Slide : Enzymes Part 3

Dr. Name : Dr.Nafez Abu Tarboosh

Sections : 1,2,3

■ Slide □ Sheet

Biochemistry

Mousa Suboh

Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

Kinetics of enzymatic reactions

Kinetics

- **Biochemical Kinetics: the science that studies rates of chemical reactions**
- **An example is the reaction (A → P), The velocity, *v*, or rate, of the reaction A → P is the amount of P formed or the amount of A consumed per unit time, *t*. That is,**

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

Reaction Rate Law

- The rate is a term of change over time
- The rate will be proportional to the conc. of the reactants
- It is the mathematical relationship between reaction rate and concentration of reactant(s)
- For the reaction (A + B → P), the rate law is

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

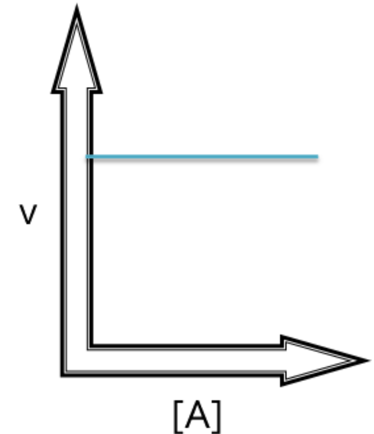
- From this expression, the rate is proportional to the concentration of A, and *k* is the rate constant

The order of the reaction & the rate constant (k)

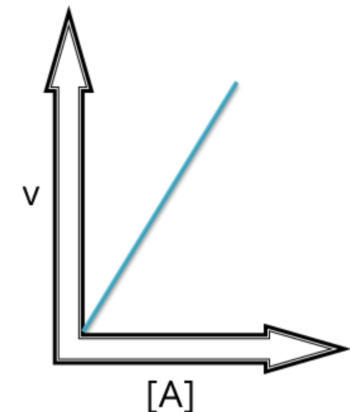
- A multistep reaction can go no faster than the slowest step

$$v = k(A)^{n1}(B)^{n2}(C)^{n3}$$

- 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
- Dimensions of k

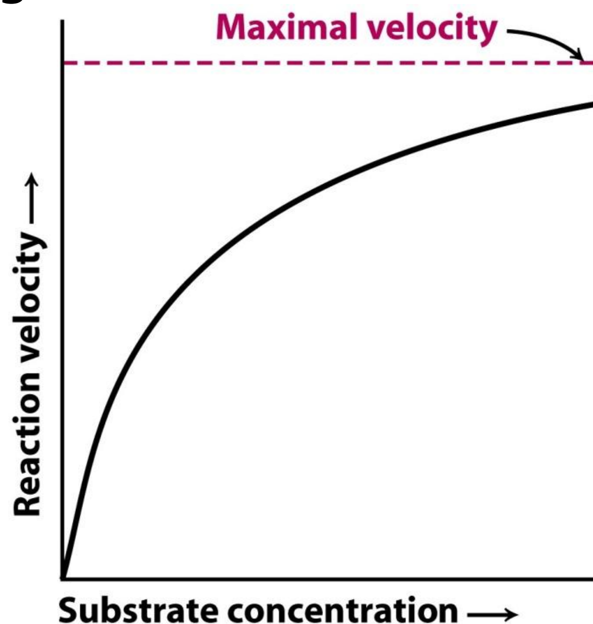


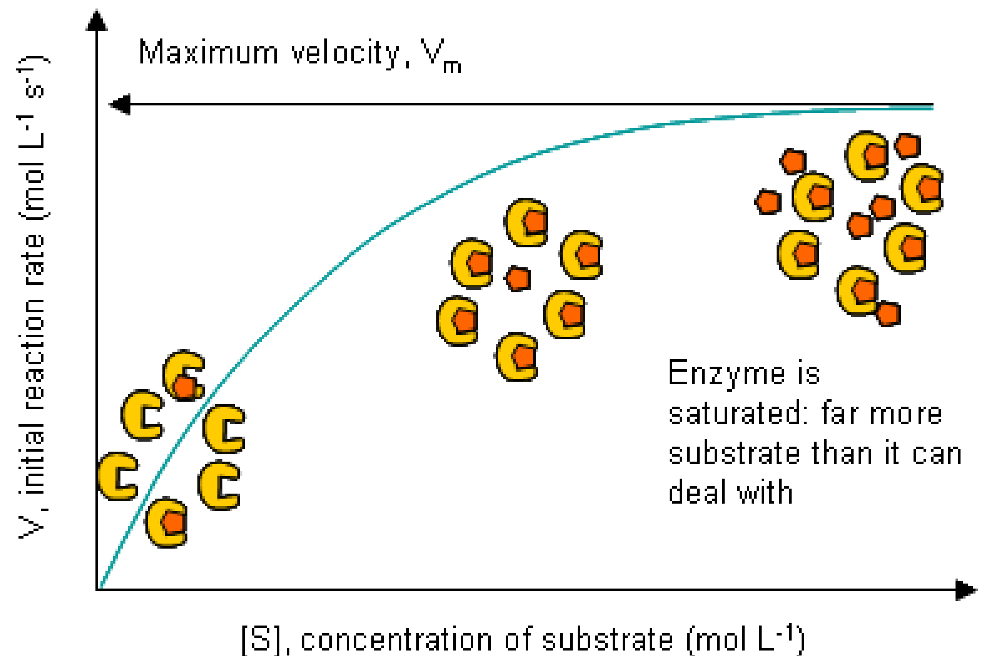
Overall order	V=	Dimentions of k
Zero	k	$(\text{conc.})(\text{time})^{-1}$
First	k(A)	$(\text{time})^{-1}$



Enzyme kinetics

- Enzymatic reactions may either have a simple behavior or complex (allosteric) behavior
- 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

