ENZYMES

Course: Biochemistry I (BIOC 230)
Textbook:
Principles of Biochemistry, 5th Ed., by L. A. Moran and others. 2014, Pearson. . Chapter 5

Enzymes – part 1

Introduction

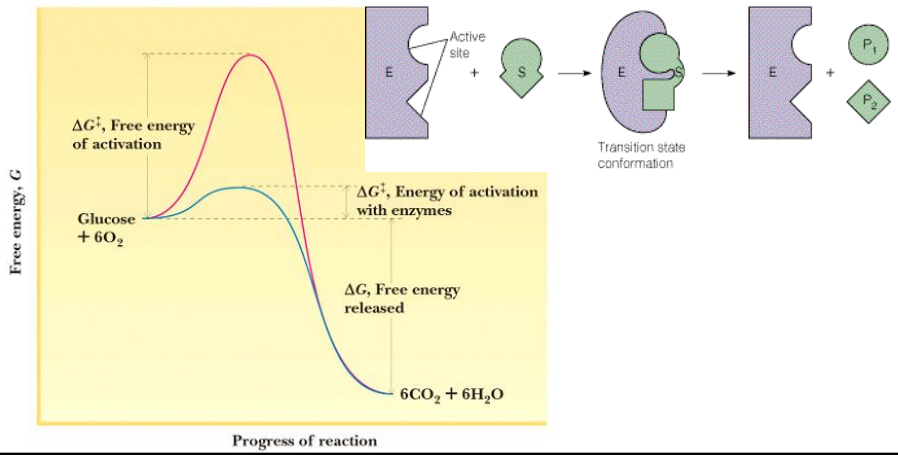
Enzymes

- **Stereospecificity**: many enzymes act on only a single stereoisomer
- **Reaction specificity:** lack of formation of wasteful by-products
- **Coupled reaction:** a common feature of many enzymes, like hydrolysis of ATP is often coupled to less favorable metabolic reaction
-

Enzymes are Catalysts

- Substance that increases rates of a chemical reaction
- Does not effect equilibrium
- Remain unchanged in overall process
- Reactants bind to catalyst, products are released

Catalysts increase product formation by
(1) lowering the energy barrier (activation energy) for the product to form
(2) increases the favorable orientation of colliding reactant molecules for product formation to be successful (stabilize transition state intermediate)



Catalytic Power

- Enzyme-catalyzed reactions are typically 10^3 to 10^{20} times faster than the corresponding uncatalyzed reactions!
- Urease is a good example:
  - Catalyzed rate: 3×10^4/sec
  - Uncatalyzed rate: 3×10^{-10}/sec
  - Ratio is 1×10^{14} !

Specificity

- Enzymes selectively recognize proper substrates over other molecules
- Enzymes produce products in very high yields – essentially 100% (?) much higher than catalyzed reactions in organic chemistry
- Specificity is controlled by structure - the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield

Nomenclature of enzymes

- Enzymes are mostly named by adding the suffix –ase to the substrates or to a descriptive term of the reaction
- Examples: Urease has urea as substrate; alcohol dehydrogenase catalyzes the removal of hydrogen from alcohol, i.e., the oxidation of alcohol
- Few enzymes like Trypsin and Amylase are known by their historic names

Classification of enzymes

REVIEW ONLY!!

- Enzymes are classified into 6 classes by IUBMB
- The IUBMB assigns a systematic name and a unique number or EC number to each enzyme.
- The EC number is a unique four-part number which identifies the reaction it catalyzes
- This book refers to enzymes by their COMMON names
- Updates of classification are available at: http://www.sbcs.qmul.ac.uk/iubmb/enzyme/

IUBMB: International Union of Biochemistry and Molecular Biology.

How the E.C. number is derived?

REVIEW ONLY!!

- Example: ATP:glucose phosphotransferase or common name "Hexokinase"

$$\text{ATP} + \text{D-glucose} \longrightarrow \text{ADP} + \text{D-glucose 6-phosphate}$$

- ATP:glucose phosphotransferase (Hexokinase)
- Its Enzyme Commission number or E.C. number is **2.7.1.1**
  - 2: Class name (Transferase)
  - 7: Subclass (phosphotransferase)
  - 1 / third number: phosphotransferase with a hydroxyl group as acceptor
  - 1 / fourth number: D-glucose as the phosphoryl group acceptor

Classes of enzymes (IUBMB classification)

1. **Oxidoreductases**
2. **Transferases**
3. **Hydrolases**
4. **Lyases**
5. **Isomerases**
6. **Ligases**

