Chem 352 – Lecture 4
Part I: Enzyme Properties

Question for the Day: Describe how to easily determine the number of reactions that one enzyme molecule is capable catalyzing in one second.

1

Introduction to Enzymes

- Enzymes are biological **catalysts**

2-1

Introduction to Enzymes

- Enzymes are biological **catalysts**

catalyst |ˈkatl-ist|

noun
a substance that increases the rate of a chemical reaction without itself undergoing any permanent chemical change.
• figurative a person or thing that precipitates an event : *the governor's speech acted as a catalyst for debate.*
ORIGIN early 20th cent.: from **catalysis** , on the pattern of *analyst.*

(New Oxford American Dictionary)

2-2

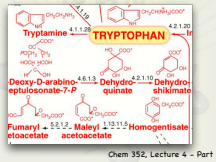
Introduction to Enzymes

- Enzyme are biological **catalysts**

3-1

Introduction to Enzymes

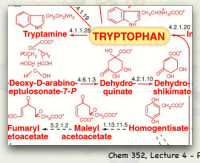
- Enzyme are biological **catalysts**
  - Nearly every reaction in a living cell is catalyzed by an enzyme.



3-2

Introduction to Enzymes

- Enzyme are biological **catalysts**
  - Nearly every reaction in a living cell is catalyzed by an enzyme.
  - Most enzymes are proteins.



3-3

Introduction to Enzymes

- Enzymes can exhibit:

4-1

Introduction to Enzymes

- Enzymes can exhibit:
  - High specificity
    - High substrate specificity
    - High reaction specificity

4-2

Introduction to Enzymes

- Enzymes can exhibit:
  - High specificity
    - High substrate specificity
    - High reaction specificity
    - Stereospecificity

4-3

Introduction to Enzymes

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4-4

Introduction to Enzymes

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  - They can couple energetically unfavorable reactions with those that are favorable.
  - They serve as control points in metabolic pathways.

4-5

Introduction to Enzymes

Question:

Explain why it should be a relatively simple task for an enzyme to distinguish between L-alanine and D-alanine.

4-6

Introduction to Enzymes

- Enzymes can exhibit:
  - High specificity
    - High substrate specificity
    - High reaction specificity
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Chem 352, Lecture 4 - Part I, Properties of Enzymes 4

4-7

Introduction to Enzymes

In this lecture will discuss

5-1

Introduction to Enzymes

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- The **classification** and **nomenclature** of enzymes

5-2

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- The **classification** and **nomenclature** of enzymes
- The analysis of the **kinetics** of enzyme-catalyzed reactions
  - and learn what this can tell us about the mechanisms of enzyme catalyzed reactions.

5-3

Introduction to Enzymes

In this lecture will discuss

- The **classification** and **nomenclature** of enzymes
- The analysis of the **kinetics** of enzyme-catalyzed reactions
  - and learn what this can tell us about the mechanisms of enzyme catalyzed reactions.
- The **regulation** of enzyme activity by inhibitors and activators

5-4

Myoglobin and Hemoglobin

While neither of these proteins are enzymes, they nicely illustrate some of the recurring themes that we will see in our discussion on enzymes.

- Hyperbolic and sigmoidal binding behaviors
- Allosteric behaviors

(Moran et al. Section 4.14)

6

Myoglobin and Hemoglobin

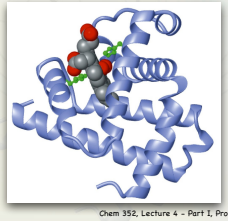
Both these proteins function to bind and transport molecular oxygen.

- But they have distinctly different roles
  - **Hemoglobin** (Hb) transports oxygen from the lungs to the tissues.
  - **Myoglobin** (Mb) accepts the oxygen from Hb and stores it in the tissues

7

Myoglobin and Hemoglobin

Hb and Mb share similar I°, II° and III° structures



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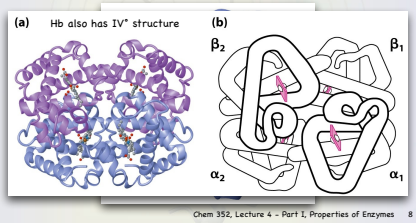

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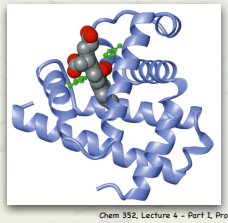

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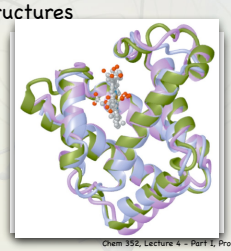

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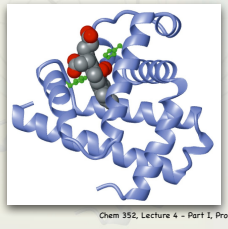

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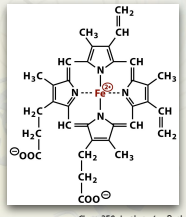

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Myoglobin and Hemoglobin

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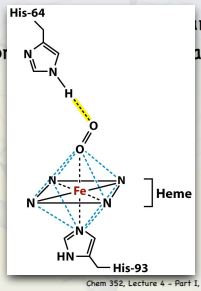

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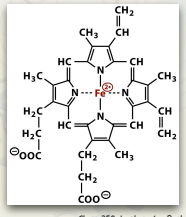

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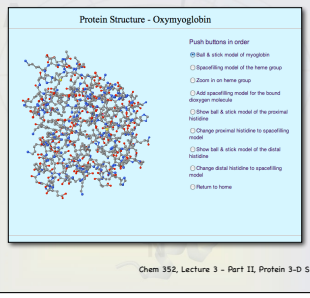

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Myoglobin and Hemoglobin

The quaternary structure for hemoglobin produces a distinctly different oxygen binding behavior than observed for myoglobin.

