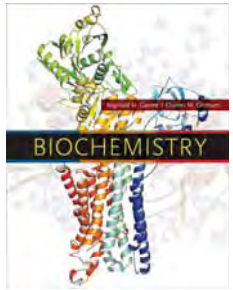
Chapter 13



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Enzyme Kinetics

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Essential Questions

- Before this class, ask your self the following questions:
  - What are enzymes?
  - How do enzymes work?
  - How many enzymes you know?
  - What is kinetics?
  - What is enzyme inhibitor?

Outline

- Part I: Get to know enzymes
  - Enzyme Features
  - Enzyme nomenclature
  - Activity
  - Unusual enzymes..
- Part II: Enzyme kinetics
- Part III: Inhibitor and bimolecular kinetics

Virtually All Reactions in Cells Are Mediated by Enzymes

- Enzymes catalyze thermodynamically favorable reactions, causing them to proceed at extraordinarily rapid rates (see Figure 13.1)
- Enzymes provide cells with the ability to exert *kinetic control over thermodynamic potentiality*
- Living systems use enzymes to accelerate and control the rates of vitally important biochemical reactions
- Enzymes are the agents of metabolic function

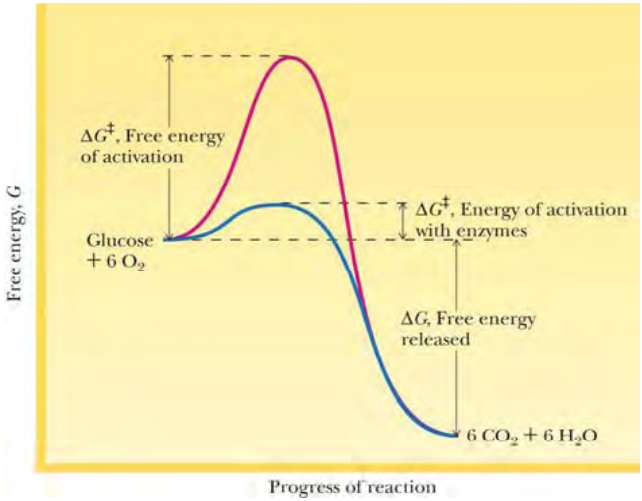


Figure 13.1 Reaction profile showing the large free energy of activation for glucose oxidation. Enzymes lower $\Delta G^{\ddagger}$, thereby accelerating rate.

13.1 What Characteristic Features Define Enzymes?

- Catalytic power
- Specificity
- Regulation
- Enzyme nomenclature
- Coenzymes and cofactors

Catalytic Power

- Catalytic power is defined as the ratio of the enzyme-catalyzed rate of a reaction to the uncatalyzed rate
- Enzymes can accelerate reactions as much as 10^{16} over uncatalyzed rates

- Urease is a good example:
  - Catalyzed rate: $3x10^{4}$/sec
  - Uncatalyzed rate: $3x10^{-10}$/sec
  - Ratio is $1x10^{14}$

Specificity

- Enzymes selectively recognize proper substrates over other molecules
- Enzymes produce products in very high yields - often much greater than 95%
- Specificity is controlled by structure - the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield

90% yield in each step; 35% over 10 steps

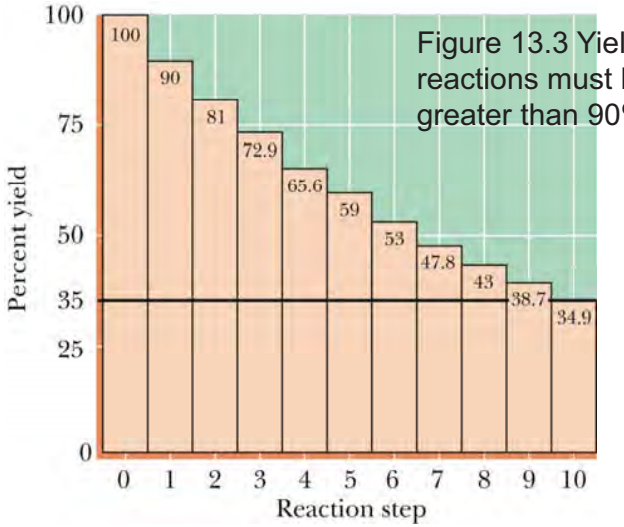


Figure 13.3 Yields in biological reactions must be substantially greater than 90%.

Regulation

- Enzymes are the Agents of Metabolic Function
- Regulation of enzyme activity ensures that the rate of metabolic reactions is appropriate to cellular requirements

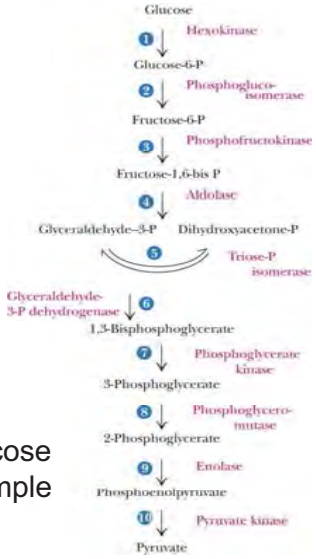


Figure 13.2 The breakdown of glucose by *glycolysis* provides a prime example of a metabolic pathway.

Enzyme nomenclature

- Common name:
  - Suffix –ase to substrate
    - Urease: urea hydrolyzing enzyme
    - Phosphatase: hydrolyzing phosphoryl group
  - What is the activity of following enzymes?
    - Catalase
    - Trypsin
    - Pepsin
- Systematic classification
  - 6 major classes of reaction

Enzyme Nomenclature Provides a Systematic Way of Naming Metabolic Reactions

TABLE 13.1 Systematic Classification of Enzymes According to the Enzyme Commission

E.C. Number	Systematic Name and Subclasses	E.C. Number	Systematic Name and Subclasses
1	*Oxidoreductases* (oxidation–reduction reactions)	4	*Lyases* (addition to double bonds)
1.1	Acting on CH—OH group of donors	4.1	C—C lyases
1.1.1	With NAD or NADP as acceptor	4.1.1	Carboxy lyases
1.1.3	With O_2 as acceptor	4.1.2	Aldehyde lyases
1.2	Acting on the >C=O group of donors	4.2	C—O lyases
1.2.3	With O_2 as acceptor	4.2.1	Hydrolases
1.3	Acting on the CH—CH group of donors	4.3	C—N lyases
1.3.1	With NAD or NADP as acceptor	4.3.1	Ammonia-lyases
2	*Transferases* (transfer of functional groups)	5	*Isomerases* (isomerization reactions)
2.1	Transferring C-1 groups	5.1	Racemases and epimerases
2.1.1	Methyltransferases	5.1.3	Acting on carbohydrates
2.1.2	Hydroxymethyltransferases and formyltransferases	5.2	*Cis-trans* isomerases
2.1.3	Carboxyltransferases and carbamoyltransferases	6	*Ligases* (formation of bonds with ATP cleavage)
2.2	Transferring aldehydic or ketonic residues	6.1	Forming C—O bonds
2.3	Acyltransferases	6.1.1	Amino acid–RNA ligases
2.4	Glycosyltransferases	6.2	Forming C—S bonds
2.6	Transferring N-containing groups	6.3	Forming C—N bonds
2.6.1	Aminotransferases	6.4	Forming C—C bonds
2.7	Transferring P-containing groups	6.4.1	Carboxylases
2.7.1	With an alcohol group as acceptor		
3	*Hydrolases* (hydrolysis reactions)		
3.1	Cleaving ester linkage		
3.1.1	Carboxylic ester hydrolases		
3.1.3	Phosphoric monoester hydrolases		
3.1.4	Phosphoric diester hydrolases		

Example:

- Classification of the enzyme catalyzing the following reaction:
- ATP + D-glucose → ADP + D-glucose-6-phosphate
- Phosphate group transferred
  - Transferase (class 2)
    - transferring P-containing group (subclass 7)
      - With an alcohol group as acceptor (sub-subclass 1)
        - » Entry 2: glucokinase (E.C.2.7.1.2.)
        - » Entry 1: hexokinase (E.C.2.7.1.1)
- What is kinase?