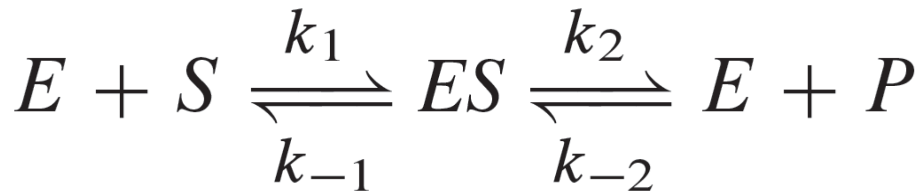


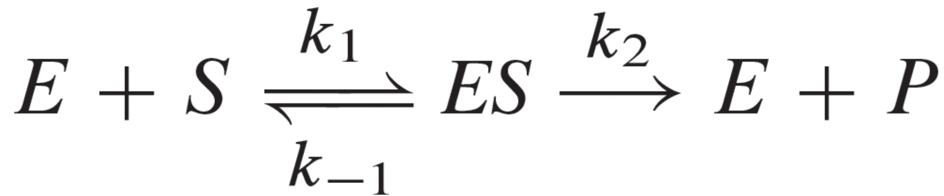


Enzyme kinetics

- The maximal rate, V_{max}, is achieved when the catalytic sites on the enzyme are saturated with substrate
- V_{max} reveals the turnover number of an enzyme
  - The number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate
- At V_{max}, the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction

Expression of enzyme kinetic reactions "Steady State Assumption"





$$v = k_2\, ES$$

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

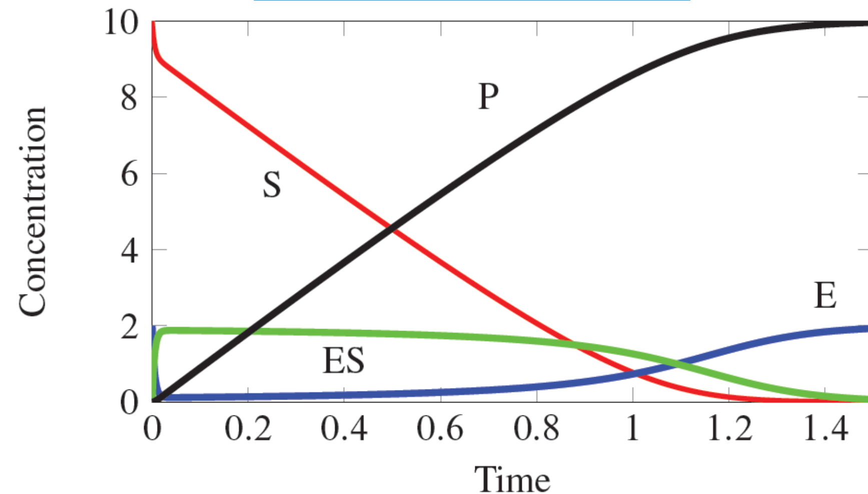
$$0 = k_1\, E\, .\, S - k_{-1}\, ES - k_2\, ES$$

$$E_t = E + ES$$

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

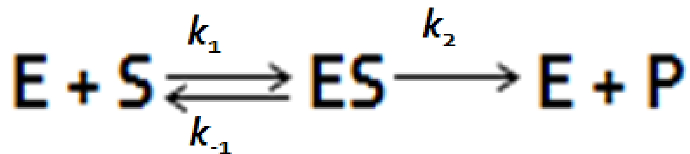
$$v = \frac{E_t\, k_2\, S}{(k_{-1} + k_2)/k_1 + S}$$

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



The Michaelis constant (K_m)

- For a reaction:



STEADY STATE APPROXIMATION

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

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

- K_m, called the Michaelis constant is $K_M = \frac{k_{-1} + k_2}{k_1}$

- In other words, K_m is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex

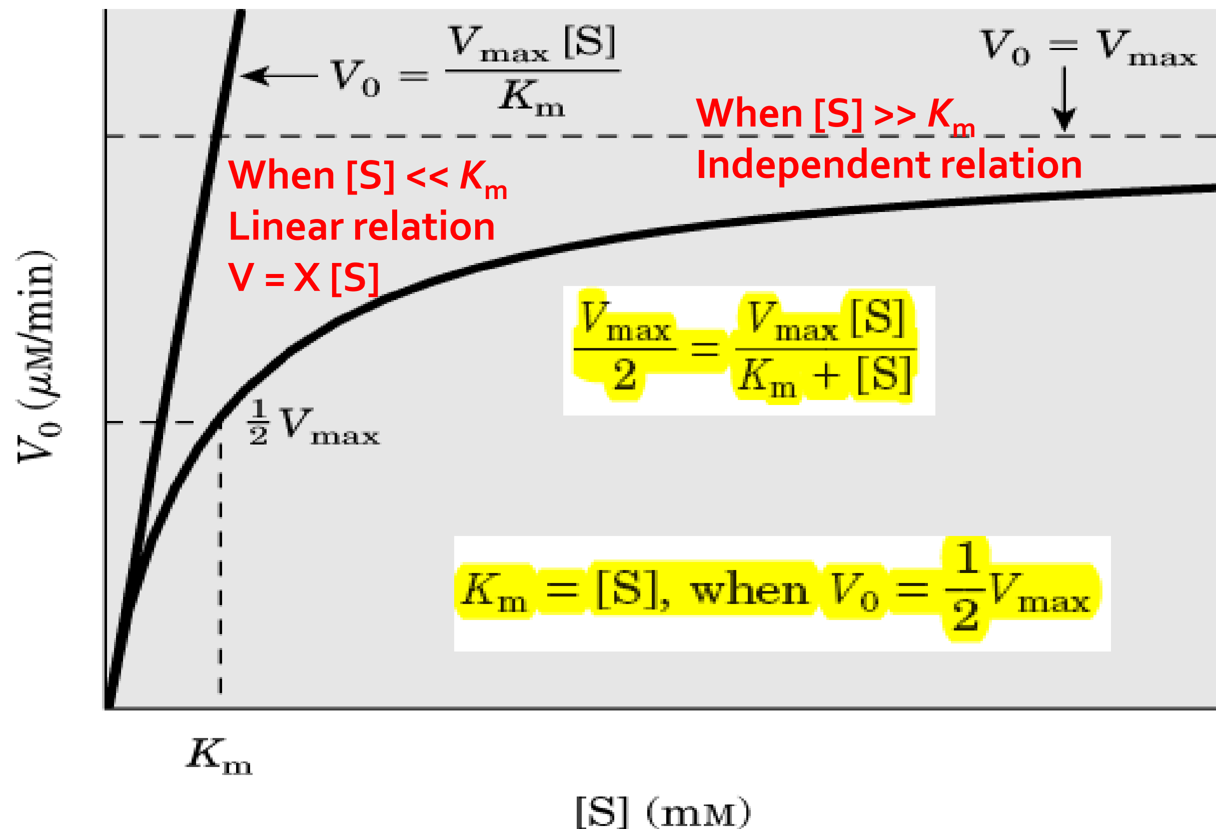
- K_m describes the affinity of enzyme for the substrate

