Chem 352 - Lecture 6
Enzymes: Biological Catalysts

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

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Outline

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8.1 Enzymes as Biological Catalysts

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Enzymes as Catalysts

Enzymes are biological catalysts

catalyst |'katl-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)

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Enzymes as Catalysts

Enzyme are biological catalysts

- Nearly every reaction in a living cell is catalyzed by an enzyme.
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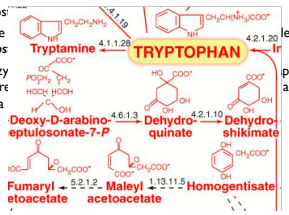

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Enzymes as Catalysts

Enzymes can exhibit:

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

Question:

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

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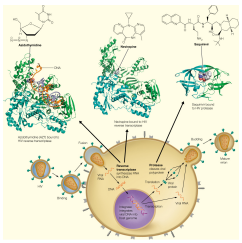
Enzymes as Catalysts

Enzymes are often the target for drug therapies.



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Enzymes as Catalysts

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
- A case study: Serine Proteases

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8.1 The Diversity of Enzyme Function

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Enzyme Classes and Nomenclature

Common names use the suffix ***-ase***

- The common name often describes the substrate as well as the reaction that the enzyme catalyzes.
- For example:
  - ***pyruvate kinase*** - catalyze the transfer of a phosphate group (kinase) from/to pyruvate and ATP
  - ***alcohol dehydrogenase*** - removes an equivalent of a hydrogen molecule, H_2, from an alcohol such as ethanol.

nomenclature |ˈnōmənˌklā ch ər| |ˈnoomənˈkleɪtʃ(ə)rəl| |-ˈklatʃ(ə)r(ə)l| |-kləˈtʃoər(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 Classes and Nomenclature

There is also a systematic nomenclature that names of enzymes using numbers

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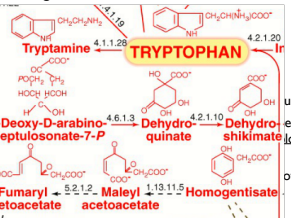

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Enzyme Classes and Nomenclature

TABLE 6.1 Examples of each of the major classes of enzymes

Class	Example (reaction type)	Reaction Catalyzed
1. Oxidoreductases	Alcohol dehydrogenase (oxidation with NAD^+)	Ethanol → Acetaldehyde
2. Transferases	Hexokinase (phosphorylation)	α-D-Glucose → α-D-Glucose-6-phosphate
3. Hydrolases	Carboxypeptidase A (peptide bond cleavage)	C-terminus of polypeptide → Shortened polypeptide + C-terminal residue
4. Lyases	Pyruvate decarboxylase (decarboxylation)	Pyruvate → Acetaldehyde
5. Isomerases	Maleate isomerase (cis–trans isomerization)	Maleate → Fumarate
6. Ligases	Pyruvate carboxylase (carboxylation)	Pyruvate → Oxaloacetate

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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 H2 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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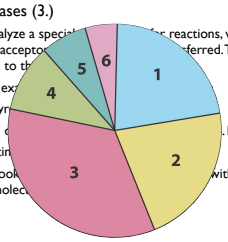
Enzyme Classes

Hydrolases (3.)

- Catalyze a specia… for reactions, where water is the acceptor… ferred. The reactions lead to th…
- For ex…
  - pyr…
    - …1.1.1)
- Distin…
  - Look… with another molec…



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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 bond

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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 Pi
- 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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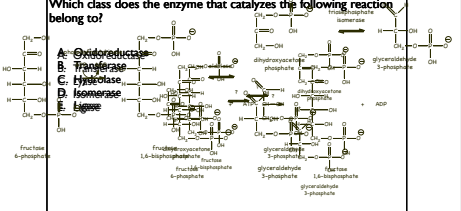
Enzyme Classes

Question:

Which class does the enzyme that catalyzes the following reaction belong to?