Coenzymes and Cofactors

- Nonprotein components essential to enzyme activity
- Cofactor = vitamin?
- Prosthetic group?
- Holoenzyme vs. apoenzyme

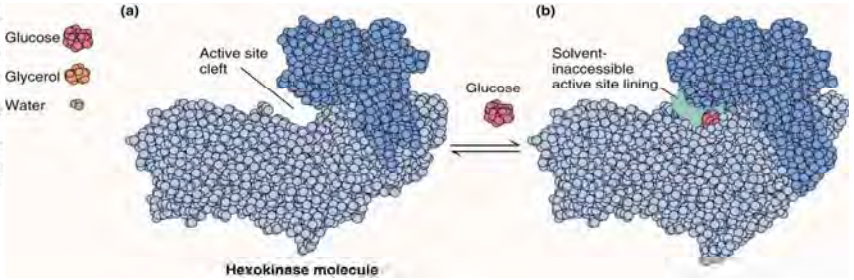
TABLE 13.2 Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

Metal Ions and Some Enzymes That Require Them		Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups		
Metal Ion	Enzyme	Coenzyme	Entity Transferred	Representative Enzymes Using Coenzymes
Fe^{2+} or Fe^{3+}	Cytochrome oxidase Catalase Peroxidase	Thiamine pyrophosphate (TPP) Flavin adenine dinucleotide (FAD) Nicotinamide adenine dinucleotide (NAD)	Aldehydes Hydrogen atoms Hydride ion (H^-)	Pyruvate dehydrogenase Succinate dehydrogenase Alcohol dehydrogenase
Cu^{2+} Zn^{2+}	Cytochrome oxidase DNA polymerase Carbonic anhydrase Alcohol dehydrogenase	Coenzyme A (CoA) Pyridoxal phosphate (PLP)	Acyl groups Amino groups	Acetyl-CoA carboxylase Aspartate aminotransferase
Mg^{2+}	Hexokinase Glucose-6-phosphatase	5′-Deoxyadenosylcobalamin (vitamin B_{12})	H atoms and alkyl groups	Methylmalonyl-CoA mutase
Mn^{2+} K^+	Arginase Pyruvate kinase (also requires Mg^{2+})	Biotin (biocytin) Tetrahydrofolate (THF)	CO_2 Other one-carbon groups, such as formyl and methyl groups	Propionyl-CoA carboxylase Thymidylate synthase
Ni^{2+}	Urease			
Mo	Nitrate reductase			
Se	Glutathione peroxidase			

Why Enzymes Are So Specific?

- The "Lock and key" hypothesis
- The "Induced fit" hypothesis
- Induced fit favors formation of the transition-state
- Specificity and reactivity are often linked.
  - hexokinase example
    - binding of glucose in the active site induces a conformational change in the enzyme
    - causes the two domains of hexokinase to close around the substrate, creating the catalytic site

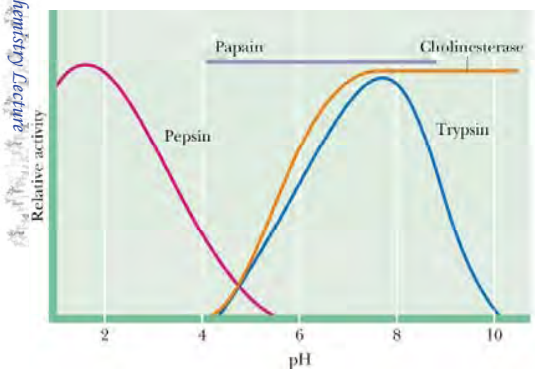
Catalysis of Hexokinase



Enzymatic Activity and pH

- Enzyme-substrate recognition and catalysis are greatly dependent on pH
- Enzymes have a variety of ionizable side chains that determine its secondary and tertiary structure and also affect events in the active site
- Substrate may also have ionizable groups
- Enzymes are usually active only over a limited range of pH

Enzymatic Activity is Strongly Influenced by pH



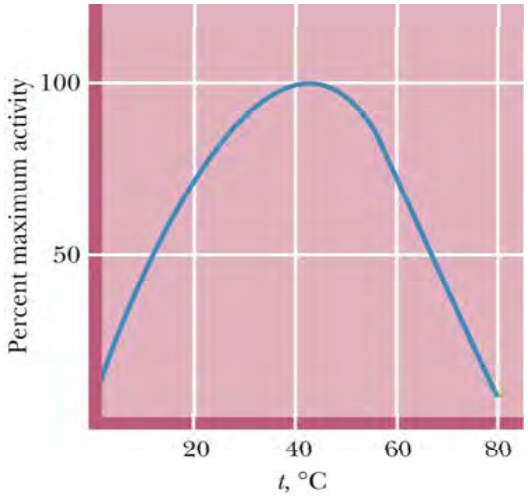
Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

Figure 13.11 The pH activity profiles of four different enzymes.

The Response of Enzymatic Activity to Temperature

- Rates of enzyme-catalyzed reactions generally increase with increasing temperature
- However, at temperatures above 50° to 60° C, enzymes typically show a decline in activity
- Two effects here:
  - Enzyme rate typically doubles in rate for ever 10°C as long as the enzyme is stable and active
  - At higher temperatures, the protein becomes unstable and denaturation occurs

The effect of temperature on enzyme activity



Unusual Enzymes

- Ribozymes - segments of RNA that display enzyme activity in the absence of protein
  - Examples: RNase P and peptidyl transferase
- Abzymes - antibodies raised to bind the transition state of a reaction of interest
  - For a good review of abzymes, see Science, Vol. 269, pages 1835-1842 (1995)
  - Transition states are covered in more depth in Chapter 14

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RNA Molecules That Are Catalytic Have Been Termed "Ribozymes"

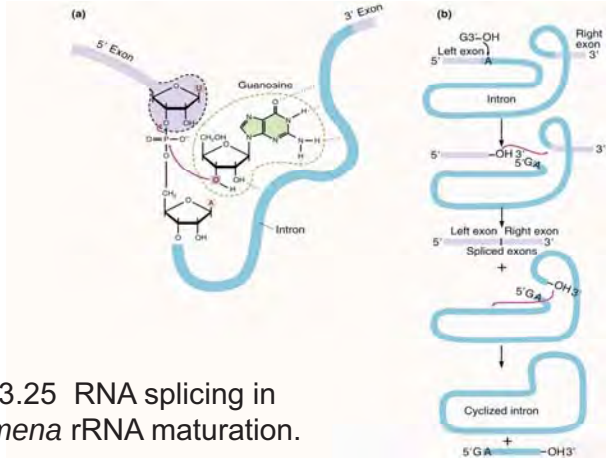

Figure 13.25 RNA splicing in *Tetrahymena* rRNA maturation.