Expression of enzyme kinetic reactions Michaelis-Menten equation

- **A quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_o) & substrate concentration [S]**
  - ✓ **The rate constant (K_m) and maximal velocity (V_{max})**

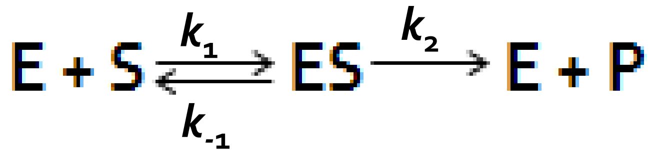
$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

The substrate concentration at which V_o is half maximal is K_m



V_{max} & k_{cat}

- For the enzymatic reaction



Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

- The maximal rate, V_{max}, is equal to the product of k_2, also known as k_{cat}, and the total concentration of enzyme

$$V_{max} = k_2\,[E]_T$$

- k_{cat}, the turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated

$$k_{cat} = V_{max} / [E]_T$$

- In other words, the maximal rate, V_{max}, reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known

The Michaelis constant (K_m)

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

- The lower the K_m of an enzyme towards its substrate, the higher the affinity
- When more than one substrate is involved? Each will have a unique K_m & V_{max}
- In multi-substrate reaction, the sequence of substrate binding & product release affect the rate equation
- K_m values have a wide range. Mostly between (10^{-1} & 10^{-7} M)

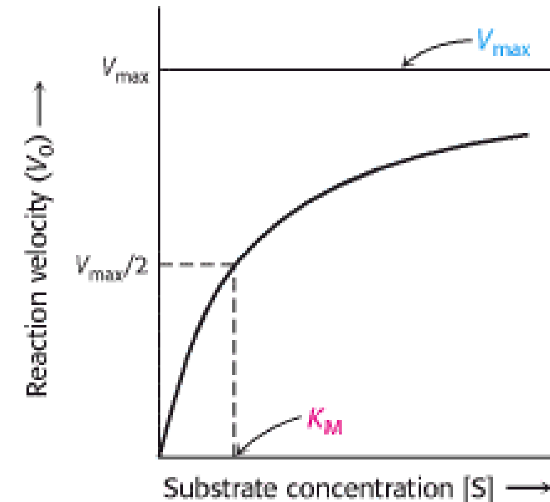
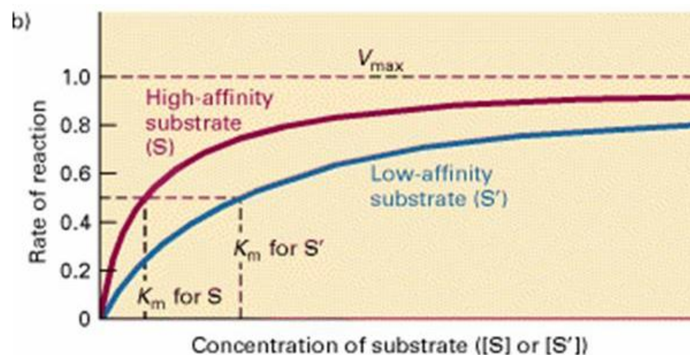


table 8–6

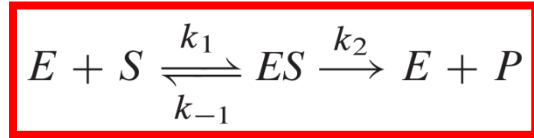
K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
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