Classes of enzymes (IUBMB classification)/I

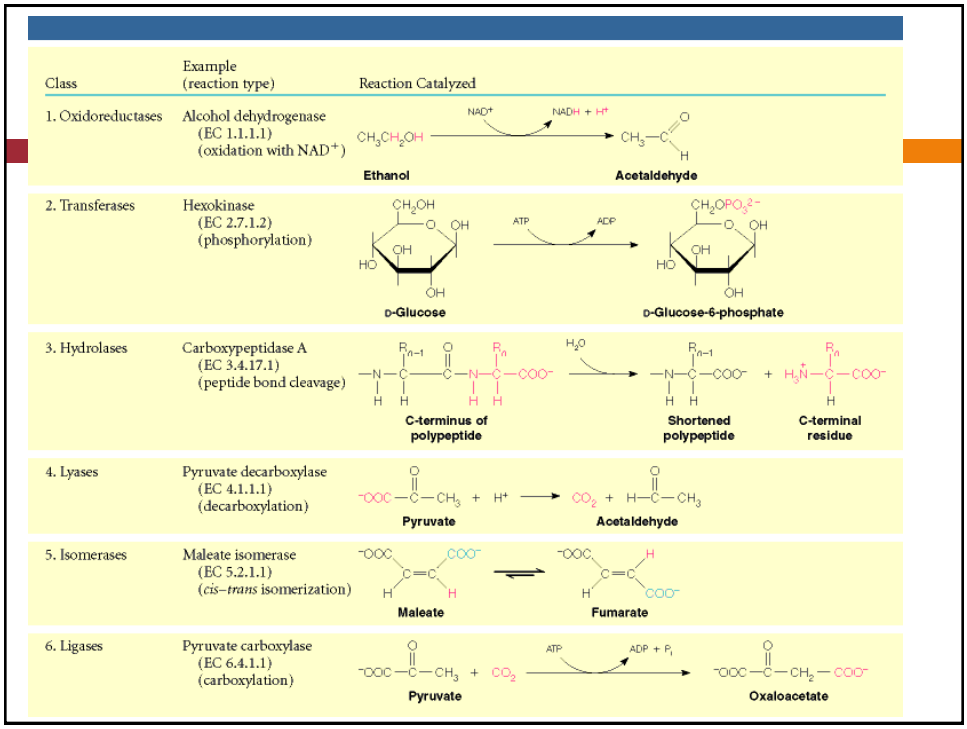
- **Oxidoreductases**:
  - catalyze oxidation-reduction reactions (NADH),
  - mostly referred as dehydrogenases,
  - others include oxidases, peroxidases, oxygenases and reductases
- **Transferases:**
  - catalyze transfer of functional groups from one molecule to another.
  - In group transfer reactions, a portion of the substrate usually binds covalently to the enzyme or its coanzyme
  - Includes kinases

Classes of enzymes /II

- **Hydrolases:**
  - catalyze hydrolytic cleavage.
  - Water serving as the acceptor of the group transferred.
- **Lyases:**
  - catalyze removal of a group from or addition of a group to a double bond, or other cleavages involving electron rearrangement.
  - these are nonhydrolytic, nonoxidative, elimination reaction
  - **Synthases.**

Classes of enzymes /III

- **Isomerases:**
  - catalyze intramolecular rearrangement.
- **Ligases:**
  - catalyze reactions in which two molecules are joined.
  - Uses ATP as an energy source.
  - Synthetases.



Co-enzymes

- Non-protein molecules that help enzymes function
- Associate with active site of enzyme
- Enzyme + Co-enzyme = holoenzyme (Functional form)
- Enzyme alone = apoenzyme
- Organic co-enzymes – thiamin, riboflavin, niacin, biotin
- Inorganic co-enzymes – Mg^{++}, Fe^{++}, Zn^{++}, Mn^{++}

Kinetics

- Study of reaction rate
- Determines number of steps involved
- Determines mechanism of reaction
- Identifies “rate-limiting” step

Enzymes – part 2

Kinetics

Rate constants and reaction order

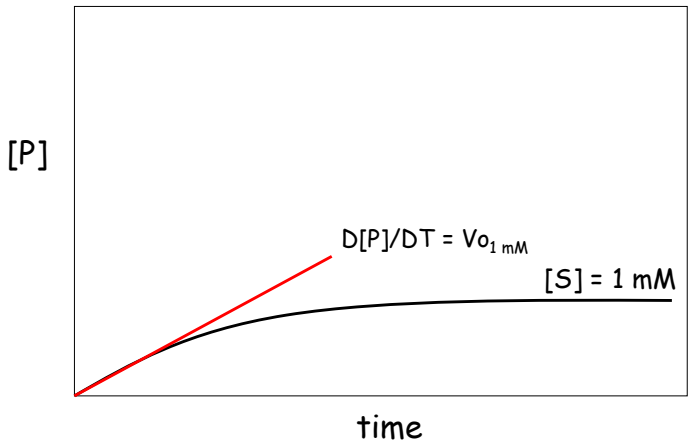
- Rate constant (k) measures how rapidly a rxn occurs

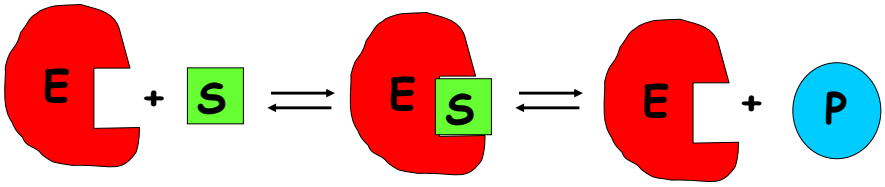
$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B + C$$