A. Oxidoreductase
B. Transferase
C. Hydrolase
D. Isomerase
E. Ligase



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8.3 Chemical Reaction Rates and Effects of Catalysts

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Reaction Rates and Catalysts

Observing the kinetics of a reaction can reveal details about the mechanism of the reaction

Starting with the kinetics of non-catalyzed reactions

- The rate, or velocity, v, of a reaction, is monitored by observing the change in the concentration of either the substrate (reactant) or the product of the reaction.

$$A \rightarrow B$$

$$Rate = v = \frac{\text{change in concentration of A or B}}{\text{change in time}}$$

$$v = \frac{d\text{B}}{dt} = -\frac{d\text{A}}{dt}$$

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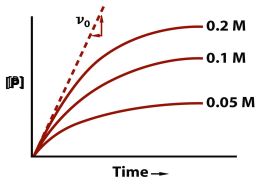
Reaction Rates and Catalysts

Kinetics of non-catalyzed reactions

- Typically it is the substrate dependence of the initial rate, v_o, that is determined.

$$v_o = \frac{d[\text{B}]}{dt} \text{ at } t = 0$$



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Reaction Rates and Catalysts

The rate equation for this relationship can take on different forms with respect to the substrate or product concentrations, for example:

$$v = \frac{d[\text{B}]}{dt} = -\frac{d[\text{A}]}{dt} = k_1[\text{A}]^n$$

- where k_1 is the rate constant
- and n is the order of the reaction
  - n = 1, first order,
  - n = 2, second order
  - n = 0, zero order

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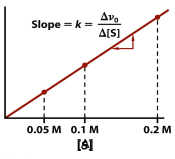
Reaction Rates and Catalysts

Kinetics of non-catalyzed reactions

- For first-order reactions, v_o is proportional to [A].

$$v_o = k_1[\text{A}]$$



- In this case, k_1 is called the first order rate constant and has dimension of s^{-1}.

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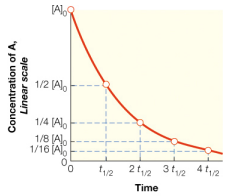
Reaction Rates and Catalysts

Kinetics of non-catalyzed reactions

- Deriving an expression that gives [A] as function of time for a first order reaction.



$$\ln([A]_t) - \ln([A]_o) = -k_1 t$$

$$\ln\left(\frac{[A]_t}{[A]_o}\right) = -k_1 t$$

$$[A]_t = [A]_o e^{-k_1 t}$$

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Reaction Rates and Catalysts

Kinetics of non-catalyzed reactions

- For a second order reaction, v_o has a quadratic dependence on [A].

$$A + A \rightarrow C$$
$$v_o = k_1[A]^2$$

or

$$A + B \rightarrow C$$
$$v_o = k_1[A][B]$$

  - v_o is the second order rate constant and has units of $M^{-1}s^{-1}$.
- For a zero order reaction, v_o is independent of [A]
  - $v_o = k_1$

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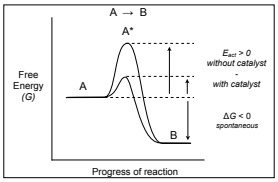
Reaction Rates and Catalysts

Catalysts speed up reactions by lowering the free energy of the transition state.



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8.4 How Enzymes Act as Catalysts

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Catalysts Speed Up Reactions

We will focus on two ways that enzyme catalysts do this.

- Chemical Mode: The enzyme provides **chemical catalysts**
- Binding Mode: The **binding of substrates** and **transition state intermediates** lowers the entropy for the reaction and helps to stabilizes the transition states.

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Chemical Modes of Enzymatic Catalysis

Functional groups present at the active site of an enzyme can play a catalytic role.

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Chemical Modes of Enzymatic Catalysis

The most common catalytic groups come from the polar amino acid side chains, which are embedded in a non-polar environment of the active site

TABLE 6.2 Typical p*K*a values of ionizable groups of amino acids in proteins

Group	p*K*a
Terminal α-carboxyl	3–4
Side-chain carboxyl	4–5
Imidazole	6–7
Terminal α-amino	7.5–9
Thiol	8–9.5
Phenol	9.5–10
ε-Amino	~10
Guanidine	~12
Hydroxymethyl	~16

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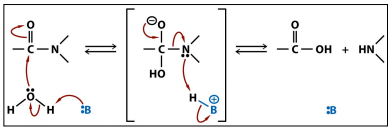
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Example: General base catalysis can assist in the cleavage of a peptide bond.