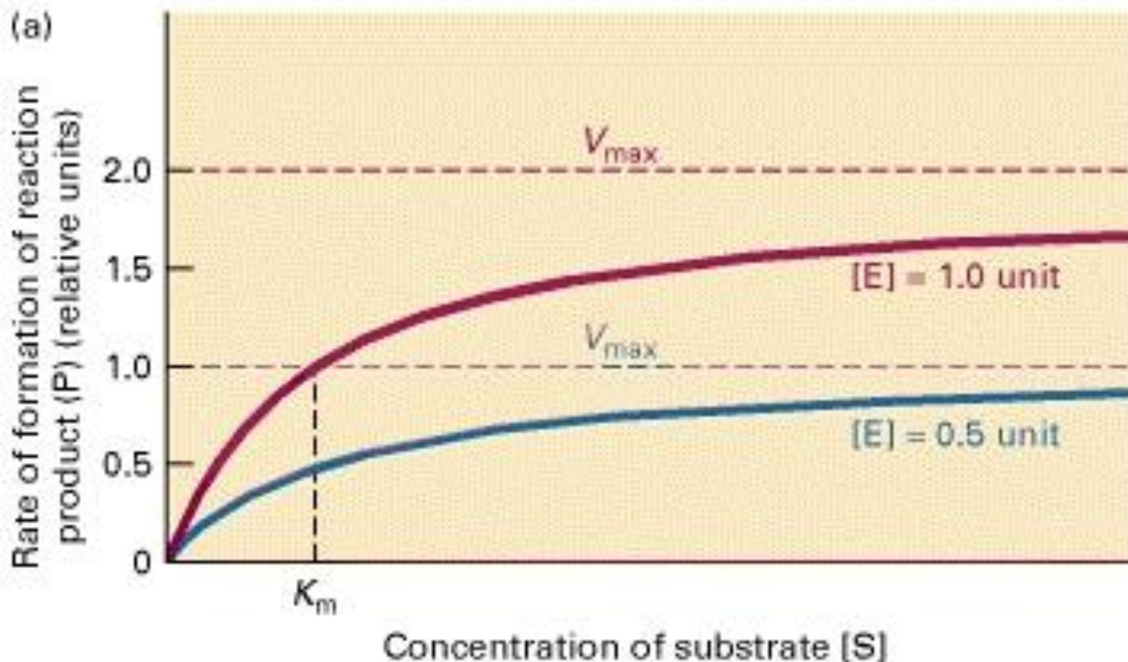


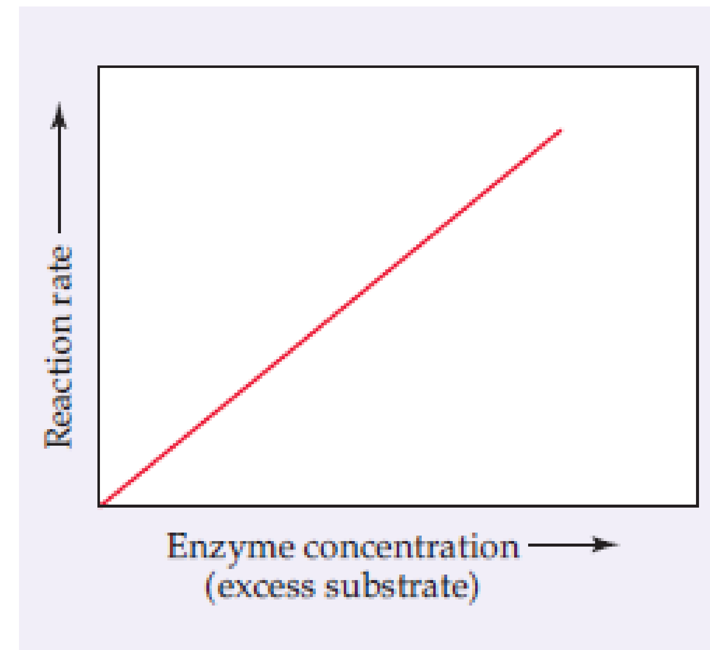
K_M & K_D
[E], K_M & V_{max}

- K_D: dissociation constant, The actual measure of the affinity
- $K_D = (k_{-1}/k_1)$


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

- When you increase the enzyme concentration, what will happen to V_{max} & K_m?





Example

- a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate
  - ✓ Hence, k_{cat} is $6 \times 10^5\ s^{-1}$
  - ✓ $10^4\ min^{-1}$
- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is 1.7 μs for carbonic anhydrase
- The turnover numbers of most enzymes with their physiological substrates fall in the range from $1\text{-}10^4\ (s^{-1})$

Fractional Saturation: Physiological [S]/K_M Specificity & Efficiency

- Most enzymes are not normally fully saturated
- Under physiological conditions, the [S]/K_M ratio is typically between 0.01 and 1.0

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

- Specificity constant (k_{cat}/K_M): determines the relative rate of the reaction at low [S]

$$V = (k_{cat}/K_M)[E][S]$$

- k_{cat}/K_M (M^{-1} min^{-1}) is indicative of:
  - ✓ Enzyme's substrate specificity: the higher the ratio, the higher the specificity
  - ✓ Enzyme's catalytic efficiency: the higher the ratio, the more efficient the enzyme

Table 6.2

Turnover Numbers and Km for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M**
Catalase	Conversion of H_2O_2 to H_20 and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

k_{cat} values vary over a range of nearly 3000

K_M values vary over a range of nearly 300

K_{cat}/K_M, the range is only 4

Reaction rate (v); Enzyme activity; Specific activity; Turnover number

- Reaction rate; measures the concentration of substrate consumed (or product produced) per unit time (mol/{L.s} or M/s)
- Enzyme activity; measures the number of moles of substrate consumed (or product produced) per unit time (mol/s)
  - ✓ Enzyme activity = rate of reaction × reaction volume
- Specific activity; measures moles of substrate converted per unit time per unit mass of enzyme (mol/{s.g})
  - ✓ Specific activity = enzyme activity / actual mass of enzyme
  - ✓ This is useful in determining enzyme purity after purification
- Turnover number; measures moles of substrate converted per unit time per moles of enzyme (min^{-1} or s^{-1})
  - ✓ Turnover number = specific activity × molecular weight of enzyme

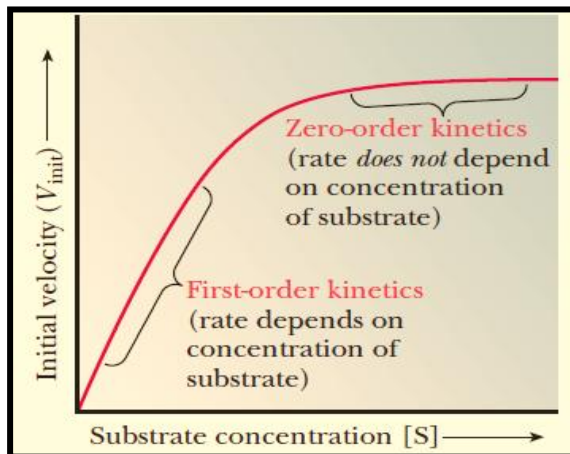
Sample calculations:

A solution contains initially **25.0 × 10^{-4} mol L^{-1}** of peptide substrate and **1.50 μg chymotrypsin**, in **2.5 mL** volume. **After 10 minutes, 18.6 × 10^{-4} mol L^{-1}** of peptide substrate remain. Molar mass of chymotrypsin is **25,000 g mol^{-1}**.

peptide substrate consumed	= 6.4 x 10^{-4} mol L^{-1} in 10 minutes
Rate of reaction	**= 6.4 x 10^{-5} mol L^{-1} min^{-1}**
Enzyme activity	= 6.4 x 10^{-5} mol L^{-1} min^{-1} x 2.5 x 10^{-3} L
(rate × volume)	**= 1.6 x 10^{-7} mol min^{-1}**
Specific activity	= 1.6 x 10^{-7} mol min^{-1} / 1.50 μg
(activity / mass)	**= 1.1 x 10^{-7} mol $μg^{-1}$ min^{-1}**
Turnover number	= 1.1 x 10^{-7} mol $μg^{-1}$ min^{-1} x 25,000 g mol^{-1} x 10^{6} μg g^{-1}
(sp. act. × molar mass)	= 2.7 x 10^{3} min^{-1} = **45 s^{-1}**

Disadvantage of Michaelis-Menten equation & Lineweaver-Burk or double-reciprocal plot

