substrate
- Induced enzyme ≡ present in the cell ONLY when substrate activates gene mechanisms causing intracellular release of active enzyme.

Practical Conditions

- To Study Enzymes:
- Substrate (S) must be converted to product (P) by the enzyme (E) under the following conditions:
  - The reaction is thermodynamically feasible
  - S goes through and above the appropriate E_a for P to form

Example

Reaction	Catalyst	Ea (@ RT)
$2H_2O_2 \rightarrow 2H_2O + O_2$	None	319.2 kcal/mol
$2H_2O_2 \rightarrow 2H_2O + O_2$	I^-	239.4 kcal/mol
$2H_2O_2 \rightarrow 2H_2O + O_2$	Catalase	33.6 kcal/mol

Efficiency of Enzymes

- Increases rate of reaction without being consumed themselves
- Lower the E_a; no effect on K_{eq}
- Permit reactions to reach equilibrium quicker
- Have pH and temperature requirements
- Cause reactions to go within seconds as opposed to lab reactions that may take years
- Necessity to/for life

  E.g., $CO_2 + H_2O \leftrightarrow H_2CO_3$
- Catalyzed by carbonic anhydrase at a rate of $6*10^5$ molecules of CO_2 condensed per second

Specificity of Enzymes

- In reaction types catalyzed
- In substance involved in the reaction (S)
  - Absolute specificity ≡ catalyzes reaction with only one S
  - Relative specificity ≡ catalyzes reaction of substrates with similar structures
  - Stereochemical specificity ≡ D vs L – more later in class

Enzyme Regulation

- Cell regulates which enzymes function and when, i.e., not ALL enzymes are working at the same time
- Some catalyze uni-directional; some catalyze bi-directional

Enzyme Activity

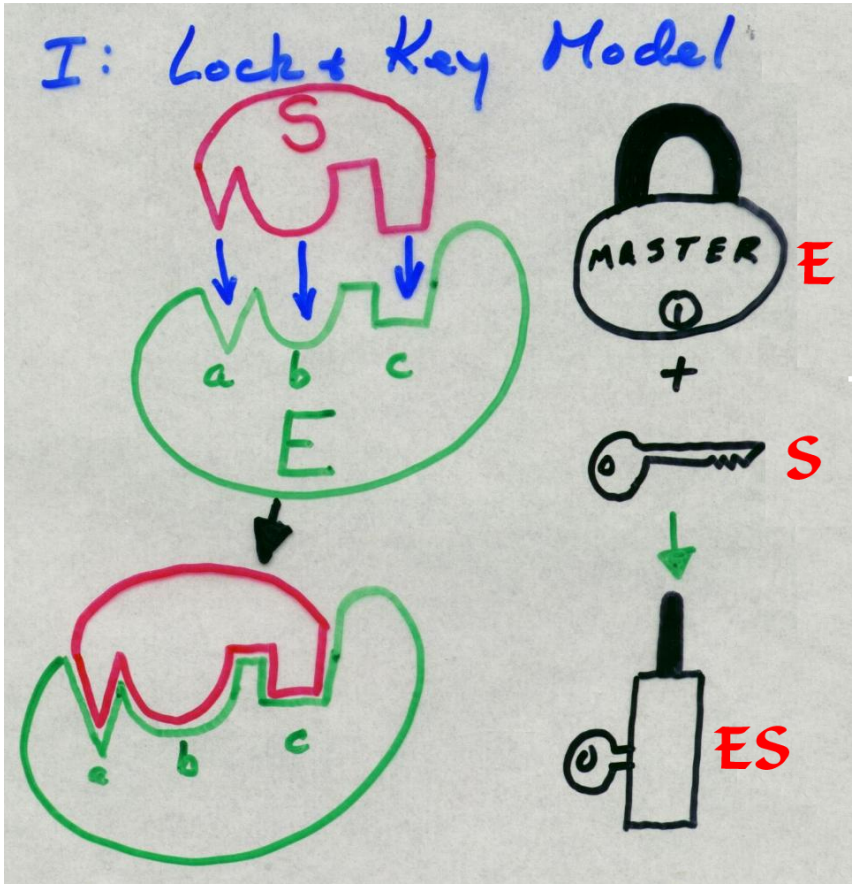
- ≡ catalytic capacity of enzyme to increase reaction rate
- Turnover number ≡ # of S molecules acted upon by ONE enzyme molecule per minute
- Enzyme assays ≡ measure enzyme activity

Enzyme Activity

- International Units ≡ IU
  - The amount of enzyme that catalyzes 1 μmol of substrate to be altered to product per minute at a given pH, T and [S].
  - It measures the amount of enzyme present, therefore, an enzyme level of 150 IU = an enzyme concentration 150 times greater than the standard – useful in diagnosing diseases (more on this later).

Enzyme Models

- Of significance, of course, is the fact that the shape of the enzyme gives it its function (the shape of a protein gives it its function).
- Enzymes speed up the reaction rate in biological systems 100,000 - 1,000,000 fold!
- Some are known to increase the reaction rate > 10^{20}-fold!
- Enzymes have specific substrates (chemical group upon which the enzyme works), but can work on limited kinds of substrates.
- There are two generally accepted models for the functioning of enzymes: the lock and key model and the induced fit model.
- We will address the lock and key model first.

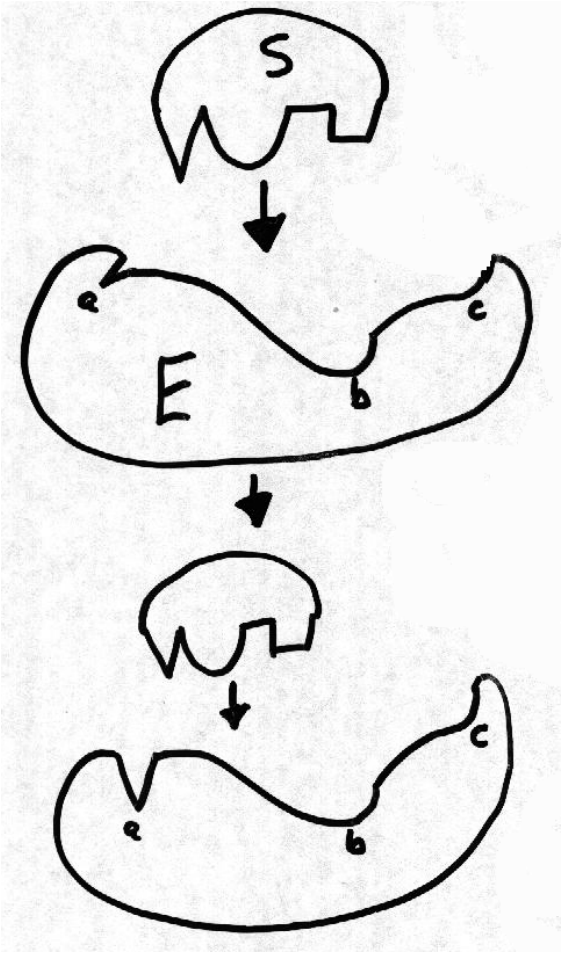


Model #1: Lock-n-Key

- In this model, see graphic, above, the substrate (S) is complimentary to the binding/active site in the enzyme (E). This is likened to the lock and key, where the lock is complimentary to the key. As the E and S bind, they form the Enzyme-Substrate complex (ES). This is an intermediate in the reaction that will cause S to be changed into a product (P).
- The enzyme acts as a sort of scaffold, holding the substrate so that one specific reaction may occur.
- In this case, a bond (or bonds) is (are) broken as the enzyme changes its shape ever so slightly, causing the substrate to break exactly where it's supposed to, releasing the new products and the enzyme for use, again.
- Remember that the active sites (a, b, c) of the enzyme are complimentary to the SHAPE of the substrate.

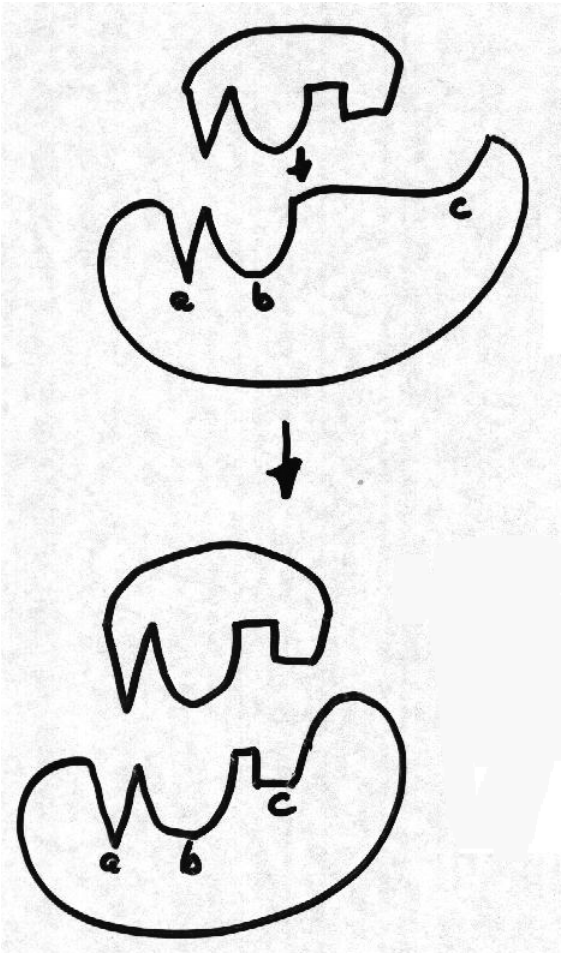
Model #2: Induced Fit

- The second model is called the induced fit model.
- This means that as the S gets closer to the E, the E actually undergoes a conformational change (shape change) to fit the S, i.e., its shape is INDUCED to change by the presence of the substrate.