General acid-base catalysis involves a molecule besides water that acts as a proton donor or acceptor during the enzymatic reaction.

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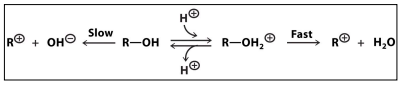
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Example: General acid catalysis can assist in a dehydration reaction.
- OH_2 makes a better leaving group than OH^-



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Chemical M ic Catalysis

Acid/Base catalys

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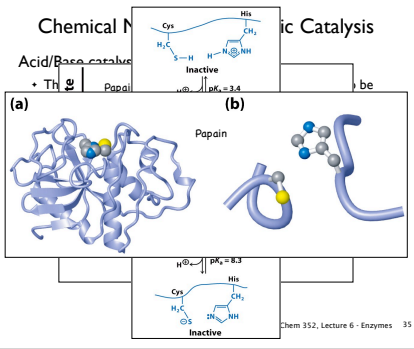

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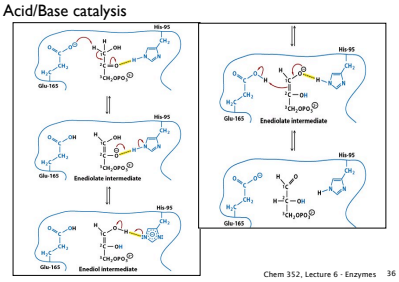
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis



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Chemical Modes of Enzymatic Catalysis

Covalent bond catalysis

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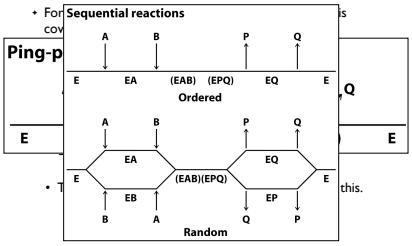

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Chemical Modes of Enzymatic Catalysis

- Acid/Base catalysis and covalent bond catalysis can account for approximately 10 to 100 fold increases in the reaction rates
  - However, 10^8 fold increases are observed
- The Binding Modes of Enzyme Catalysis can account for this difference.
  - Enzymes can bind substrates and orient them relative to one another and to catalytic groups on the enzyme.
  - They can also help stabilize the transition state.

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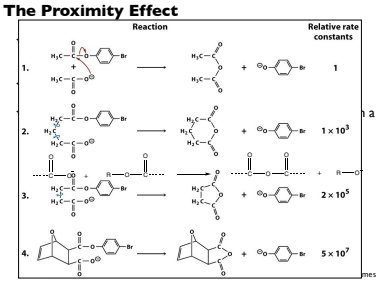
Binding Modes of Enzymatic

The Proximity Effect



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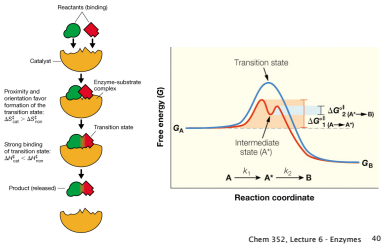
Binding Modes of Enzymatic Catalysis

- The favorable binding of the transition state helps to lower the activation barrier and, therefore, speed up a reaction.



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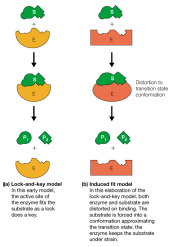
Binding Modes of Enzymatic Catalysis

"Lock and Key" vs "Induced Fit" models

- In the late 1890's Emil Fischer, with his "lock and key" model, predicted what we know now to be the contribution of substrate binding to enzyme catalysis.
- In the 1958, Daniel Koshland proposed an alternative "induced fit" model, which envisages the enzyme distorting the shape of the substrate to more closely resemble the transitions state.