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Myoglobin and Hemoglobin

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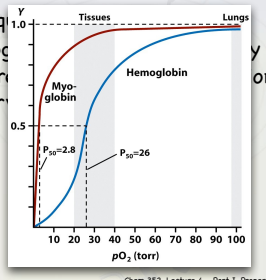

11-2

Myoglobin and Hemoglobin

The quaternary structure for hemoglobin produces a distinctly different oxygen binding behavior than observed for myoglobin.

11-3

Myoglobin and Hemoglobin

The four oxygen binding sites in hemoglobin communicate with one another.

- O_2 binding at one site, increasing the binding affinity at the remaining sites.

12-1

Myoglobin and Hemoglobin

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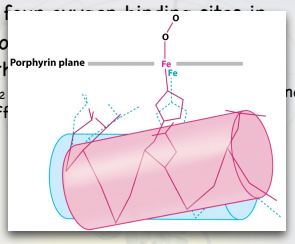

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Myoglobin and Hemoglobin

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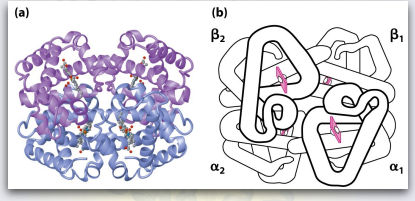

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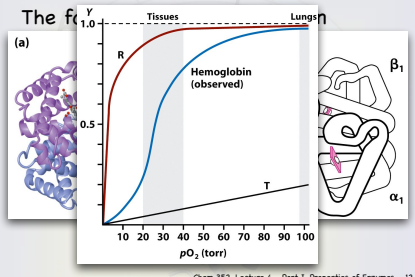

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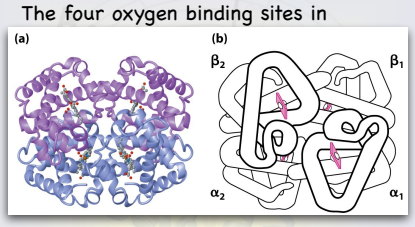

12-5

Myoglobin and Hemoglobin

The four oxygen binding sites in hemoglobin communicate with one another.

- O_2 binding at one site, increasing the binding affinity at the remaining sites.

12-6

Myoglobin and Hemoglobin

Hemoglobin also provides an example of allosteric regulation.

- The binding of protons (Bohr effect) and the binding of 2,3-bisphosphoglycerate both lower the affinity of Hb for O2.

13-1

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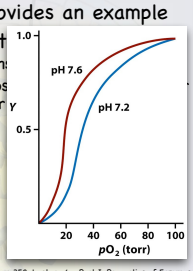

13-2

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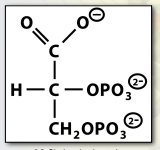

2,3-Bisphosphoglycerate

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Myoglobin and Hemoglobin

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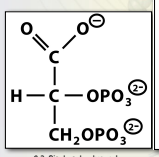
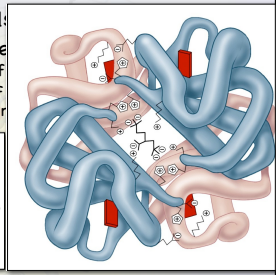


2,3-Bisphosphoglycerate



15-3

Myoglobin and Hemoglobin

- Hemoglobin also provides an example of allosteric regulation.
  - The binding of protons (Bohr effect) and the binding of 2,3-bisphosphoglycerate both lower the affinity of Hb for O_2.

15-4

Enzyme Nomenclature

nomenclature |ˈnōmənˌklā ch ər|

noun
the devising or choosing of names for things, esp. in a science or other discipline.
• the body or system of such names in a particular field : *the nomenclature of chemical compounds.*
• formal the term or terms applied to someone or something : *"customers" was preferred to the original nomenclature "passengers."*
DERIVATIVES
nomenclatural |ˌnōmənˈklā ch ərəl| |ˈnoʊmənˈkleɪtʃ(ə)rəl| |-ˈklatʃ(ə)r(ə)l| |-kləˈtʃʊər(ə)l| adjective
ORIGIN early 17th cent.: from French, from Latin ***nomenclatura***, from ***nomen 'name'*** + ***clatura 'calling, summoning'*** (from ***calare 'to call'***).

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Enzyme Nomenclature

The Systematic names of enzymes use numbers

17-1

Enzyme Nomenclature

The Systematic names of enzymes use numbers

- The numbers describes

17-2

Enzyme Nomenclature

The Systematic names of enzymes use numbers

- The numbers describes
  - the class of reaction

17-3

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17-4

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  - the substrates used in the reaction
  - the products produced in the reaction

17-5

Enzyme Nomenclature

The Systematic names of enzymes use numbers

- The numbers describes
  - the class of reaction
  - the substrates used in the reaction
  - the products produced in the reaction
- The scheme for deriving these number is defined by the International Union of Biochemistry and Molecular Biology (IUBMB)

17-6

Enzyme Nomenclature

- Systematic names use numbers
  - pyruvate kinase - EC 2.7.1.40
  - alcohol dehydrogenase - EC 1.1.1.1

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Enzyme Classes

- Oxidoreductases (1.)
  - Catalyze oxidation-reduction reactions
  - For example
    - lactate dehydrogenase
      - lactate:NAD oxidoreductase (EC 1.1.1.27)

- Distinguishing features:
  - Many (dehydrogenases) involve addition or removal of H_2 as $H:^- + H^+$

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Enzyme Classes

- Transferases (2.)
  - Catalyze the transfer of a group from one molecule to another
  - For example
    - L-alanine amino transferase
      - L-alanine:2-oxyglutarate aminotransferase (EC 2.6.1.2)
- Distinguishing features:
  - Look for double-replacement reactions

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Enzyme Classes

- Hydrolases (3.)
  - Catalyze a special case of transfer reactions, where water is the acceptor of the group being transferred. The reactions lead to the splitting of molecules in two.
  - For example
    - pyrophosphatase
      - diphosphate phosphohydrolase (EC 3.6.1.1)
  - Distinguishing features:
    - Look for water splitting in conjunction with another molecule splitting