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RNA Molecules That Are Catalytic Have Been Termed "Ribozymes"

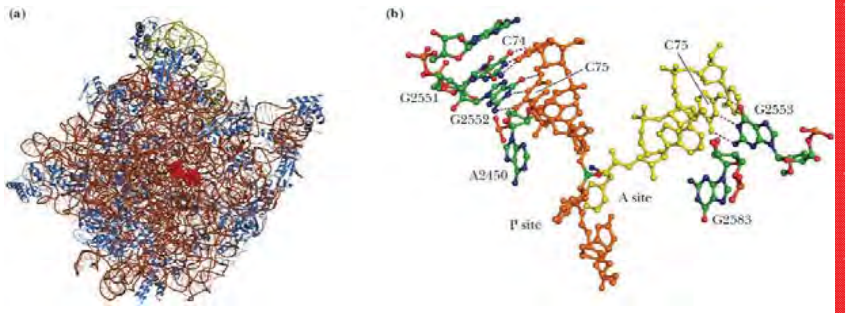

Figure 13.26 (a) The 50S subunit from *H. marismortui*. (b) The aminoacyl-tRNA (yellow) and the peptidyl-tRNA (orange) in the peptidyl transferase active site.

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RNA Molecules That Are Catalytic Have Been Termed "Ribozymes"

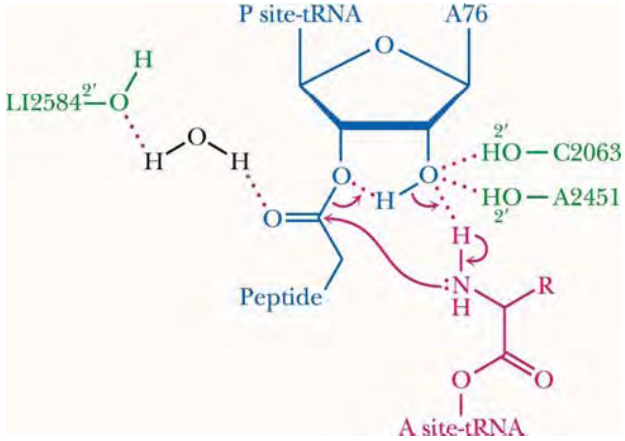


Figure 13.27 The peptidyl transferase reaction.

Antibody Molecules Can Have Catalytic Activity

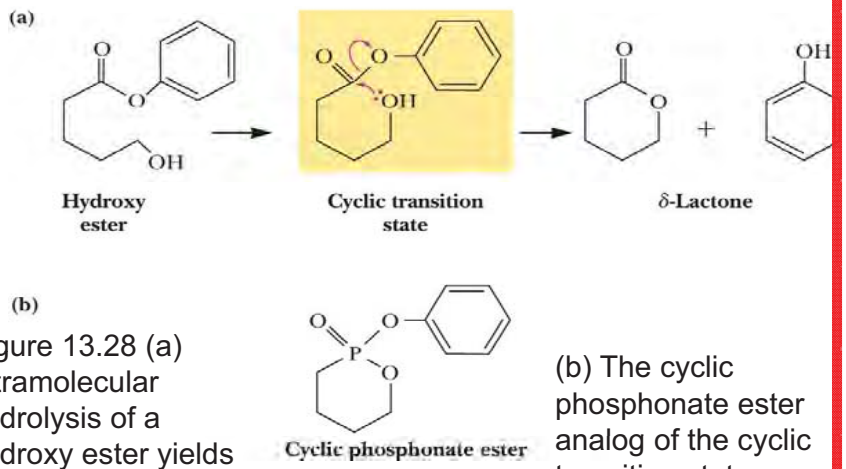


Figure 13.28 (a) Intramolecular hydrolysis of a hydroxy ester yields a δ-lactone. (b) The cyclic phosphonate ester analog of the cyclic transition state.

13.8 Is It Possible to Design An Enzyme to Catalyze Any Desired Reaction?

- A known enzyme can be "engineered" by in vitro mutagenesis, replacing active site residues with new ones that might catalyze a desired reaction
- Another approach attempts to design a totally new protein with the desired structure and activity
  - This latter approach often begins with studies "in silico" – i.e., computer modeling
  - Protein folding and stability issues make this approach more difficult
  - And the cellular environment may provide complications not apparent in the computer modeling

13.8 Is It Possible to Design An Enzyme to Catalyze Any Desired Reaction?

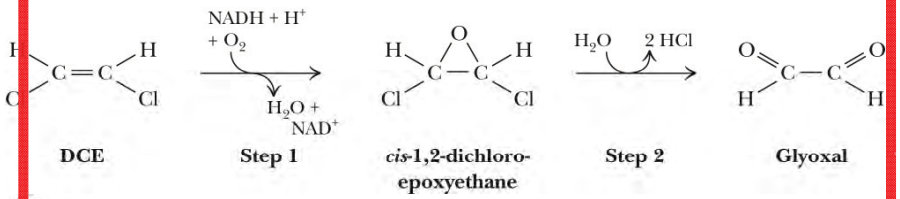


Figure 13.29 *cis*-1,2-Dichloroethylene (DCE) is an industrial solvent that poses hazards to human health.

Site-directed mutations have enabled the conversion of a bacterial epoxide hydrolase to catalyze the chlorinated epoxide hydrolase reaction.

End of Part 1

- Ask yourself…
  - What are general features of an enzyme?
  - Why and how an enzyme could be so specific?
  - Are all enzymes proteins?
  - What kind of enzyme can you buy and use in your daily life? Where are they come from?
  - Can we design an enzyme by ourselves?

13.2 Can the Rate of an Enzyme-Catalyzed Reaction Be Defined in a Mathematical Way?

- What is Kinetics?
  - the branch of science concerned with the rates of reactions
- What can we learn from enzyme kinetics?
  - to determine the maximum reaction velocity and binding affinities for substrates and inhibitors
- Why we have to learn about enzyme kinetics?
  - To know insights of enzyme mechanisms and metabolic pathways
  - This information can be exploited to control and manipulate the course of metabolic events (Pharmaceutical purpose)

Several kinetics terms to understand

- rate or velocity
- rate constant
- rate law
- order of a reaction
- molecularity of a reaction

Before we learn enzyme kinetics…

- Let's review some general ideas…
  - Chemical kinetics
  - Activation energy
  - Transition state
  - Reaction rate

Chemical Kinetics Provides a Foundation for Exploring Enzyme Kinetics

- Enzyme kinetics is based on chemical kinetics:
- First-order reaction = unimolecular reaction → molecularity =1
  - The simple elementary reaction of A→P is a first-order reaction
  - Examples
    - Intramolecular rearrangement reaction
    - Isotope decay

Chemical Kinetics: First-order reaction

- Consider a reaction of overall stoichiometry as shown:

$$A \rightarrow P$$

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

$$v = \frac{-[A]}{dt} = k[A]$$

- The rate is proportional to the concentration of A

The Time-Course of a First-Order Reaction

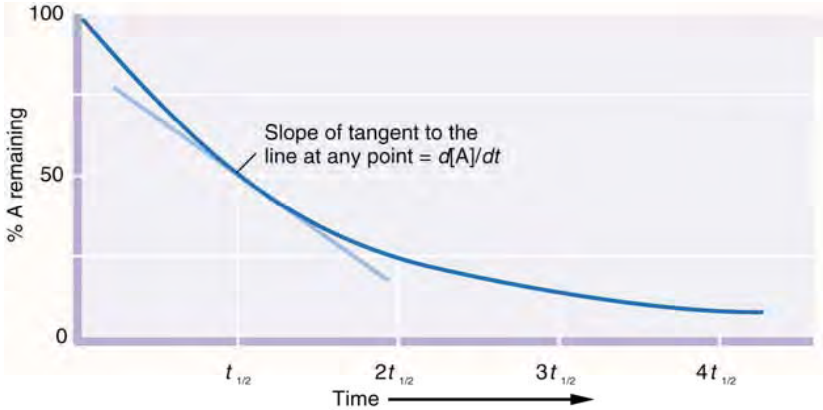


Figure 13.4 Plot of the course of a first-order reaction. The half-time, $t_{1/2}$ is the time for one-half of the starting amount of A to disappear.