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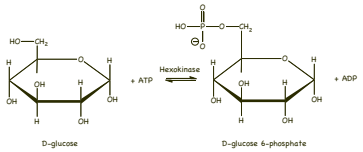
Binding Modes of Enzymatic Catalysis

"Induced fit" model

- In the "induced fit" model, substrate binding induces conformational changes in the enzyme.
- Hexokinase provides a good example of "induced fit"



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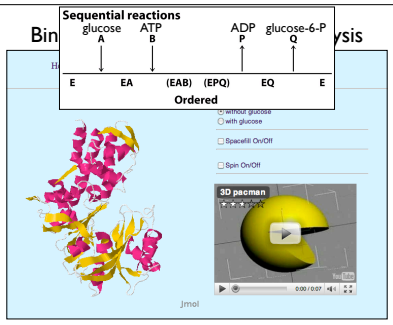
Bin…ysis



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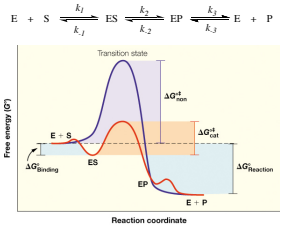
Binding Modes of Enzymatic Catalysis

Flattening the Transition State



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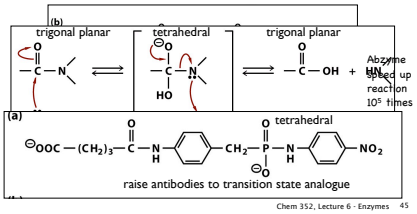
Binding Modes of Enzymatic Catalysis

Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.



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Serine Proteases - A Case Study

Case studies of enzyme catalyzed reactions presented in the textbook:

- **Lysozyme**
  - Cleaves the polysaccharide found in bacterial cell walls.
- **Chymotrypsin**
  - A Serine protease that cleaves the polypeptide backbone during protein digestion.

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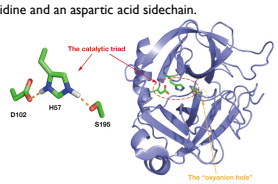
Serine Proteases - A Case Study

Serine proteases are a group of enzymes that cleave peptide bonds.

- There are many different serine proteases
- All contain a serine side chain in their active site, along with a histidine and an aspartic acid sidechain.



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Serine Proteases - A Case Study

Serine proteases nicely illustrate many of the tricks that can be used to speed up chemical reactions

- Catalytic modes of enzymatic catalysis
  - Acid/base catalysis
  - Covalent catalysis
- Binding modes of enzymatic catalysis
  - Proximity effect
  - Transition state stabilization

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Serine Proteases - A Case Study

They also illustrate

- Importance of protein folding in creating a functional protein
- Substrate specificity
- Activation through irreversible covalent modifications

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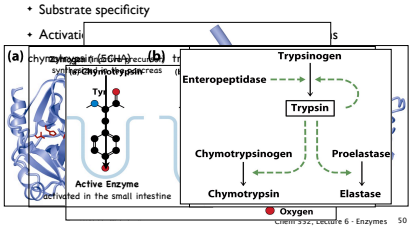
Serine Proteases - A Case Study

They also illustrate

- Importance of folding to creating a functional protein
- Substrate specificity
- Activati s



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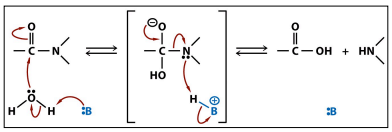
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

- The serine protease breaks the overall reaction down into two cycles with two transition state.



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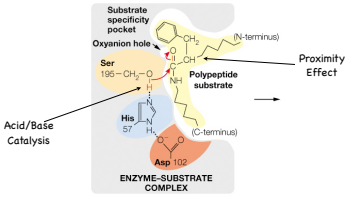
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



1 Polypeptide substrate binds noncovalently in the enzyme active site. The catalytic triad includes a reactive Ser nucleophile that attacks the electrophilic amide C atom.

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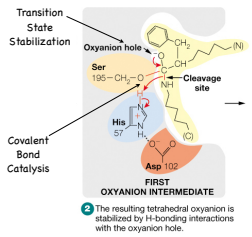
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



2 The resulting tetrahedral oxyanion is stabilized by H-bonding interactions with the oxyanion hole.

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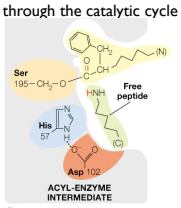
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



3 Collapse of the tetrahedral intermediate and H^+ transfer from His57 lead to cleavage of the C—N bond. The N-terminal peptide is bound through acyl linkage to serine.