- Rate (v, velocity) = (rate constant) (concentration of reactants)
  - $v = k_1 [A]$
- 1^{st} order rxn (rate dependent on concentration of 1 reactant)
  - $v = k_{-1}[B][C]$
- 2^{nd} order rxn (rate dependent on concentration of 2 reactants)
- Zero order rxn (rate is independent of reactant concentration)

Initial Velocities or rates

Hold [E] constant; [S] is much greater than [E]

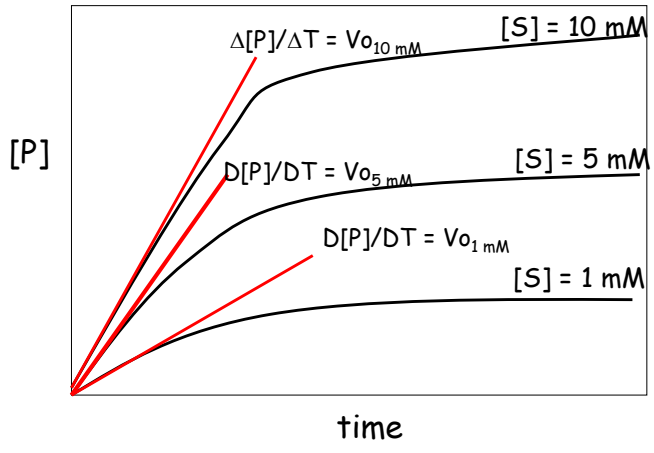




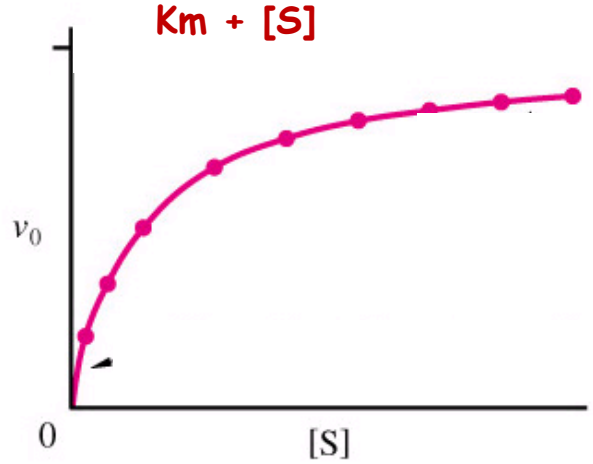
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Initial Velocities

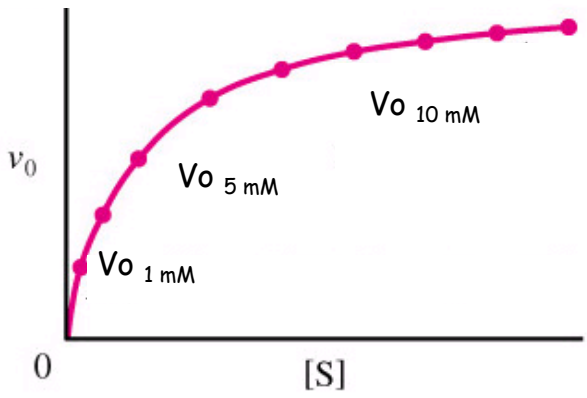
[P] increases as the rxn proceeds.
Initial velocity (v_0) is the slope of the initial linear part of the curve.

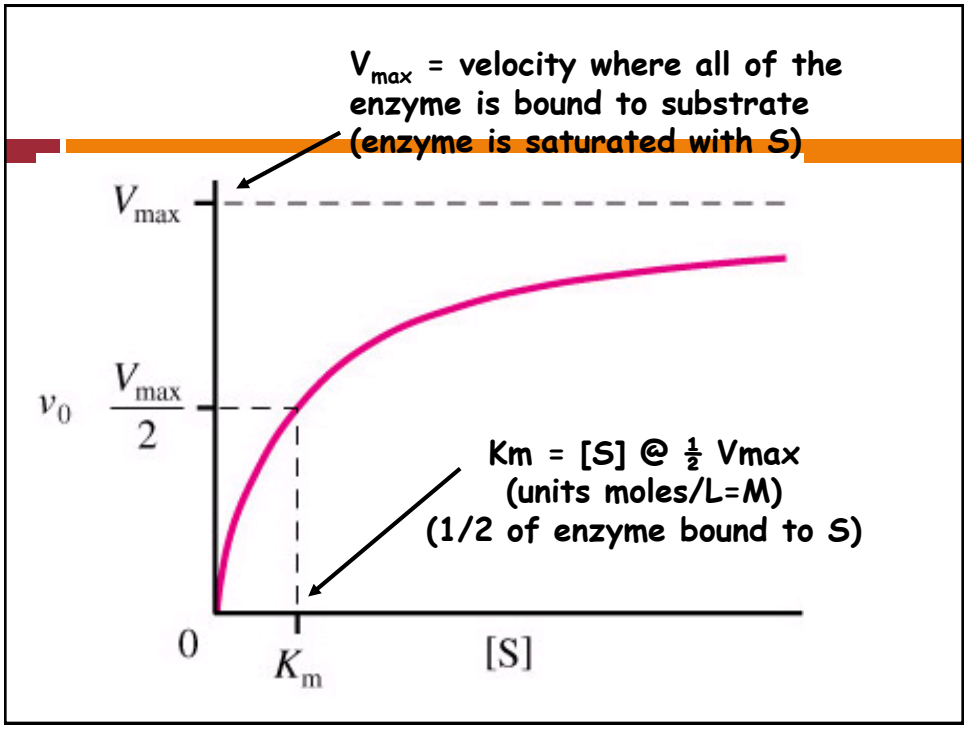


- Michaelis-Menton Equation
- Describes rectangular hyperbolic plot
- **Vo = $\frac{Vmax\ [S]}{Km + [S]}$**