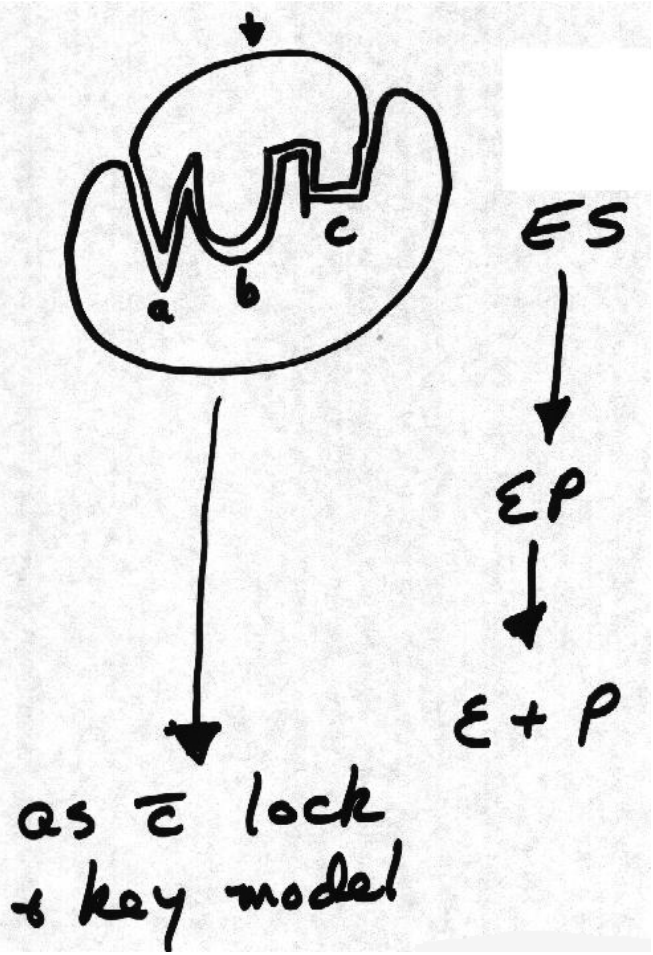
Model #2: Induced Fit

- Note that as S gets closer to E, the active site "a" changes shape to match the complimentary site on S.
- As S continues to get even closer, site "b" shifts its shape, as does site "c" when S is all but bound to the enzyme.



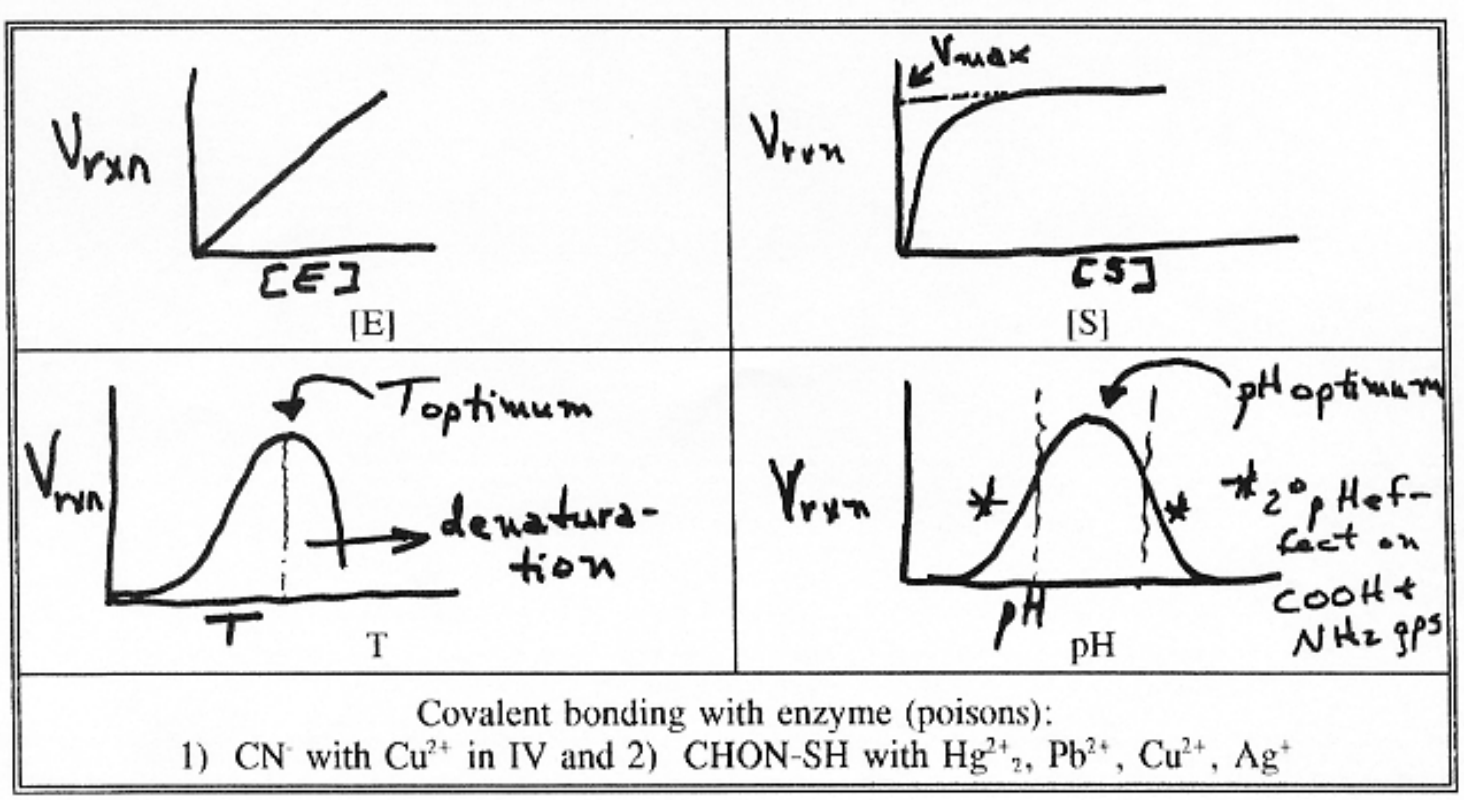
Model #2: Induced Fit

- Once ES is formed, this model conforms to the remainder of the lock and key theory of enzyme-substrate binding.



What Effects Enzyme Activity?

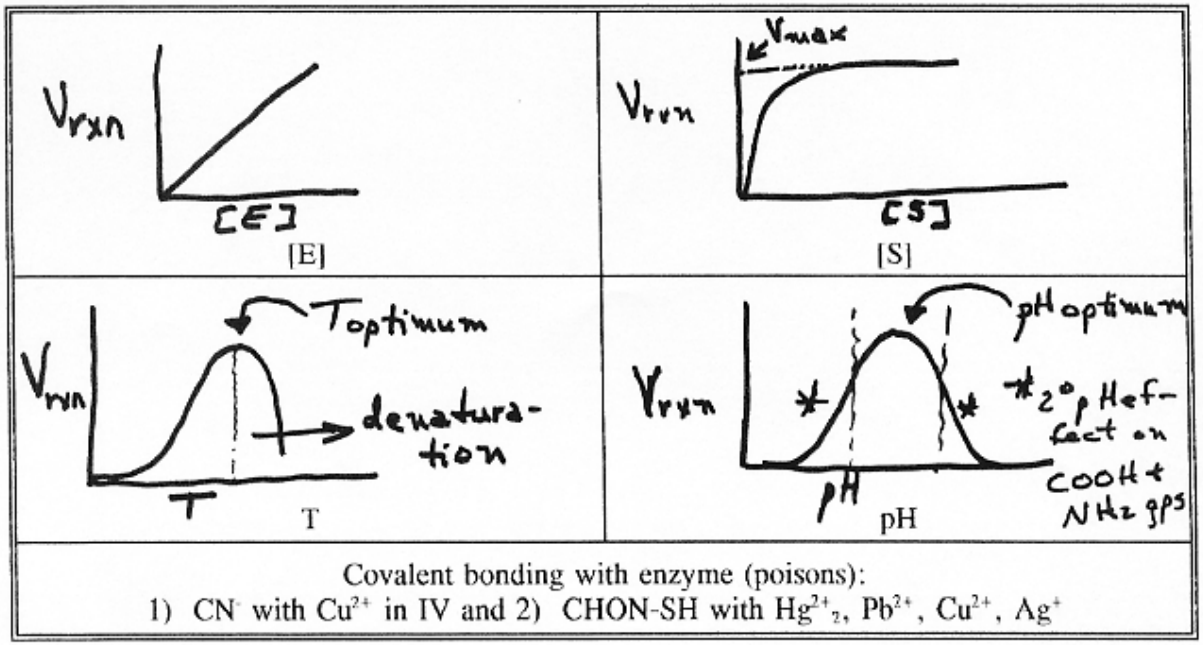
Easy Answer: [E], [S], T, pH and covalent bonding



Covalent bonding with enzyme (poisons):
1) CN^- with Cu^{2+} in IV and 2) CHON-SH with $Hg^{2+}{}_2$, Pb^{2+}, Cu^{2+}, Ag^+