21-1

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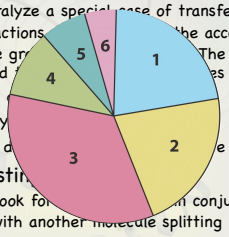

21-2

Enzyme Classes

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      - diphosphate phosphohydrolase (EC 3.6.1.1)
  - Distinguishing features:
    - Look for water splitting in conjunction with another molecule splitting

21-3

Enzyme Classes

- Lyases (4.)
  - Catalyze a lysis of a subtrate, which is nonoxidative, nonhydrolytic and generates a double bond.
  - For example
    - pyruvate decarboxylase
      - pyruvate decarboxylase, 2-oxo-acid carboxy-lyase (EC 4.1.1.1)
  - Distinguishing features:
    - Look for addition and elimination reactions involving the loss or formation of a double

22

Enzyme Classes

- Isomerase (5.)
  - Catalyze a conversion between two isomers.
    - Sometimes referred to as an rearrangement reaction.
  - For example
    - alanine racemase
      - alanine racemase (EC 5.1.1.1)
  - Distinguishing features:
    - Look for reactions that have only a single substrate and a single product.

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Enzyme Classes

- Ligase (6.)
  - Catalyze the ligation or joining of two molecules.
    - This reaction usually requires a source of chemical energy, which is often provided by the hydrolysis of ATP to ADP and P_i
  - For example
    - glutamine synthetase
      - L-glutamate:ammonia ligase (EC 6.3.1.2)
  - Distinguishing features:
    - Look for a combination of two reactions

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Enzyme Kinetics

- The **kinetics** of a reaction can tell you details about the mechanism of the reaction
- Kinetics of non-catalyzed reactions
  - The **rate**, or **velocity**, of a reaction is monitored as a function of substrate (reactant) conc.

$$S \longrightarrow P$$

$$Rate = \frac{\text{change in concentration}}{\text{change in time}} = \frac{dP}{dt} = -\frac{dS}{dt}$$

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Enzyme Kinetics

- The rate equation for this relationship can take on different forms with respect to the substrate or product concentration:
  - first order
  - second order
  - zero order

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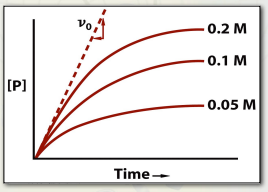
Enzyme Kinetics

- Kinetics of non-catalyzed reactions
  - Typically it is the substrate dependence of the initial rate, v_o, that is determined.



27-1

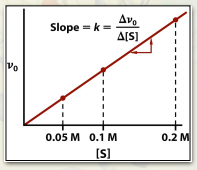
Enzyme Kinetics

- Kinetics of non-catalyzed reactions
  - Typically it is the substrate dependence of the initial rate, v_o, that is determined.

27-2

Enzyme Kinetics

- Kinetics of non-catalyzed reactions
  - For a first-order reaction, v_o has a straight-line dependence on [S].
    - $v_o = k_1[S]$ (k_1 is first order rate constant)



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Enzyme Kinetics

- Kinetics of non-catalyzed reactions
  - For a second order reaction, v_o has a quadratic dependence on [S].
    - $v_o = k_2[S]^2$ (k_2 is second order rate constant)
  - For a zero order reaction, v_o is independent of [S]
    - $v_o = k_0$ (k_0 is the zero order rate constant)

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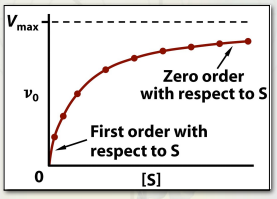
Enzyme Kinetics

- The kinetics of an **enzyme catalyzed reaction** looks different.



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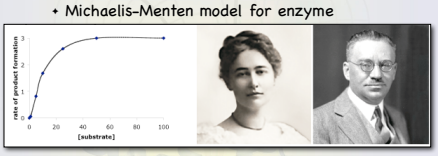
Enzyme Kinetics

- Kinetics of enzyme-catalyzed reactions
  - Michaelis-Menten model for enzyme



Maud Menten (1879-1960) Leonor Michaelis (1875-1949)

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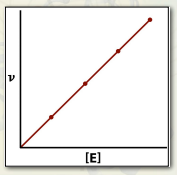
Enzyme Kinetics

- For enzyme-catalyzed reactions
  - See first-order enzyme concentration, [E], dependence at high substrate concentrations [S], where kinetics is zero order.



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Enzyme Kinetics

- Proposed the following mechanism for an enzyme catalyzed reaction:

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

  - The overall rate of the reaction is therefore determined by the conversion of enzyme-substrate complex, ES, to product:

$$v_o = k_2[ES]$$

- Came up with an expression for [ES] as a function of [S]
  - Substitution in the the above expression for v_o will then give v_o as a function of [S].

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Enzyme Kinetics

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

- Proposed that the concentration of ES quickly reaches a steady state, in which the rate at which ES is formed, ($=k_1[E][S]$), is equal to the rate at which ES is consumed ($=k_{-1}[ES] + k_2[ES]$):

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

  - Solving for [ES] gives:

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

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Enzyme Kinetics

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

  - The rate constants are combined to produce a single constant, K_M, called the Michaelis-Menten constant.