Chemical Kinetics: Second-order reaction

- Bimolecular reaction = second-order reaction
- Molecularities = 2
- A + B → P + Q
  - the rate law is

$$v = k\,[A][B]$$

- 2 A → P + Q

$$v = k\,[A]^2$$

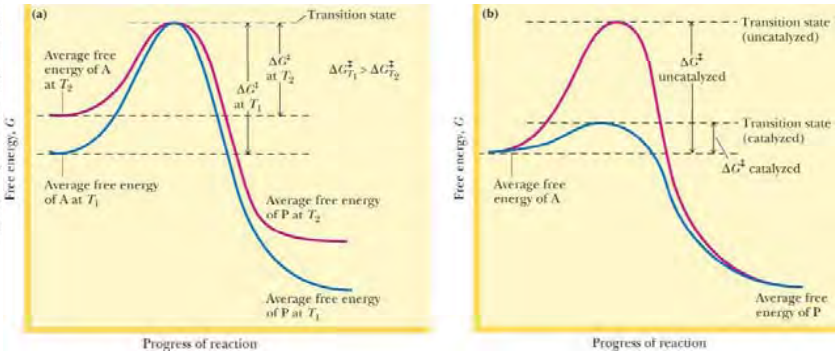
- Question: What is the unit of rate constant (k)?

Summary of Chemical Kinetics

- Molecularities > 2 is rare and never greater 3!
- Remember: Kinetics cannot prove a reaction mechanism. They can only rule out various alternative hypotheses!

Two ways to increase chemical reaction rate!

Energy diagram for a chemical reaction (A→P)



(a) raising the temperature from T_1 to T_2
(b) adding a catalyst.

Transition state

- The transition state sits at the apex of the energy profile in the energy diagram
- A typical enzyme-catalyzed reaction must pass through a transition state
- The reaction rate is proportional to the concentration of reactant molecules with the transition-state energy

Activation energy

- Free energy of activation, $\Delta G^{\ddagger}$
  - the energy required to raise the average energy of 1 mol of reactant to the transition state energy (at a given temperature).
- Decreasing $\Delta G^{\ddagger}$ increases the reaction rate
- The activation energy is related to the rate constant by Arrhenius equation:

$$k = Ae^{-\Delta G/RT}$$

Note *the difference between* ΔG *and* $\Delta G^{\ddagger}$

13.3 What Equations Define the Kinetics of Enzyme-Catalyzed Reactions?

- Enzyme is not involved in the chemical kinetics of the reaction!
  - True or False?
- A simple first-order reactions
  - display a plot of the reaction rate vs. reactant concentration as a straight line (Figure 13.6)
  - If the same reaction with an enzyme involved, is anything changed?

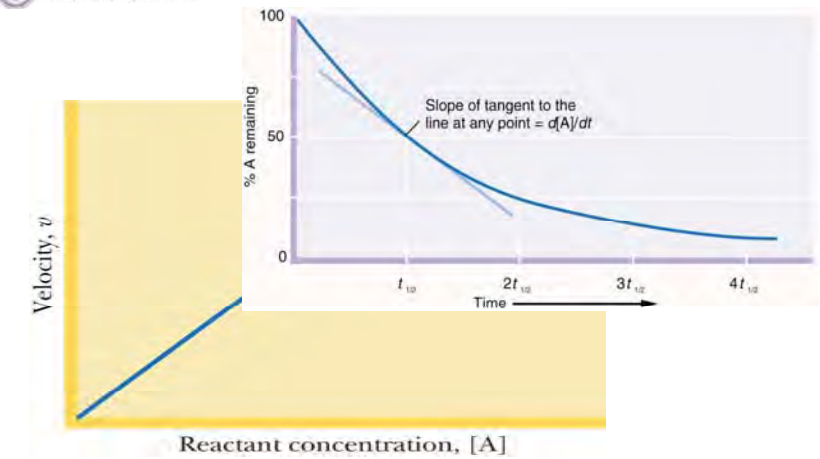
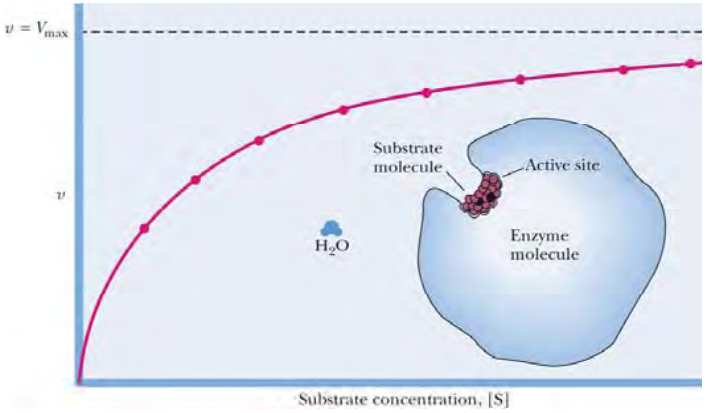


Figure 13.6
A plot of *v* vs. [A] for the unimolecular chemical reaction, A→P, yields a straight line having a slope equal to k.

Enzyme did change the chemical kinetics

- Truth is more complicated!
- At low concentrations of the substrate
  - The rate is proportional to S, as in a **first-order** reaction
- At higher concentrations of substrate
  - The enzyme reaction approaches **zero-order kinetics**

Saturation Effect



As [S] increases, kinetic behavior changes from 1st order to zero-order kinetics

We need a new equation for enzyme-catalyzed chemical kinetics!

- Louis Michaelis and Maud Menten invented a new equation which became the fundamental equation of enzyme kinetics.
- What can we learn from M-M equation?
  - We could know two major indexes of the enzyme: K_m and V_{max}!

The Michaelis-Menten Equation

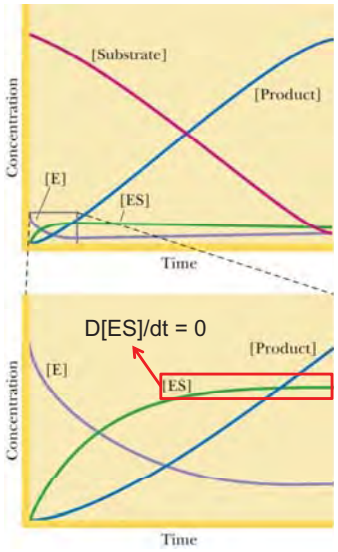
- Assumptions of M-M enzyme reaction
  1. It assumes the formation of an enzyme-substrate complex
  2. It assumes that the ES complex is in rapid equilibrium with free enzyme
  3. Breakdown of ES to form products is assumed to be slower than
     1) formation of ES and
     2) breakdown of ES to re-form E and S

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

Two More Assumptions of M-M equation

4. [ES] Remains Constant: also known as steady state assumption by Briggs & Haldane in 1925
5. Rate measurement is finished right after Substrate added
   – To ignore E + P → ES

- These assumption would simplify the following calculating



Understanding K_m

- K_m is the "kinetic activator constant" derived from rate constants
- Learning Point!
  – Three meanings of K_m!
  – How to get K_m of an enzyme?
- Please pay attention!