[E] – longer answer

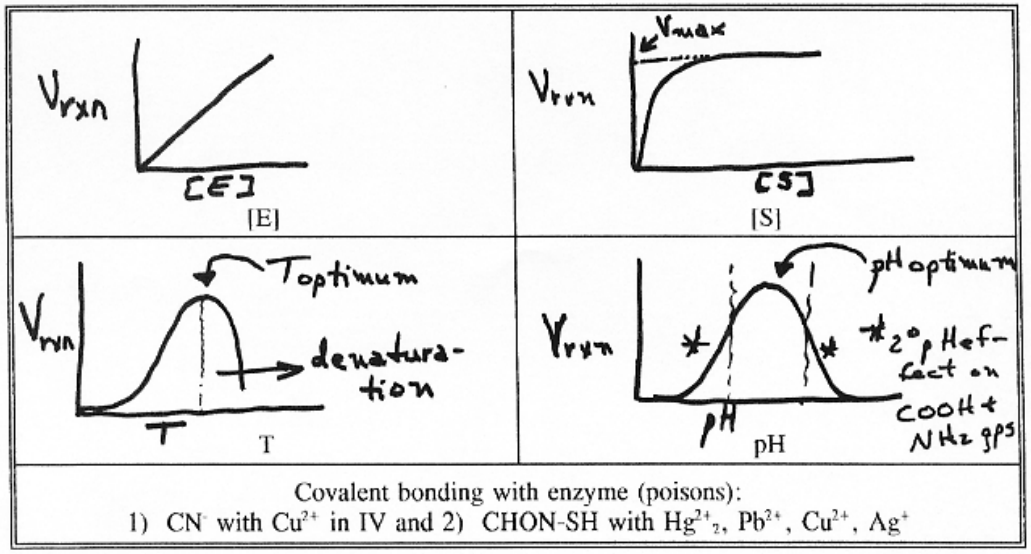
3 [E] = 3 V_{rxn}



Covalent bonding with enzyme (poisons):
1) CN^- with Cu^{2+} in IV and 2) CHON-SH with $Hg^{2+}{}_2$, Pb^{2+}, Cu^{2+}, Ag^+

[S] – Longer Answer

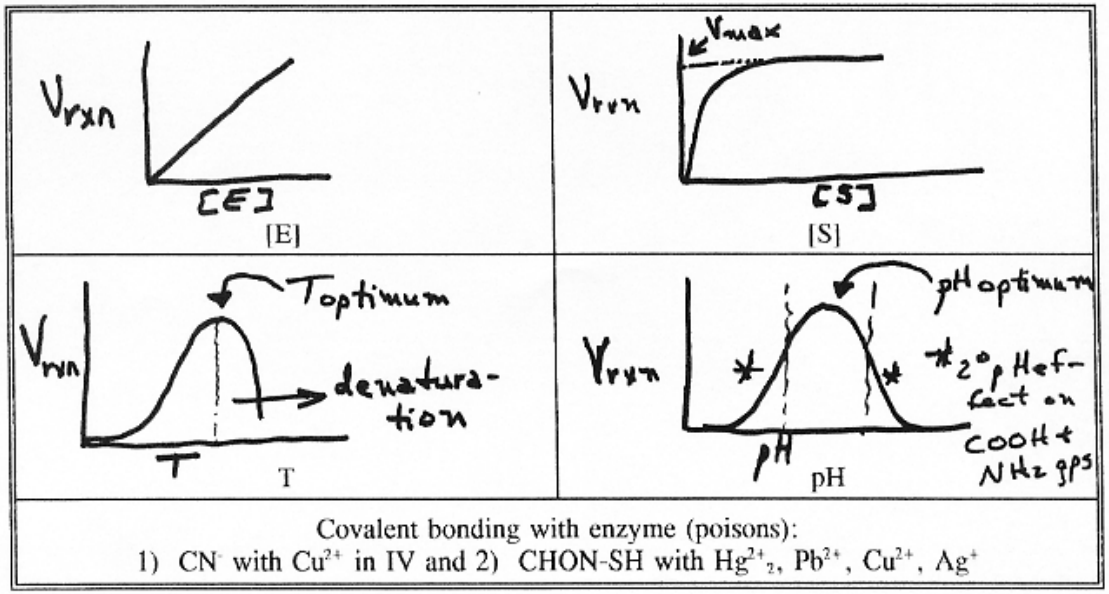
- With increasing [S], causes S to bind at activation site causing conformational changes so that the active site binds S. At some [S], E is sat'd with S and will not work any faster. This rate is called the V_{max}.



Covalent bonding with enzyme (poisons):
1) CN^- with Cu^{2+} in IV and 2) CHON-SH with $Hg^{2+}{}_2$, Pb^{2+}, Cu^{2+}, Ag^+

T – Longer Answer

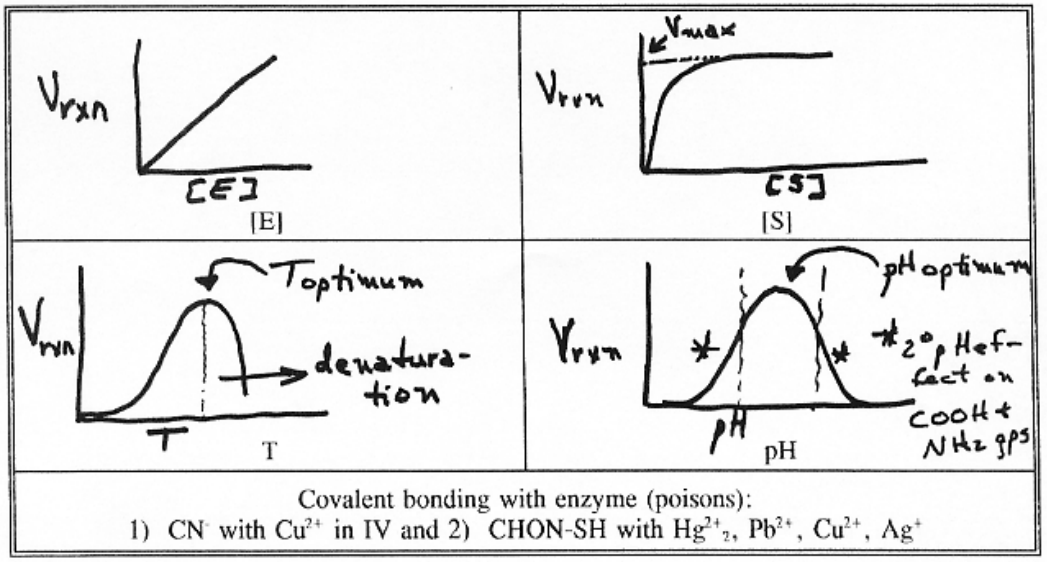
- Increases the rate of the reaction (V_{rxn}) to a point where activity drops off (denaturation)



Covalent bonding with enzyme (poisons):
1) CN^- with Cu^{2+} in IV and 2) CHON-SH with $Hg^{2+}{}_2$, Pb^{2+}, Cu^{2+}, Ag^+

pH – Longer Answer

- pH optimum is where enzyme has greatest activity; at pH's above and below this pH, still has some activity until pH extremes are reached. This causes enzyme denaturation at either end of extremes.



Covalent bonding with enzyme (poisons):
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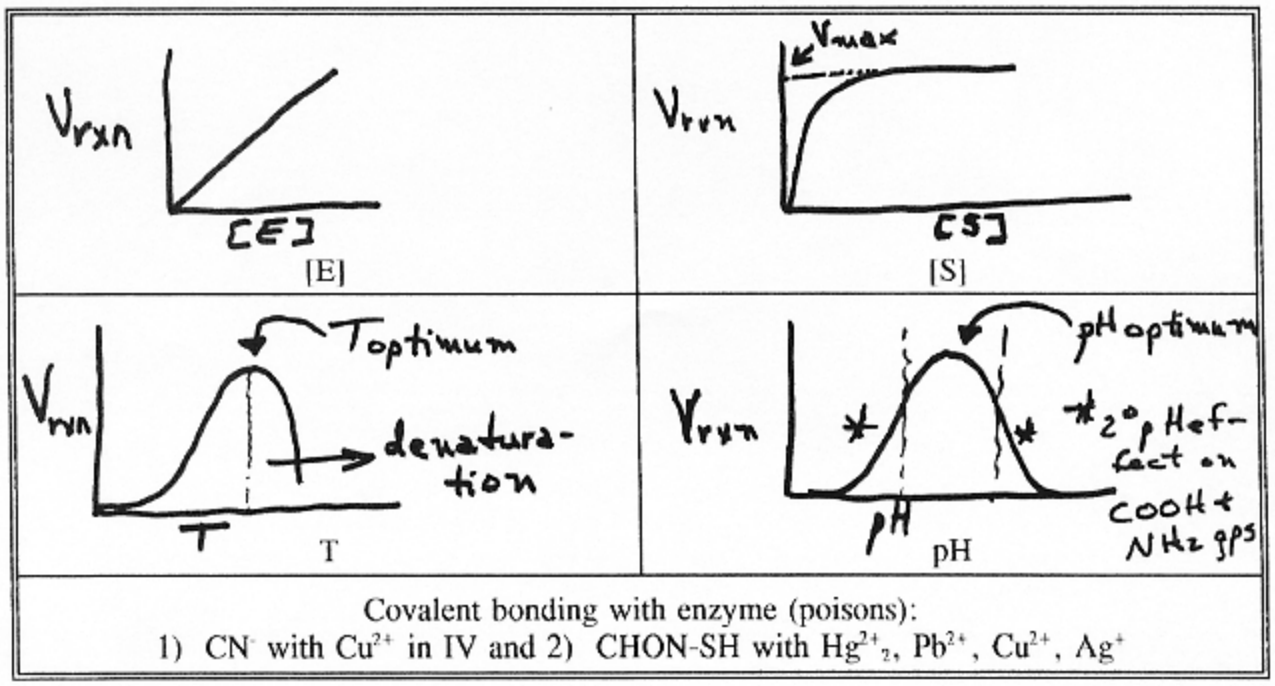
Covalent Bonding – Longer Answer



Covalent bonding with enzyme (poisons):
1) CN^- with Cu^{2+} in IV and 2) CHON-SH with $Hg^{2+}{}_2$, Pb^{2+}, Cu^{2+}, Ag^+

Anything Else?