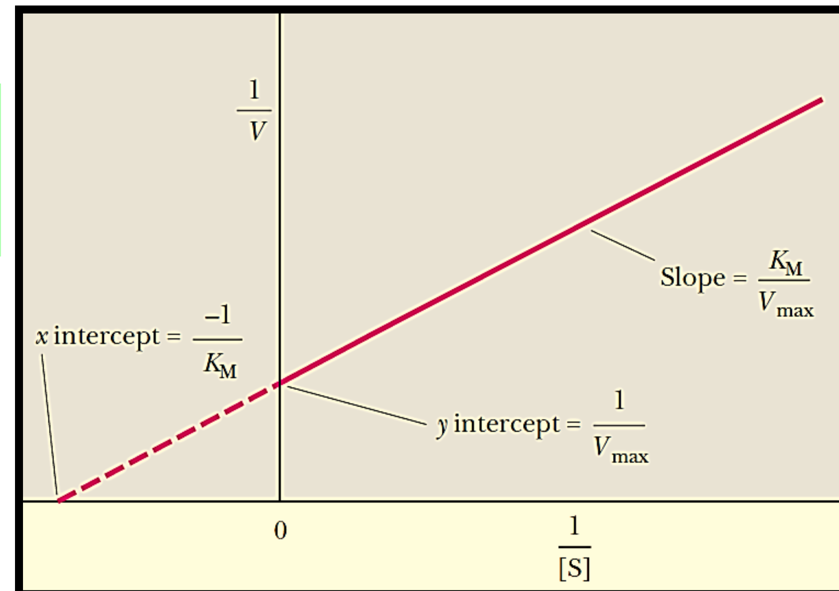
- Determining the K_m from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach V_{max}
- This prevents the calculation of both V_{max} & K_m
- Lineweaver-Burk plot: A plot of $1/v_o$ versus $1/[S]$ (double-reciprocal plot), yields a straight line with an y-intercept of $1/V_{max}$ and a slope of K_M/V_{max}
- The intercept on the x-axis is $-1/K_M$



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

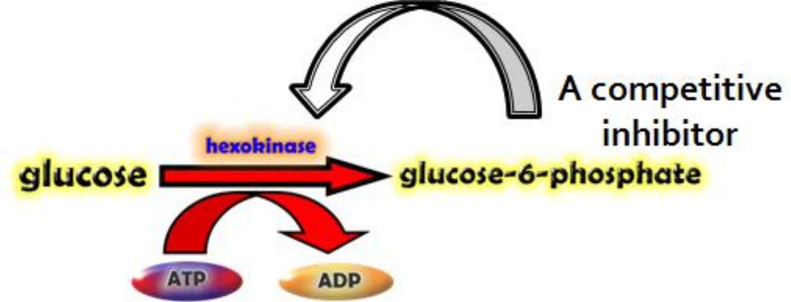
ص=أس+ب

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

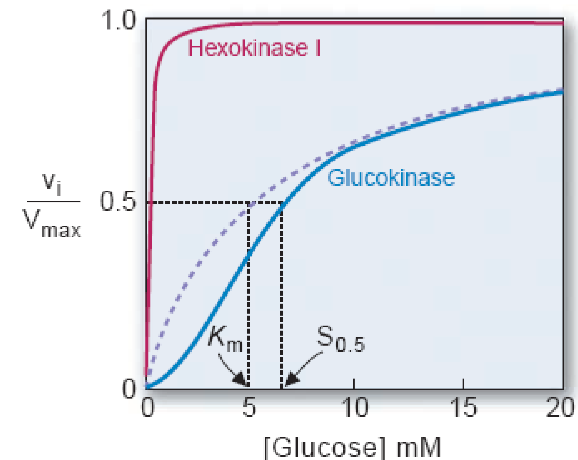


Enzymes Regulation

1. Isozymes (isoenzymes) The Differential K_M Value "Hexokinase"

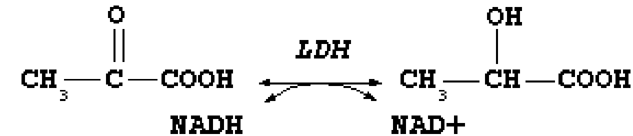


- What are isozymes? Same substrate & product, different gene, <u>different localization</u>, <u>different parameters (K_m, V_{max}, k_{cat})</u>
- Hexokinase found in RBCs & in the liver
- Catalyzes the first step in glucose metabolism
- Hexokinase I (RBCs): K_M (glucose) ≈ 0.05 mM
- Hexokinase IV (glucokinase, liver, pancreas) ≈ 5-6 mM
- RBCs: when blood glucose falls below its normal fasting level (≈ 5 mM), RBCs could still phosphorylate glucose at rates near V_{max}
- Liver: rate of phosphorylation increases above fasting levels (after a high-carbohydrate meal)
  - High K_M of hepatic glucokinase promotes storage of glucose
- Pancreas: works as a sensor

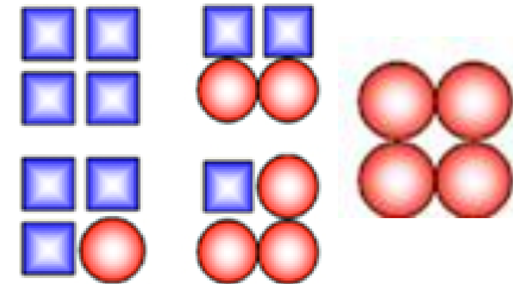


Isoenzymes (isozymes), LDH

- lactate dehydrogenases (LDHs);



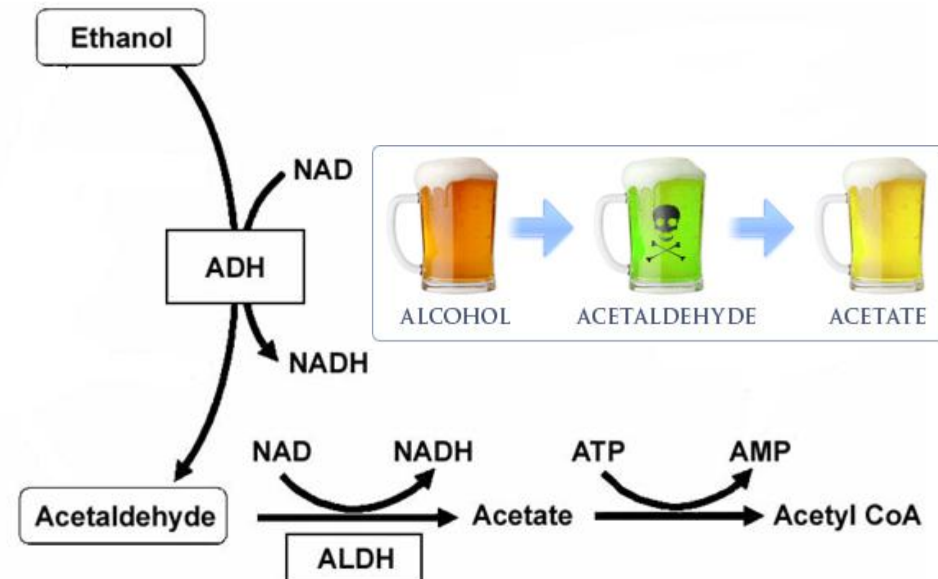
  - Tetramer; H & M subunits; 5 isozymes; all H (heart); all M (muscle); different combinations
  - Different efficiencies
  - M4 functions anaerobically (pyruvate into lactate); H4 functions aerobically (reverse reaction)
  - H4 (low K_m for pyruvate & inhibited by high levels of pyruvate); M4 (higher K_m for pyruvate & is not inhibited by pyruvate)