K_m Step 1

- According to M-M assumption, enzyme reaction could be presented as…

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

- The synthesis rate of ES

$$V_f = k_1 [E] [S]$$

- Therefore, the dissociation of ES

$$V_d = k_{-1} [ES] + k_2 [ES]$$

K_m Step 2

- Briggs & Haldane assumed that $V_f = V_d$ under steady-state!
- Therefore….

$V_f = k_1 [E] [S] = k_{-1} [ES] + k_2 [ES] = V_d$ (*Eq1*)

- Please remember
  - [E]: concentration of enzyme without substrate binding!
  - [ES]: concentration of enzyme binding with substrate!
  - We don't know [E] and [ES], actually. We only know $[E_T]$: concentration of total enzyme!

K_m Step 3

- Because $[E_T] = [E] + [ES]$

  Therefore $[E] = [E_T] - [ES]$
- Try to reduce unknown value in *Eq*1

$$k_1 [E] [S] = k_{-1} [ES] + k_2 [ES]$$

$$k_1([E_T] - [ES]) [S] = (k_{-1} + k_2) [ES]$$

- Move all constants to one side, then….

$$\frac{([E_T] - [ES]) [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m \quad (Eq2)$$

The First Meaning of K_m

$$K_m = \frac{k_{-1} + k_2}{k_1} = \frac{\text{Sum of ES dissociation rate constants}}{\text{ES synthesis rate constant}}$$

- An enzyme with bigger K_m:
  - Dissociation rate constants are larger or synthesis rate constant is smaller
  - Literally, substrate and enzyme are difficult to bind!
- What if an enzyme with small K_m?
- Therefore, K_m is related to the enzyme-substrate binding capacity!

K_m Step 4

- In all kinds of kinetics studies, the most important issue is reaction rate, "*V*" !

$$V = k_2 [ES] \quad (Eq3)$$

- We don't know [ES] exactly, but we know…

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

- Try to move [ES] to one side…

The Second Meaning of K_m

- After [ES] moved to one side, *Eq*2 becomes..

$$[ES] = \frac{[E_T] \bullet [S]}{K_m + [S]} \quad (Eq4)$$

- Now combine *Eq*3 and *Eq*4, you will get

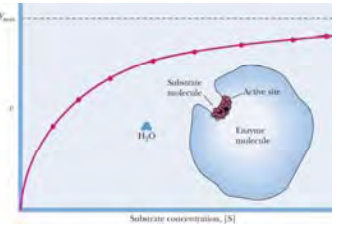
$$V = \frac{k_2 [E_T] \bullet [S]}{K_m + [S]} \quad (Eq5)$$

- K_m is related to the reaction rate!
  - An enzyme with smaller K_m may has higher reaction rate!

K_m Step 5

$$V = \frac{k_2 [E_T] \bullet [S]}{K_m + [S]} \quad (Eq5)$$

- As you see in *Eq*5, *V* increases when [S] increased.
- If [S] becomes very high, what happened to *V*?
  - Enzyme will be saturated!
  - $K_m + [S] \rightarrow [S]$
  - $V \rightarrow V_{max}$



K_m Step 6

- Now Eq5 becomes

$$V_{max} = \frac{k_2 [E_T] \bullet [S]}{[S]} = k_2 [E_T] \quad (Eq6)$$

- We could also combine *Eq*5 and *Eq*6, make it simpler!

$$V = \frac{V_{max} \bullet [S]}{K_m + [S]} \quad (Eq7)$$

The Third Meaning of K_m

- From *Eq*7, we could redefine the meaning of K_m as..

$$K_m = \frac{[S] \bullet (V_{max} - V)}{V} = [S] \bullet \left(\frac{V_{max}}{V} - 1\right) \quad (Eq8)$$

- That means when $\frac{V_{max}}{V} - 1 = 1$, $K_m = [S]$
  - Also means $\frac{V_{max}}{V} = 2 \rightarrow V = \frac{1}{2} V_{max}$

- When reaction rate is ½ V_{max}, the corresponding [S] equals to K_m!

Take a look at the K_m values for some enzymes and their substrates

TABLE 13.3 K_m Values for Some Enzymes

Enzyme	Substrate	K_m (mM)
Carbonic anhydrase	CO_2	12
Chymotrypsin	*N*-Benzoyltyrosinamide	2.5
	Acetyl-L-tryptophanamide	5
	N-Formyltyrosinamide	12
	N-Acetyltyrosinamide	32
	Glycyltyrosinamide	122
Hexokinase	Glucose	0.15
	Fructose	1.5
β-Galactosidase	Lactose	4
Glutamate dehydrogenase	NH_4^+	57
	Glutamate	0.12
	α-Ketoglutarate	2
	NAD^+	0.025
	NADH	0.018
Aspartate aminotransferase	Aspartate	0.9
	α-Ketoglutarate	0.1
	Oxaloacetate	0.04
	Glutamate	4
Threonine deaminase	Threonine	5
Arginyl-tRNA synthetase	Arginine	0.003
	$tRNA^{Arg}$	0.0004
	ATP	0.3
Pyruvate carboxylase	HCO_3^-	1.0
	Pyruvate	0.4
	ATP	0.06
Penicillinase	Benzylpenicillin	0.05
Lysozyme	Hexa-*N*-acetylglucosamine	0.006

Other indexes for an enzyme

- k_{cat}, the turnover number
  - A measure of catalytic activity
  - Defines the activity of one molecule of enzyme
  - If the M-M model fits, $k_2 = k_{cat} = V_{max}/E_t$
  - Values of k_{cat} range from less than 1/sec to many millions per sec

The Turnover Number Defines the Activity of One Enzyme Molecule

TABLE 13.4 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

Other indexes for an enzyme

- The Ratio k_{cat}/K_m
  - Defines the catalytic efficiency of an enzyme
  - An estimate of "how perfect" the enzyme is
  - k_{cat}/K_m is an apparent second-order rate constant
  - It measures how the enzyme performs when S is low
  - The upper limit for k_{cat}/K_m is the diffusion limit - the rate at which E and S diffuse together

The Ratio k_{cat}/K_m Defines the Catalytic Efficiency of an Enzyme

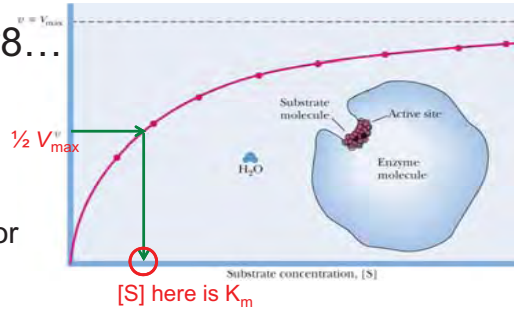
TABLE 13.5 Enzymes Whose k_{cat}/K_m Approaches the Diffusion-Controlled Rate of Association with Substrate

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m (M^{-1} sec^{-1})
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	0.012	8.3×10^7
	HCO_3^-	4×10^5	0.026	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	800	5×10^{-6}	1.6×10^8
	Malate	900	2.5×10^{-5}	3.6×10^7
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	4.3×10^3	1.8×10^{-5}	2.4×10^8
β-Lactamase	Benzylpenicillin	2×10^3	2×10^{-5}	1×10^8

Catalytic perfection

How to measure the K_m?

- The nature of Michaelis-Menten equation:
  - *– Combination of 0-order and 1st-order kinetics*
  - – Describes a rectangular hyperbolic dependence of v on S
- According to *Eq*8…



But this is not good for calculating…

[S] here is K_m

Because of V_{max}

- V_{max} is a *theoretical maximal velocity* and is a constant
- V_{max} is NEVER achieved in reality

- For example:

  To reach a rate of 99% V_{max}

$$\frac{V}{V_{max}} = 0.99 = \frac{[S]}{K_m + [S]}$$

- That means you have to prepare substrate [S] = 99 K_m
- It is not possible practically.