- YES!!!!

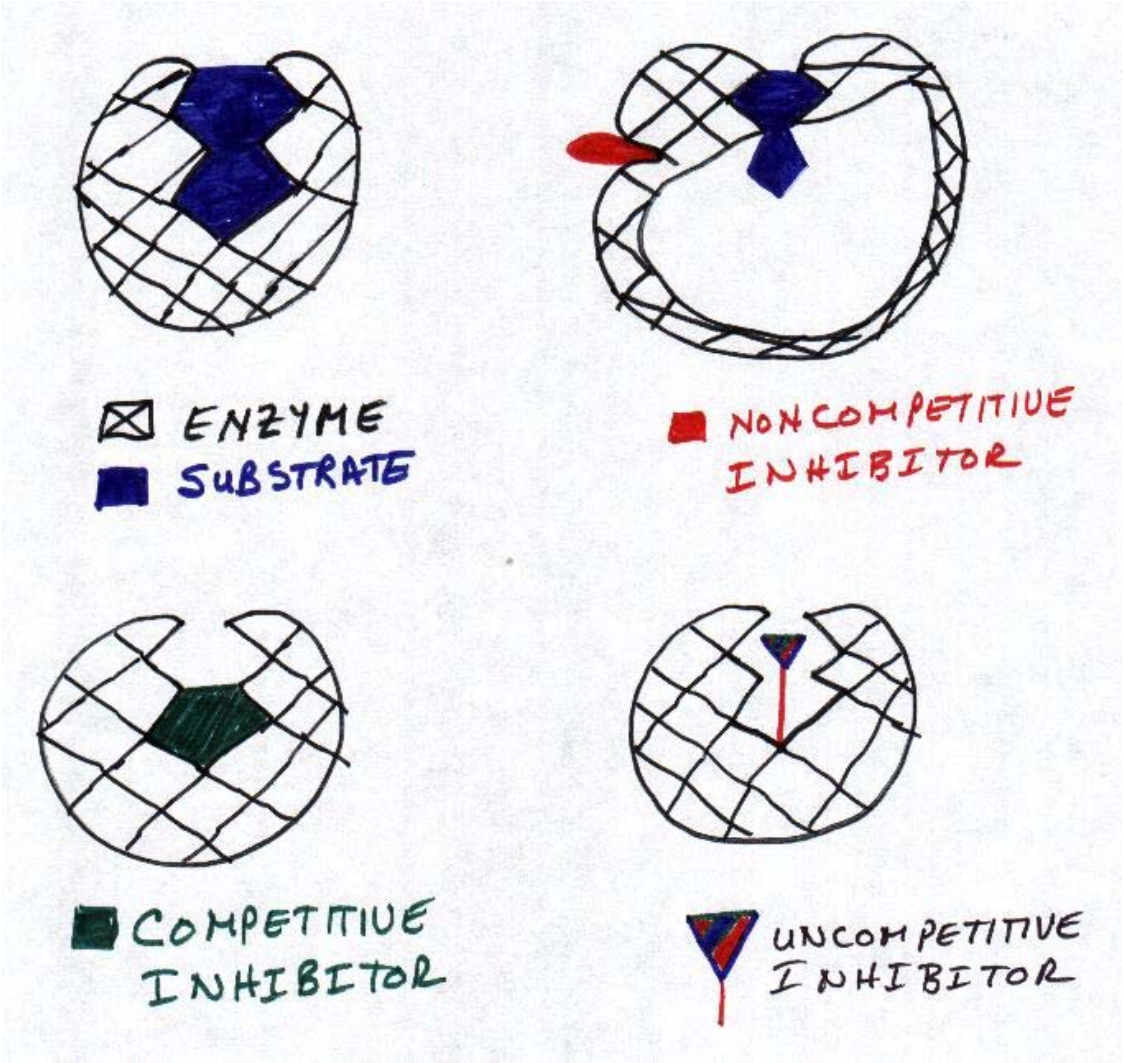
- Effectors
  - Non-substrate that turns on E, e.g., calmodulin (in most cells) and troponin (in muscle cells {skeletal and cardiac}). To activate E's, both must bind Ca^{2+}

Enzyme Inhibition

Descriptive Introduction

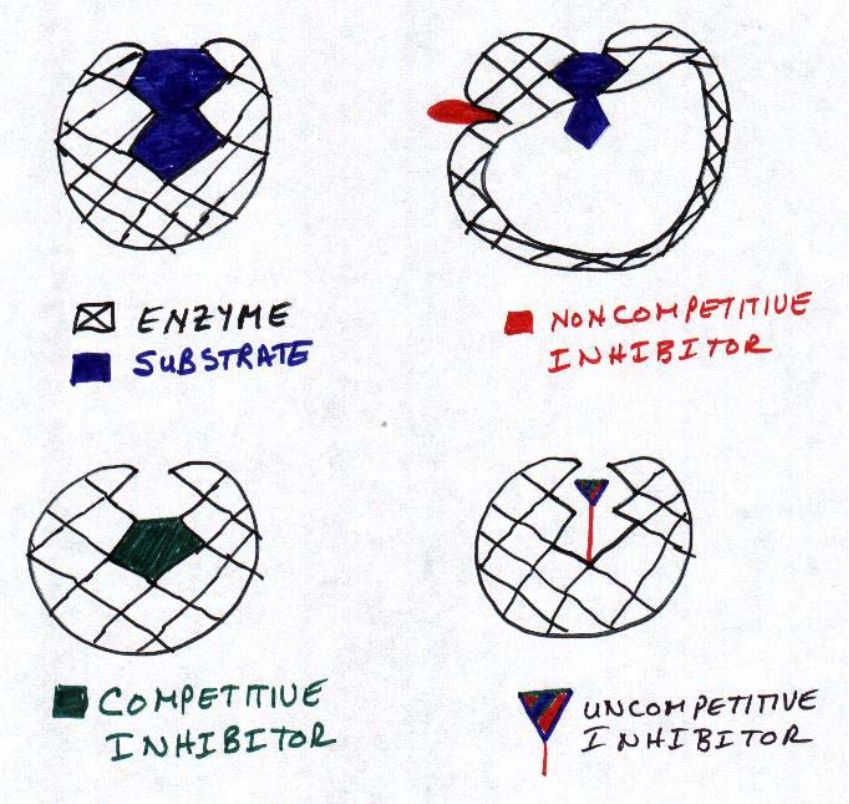
Normal

- The upper left graphic represents the normal ES complex.



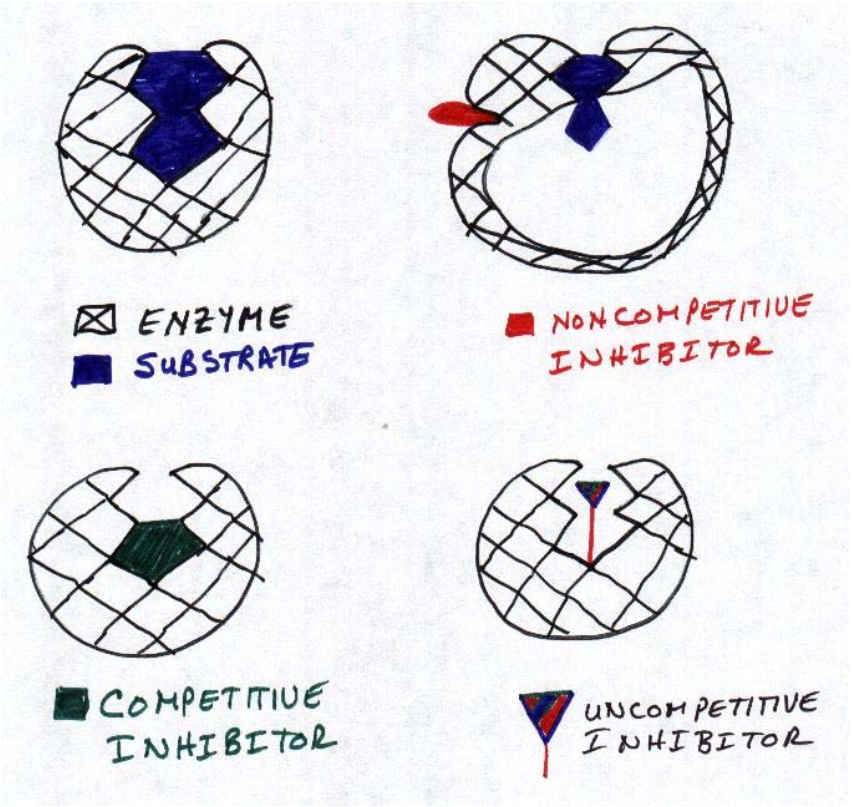
Competitive Inhibition

- The lower left graphic represents competitive inhibition of an enzyme, i.e., an inhibitor specific to this enzyme COMPETES with the substrate for the active site of this enzyme.
- It is reversible; will block S from binding.
- One example of this sort of inhibition is carbamoyl choline that competitively inhibits acetylcholinesterase.