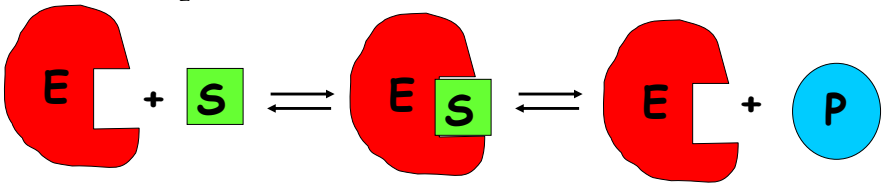
Plot V_0 vs. [S]





Initial Velocity Assumption

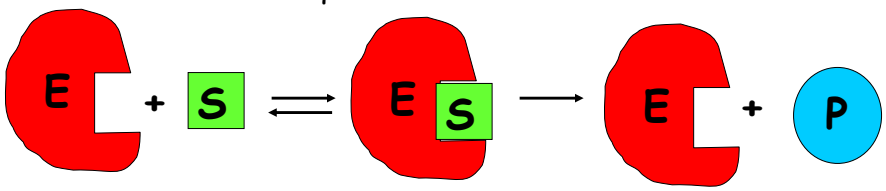
1) Measurements made to measure initial velocity (v_o). At v_o very little product formed. Therefore, the rate at which E + P react to form ES is negligible and k_{-2} is 0. Therefore



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

Steady State Assumption

Steady state Assumption = [ES] is constant. The rate of ES formation equals the rate of ES breakdown



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

What does Km mean?

- Km represents the amount of substrate required to bind ½ of the available enzyme (binding constant of the enzyme for substrate)
- Km can be used to evaluate the specificity of an enzyme for a substrate (if obeys M-M)
- Small K_m means tight binding; high K_m means weak binding

Hexose Kinase
Glucose + ATP <-> Glucose-6-P + ADP

Glucose	Km = 8 X 10^{-6}
Allose	Km = 8 X 10^{-3}
Mannose	Km = 5 X 10^{-6}

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Table 6-6
Lehninger Principles of Biochemistry, Sixth Edition

Applications of MM equation

- Interpreting Vmax and Km:
- Km can vary greatly from enzyme to enzyme and even for different substrates of same enzyme (see Table 6-6)
- Km is sometimes used as indicator of enzyme affinity for substrate, but the actual meaning depends on the specific aspects of the reaction mechanism
- Km can also be used to evaluate the Specificity of action of a given enzyme toward similar substrates. The general rule is, the lower the Km value, the better (more preferred) is the substrate,

Applications of MM equation

- Interpreting Vmax and Km:
- Vmax varies greatly from one enzyme to another
- Vmax is an expression of the upper limit efficiency of operation for a given amount of an enzyme

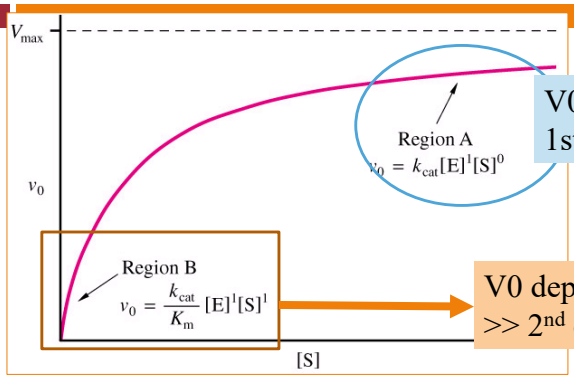
EXAM-TYPE QUESTION

- **In deriving the Michaelis-Menten equation for enzyme-mediated reactions, which of the following did we assume?**
  a. The concentration of S is reduced by formation of ES.
  b. The rate of the reaction is limited by ES dissociation to form free enzyme and substrate S.
  c. ES breakdown to form E + S is slower than ES breakdown to form E + P.
  d. An intermediate complex, EP is involved in the reaction.
  e. The reverse reaction is insignificant*

What does k_{cat} mean?

- Catalytic constant (k_{cat}) is the 1st order rate constant describing ES → E+P
- Also known as the turnover # because it describes the number of rxns a molecule of enzyme can catalyze per second under optimal condition.
- Kcat describes how quickly a given enzyme can catalyze a specific reaction, a very useful way of describing the effectiveness of an enzyme.
- Most enzymes have k_{cat} values between 10^2 and 10^3 s^{-1}
- For simple reactions $k_2 = k_{cat}$, for multistep rxns k_{cat} = rate limiting step

What does k_{cat} mean?