I	Heart
II	Endothelium
III	Lungs
IV	Kidneys, pancreas
V	Liver, muscles

Example of a physiological significance of K_M; Effect of alcohol

- Sensitivity of individuals to ethanol vary
- More sensitive people will exhibit facial flushing and rapid heart rate (tachycardia) after ingesting even small amounts
- Alcohol dehydrogenase followed by aldehyde dehydrogenase
- Acetaldehyde (the cause of the symptoms)
- Most people have two forms of the ALDH:
  - ✓ 1. Mitochondrial (low K_m)
  - ✓ 2. Cytosolic (high K_m)
- Sensitive people have a mutation in 1 (less active)
- 2 is less active: Acetaldehyde escape to blood stream



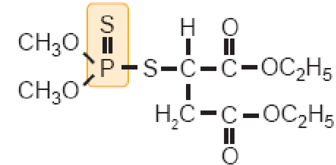
2. Inhbition

2.1 Mechanism-Based Inhibitors

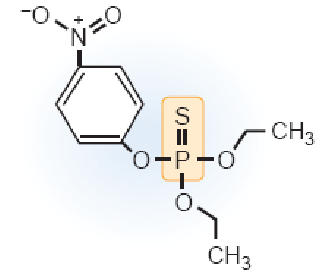
- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction
- The term includes:
- A. Covalent inhibitors
- B. Transition state analogs
- C. Heavy metals

- The kinetic effect of irreversible inhibitors is to <u>decrease the concentration of active enzyme</u>

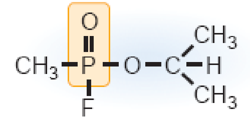
2.1.A. Covalent Inhibitors



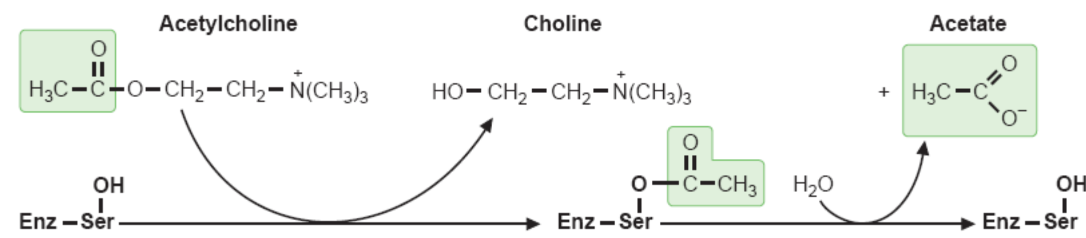
- Covalent or extremely tight bonds with aactive site mino acids
- Amino acids are targeted by drugs & toxins

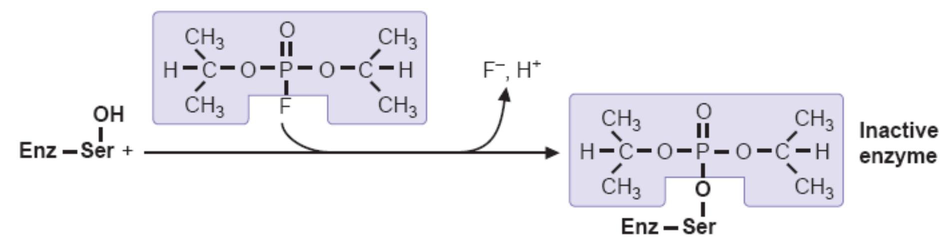


- The lethal compound [DFP] is an organophosphorus compound that served as a prototype for:
  - The nerve gas sarin
  - The insecticides malathion & parathion



- DFP also inhibits other enzymes that use serine (ex. serine proteases), but the inhibition is not as lethal

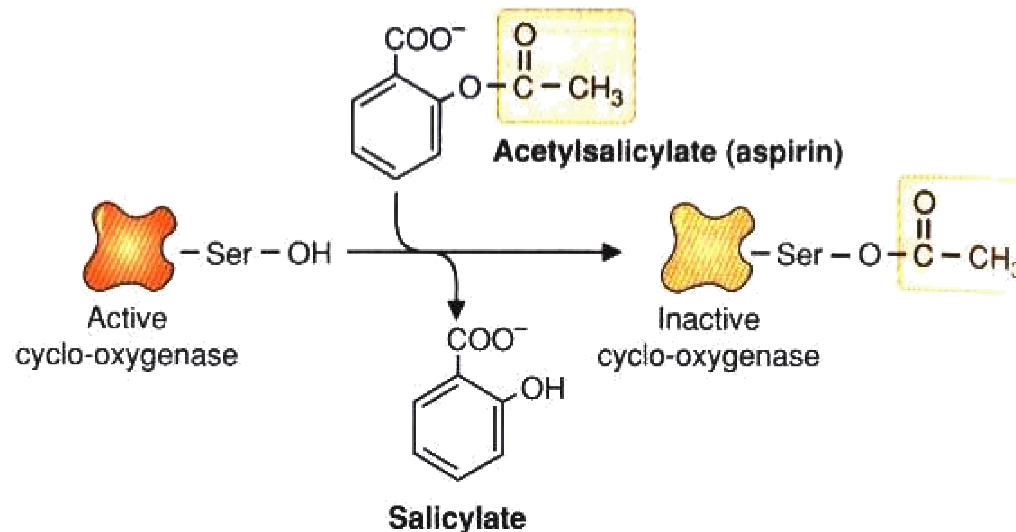




2.1.A. Covalent Inhibitors

- **Aspirin (acetylsalicylic acid): covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase)**