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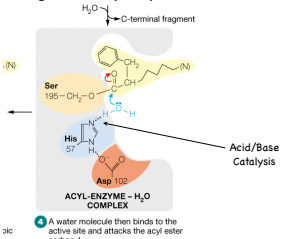
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



4 A water molecule then binds to the active site and attacks the acyl ester carbonyl.

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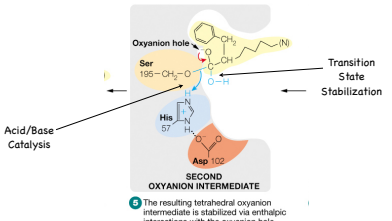
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



5 The resulting tetrahedral oxyanion intermediate is stabilized via enthalpic interactions with the oxyanion hole.

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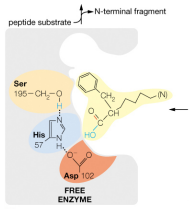
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



6 The second peptide fragment is released, and the enzyme returns to its initial state.

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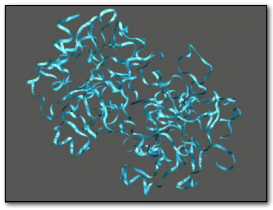
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle



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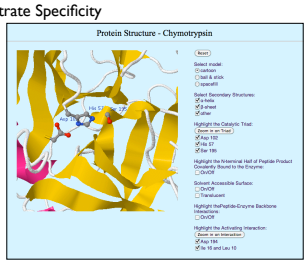
Serine Proteases - A Case Study

Substrate Specificity



59

8.5 Coenzymes, Vitamins, and Essential Metals

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Coenzymes

Enzyme cofactors are non-protein components of enzymes that contributes to an enzyme's activity.

They include,

- Metal ions
- Coenzymes that are organic molecules
  - Many of which are derived from vitamins

TABLE 8.3 Some important coenzymes and related vitamins

Vitamin	Coenzyme	Reactions involving the coenzyme	Section in which coenzyme is introduced
Thiamine (vitamin B_1)	Thiamine pyrophosphate	Activation and transfer of aldehydes	Section 12.3
Riboflavin (vitamin B_2)	Flavin mononucleotide; flavin adenine dinucleotide	Oxidation-reduction	Section 13.2
Niacin (vitamin B_3)	Nicotinamide adenine dinucleotide; nicotinamide adenine dinucleotide phosphate	Oxidation-reduction	Section 11.3
Pantothenic acid (vitamin B_5)	Coenzyme A	Acyl group activation and transfer	Section 13.2
Pyridoxine (vitamin B_6)	Pyridoxal phosphate	Various reactions involving amino acid activation	Section 18.4
Biotin (vitamin B_7)	Biotin	CO_2 activation and transfer	Section 12.5
Lipoic acid	Lipoamide	Acyl group activation; oxidation-reduction	Section 13.2
Folic acid (vitamin B_9)	Tetrahydrofolate	Activation and transfer of single-carbon functional groups	Section 18.4
Vitamin B_{12}	Adenosyl cobalamin; methyl cobalamin	Isomerizations and methyl group transfers	Section 18.4

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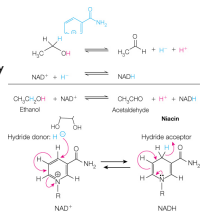
Coenzymes

Example: Nicotinamide Adenine Dinucleotide (NAD^+)

- It comprises two ribonucleotides
  - Adenosine
  - Nicotinamide
- They are joined at their 5' ends by a pyrophosphate bridge
- The nicotinamide base is derived from the B vitamin niacin
- NAD^+ serves in oxidation/ reduction reactions as the oxidation agent
  - For example, the oxidation of ethanol to acetaldehyde by the enzyme alcohol dehydrogenase.



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Metals as Enzyme Cofactors

Metal ions also play important roles in facilitating enzyme catalyzed reactions.