V0 depends on [E] >> 1st order rxn

V0 depends on [S] & [E] >> 2nd order rxn

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

Note: limiting step is ES->E+P
Kcat=K2

- Kcat describes how quickly a given enzyme can catalyze a specific reaction, a very useful way of describing the effectiveness of an enzyme.
- Kcat is also known as the turnover # because it describes the number of rxns a molecule of enzyme can catalyze per second under optimal condition.

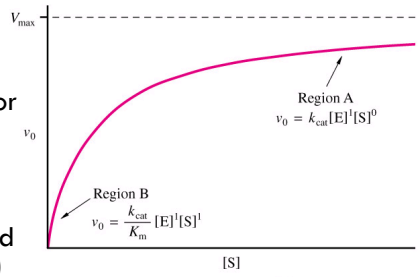
Table 14.4

Values of k_{cat} (Turnover Number) for Some Enzymes

Enzyme	k_{cat} (sec^{-1})
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

What does k_{cat}/K_m mean?

- It measures how the enzyme performs when S is low
- k_{cat}/K_m describes an enzymes preference or catalytic proficiency for different substrates = specificity constant
- The upper limit for k_{cat}/K_m is the diffusion limit - the rate at which E and S diffuse together (10^8 to 10^9 m^{-1} s^{-1})
- Catalytic perfection when k_{cat}/K_m = diffusion rate
- More physiological than kcat



Region B: $E + S \xrightarrow{\frac{k_{cat}}{K_m}} E + P$

Enzyme	Reaction Catalyzed	K_M (mol/L)	$k_{cat}(s^{-1})$	k_{cat}/K_M $[(mol/L)^{-1} s^{-1}]$
Chymotrypsin	Ac–Phe–Ala $\xrightarrow{H_2O}$ Ac–Phe + Ala	1.5×10^{-2}	0.14	9.3
Pepsin	Phe–Gly $\xrightarrow{H_2O}$ Phe + Gly	3×10^{-4}	0.5	1.7×10^3
Tyrosyl-tRNA synthetase	Tyrosine + tRNA $\longrightarrow$ tyrosyl-tRNA	9×10^{-4}	7.6	8.4×10^3
Ribonuclease	Cytidine 2′, 3′ cyclic phosphate $\xrightarrow{H_2O}$ cytidine 3′-phosphate	7.9×10^{-3}	7.9×10^2	1.0×10^5
Carbonic anhydrase	$HCO_3^- + H^+ \longrightarrow H_2O + CO_2$	2.6×10^{-2}	4×10^5	1.5×10^7
Fumarase	Fumarate $\xrightarrow{H_2O}$ malate	5×10^{-6}	8×10^2	1.6×10^8

Limitations of M-M

1. Some enzyme catalyzed rxns show more complex behavior E + S<->ES<->EZ<->EP<-> E + P
   With M-M can look only at rate limiting steps
2. Often more than one substrate
   $E+S_1 <-> ES_1 + S_2 <-> ES_1S_2 <-> EP_1P_2 <-> EP_2 + P_1 <-> E + P_2$ Must optimize one substrate then calculate kinetic parameters for the other
3. Assumes $k_{-2} = 0$
4. Assume steady state conditions

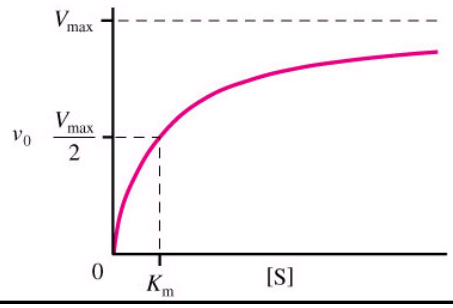
Enzymes – part 3

Kinetics/cont'd

Michaelis-Menton

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

- $Vo = \frac{Vmax\ [S]}{Km + [S]}$



- V_{max}
- K_m
- k_{cat}
- k_{cat}/K_m

How do you get values for V_{max}, K_m and k_{cat}?

- Can determine K_m and V_{max} experimentally
- Km can be determined without an absolutely pure enzyme
- K_{cat} values can be determined if V_{max} is known and the absolute concentration of enzyme is known

$$V_{max} = k_{cat}[E_{total}]$$

Exam-type questions

- An enzyme has a Km for substrate (S) of 10 mM and Vm of 5 μmol.L^{-1}.sec^{-1} at a total enzyme concentration of 1 nM. At [S] = 10 mM, kcat is:
  a. 2500 per M per sec
  b. 5000 per M per sec
  c. 2500 per sec
  d. 5000 per sec*

How to solve the M-M equation of Vmax & Km?