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

  - Therefore the expression for [ES] becomes

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

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Enzyme Kinetics

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

- Before this expression for [ES] can be substituted into the expression for v_o, the variable [E] needs to be eliminated.
  - [E] is the free enzyme concentration, which is equal to the total enzyme concentration, $[E]_T$, minus the enzyme-substrate concentration, [ES].

$$[E] = [E]_T - [ES]$$

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Enzyme Kinetics

- Substitution of this expression for [E] into the one derived before for [ES] gives an expression for v_o as a function of [S]:

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

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

$$[ES]\left(1 + \frac{[S]}{K_M}\right) = \frac{[E]_T[S]}{K_M}$$

$$[ES]\left(\frac{K_M + [S]}{K_M}\right) = \frac{[E]_T[S]}{K_M}$$

$$[ES] = \left(\frac{K_M}{K_M + [S]}\right)\frac{[E]_T[S]}{K_M}$$

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

37

Enzyme Kinetics

- Substitution of this expression for [ES] into the one for v_o gives us the Michaelis-Menton equation:

$$v_o = k_2[ES]$$

$$v_o = \frac{k_2[E]_T[S]}{K_M + [S]}$$

At very high substrate concentration ([S] >> K_M),

$$v_o = k_2[E]_T \qquad \text{(as [S] becomes large)}$$

- Which is a constant equal to the maximum velocity, V_{max}

$$V_{max} = k_2[E]_T, \qquad v_o = \frac{V_{max}[S]}{K_M + [S]}$$

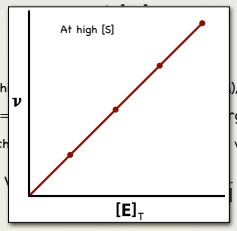
38-1

Enzyme Kinetics

- Substitution of this expression for [ES] into the one for v_o gives us the Michaelis-Menton equation:

At very h), ge)

- Which velocity, V_{max}



38-2

Enzyme Kinetics

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- Which is a constant equal to the maximum velocity, V_{max}

$$V_{max} = k_2[E]_T, \qquad v_o = \frac{V_{max}[S]}{K_M + [S]}$$

38-3

Enzyme Kinetics

k_{cat}

- Represented by the zero-order rate constant at high [S] ($k_{cat} = k_2$)
- It is determined from V_{max} ($k_{cat} = V_{max}/[E]_T$)
- Has units of frequency (1/sec, 1/min) and represents the number of catalytic cycles an enzyme can carry out per unit time when fully saturated with substrate.
- Also called the turnover number

39-1

Enzyme Kinetics

k_{cat}

- Repres… nstant at high…
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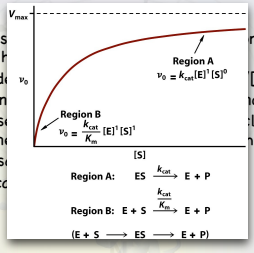

39-2

Enzyme Kinetics

k_{cat}

- Represen… constant at high [S…
- It is dete… ax/[E]$_T$)
- Has units… and represent… cycles an enzyme c… when fully satu…
- Also calle…

TABLE 5.1 Examples of catalytic constants

Enzyme	$k_{cat}(s^{-1})$*
Papain	10
Ribonuclease	10^2
Carboxypeptidase	10^2
Trypsin	10^2 (to 10^3)
Acetylcholinesterase	10^3
Kinases	10^3
Dehydrogenases	10^3
Transaminases	10^3
Carbonic anhydrase	10^6
Superoxide dismutase	10^6
Catalase	10^7

*The catalytic constants are given only as orders of magnitude.

39-3

Enzyme Kinetics

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- It is determined from V_{max} ($k_{cat} = V_{max}/[E]_T$)
- Has units of frequency (1/sec, 1/min) and represents the number of catalytic cycles an enzyme can carry out per unit time when fully saturated with substrate.
- Also called the turnover number

39-4

Enzyme Kinetics

K_M

- When $k_2 << k_{-1}$, K_M is equal to the dissociation constant for the enzyme-substrate complex

$$ES \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} E + S \qquad K_d = \frac{k_{-1}}{k_1} \approx K_M$$

  - Small K_M indicates strong binding of the substrate to enzyme
  - Large K_M indicate weak binding of substrate to enzyme.

40

Enzyme Kinetics

·Combining k_{cat} and K_M

- k_{cat}/K_M is called the **catalytic efficiency**.
- It represents the second order rate constant at low [S]
- Higher values of k_{cat}/K_M indicate an enzyme that is both good at forming the ES complex, and then once formed, good at converting the bound substrate to product.

41-1

Enzyme Kinetics

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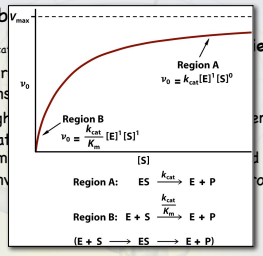

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41-2

Enzyme Kinetics

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41-3

Enzyme Kinetics

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  - It represents the second order rate constant at low [S]
  - Higher values of k_{cat}/K_M indicate an enzyme that is both good at forming the ES complex, and then once formed, good at converting the bound substrate to product.

41-4

Enzyme Kinetics

k_{cat}/K_M – **catalytic efficiency**

- It is used to assess **catalytic perfection**
  - When $k_{cat}/K_M > 10^8$ $s^{-1}M^{-1}$ it says that the reaction is diffusion-rate limited
  - This means that there are no changes that can be made to the enzyme to make the reaction go any faster.

42-1

Enzyme Kinetics

k_{cat}/K_M – **catalytic efficiency**

- It is
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TABLE 5.2 Catalytic efficiencies of some enzymes

	Enzymatic rate constant (k_{cat}/K_m in $M^{-1}s^{-1}$)
Carbonic anhydrase	7×10^6
Chymotrypsin	9×10^7
Chorismate mutase	2×10^6
Triose phosphate isomerase	4×10^8
Cytidine deaminase	3×10^6
Adenosine deaminase	10^7
Mandelate racemase	10^8
β-Amylase	10^7
Fumarase	10^8
Arginine decarboxylase	10^6
Alkaline phosphatase	3×10^7
Orotidine 5′-phosphate decarboxylase	6×10^7

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42-2

Enzyme Kinetics

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  - This means that there are no changes that can be made to the enzyme to make the reaction go any faster.