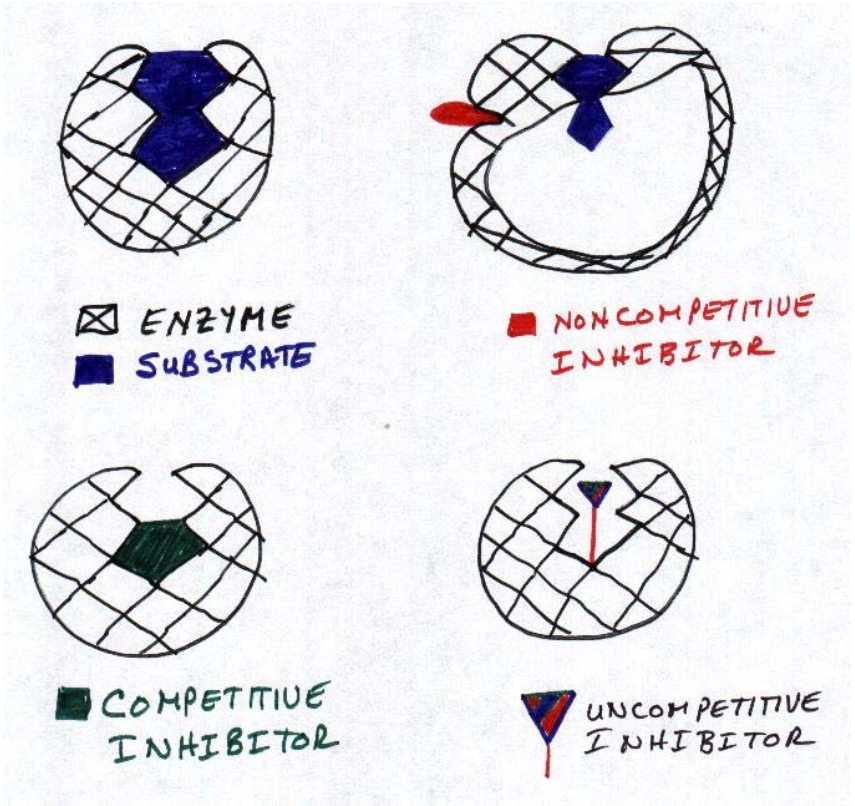
Uncompetitive Inhibition

- The lower right graphic represents uncompetitive inhibition.
- This sort of inhibition involves covalently bound inhibitor and inactivates the enzyme irreversibly.
- Two examples of this sort of inhibitor are nerve gas and organophosphates that inhibit acetylcholinesterase.
- Organophosphate poisoning may be reversed by injecting a drug called 2-PAM.
- Valium and atropine are useful to treat muscle spasms and breathing difficulties, as well.



Non-Competitive Inhibition

- The upper right graphic represents noncompetitive inhibition.
- Note that the inhibitor does NOT bind to the active site of the enzyme, rather it has its own unique binding site.
- When a noncompetitive inhibitor binds to an enzyme, it causes the enzyme to change shape and shuts off its activity reversibly by not allowing S to bind completely.
- This sort of inhibition is also referred to as allosteric inhibition and plays major roles in metabolic regulation.
- An example of a noncompetitive inhibitor is aspirin.
- Aspirin inhibits cyclo-oxygenase which is the main enzyme in prostaglandin biosynthesis.
- Prostaglandins mediate pain, inflammation, blood pressure, gastric mucous secretion, blood clotting, labor and delivery, to name a few.



Apply This to Enzymes

- Enzymes are, with a couple of exceptions, proteins
- Enzymes are biological catalysts
- Enzymes speed up biological reactions incredibly
- For this discussion:
  - E = enzyme,
  - S = substrate and
  - P = product

$$E + S \xrightarrow{k_1} ES \qquad FAST$$

$$ES \xrightarrow{k_{-1}} E + S \qquad FAST$$

$$ES \xrightarrow{k_2} E + P \qquad SLOW$$

$$AND$$

$$\frac{d\,[ES]}{d\,t} = -\frac{d\,[ES]}{d\,t}$$

Short Method

$$k_1[E][S] = k_{-1}[ES]$$

Solve for $[ES]$:

$$\frac{k_1}{k_{-1}}[E][S] = [ES]$$

Stop temporarily

- Rate limiting step is step 3
- Rate equation is: k_2 [ES]
- Substitute as before:

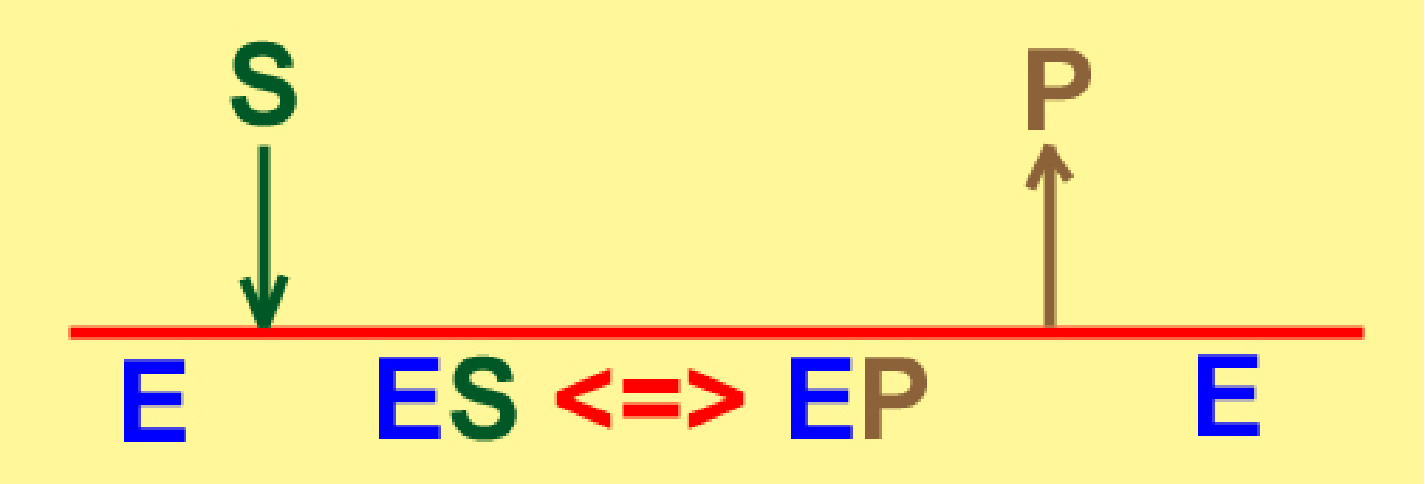
$$\left(\frac{k_1 k_2}{k_{-1}}\right)[E][S] = k[E][S]$$

$$Write\ the\ overall\ reaction:$$

$$E + S \Leftrightarrow ES \rightarrow E + P$$

$$This\ is\ a\ Uni - Uni\ Rxn$$

Uni-Uni Reaction



Ter-molecular Reactions

- An enzyme catalyzed reaction may utilize 2 substrates.
- This reaction is always SEQUENTIAL, however,
- May be
  - ORDERED or
  - RANDOM

E.g., Ordered Sequential Reaction

$$E + X + Y \rightarrow E + R + S$$

E is still enzyme

X and Y are substrates

R and S are products

Putative Mechanism

$E + X \rightarrow EX$

$EX + Y \rightarrow EXY$

$EXY \rightarrow ERS$ SLOW STEP

$ERS \rightarrow ER + S$

$ER \rightarrow E + S$

And:

$$\frac{d[ERS]}{dt} = -\frac{d[EXY]}{dt}$$

$$\frac{d[ERS]}{dt} = k_1[E][X]k_2[EX][Y] \qquad -\frac{d[EXY]}{d\ t} = k_3[EXY]$$

Equate

$$k_1[E][X]k_2[EX][Y] = k_3[EXY]$$

Solve for $[EXY]$

$$\left(\frac{k_1 k_2}{k_3}\right)[E][X][Y][EX] = [EXY]$$

Stop here temporarily

- Rate Limiting Step is: k_3 [EXY]
- Substitute:

$$k_3 \frac{k_1 k_2}{k_3}[E][X][Y][EX] = k_1 k_2 \ [E][X][Y][EX]$$

$$= k \ [E][X][Y][EX]$$

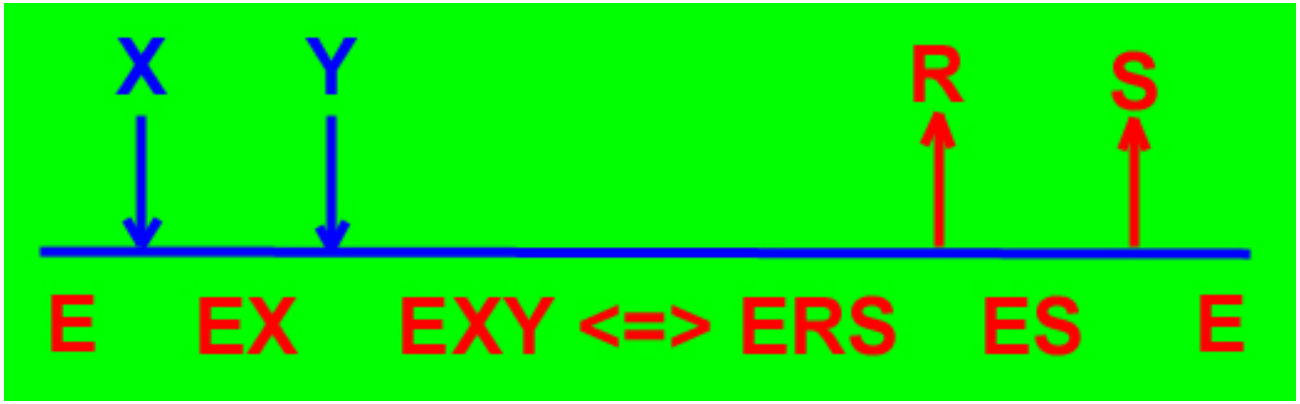
Kinetic Data Tells us:

- The following sequence MUST be taking place:

E + X + Y → EX (FIRST!) → EXY (SECOND!) → ERS → ES + R → E + S

- And is an Ordered Bi Bi Reaction

Ordered Bi Bi Reaction



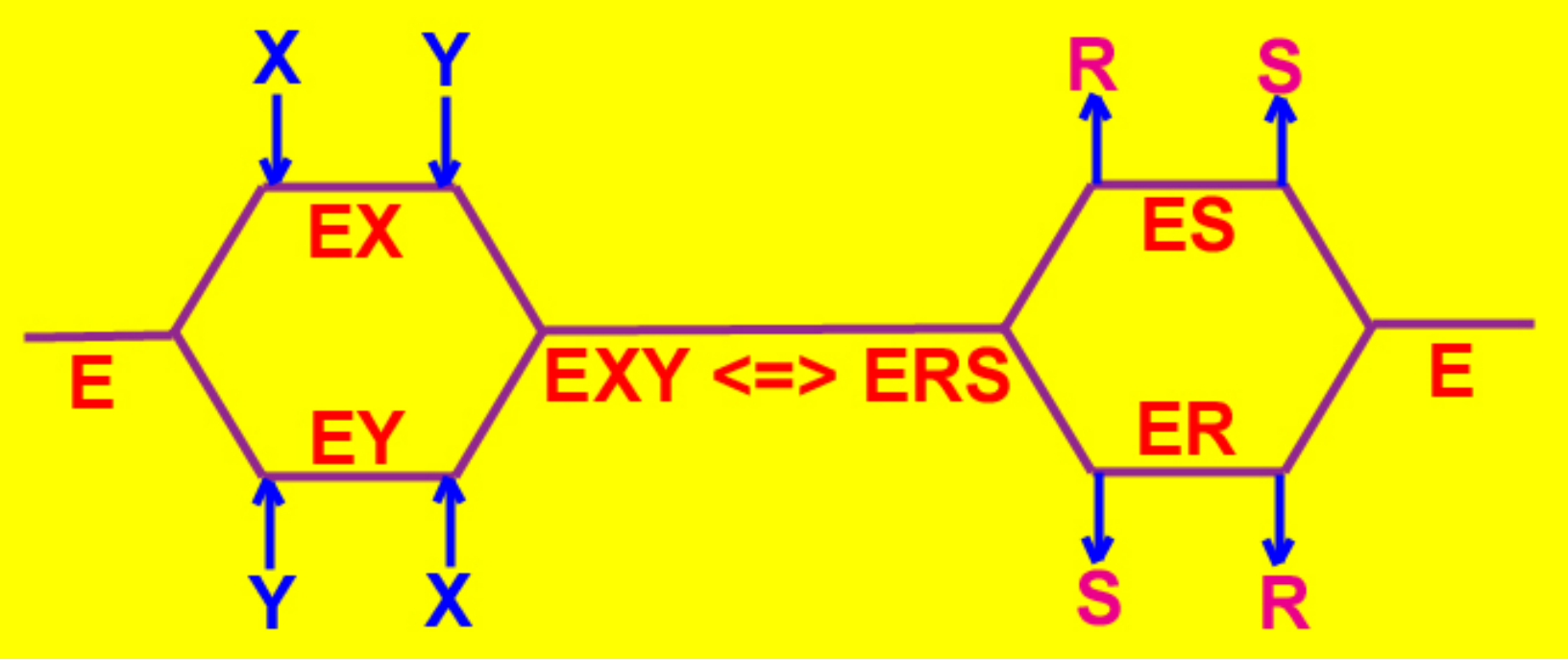
- In the case where separate experiments about the same system give 2 different rate equations, e.g.,

$$k [E] [X] [Y] [EX]$$

And

$$k [E] [X] [Y] [EY]$$

Mechanism = Random Sequential



- What, though, if an enzyme catalyzed a reaction that bound one substrate, released its product, then binds a SECOND substrate and releases ITS product?

Overall Reaction is: $E + X + Y \rightarrow E + R + S$

Mechanism

$E + X \rightarrow EX$

$EX \rightarrow ER$ 1^{st} rate limiting step

$ER \rightarrow E + R$

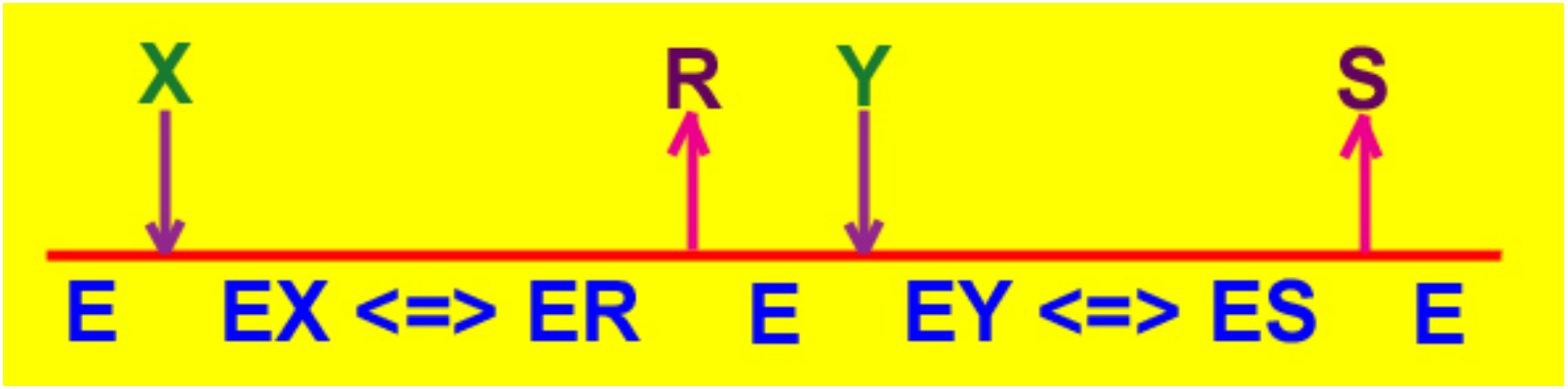
$E + Y \rightarrow EY$

$EY \rightarrow ES$ 2d rate limiting step

$ES \rightarrow E + S$

- Note: while written unidirectionally, in many cases the reactions are reversible
- With 2 rate limiting steps, this reaction and its kinetics get ugly fast.
- This sort of reaction between 2 substrates and the 1 enzyme act like a ping pong game.

Ping Pong Mechanism



Enzyme Inhibition

An Introduction to Quantitative Enzymology

- From this point on, the arithmetic manipulations to study enzymes are all based on the following reaction sequence:

$$E + S \leftrightarrow ES \rightarrow EP \rightarrow E + P$$

- Inherent to this sort of study are 3 assumptions:
  1. [E] << [S]

  2. P ≠ S

  3. ES forms either
     1. E + S OR
     2. E + P

Fundamental Reaction and Constants

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

We can use the above sequence to write a statement about the rate of the reaction (V) which is proportional to [E], [S], and k_1, k_2 and k_3 (these latter three rate constants are “fudge factors” – more on them in CHEM 122 – they can also be used to determine K_{eq} as we’ll see in a bit).

Catalytic Velocity

≡ product of [ES] and k_3 (rate limiting step is rate of P formation and, hence, ES loss) or

$$V = k_3 [ES]$$

- Can we write equation[s] to tell us about [ES] in terms of measurable [E], [S] and [P]? YES!!!!!!

$$V_{\text{formation [ES]}} = k_1 [E] [S]$$

$$V_{\text{loss of [ES]}} = (k_2 + k_3) [ES]$$

!!! ES = INTERMEDIATE in reaction sequence!!!

- When $V_{formation} = V_{loss}$,
  - [ES] is a constant as [S] decreases and [P] increases.
- We may write that arithmetically as

$$k_1 [E] [S] = (k_2 + k_3) [ES]$$

Steady State Approximation

- This approximation is so called because the [intermediate] does not change.

- Let's solve for [ES] since it DOES remain constant:

$$[ES] = \frac{k_1[E][S]}{(k_2 + k_3)} \Leftrightarrow \frac{\frac{[E][S]}{(k_2 + k_3)}}{(k_1)}$$

- Remember that equilibrium constants express what is going on in a reaction in terms of products and reactants.
- They can also be expressed in terms of rate constants. In this case, the equilibrium constant is given a special name and icon: K_M – the Michaelis-Menton constant.

K_M Equals

$$K_M = \frac{k_2 + k_3}{k_1}$$

$$\therefore$$

with substitution

$$[ES] = \frac{[E][S]}{K_M}$$

Is There A Way to Determine [E]?

- YES!!!!!!