- **Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme**

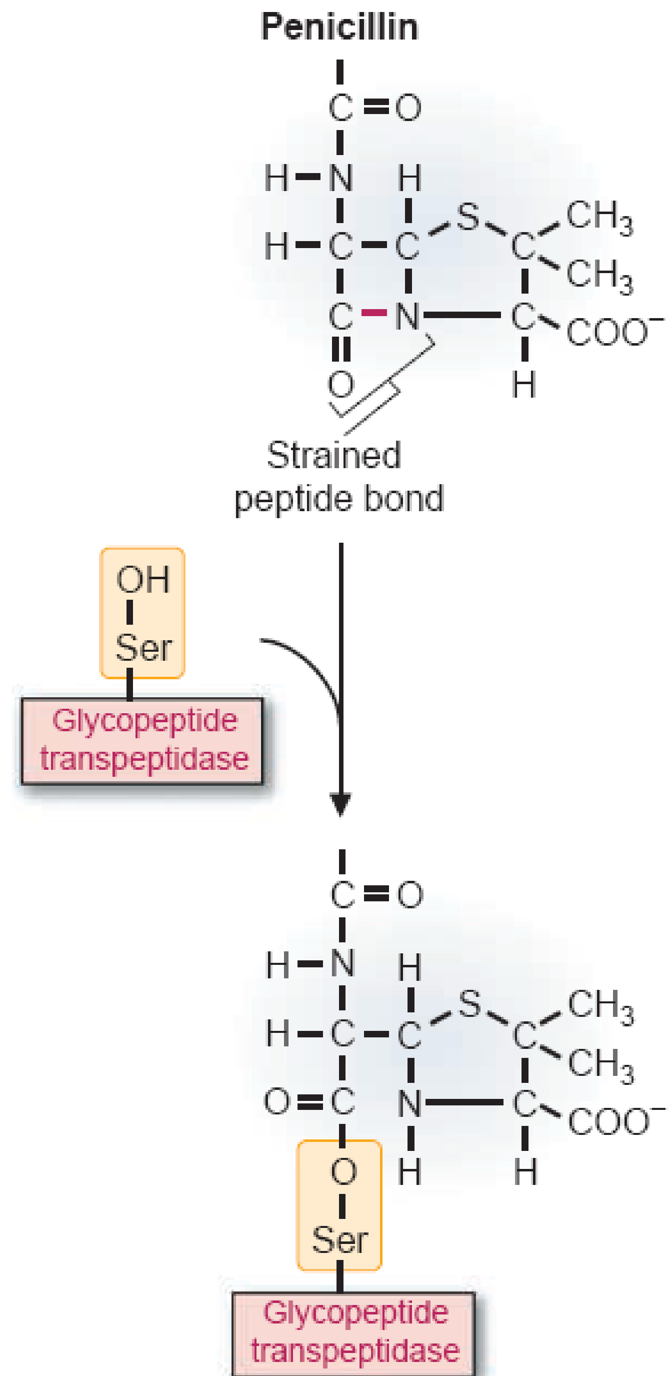


2.1.B. Transition-State Analogs & Compounds that Resemble Intermediate Stages of the Reaction

- Transition-state analogs: extremely potent inhibitors (bind more tightly)
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure)
- Substrate analogs: even though they bind more tightly than substrates

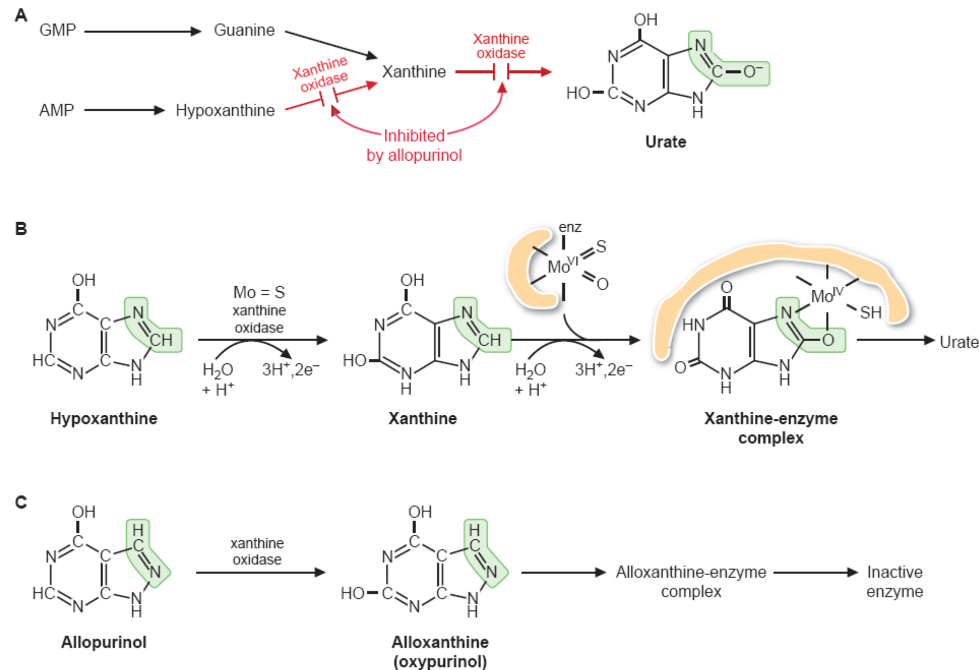
2.1.B.1 PENICILLIN

- A transition-state analog to *glycopeptidyl transferase*
- Required by bacteria for synthesis of the cell wall
- The reaction is favored by the strong resemblance between the peptide bond in the β-lactam ring of penicillin & the transition-state complex of the natural transpeptidation reaction
- Inhibitors that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed *suicide inhibitors*



2.1.B.2 ALLOPURINOL

- A drug used to treat gout
- Decreases urate production by inhibiting xanthine oxidase
- The enzyme commits suicide by converting the drug to a transition-state analog
- The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions
- Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, a compound that binds very tightly to a molybdenum–sulfide complex in the active site



2.1.C. Heavy Metals

- Tight binding of a metal to a functional group in an enzyme
- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)
- Relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity
- Mercury: binds to so many enzymes, often at reactive sulfhydryl groups in the active site
  - It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity
- Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc
  - Its developmental & neurologic toxicity may be caused by its ability to replace Ca^{+2} in several regulatory proteins that are important in the central nervous system and other tissues