42-3

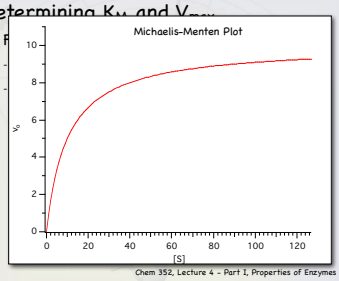
Enzyme Kinetics

•Determining K_M and V_{max}.

- From the v_o versus [S] plot
  - V_{max} is the maximum v_o at high [S]
  - K_M is the [S] value when v_o is at the half maximum, $v_o = V_{max}/2$

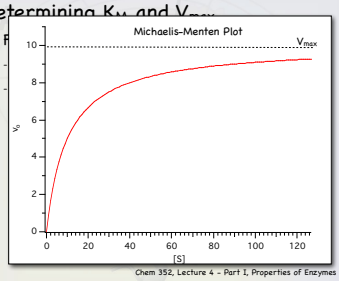
43-1

Enzyme Kinetics



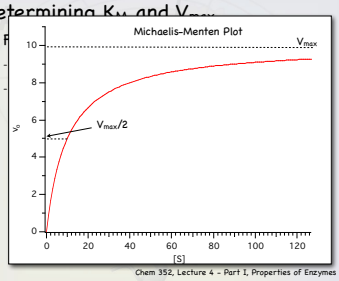
43-2

Enzyme Kinetics



43-3

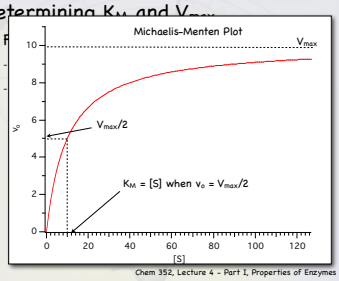
Enzyme Kinetics



43-4

Enzyme Kinetics

·Determining K_M and V_{max}.



43-5

Enzyme Kinetics

Problem:

Initial velocities have been measured for the reaction of α-chymotrypsin with tyrosine benzyl ester [S] at six different substrate concentration. Use the data below to make a reasonable estimate of the V_{max} and K_M for this substrate.

[S] {mM}	v_o {mM/min}
0.008	40
0.01	45
0.04	75
0.1	87
2	90
10	95

44

Enzyme Kinetics

·Determining K_M and V_{max}.

- From the double-reciprocal plot (Lineweaver-Burk plot)
- Taking the reciprocal of the Michaelis-Menten equation and plotting $1/v_o$ versus $1/[S]$ produces a straight line

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

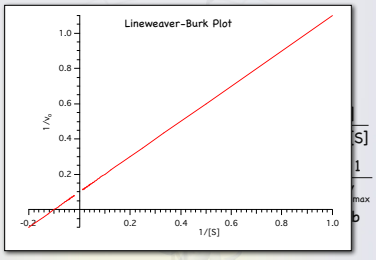
$$= \frac{K_M}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

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

$$y = m \bullet x + b$$

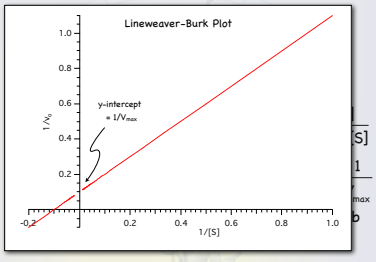
45-1

Enzyme Kinetics



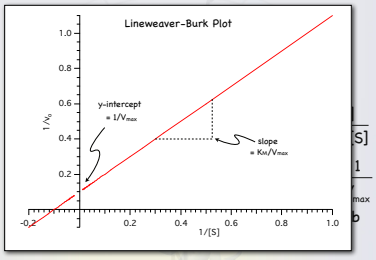
45-2

Enzyme Kinetics



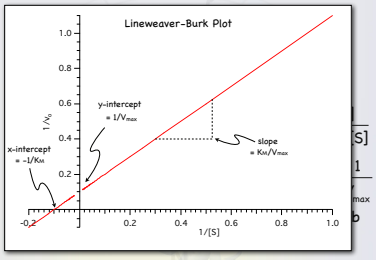
45-3

Enzyme Kinetics



45-4

Enzyme Kinetics



45-5

Enzyme Kinetics

Summary:

V_{max}

- It is the velocity observed when an enzyme is fully saturated with substrate at high [S]
- Is the maximum velocity in the Michaelis-Menten plot.
- It can be used to determine k_{cat}.
- It can be determined from the y-intercept in a Lineweaver-Burk plot ($y\text{-intercept} = 1/V_{max}$).

46

Enzyme Kinetics

Summary:

K_M

- It is a measure of how strongly an enzyme is able to bind to the substrate.
- It is equal to the substrate concentration that produces a half-maximum velocity ($v_o = V_{max}/2$) in the Michaelis-Menten plot.
- It can be determined from either the slope/ y-intercept, or from the x-intercept of a Lineweaver-Burk plot($x\text{-intercept} = -1/K_M$).

47

Enzyme Kinetics

Summary:

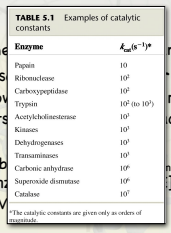
k_{cat}

- It is the catalytic rate constant (k_2).
- It is also called the **turnover number** and tells how often each enzyme molecule converts a substrate to product per unit of time.
- It can be determined from V_{max} and the total enzyme concentration $[E]_T$, ($k_{cat} = V_{max}/[E]_T$).

48-1

Enzyme Kinetics

Summary:



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Trypsin	10^2 (to 10^3)
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Kinases	10^3
Dehydrogenases	10^3
Transaminases	10^3
Carbonic anhydrase	10^6
Superoxide dismutase	10^6
Catalase	10^7

*The catalytic constants are given only as orders of magnitude.

- It is the nt (k_2).
- It is als **number** and tells ho molecule converts ct per unit of time.
- It can b ax and the total en $]_T$, (k_{cat} = V

48-2

Enzyme Kinetics

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- It is also called the **turnover number** and tells how often each enzyme molecule converts a substrate to product per unit of time.
- It can be determined from V_{max} and the total enzyme concentration $[E]_T$, ($k_{cat} = V_{max}/[E]_T$).