  $[E] = [E_T] - [ES]$ which is the UNCOMBINED E

- By substituting $[E_T] - [ES]$ for [E], we can obtain the following equation on the next slide

$$[ES] = \frac{([E_T] - [ES])[S]}{K_M} = \frac{[E_T][S]}{K_M} - \frac{[ES][S]}{K_M} = \frac{[E_T][S] - [ES][S]}{K_M}$$

By Rearranging, We Get

$$K_M\,[ES] = [E_T]\,[S] - [ES]\,[S]$$

$$[ES]\,K_M + [ES]\,[S] = [E_T]\,[S]$$

$$[ES]\,(K_M + [S]) = [E_T]\,[S]$$

And

$$[ES] = \frac{[E_T][S]}{K_M + [S]}$$

Remember, now

- The rate limiting step equation is:

$$V = k_3 [ES]$$

So let's substitute for [ES]

On the next slide

$$V = \frac{k_3[E_T][S]}{K_M + [S]}$$

$$When [S] >>>> K_M,$$

$$\frac{[S]}{K_M + [S]} \rightarrow 1$$

This means

- E is saturated with S at AND causing V_{max} and

$$V_{max} = k_3 [E_T]$$

- When we substitute on the next slide, we get:

Michaelis-Menton Equation

$$V = \frac{V_{\max}[S]}{K_M + [S]}$$

Application #1 – when [S] = K_M

$$V = \frac{V_{\max}[S]}{K_M + [S]} = \frac{V_{\max} K_M}{2 K_M} = \frac{V_{\max}}{2}$$

hence

$$K_M = [S] \text{ @ } \frac{V_{\max}}{2}$$

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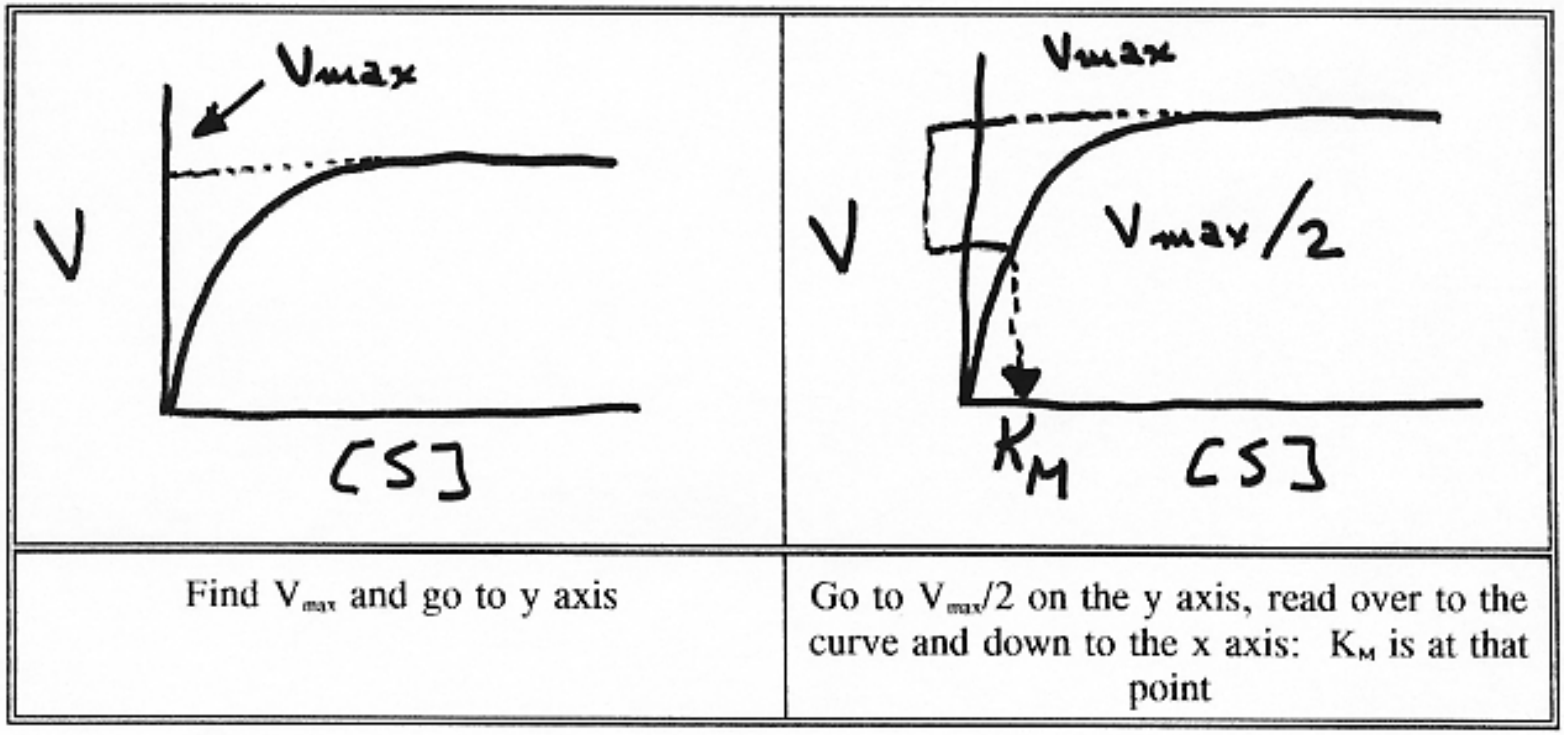


Find V_{max} and go to y axis	Go to $V_{max}/2$ on the y axis, read over to the curve and down to the x axis: K_M is at that point

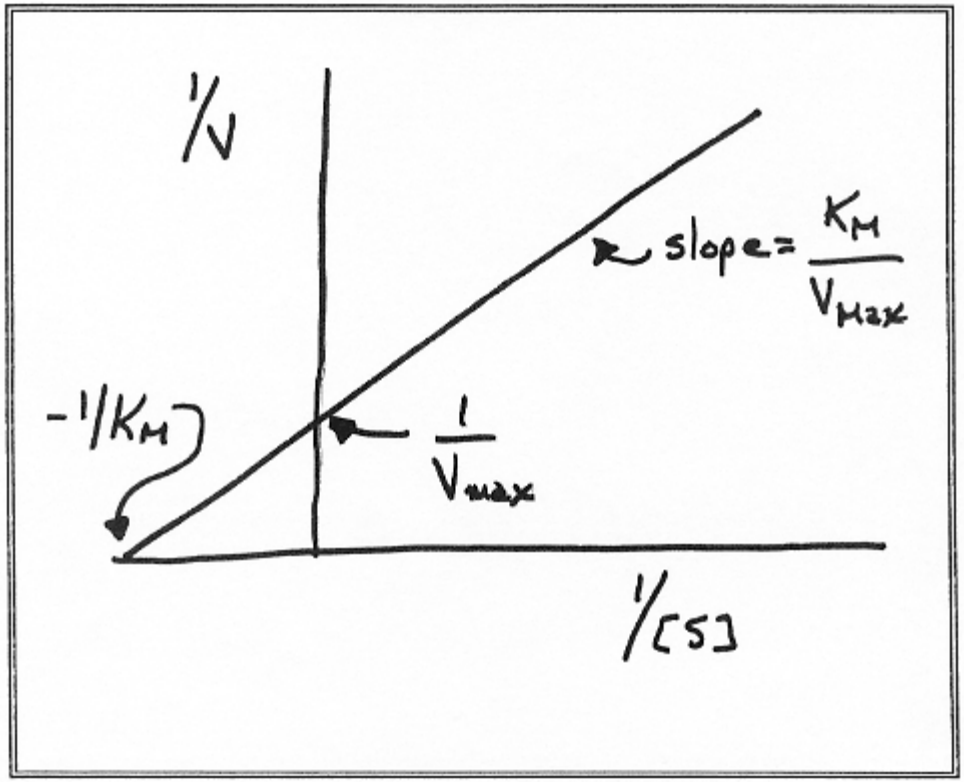
This is in the form of a rectangular hyperbola -- not very useful to the enzymologist.

Lineweaver & Burke Modification

- They took double reciprocals (the other name of this method) and obtained the following equation:

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}[S]}$$

Graphically



This is very useful! -- more to come later.

Application #2

- When $k_2 >>> k_1$ (for most – not all – enzymes)

$$K_M = \frac{k_2}{k_1}$$

Cont'd

- When K_M is high, k_2 is increased and/or k_1 is decreased and favors ES $\rightarrow$ E + S
- High K_M = weak bonding of S with E to form ES
- When K_M is low, k_2 is reduced and/or k_1 is increased and favors ES $\rightarrow$ E + P
- Low K_M = strong bonding of S with E to form ES

Cont'd – E.g.