2.2 Reversible Inhibitors

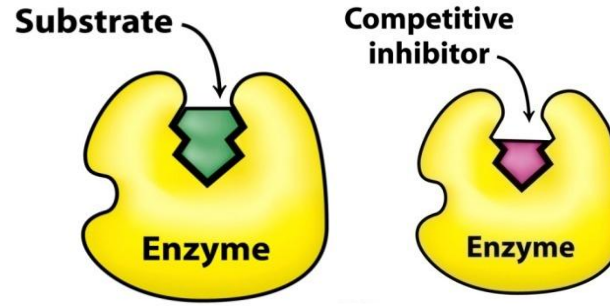
- **Characterized by a rapid dissociation of the enzyme-inhibitor complex**

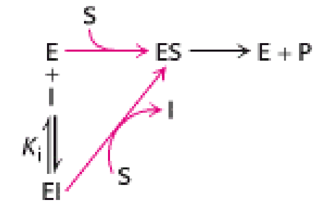
- **Usually these inhibitors bind through non-covalent interactions & inhibitor maintains a reversible equilibrium with the enzyme**

- **Reversible inhibitors can be divided into two classes: competitive & noncompetitive**

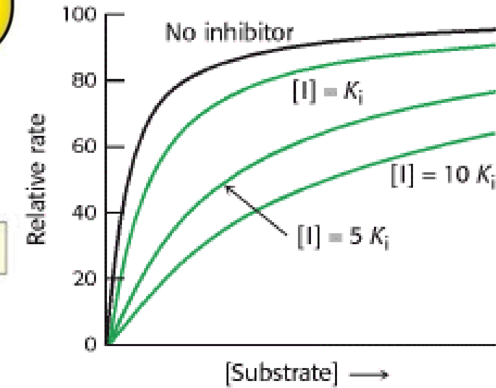
- **The double-reciprocal plots are highly useful for distinguishing among these inhibitors**

2.2.A. Competitive inhibition

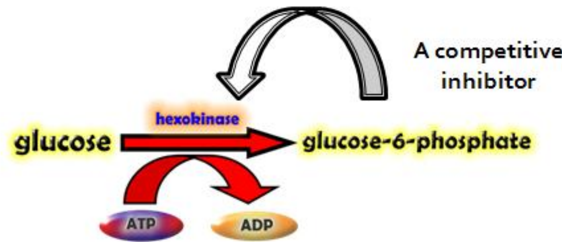




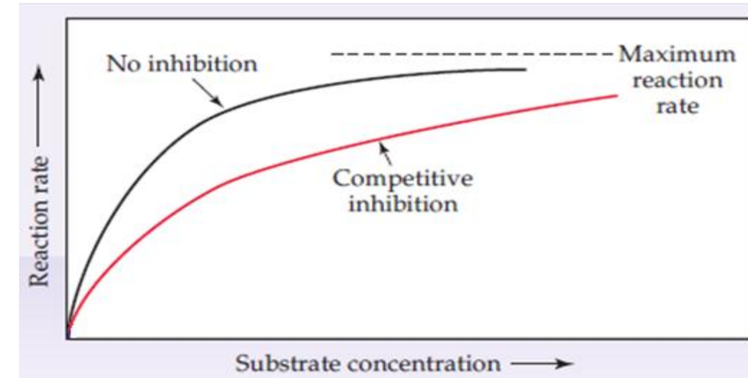
- The inhibitor competes with substrate

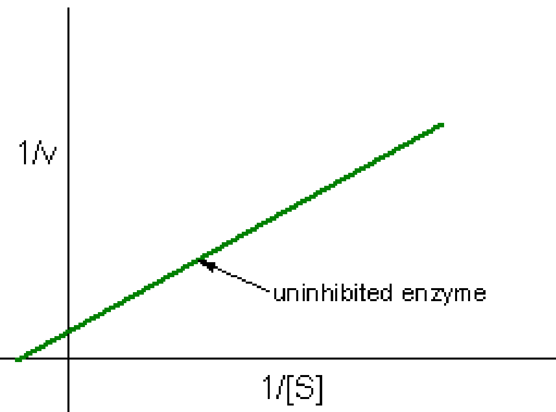


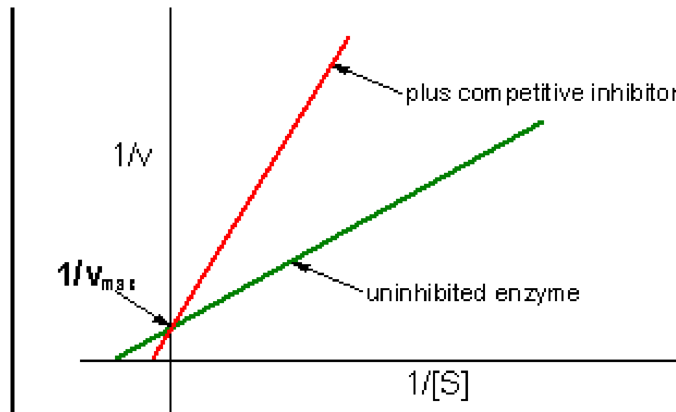
- Increasing [S] can overcome the inhibition (V_{max})
- Does K_M change?

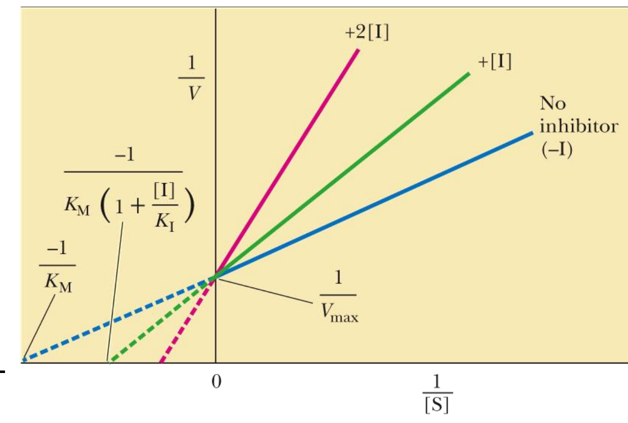


- Significance (ex. Hexokinase)

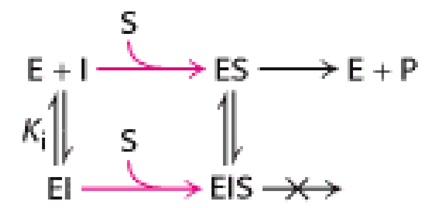








2.2.B. Noncompetitive inhibition



- The inhibitor binds at a site other than the active site
- The complex does not proceed to form product or has a lower efficiency
- V_{max} vs. K_M
- Can we reach V_{max}?