48-3

Enzyme Kinetics

Summary:

k_{cat}/K_M

- It is a measure of the catalytic efficiency for an enzyme and incorporates both how readily an enzyme binds its substrate to form the enzyme-substrate complex ($1/K_M$), and once formed, how readily it converts it to product (k_{cat}).
- It is used to assess **catalytic perfection**
  - $k_{cat}/K_M > 10^8$ $s^{-1}M^{-1}$ (The theoretical, diffusion rate limit.)

49-1

Enzyme Kinetics

Summary:

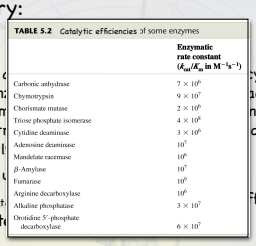

TABLE 5.2 Catalytic efficiencies of some enzymes

	Enzymatic rate constant (k_{cat}/K_m in $M^{-1}s^{-1}$)
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Chymotrypsin	9×10^7
Chorismate mutase	2×10^6
Triose phosphate isomerase	4×10^8
Cytidine deaminase	3×10^6
Adenosine deaminase	10^7
Mandelate racemase	10^6
β-Amylase	10^7
Fumarase	10^9
Arginine decarboxylase	10^6
Alkaline phosphatase	3×10^7
Orotidine 5′-phosphate decarboxylase	6×10^7

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49-2

Enzyme Kinetics

Summary:

k_{cat}/K_M

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- It is used to assess **catalytic perfection**
  - $k_{cat}/K_M > 10^8$ $s^{-1}M^{-1}$ (The theoretical, diffusion rate limit.)

49-3

Enzyme Kinetics

Summary:

$(k_{cat}/K_M)/k_n$

- Is called the **catalytic proficiency** and is a measure of how much an enzyme increases the rate of a reaction over its non-catalyzed rate (k_n).

50-1

Enzyme Kinetics

Summary:

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TABLE 5.2 Catalytic proficiencies of some enzymes

	Nonenzymatic rate constant (k_n in s^{-1})	Enzymatic rate constant (k_{cat}/K_m in $M^{-1}s^{-1}$)	Catalytic proficiency
Carbonic anhydrase	10^{-1}	7×10^6	7×10^7
Chymotrypsin	4×10^{-9}	9×10^7	2×10^{16}
Chorismate mutase	10^{-5}	2×10^6	2×10^{11}
Triose phosphate isomerase	4×10^{-6}	4×10^8	10^{14}
Cytidine deaminase	10^{-10}	3×10^6	3×10^{16}
Adenosine deaminase	2×10^{-10}	10^7	5×10^{16}
Mandelate racemase	3×10^{-13}	10^6	3×10^{18}
β-Amylase	7×10^{-14}	10^7	10^{20}
Fumarase	10^{-13}	10^9	10^{22}
Arginine decarboxylase	9×10^{-16}	10^6	10^{21}
Alkaline phosphatase	10^{-15}	3×10^7	3×10^{22}
Orotidine 5′-phosphate decarboxylase	3×10^{-16}	6×10^7	2×10^{23}

50-2

Enzyme Kinetics

Summary:

$(k_{cat}/K_M)/k_n$

- Is called the **catalytic proficiency** and is a measure of how much an enzyme increases the rate of a reaction over its non-catalyzed rate (k_n).

50-3

Enzyme Kinetics

Problem:

Initial velocities have been measured for the reaction of α-chymotrypsin with tyrosine benzyl ester [S] at six different substrate concentration. Use the data below to make a reasonable estimate of the V_{max} and K_M for this substrate.

[S] {mM}	v_o {mM/min}
0.008	40
0.01	45
0.04	75
0.1	87
2	90
10	95

51

Enzyme Kinetics

Problem:

Given the enzyme concentration used in this experiment was 72μM,

A. What is the turnover number for chymotrypsin when it is fully saturated with the substrate?
B. Is chymotrypsin, under the conditions used in this experiment, displaying catalytic perfection?

52

Enzyme Kinetics

Enzyme Inhibition

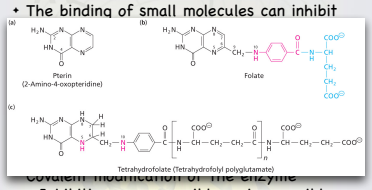
- The binding of small molecules can inhibit enzyme activity
  - Metabolites
    - Can be part of normal metabolic regulation.
    - Allosteric inhibition
  - Drugs
  - Toxins
- Covalent modification of the enzyme
  - Inhibition can reversible or irreversible

53-1

Enzyme Kinetics

Enzyme Inhibition

- The binding of small molecules can inhibit



- Inhibition can reversible or irreversible

53-2

Enzyme Kinetics

Enzyme Inhibition

- The binding of small molecules can inhibit

- Inhibition can reversible or irreversible

53-3

Enzyme Kinetics

Enzyme Inhibition

- The binding of small molecules can inhibit

- Inhibition can reversible or irreversible

53-4

Enzyme Kinetics

Enzyme Inhibition

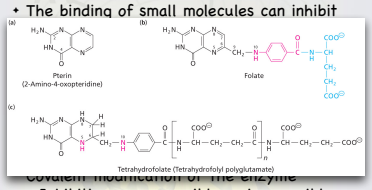
- The binding of small molecules can inhibit

53-5

Enzyme Kinetics

Enzyme Inhibition

- The binding of small molecules can inhibit



  - Inhibition can reversible or irreversible

53-6

Enzyme Kinetics

Enzyme Inhibition

- The binding of small molecules can inhibit enzyme activity
  - Metabolites
    - Can be part of normal metabolic regulation.
    - Allosteric inhibition
  - Drugs
  - Toxins
- Covalent modification of the enzyme
  - Inhibition can reversible or irreversible