- A simple trick to solve problems for Vmax & Km, is the reciprocal of M-M equation:

$$\frac{1}{v} = \frac{K_m}{V_m[S]} + \frac{[S]}{V_m[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_m}\frac{1}{[S]} + \frac{1}{V_m}$$

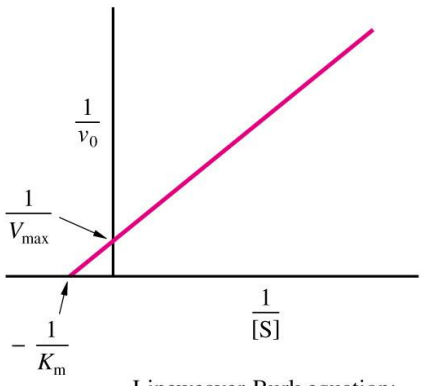
This is a linear equation with the familiar form,

$$y = \text{slope} * x + \text{intercept}$$

thus making: 1/v = y and, 1/[S] = x

- A plot of 1/V versus 1/[S] will produce a straight line with positive slope= Km/Vm and y-intercept= 1/Vmax

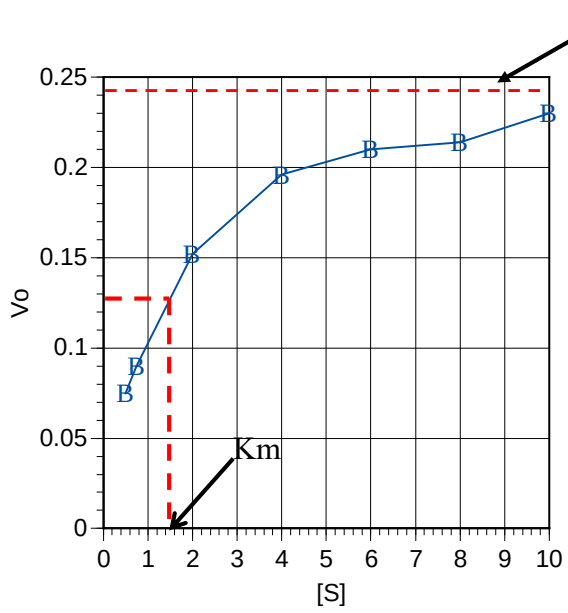
Lineweaver-Burke Plots
(double reciprocal plots)



Lineweaver-Burk equation:

$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

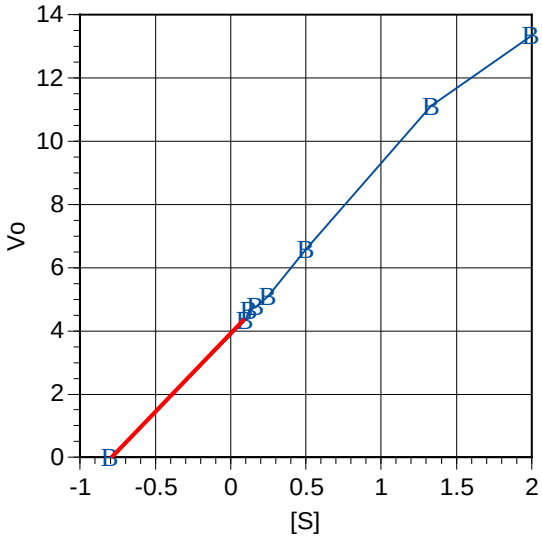
- Plot 1/[S] vs $1/V_o$
- L-B equation for straight line
- X-intercept = -1/Km
- Y-intercept = 1/Vmax
- Easier to extrapolate values w/ straight line vs hyperbolic curve



[S]	Vo
0.5	0.075
0.75	0.09
2	0.152
4	0.196
6	0.21
8	0.214
10	0.23

Km ~ 1.3 mM

Vmax ~ 0.25



[S]	Vo
2.000	13.333
1.333	11.111
0.500	6.579
0.250	5.102
0.167	4.762
0.125	4.673
0.100	4.348

-1/Km = -0.8

Km = 1.23 mM

1/Vmax = 4.0

Vmax = 0.25

Exam-type questions

- With the following enzyme activity results, determine:
- a) Vmax
- b) why is the velocity v constant at [S] greater than 2 x 10-3 M?
- c) what is the free [E] at [S] = 2 x 10-2 M?

[S] (mol/L)	v (µmol/min)
2×10^{-1}	60.00
2×10^{-2}	60.00
2×10^{-3}	60.00
2×10^{-4}	48.00
$1{,}5 \times 10^{-4}$	45.00
$1{,}3 \times 10^{-5}$	12.00

Enzyme Inhibition

- Inhibitor – substance that binds to an enzyme and interferes with its activity
- Can prevent formation of ES complex or prevent ES breakdown to E + P.
- Irreversible and Reversible Inhibitors
- Irreversible inhibitor binds to enzyme through covalent bonds (binds irreversibly)
- Reversible Inhibitors bind through non-covalent interactions (disassociates from enzyme)
- Why important?

Reversible Inhibitors

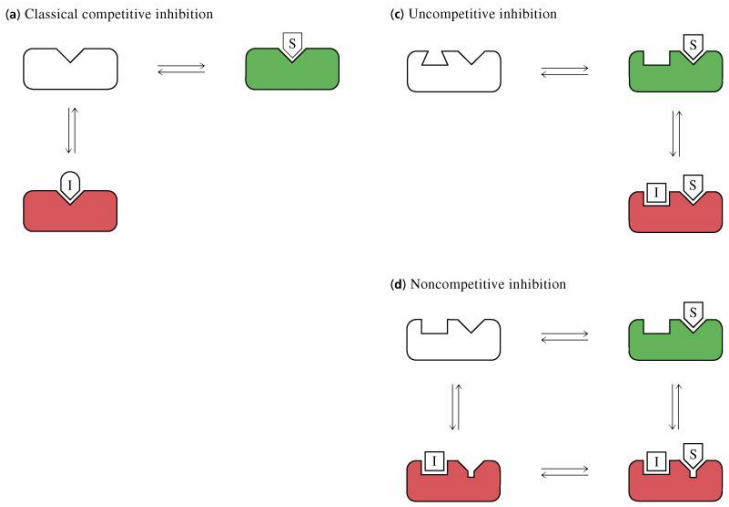
$$E + S \leftrightarrow ES \rightarrow E + P$$