Linear Plots Can Be Derived from the Michaelis-Menten Equation

$$V = \frac{V_{max} \bullet [S]}{K_m + [S]} \quad (Eq7)$$

- Rearrange to obtain the Lineweaver-Burk equation:

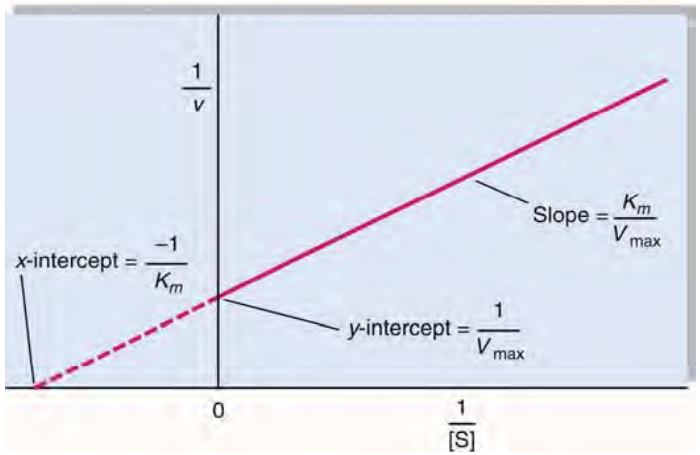
$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

- A plot of 1/v versus 1/[S] should yield a straight line

The Lineweaver-Burk double-reciprocal plot

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

Y = aX + b



Hanes-Woolf Linear Plot

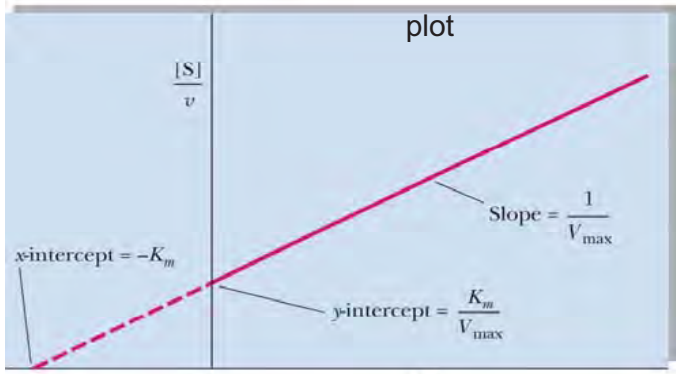
- Begin with Lineweaver-Burk and divide both sides by [S] to obtain:

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

Hanes-Woolf Plot is Better - Why?

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

- Hanes-Woolf plot
  - [S]/v versus [S]
  - ┬smaller and more consistent errors across the plot



More about kinetics

- pH may effect on K_m or V_{max} or both in enzyme kinetics
- What if the plot is not linear?
  - – Regulatory enzymes (allosteric enzymes)

End of Part 2

- Ask yourself…
  - How many indexes you have learned to describe the kinetic properties of an enzyme?
  - What is M-M equation? Do you know what's different from enzyme kinetics and chemical kinetics?
  - Do you understand the three meanings of K_m and know how to conduct from *Eq*1 to *Eq*8?
  - Do you understand how to plot Lineweaver-Burk plot, and estimate K_m and V_{max} from it?

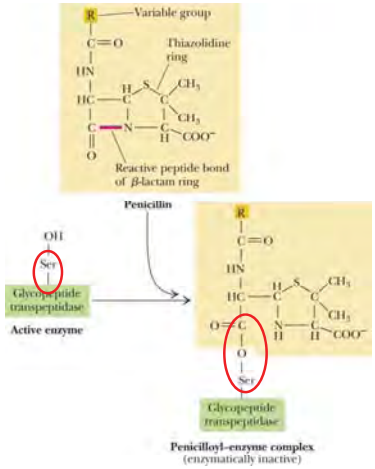
13.4 What Can Be Learned from the Inhibition of Enzyme Activity?

- Enzymes may be inhibited reversibly or irreversibly
- Reversible inhibitors
  - may bind at the active site or at some other site
    - Competitive inhibition
    - Noncompetitive inhibition
    - Uncompetitive inhibition
- Irreversible inhibitors
  - Covalent modification
    - Kinetically similar to ___cometitive inhibition!

Irreversible inhibitor

Example:

- Penicillin is an irreversible inhibitor of *glycopeptide transpeptidase*
  - Suicide inhibitor
  - catalyzes an essential step in bacterial cell all synthesis



Reversible Inhibitors May Bind at the Active Site or at Some Other Site

TABLE 13.6 The Effect of Various Types of Inhibitors on the Michaelis–Menten Rate Equation and on Apparent K_m and Apparent V_{max}

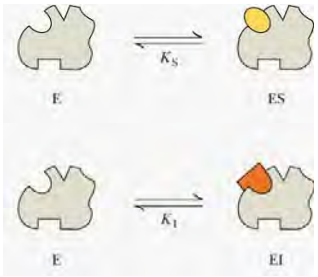
Inhibition Type	Rate Equation	Apparent K_m	Apparent V_{max}
None	$v = V_{max}[S]/(K_m + [S])$	K_m	V_{max}
Competitive	$v = V_{max}[S]/([S] + K_m(1 + [I]/K_I))$	$K_m(1 + [I]/K_I)$	V_{max}
Noncompetitive	$v = (V_{max}[S]/(1 + [I]/K_I))/(K_m + [S])$	K_m	$V_{max}/(1 + [I]/K_I)$
Mixed	$v = V_{max}[S]/((1 + [I]/K_I)K_m + (1 + [I]/K_I'[S]))$	$K_m(1 + [I]/K_I)/(1 + [I]/K_I')$	$V_{max}/(1 + [I]/K_I')$
Uncompetitive	$v = V_{max}[S]/(K_m + [S](1 + [I]/K_I'))$	$K_m/(1 + [I]/K_I')$	$V_{max}/(1 + [I]/K_I')$

Competitive inhibition

- Inhibitor, I, also binding to enzyme and compete for the same binding site with substrate

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$$

$$E + I \underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} EI$$



Kinetic of competitive inhibition Step 1

- Because $[E_T] = [E] + [ES]$ and

$$\frac{([E_T] - [ES])\,[S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m \quad (Eq2)$$

- So…

$$[ES] = \frac{[E]\,[S]}{K_m} \quad (Eq9)$$

Kinetic of competitive inhibition Step 2

- Because

$$E + I \underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} EI$$

- $V_f = V_d$ under steady-state!
- Therefore….

$$V_f = k_3\,[E]\,[I] = k_{-3}\,[EI] \quad (Eq10)$$

$$[EI] = \frac{k_3}{k_{-3}}\,[E]\,[I] = 1/k_I\,[E]\,[I] \quad (Eq11)$$

Kinetic of competitive inhibition Step 3

- Knowing $[E_T] = [E] + [ES] + [EI]$

$$[E_T] = [E] + \frac{[E]\,[S]}{K_m} + \frac{[E]\,[I]}{K_I} \quad (Eq12)$$

- Put [E] to one side….

$$[E] = \frac{K_I K_m\,[E_T]}{(K_I K_m + K_I\,[S] + K_m\,[I])} \quad (Eq13)$$

Kinetic of competitive inhibition Step 4

- Rate is still the most important!

$$V = k_2 [ES]$$

- According to *Eq9*

$$[ES] = \frac{[E]\,[S]}{K_m} \qquad (Eq9)$$

- *it could be changed to…*

$$V = \frac{k_2 [E]\,[S]}{K_m} \qquad (Eq14)$$

Kinetic of competitive inhibition Step 5

- Combine *Eq13* and *Eq14* to redefine rate!