53-7

Enzyme Kinetics

Reversible Enzyme Inhibition

- Competitive inhibition
- Uncompetitive inhibition
- Noncompetitive inhibition

$$E + I \underset{}{\overset{1/K_i}{\rightleftharpoons}} EI \quad \text{or} \quad ES + I \underset{}{\overset{1/K_i}{\rightleftharpoons}} ESI$$

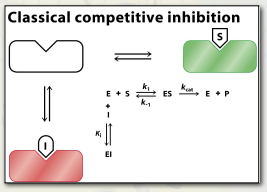
$$K_i = K_{ii} = \frac{[E][I]}{[EI]} \text{ or } \frac{[ES][I]}{[ESI]}$$

54

Enzyme Kinetics

Reversible Enzyme Inhibition

- Competitive inhibition
  - Mode used in drug design.

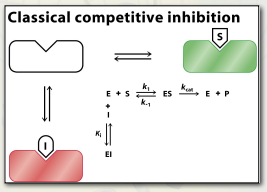


55-1

Enzyme Kinetics

Reversible Enzyme Inhibition

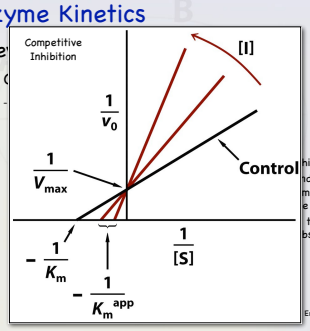
- Competitive inhibition
  - Mode used in drug design.



Inhibitor binds to the same site on the enzyme as the substrate

55-2

Enzyme Kinetics

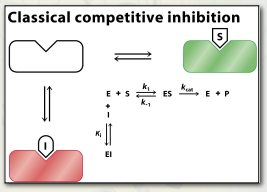


Inhibitor binds to the same site on the enzyme as the substrate

55-3

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Competitive inhibition
    - Mode used in drug design.

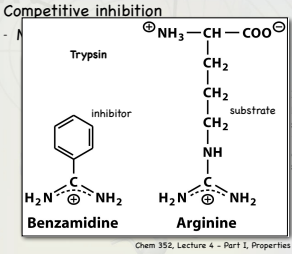


Inhibitor binds to the same site on the enzyme as the substrate

55-4

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Competitive inhibition
    - Mode used in drug design.

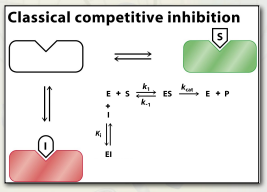


Inhibitor binds to the same site on the enzyme as the substrate

55-5

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Competitive inhibition
    - Mode used in drug design.

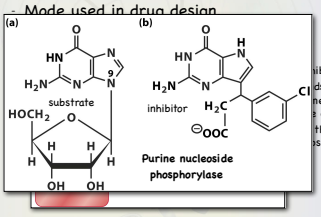


Inhibitor binds to the same site on the enzyme as the substrate

55-6

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Competitive inhibition
    - Mode used in drug design.



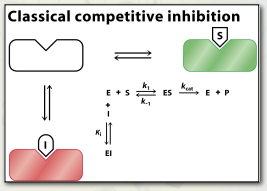
Inhibitor binds to the same site on the enzyme as the substrate

55-7

Enzyme Kinetics

·Reversible Enzyme Inhibition

- Competitive inhibition
  - Mode used in drug design.



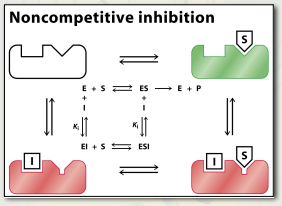
Inhibitor binds to the same site on the enzyme as the substrate

55-8

Enzyme Kinetics

·Reversible Enzyme Inhibition

- Noncompetitive inhibition
  - Mode used in allosteric inhibitioin

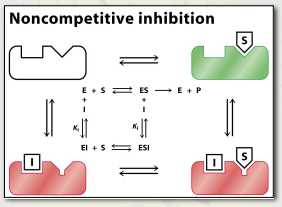


56-1

Enzyme Kinetics

·Reversible Enzyme Inhibition

- Noncompetitive inhibition
  - Mode used in allosteric inhibitioin



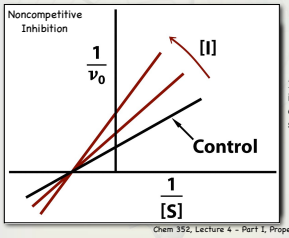
Inhibitor binds independently of the substrate

56-2

Enzyme Kinetics

·Reversible Enzyme Inhibition

- Noncompetitive inhibition



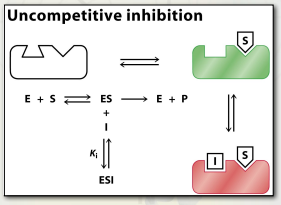
Inhibitor binds independently of the substrate