$$E + I \leftrightarrow EI$$

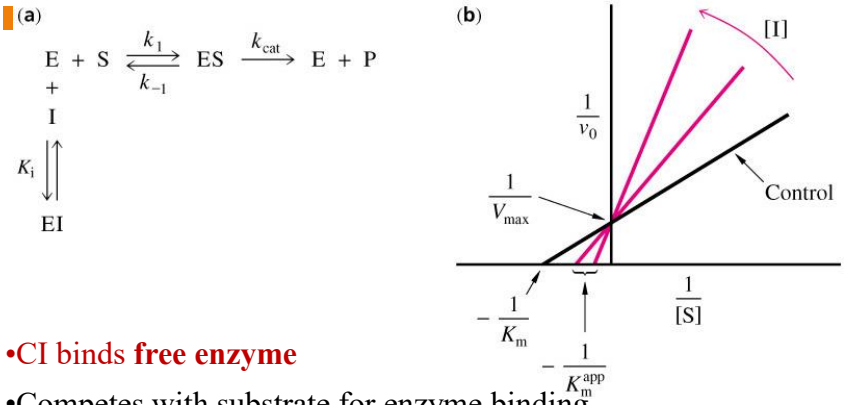
$$K_i = [E][I]/[EI]$$

- Competitive
- Uncompetitive
- Non-competitive

Types of Reversible Enzyme Inhibitors



Competitive Inhibitor (CI)

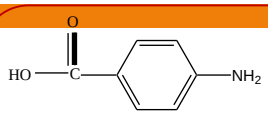


- CI binds **free enzyme**
- Competes with substrate for enzyme binding.
- Raises Km without effecting Vmax
- Can relieve inhibition with more S

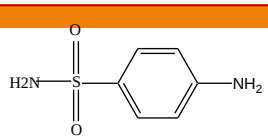
Competitive inhibition: examples

- Statin drugs are examples of competitive inhibitors:
- This group of antihyperlipidemic agents inhibit the first step in cholesterol synthesis
- Statin drugs like atorvastatin (Lipitor) and Simvastatin (Zocor) are structural analogs of the natural substrate and compete effectively to inhibit HMG CoA reductase (hydroxymethylglutaryl CoA reductase)

Competitive Inhibitors look like substrate



PABA



Sulfanilamide

PABA precursor to folic acid in bacteria

$O_2C\text{-}CH_2\text{-}CH_2\text{-}CO_2$ -------> $O_2C\text{-}CH{=}CH\text{-}CO_2$

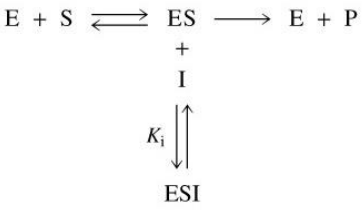
succinate fumarate

Succinate dehydrogenase (SDH)

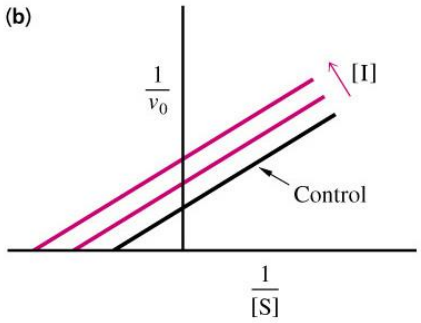
$O_2C\text{-}CH_2\text{-}CO_2$
Malonate/ CI for SDH

Uncompetitive Inhibitor (UI)

(a)

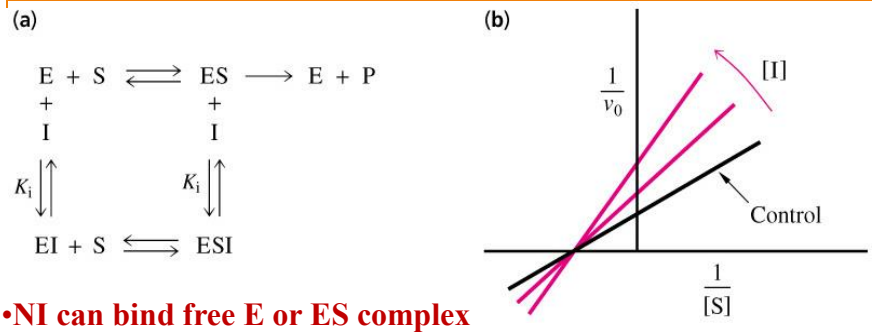


(b)