TABLE 8.4 Metals and trace elements important as enzymatic cofactors

Metal	Example of Enzyme	Role of Metal
Fe	Cytochrome oxidase	Oxidation-reduction
Cu	Ascorbic acid oxidase	Oxidation-reduction
Zn	Alcohol dehydrogenase	Helps bind NAD^+
Mn	Histidine ammonia lyase	Aids in catalysis by electron withdrawal
Co	Glutamate mutase	Co is part of cobalamin coenzyme
Ni	Urease	Catalytic site
Mo	Xanthine oxidase	Oxidation-reduction
V	Nitrate reductase	Oxidation-reduction
Se	Glutathione peroxidase	Replaces S in one cysteine in active site
Mg	Many kinases	Helps bind ATP

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8.6 The Kinetics of Enzyme Catalysis

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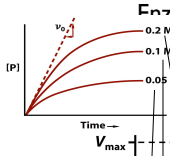
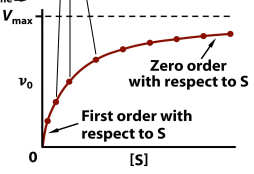
Enzyme Kinetics



me catalyzed reaction looks
ed reactions with respect to its
trate concentration, [S].
ecomes zero-ordered at high substrate



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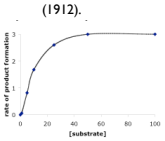
Enzyme Kinetics

A Model for kinetics of enzyme-catalyzed reactions was worked out by Maud Menten and Leonor Michaelis

- Michaelis-Menten model for enzyme catalyzed reactions (1912).



Maud Menten (1879–1960)

Leonor Michaelis (1875–1949)

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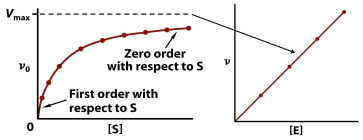
Enzyme Kinetics

They saw for enzyme-catalyzed reactions

- A first-order enzyme concentration, [E], dependence at high substrate concentrations [S], where where kinetics is zero-order with respect to [S].



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Enzyme Kinetics

- Based on these observations, they proposed the following mechanism for an enzyme catalyzed reaction:

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

- The overall rate of the reaction is therefore determined by the conversion of enzyme-substrate complex, ES, to product:
- Also, by looking at only the initial rate, v_o, the k_{-2} back reaction can be ignored, because [P] = 0.
- The overall rate will then depend on the step going from [ES] to [E] + [P]

$$v_o = k_2[ES]$$

- Michaelis and Menten next came up with an expression for [ES] as a function of [S] to substitute into this expression to come up with one for v_o as a function of [S].

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Enzyme Kinetics

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

- The also 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: $[ES] = \dfrac{k_1[E][S]}{k_{-1} + k_2}$

69

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.

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

- The expression for [ES] now 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 unknown variable [E] needs to be eliminated as well.
  - [E] is the free enzyme concentration, which is equal to the total enzyme concentration, $[E]_T$, minus the enzyme-substrate concentration, [ES].

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

71

Enzyme Kinetics

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

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

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

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

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

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

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

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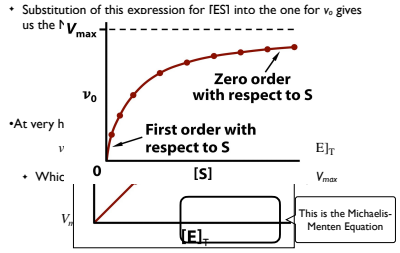
Enzyme Kinetics

- Substitution of this expression for [ES] into the one for v_o gives us the M



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73

Enzyme Kinetics

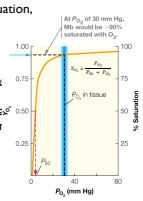
- The form of the Michaelis-Menton Equation,

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

$y = \frac{ax}{b + x}$, where a and b are

should look familiar to us, it is the equa
we saw with the O_2 binding curve for

$Y_{O_2} = \frac{P_{O_2}}{P_{50} + P_{O_2}}$, where Y_{O_2} is the fraction



- This should not surprise us since v_o is proportional to [ES], which is proportional to the fraction of enzyme that is bound with substrate.

74

Enzyme Kinetics

The const V_{max} is K_M, contain V_{max} and ie

k_{cat}

- V_{max} is u nstant, k_{cat}
- $k_{cat} = k_2$ at high substrat
  - It is d

- k_{cat} had nts the number arry out per unit tim
  - Also

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

Each catalase molecule can catalyze 10 million reactions per second!!

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

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Enzyme Kinetics

K_M

- K_M, the Michaelis Menton constant, can be used to gauge the enzyme's affinity for its substrate.