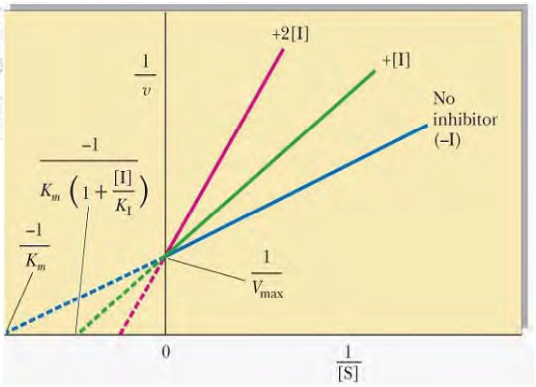
$$V = \frac{k_2 K_I \cancel{K_m} [E_T]\,[S]}{\cancel{K_m}(K_I K_m + K_I [S] + K_m [I])} \qquad (Eq15)$$

- Because $V_{max} = k_2 [E_T]$ …

$$V = \frac{V_{max} \bullet [S]}{[S] + K_m (1 + [I]/K_I)} \qquad (Eq16)$$

Effect of Competitive Inhibitors on Lineweaver-Burk plot

$$V = \frac{V_{max} \bullet [S]}{[S] + K_m (1 + [I]/K_I)}$$



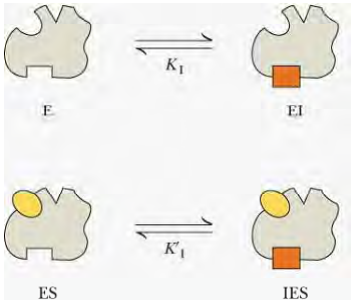
- More [I],
  - Less rate!
  - Larger Km!
  - Why?
- V_{max} unchanged!
  - Why?

Example of Competitive Inhibition

- Succinate Dehydrogenase (SDH)

Noncompetitive inhibition

- S and I bind to different sites on the enzyme
- I could bind to E or ES
- 2 K_I
- 2 types:
  - Pure noncompetition
  - Mixed noncompetition

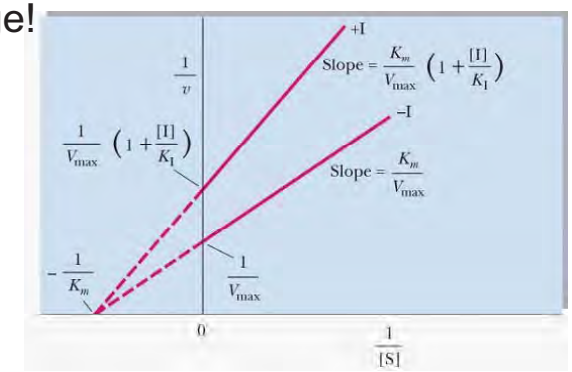


$$E + I \rightleftharpoons^{K_I} EI$$

$$ES + I \rightleftharpoons^{K_I'} IES$$

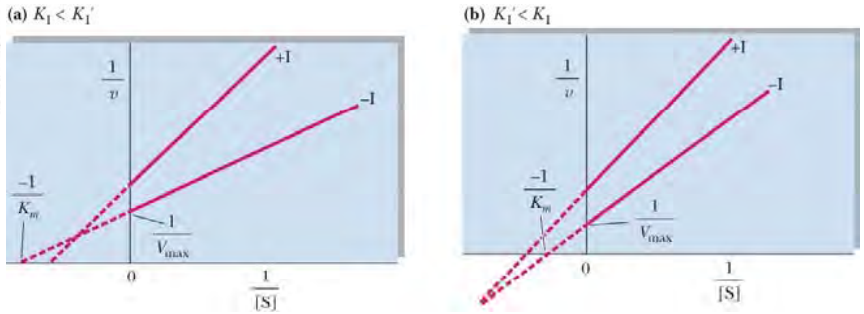
Pure Noncompetitive Inhibition

- Inhibitor binds to E or ES equally
  - $K_I = K_I'$
- K_m not change!
  - Why?
- V_{max} smaller!
  - Why?



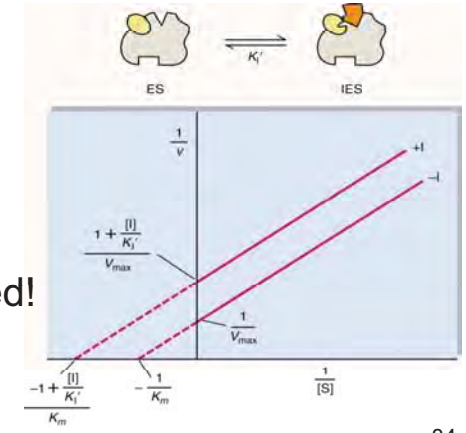
Mixed Noncompetitive Inhibition

- Binding of I influences binding of S
- 2 situations:



Uncompetitive Inhibition

- I combines only with ES and affect k_2 (k_{cat})!
- K_m smaller!
  - Why?
- V_{max} smaller!
  - Why?
- Slope is not changed!
  - Why?



Bimolecular Reactions Catalyzed by Enzymes

- Most enzymes catalyze reactions involving two (or more) substrates
- 2 Reaction mechanisms
  - Sequential (single-displacement) reactions
  - Ping-pong (double-displacement) reactions

Single-displacement Reactions

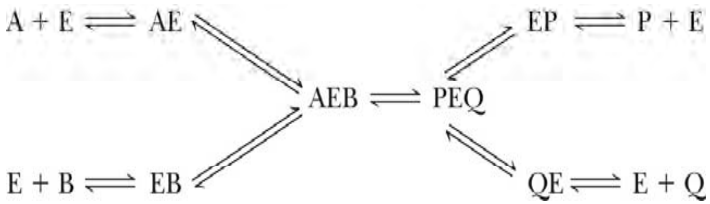
- Two distinct classes:
  - **Random**: either substrate may bind first, followed by the other substrate
  - **Ordered**: a **leading substrate** binds first, followed by the other substrate
- Similar to noncompetitive inhibition!
  - Why?

Double-reciprocal form of the rate equation:

$$\frac{1}{v} = \frac{1}{V_{max}}\left(K_m^A + \frac{K_S^A K_m^B}{[B]}\right)\left(\frac{1}{[A]} + \frac{1}{V_{max}}\left(1 + \frac{K_m^B}{[B]}\right)\right)$$

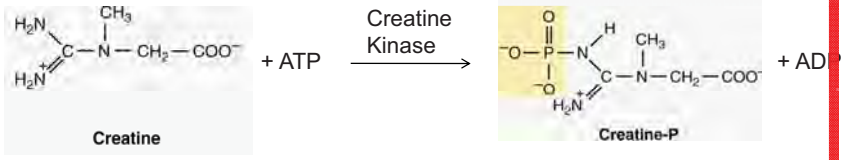
Random, Single-Displacement Reactions

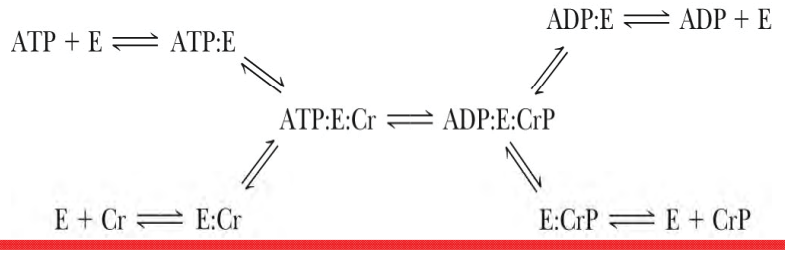
- All possible binary enzyme-substrate and enzyme-product complexes are formed rapidly and reversibly
- Conversion of AEB to PEQ is the Rate-Limiting Step
- If A has no influence on B binding → purely random!