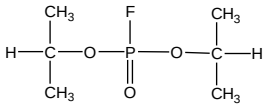
- **UI binds ES complex**
- Prevents ES from proceeding to E + P or back to E + S.
- Lowers Km & Vmax, but ratio of Km/Vmax remains the same
- Occurs with multisubstrate enzymes

Non-competitive Inhibitor (NI)

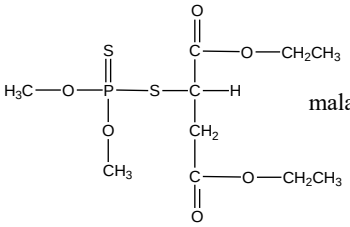


- **NI can bind free E or ES complex**
- Lowers Vmax, but Km remains the same
- NI's don't bind to S binding site therefore don't effect Km
- Alters conformation of enzyme to effect catalysis but not substrate binding

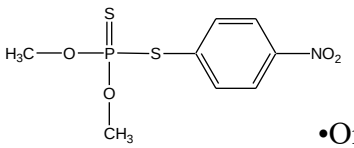
Irreversible Inhibitors



Diisopropyl fluorophosphate
(nerve gas)

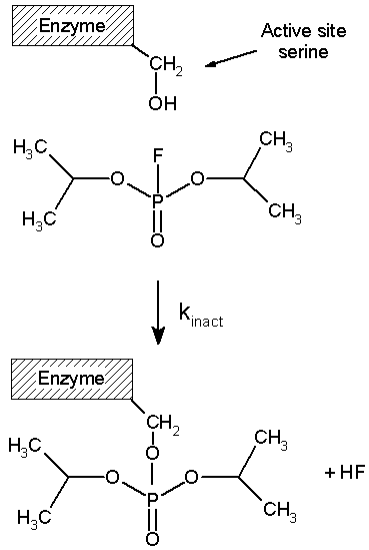


malathion



parathion

- Organophosphates
- Inhibit serine hydrolases
- Acetylcholinesterase inhibitors



Problem solving!

- The following results describe the effect of an inhibitor on enzyme activity of an enzyme, Determine:
- a) Vmax in the presence and the absence of an inhibitor
- b) Km in the presence and the absence of an inhibitor
- c) Ki
- d) type of inhibition

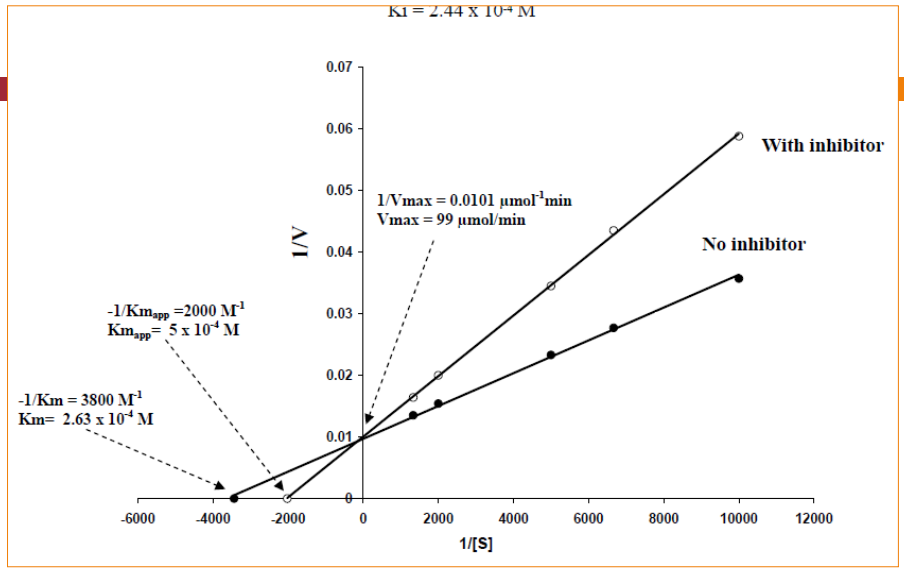
[S] (mol/L)	Without inhibitor v (µmol/min)	With inhibitor [I] = 2,2 x 10^{-4} M v (µmol/min)
1 x 10^{-4}	28.00	17.00
1,5 x 10^{-4}	36.00	23.00
2x 10^{-4}	43.00	29.00
5x 10^{-4}	65.00	50.00
7,5 x 10^{-4}	74.00	61.00

Raw data

[S] (mol/L)	Without inhibitor v (µmol/min)	With inhibitor [I] = 2,2 x 10^{-4} M v (µmol/min)
1 x 10^{-4}	28.00	17.00
1,5 x 10^{-4}	36.00	23.00
2x 10^{-4}	43.00	29.00
5x 10^{-4}	65.00	50.00
7,5 x 10^{-4}	74.00	61.00

Calculated data

1/[S] (M^{-1})	1/v ($mmol^{-1}$ x min.) No inhibitor	1/v ($mmol^{-1}$ x min.) With inhibitor
10 000	0.0357	0.0588
6 666.67	0.0277	0.0435
5 000	0.0233	0.0345
2 000	0.0154	0.0200
1 333.33	0.0135	0.0164



- Since the intersection on the y axis is the same in the presence than in the absence of the inhibitor, we can conclude that we are dealing with a competitive inhibition.