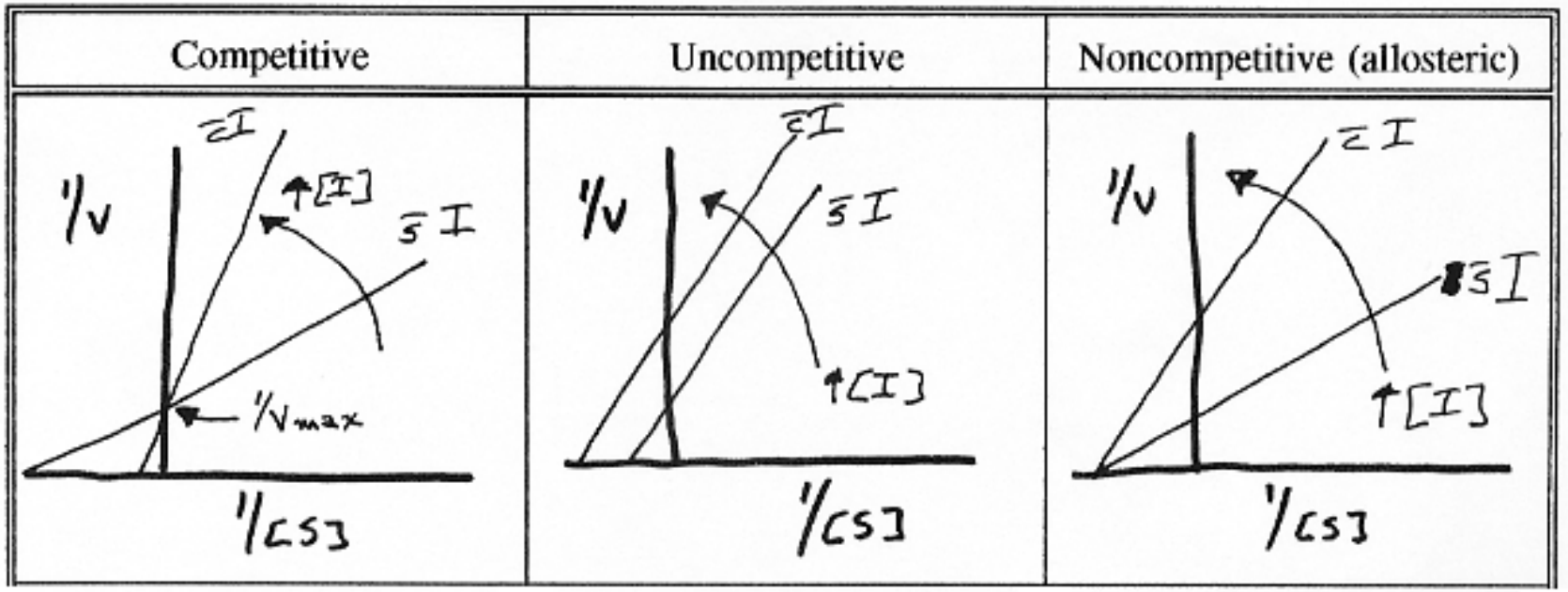
$K_M = 1*10^{-9}$ M ← low K_M

vs

$K_M = 1*10^{-3}$ M ← high K_M

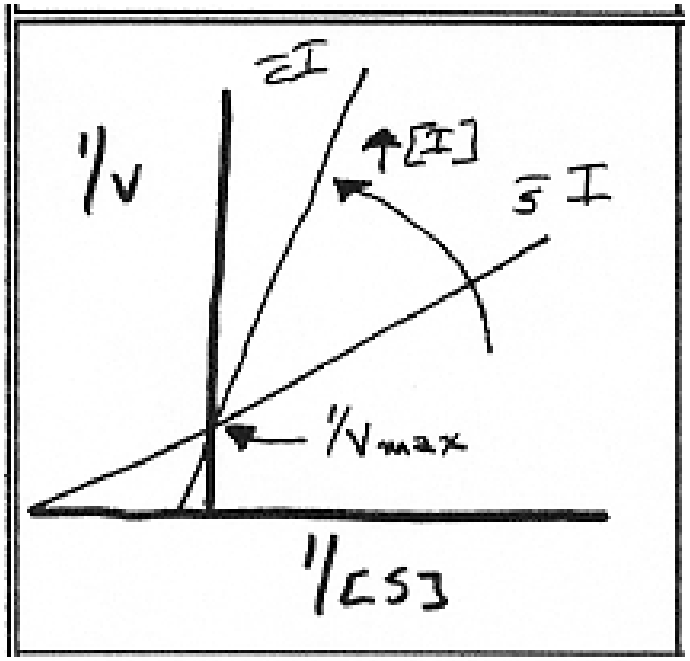
- E binds with S 1,000,000-fold tighter with the E that has the low K_M than with the E with the high K_M.

Lineweaver-Burke and Inhibitors



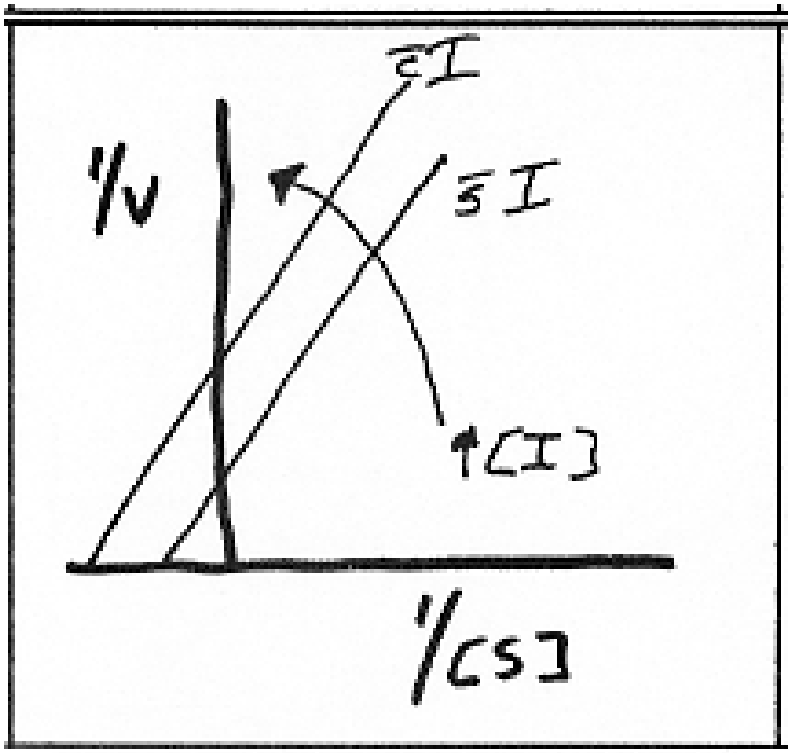
Competitive Inhibitors

- Inhibitor drives no change in V_{max}
- K_M changes with inhibitor
- Inhibition can be overcome by very high [S], i.e., “swamping out” the inhibitor
- Reversible; “temporary”
- Competitive inhibitor completely or partially identical to S molecular shape
- If effects slope and K_M = competitive inhibitor
- NO effect on x-intercept



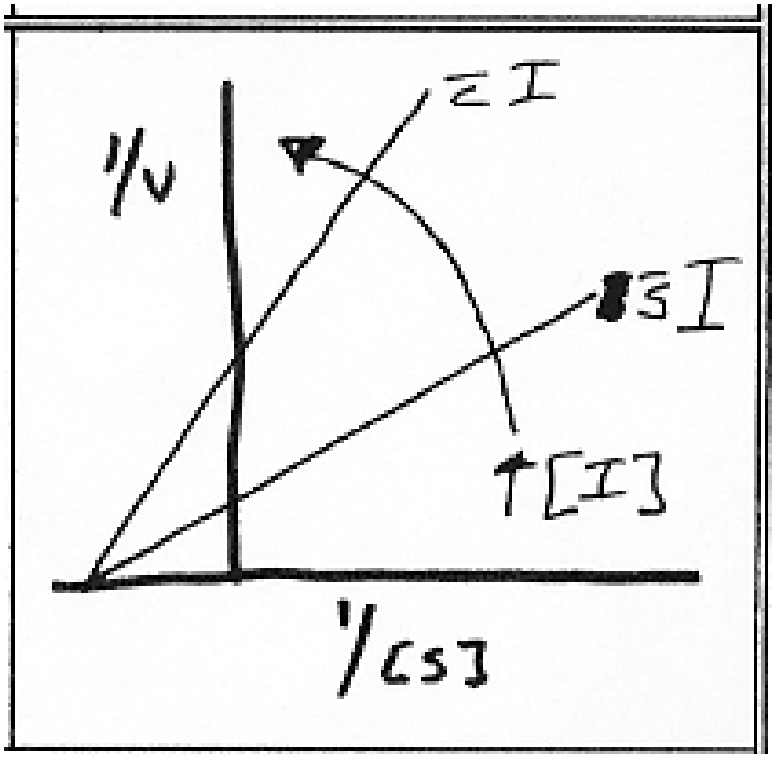
Un-Competitive Inhibitors

- V_{max} changes – decreases with inhibitor
- K_M changes
- Inhibition can not be overcome with high [S] since inhibition is covalent at active site
- Is overcome only 1) with proper drug therapy or 2) with synthesis of new enzyme
- Irreversible; “permanent”
- If effects x and y intercepts = un-competitive inhibition
- No effect on slope



Non-Competitive Inhibitors

- V max changes (decreases with inhibitor)
- No change in KM
- Inhibition can not be overcome by high [S] since inhibition does not occur at active site or E, but at surface site
- Reversible; “temporary”
- aka feedback inhibition; allosteric inhibition
- If effects slope and y intercept = non-competitive inhibition
- No effect on x-intercept



Isoenzymes/Isozymes

- ≡ multiple forms of enzymes in different tissues with the same activity;
- Have identical cofactors but slightly different apoenzymes.
- Two best examples: LDH and C[P]K

LDH – Lactate Dehydrogenase

LDH				
LD_1	LD_2	LD_3	LD_4	LD_5
H_4	H_3M	H_2M_2	HM_3	M_4
Heart	Heart (⇑); Brain = Kidney	Brain = Lung	Lung (⇑); Skeletal muscle	Liver = Skeletal Muscle
H = heart sub-units; M = muscle sub-units				

C[P]K – Creatine [phospho] kinase

C[P]K		
BB	MM	MB
1° Brain	1° Skeletal muscle	1° Heart

Medical Uses of Enzymes and Enzyme Assays

- When cells die or are injured, they dump some or all of their E's into the blood. Assays are used to make diagnoses, e.g.,
  - C[P]K, LDH 2° MI (myoglobins and troponins are being used, as well)
  - GPT (ALT) 2° liver problems
  - GOT (AST) 2° MI or liver
  - Ratios
    - GPT:GOT – normal = 0.75; viral hepatitis = 1.6
    - LD_1:LD_2 – normally < 1; 48° after MI, > 1 and is called the LD_1-LD_2 "flip"
- Calcium ion channel blockers – block calcium ion influx via channel which leads to reduced calcium ion inside the cell which leads to reduced muscle contraction which makes it easier for the heart to beat to reduce the risk of MI or death after MI.