Example of Random, Single-displacement enzyme

- Creatine Kinase (important in muscle)





Lineweaver-Burk Plot for single-displacement bisubstrate enzyme

Purely random

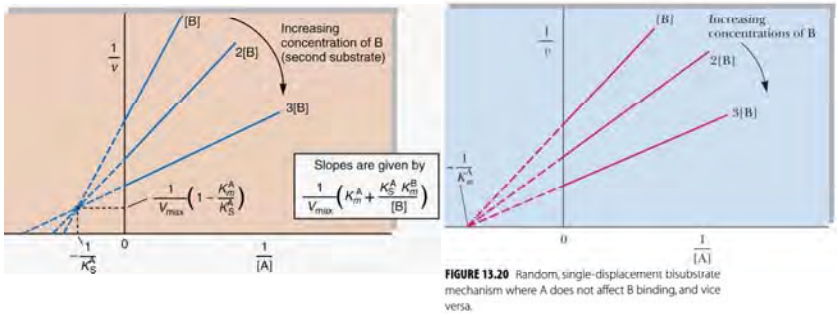
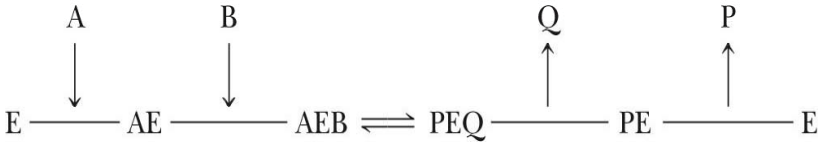


FIGURE 13.20 Random, single-displacement bisubstrate mechanism where A does not affect B binding, and vice versa.

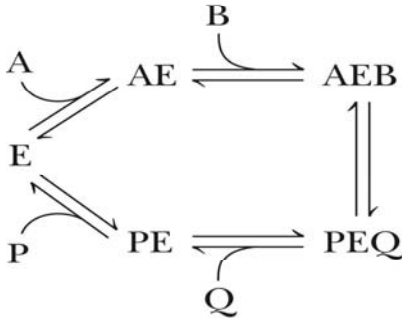
Ordered, Single-Displacement Reaction

- The leading substrate (obligatory or compulsory substrate), A, must bind first followed by B.
- Reaction between A and B occurs in the ternary complex and is usually followed by an ordered release of the products, P and Q.



An Alternative way of Portraying the Ordered, Single-Displacement Reaction

- A and P are competitive for enzyme!
- A and B are not competitve for enzyme.
- No A, no B binding!

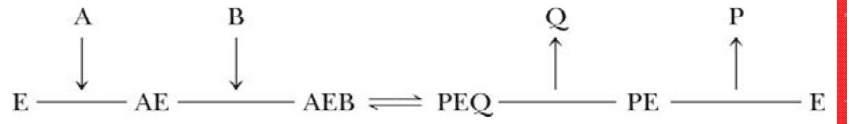


Example for Ordered, Single-Displacement Reaction

- Alcohol dehydrogenase (ADH)
  - Use NAD^+ (nicotinamide adenine dinucleotide) as coenzyme or compulsory substrate

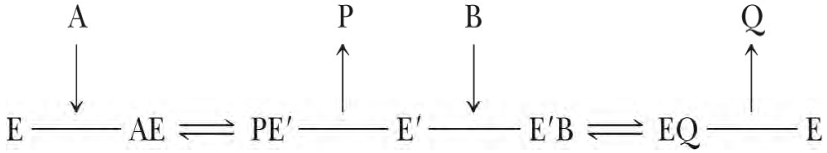
$$NAD^+ + CH_3CH_2OH \rightleftharpoons NADH + H^+ + CH_3CHO$$

(A) (B) (Q) (P)



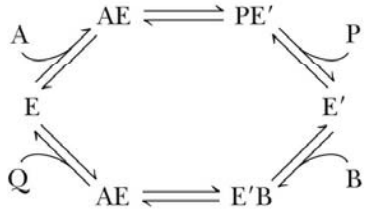
Double Displacement Reaction

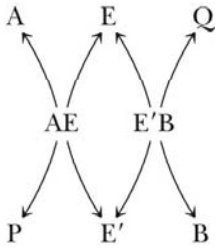
- *Aka*. Ping-Pong reactions
  - – Formation of a covalently modified enzyme intermediate.
  - – The product of the enzyme's reaction with A (called P in the above scheme) is released prior to reaction of the enzyme with the second substrate, B.



An Alternative Presentation of the Double-Displacement (Ping-Pong) Reaction

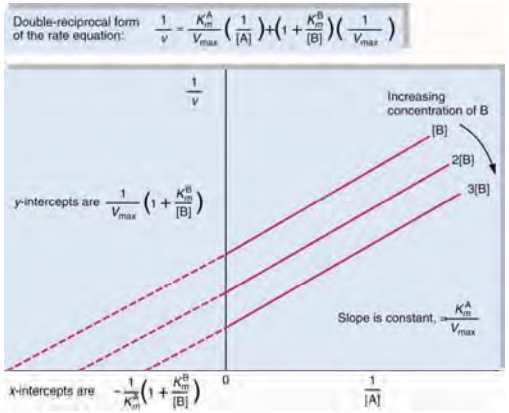
- A and Q compete for E
- P and B compete for E'





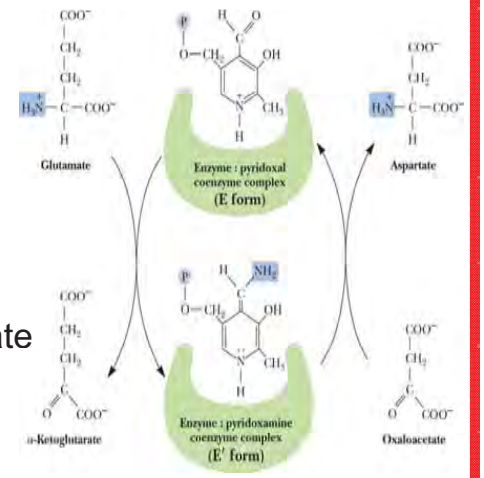
Lineweaver-Burk Plot for the Double Displacement Reaction

Similar to uncompetitive inhibition, why?



Example for the Double Displacement Reaction

- Glutamate:aspartate Aminotransferase
  - – Amino acid metabolism
  - – Enzyme bond coenzyme
  - – Pyridoxal phosphate



End of Part 3

- Ask yourself…
  - What is the difference of reversible and irreversible inhibition?
  - What is competitive, noncompetitive, uncompetitve inhibition? What are they look like in Lineweaver-Burk plot?

End of this chapter

- You should know…
  - What characteristic features define enzymes?
  - How can enzymes be so specific?
  - Are all enzymes proteins?
  - Is it possible to design an enzyme to catalyze any desired reaction?
  - Can the rate of an enzyme-catalyzed reaction be defined in a mathematical way?
  - What equations define the kinetics of enzyme-catalyzed reactions?
  - What can be learned from the inhibition of enzyme activity?
  - What is the kinetic behavior of enzymes catalyzing bimolecular reactions?