56-3

Enzyme Kinetics

·Reversible Enzyme Inhibition

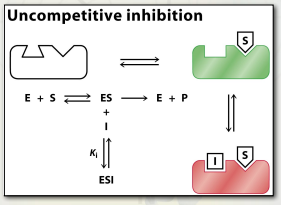
- Uncompetitive inhibition



57-1

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Uncompetitive inhibition

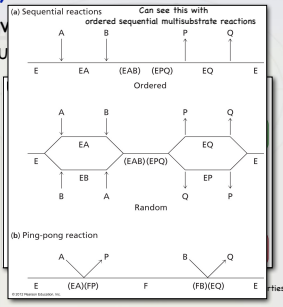


Inhibitor can only bind to the enzyme-subtrate complex

57-2

Enzyme Kinetics

- Rev
  - U

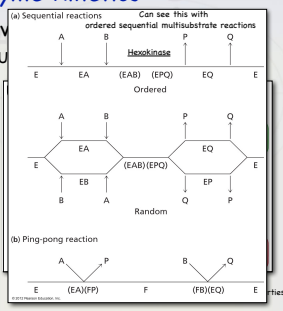


Inhibitor can only bind to the enzyme-subtrate complex

57-3

Enzyme Kinetics

- Rev
  - U

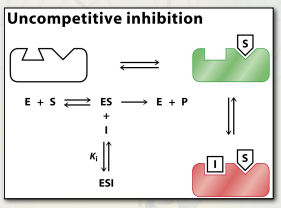


Inhibitor can only bind to the enzyme-subtrate complex

57-4

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Uncompetitive inhibition

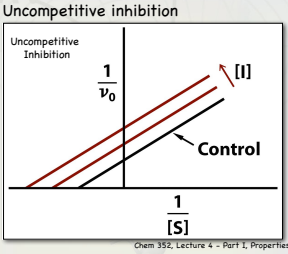


Inhibitor can only bind to the enzyme-subtrate complex

57-5

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Uncompetitive inhibition

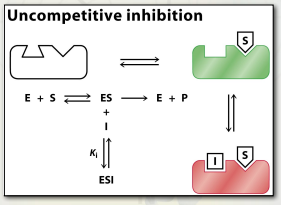


Inhibitor can only bind to the enzyme-subtrate complex

57-6

Enzyme Kinetics

- Reversible Enzyme Inhibition
  - Uncompetitive inhibition

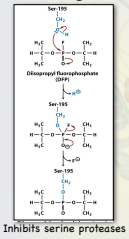


Inhibitor can only bind to the enzyme–subtrate complex

57-7

Enzyme Kinetics

- Irreversible Enzyme Inhibition through covalent modification.

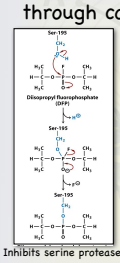


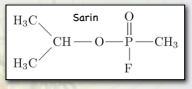
Inhibits serine proteases

58-1

Enzyme Kinetics

- Irreversible Enzyme Inhibition through covalent modification.





Inhibits serine proteases

58-2

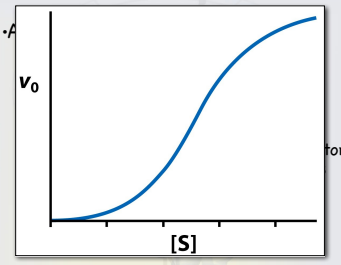
Enzyme Kinetics

- Allosteric Regulation
  - Usually catalyze key control points in a metabolic pathway.
  - Allosteric enzymes usually display cooperative substrate binding.
  - Allosteric enzyme have a second regulatory binding site for inhibitors and activators
    - Noncompeptive binding
    - Phosphofructokinase provides a good example

59-1

Enzyme Kinetics

- A



tory

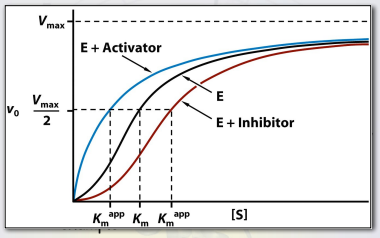
59-2

Enzyme Kinetics

- Allosteric Regulation
  - Usually catalyze key control points in a metabolic pathway.
  - Allosteric enzymes usually display cooperative substrate binding.
  - Allosteric enzyme have a second regulatory binding site for inhibitors and activators
    - Noncompeptive binding
    - Phosphofructokinase provides a good example

59-3

Enzyme Kinetics



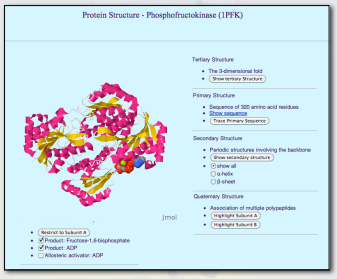
59-4

Enzyme Kinetics

- Allosteric Regulation
  - Usually catalyze key control points in a metabolic pathway.
  - Allosteric enzymes usually display cooperative substrate binding.
  - Allosteric enzyme have a second regulatory binding site for inhibitors and activators
    - Noncompeptive binding
    - Phosphofructokinase provides a good example

59-5

Enzyme Kinetics



60

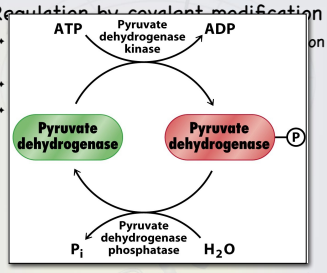
Enzyme Kinetics

- Regulation by covalent modification
  - Provides a longer term level of regulation than allosteric regulation.
  - Is reversible
  - Is often linked to hormonal control of metabolism

61-1

Enzyme Kinetics

•Regulation by covalent modification



61-2

Enzyme Kinetics

•Regulation by covalent modification

- Provides a longer term level of regulation than allosteric regulation.
- Is reversible
- Is often linked to hormonal control of metabolism

61-3

Problem:

The cytochrome P_{450} family of monooxygenases enzymes are involved in the clearance of foreign compounds (including drugs) from our body. A member of this family, P_{450}-3A4, is known to metabolize medazolam, a sedative, to a hydroxylated product. The kinetic data give below are for this reaction.

[Midazolam] {μM}	v_0 {pmol L^{-1} min^{-1}}	v_0 with 0.1 μM ketoconazole {pmol L^{-1} min^{-1}}
1	100	11
2	156	18
3	222	27
4	323	33

A. Determine the K_M and V_{max} for the uninhibited enzyme using a Lineweaver-Burk plot.

B. Ketoconazole, an antifungal, is known to cause adverse drug-drug interactions when administered with midazolam. Using the data in the table, determine the type of inhibition that ketoconazole exerts on the P_{450}-catalyzed hydroxylation of midazolam

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Next Up

•Lecture 4, Part II

- Reaction Mechanisms for enzyme catalyzed reactions (Chapter 6)

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