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

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

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

  - The assumption that $k_2 << k_{-1}$ is usually a reasonable one to make.
  - A small K_M indicates strong binding of the substrate to enzyme.
  - A large K_M indicate weak binding of substrate to enzyme.

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Enzyme Kinetics

k_{cat}/K_M

- k_{cat} and K_M can be combined to create a parameter called the ***catalytic efficiency* or *catalytic proficiency***.
- k_{cat}/K_M represents the second order rate constant at low [S]
- Higher value v_{max} hat is both good at forr e formed, good at convertin

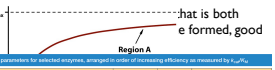

TABLE 8.5 Michaelis–Menten parameters for selected enzymes, arranged in order of increasing efficiency as measured by k_{cat}/K_M

Enzyme	Reaction Catalyzed	K_M (mol/L)	k_{cat} (s^{-1})	k_{cat}/K_M ((mol/L)$^{-1}$ s^{-1})
Chymotrypsin	$\xrightarrow{H_2O}$	1.5×10^{-2}	0.14	9.3
Pepsin	$\xrightarrow{H_2O}$	3×10^{-4}	0.5	1.7×10^3
Tyrosyl-tRNA synthetase	Tyrosine + tRNA ⟶ tyrosyl-tRNA	9×10^{-4}	7.6	8.4×10^3
Ribonuclease	$\xrightarrow{H_2O}$	7.9×10^{-3}	7.9×10^2	1.0×10^5
Carbonic anhydrase	$HCO_3^- + H^+ \longrightarrow H_2O + CO_2$	2.6×10^{-2}	4×10^5	1.5×10^7
Fumarase	$\xrightarrow{H_2O}$	5×10^{-6}	8×10^2	1.6×10^8

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Enzyme Kinetics

k_{cat}/K_M can be used to assess "catalytic perfection"

- When $k_{cat}/K_M \approx 10^8$ $M^{-1}s^{-1}$ it indicates 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.
  - The reaction rate is now dependent on how often a substrate molecule can migrate by diffusion into the active site of the enzyme.

TABLE 8.5 Michaelis–Menten parameters for selected enzymes, arranged in order of increasing efficiency as measured by k_{cat}/K_M

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Fumarase	$\xrightarrow{H_2O}$	5×10^{-6}	8×10^2	1.6×10^8

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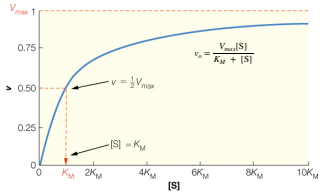
Enzyme Kinetics

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, $v_o = V_{max}/2$



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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}
0.015	5.43
0.030	10.3
0.050	16.1
0.10	27.4
0.25	47.9
0.40	59.4
0.60	68.1
0.80	73.5

Use Excel to plot these data

V_{max} = 96.9 mM/min
K_m = 0.25 mM

80

Enzyme Kinetics

Determining K_M and V_{max}.

- From the double-reciprocal plot (Lineweaver-Burke plot)
- Taking the reciprocal of the Michaelis-Menten equation and plotting $1/v_o$ versus $1/[S]$ produces a straight line,
  - where slope, $m = K_M/V_{max}$
  - and y-intercept, $b = 1/V_{max}$

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

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

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

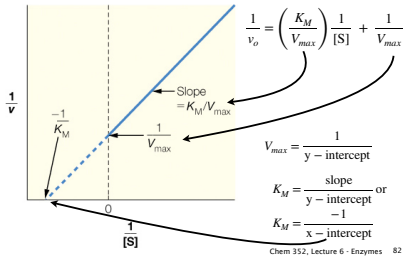
$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

$y = m \cdot x + b$

81

Enzyme Kinetics

Lineweaver-Burke Plot



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

$$V_{max} = \frac{1}{\text{y} - \text{intercept}}$$

$$K_M = \frac{\text{slope}}{\text{y} - \text{intercept}} \text{ or}$$

$$K_M = \frac{-1}{\text{x} - \text{intercept}}$$

82

Enzyme Kinetics

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0.050	16.1
0.10	27.4
0.25	47.9
0.40	59.4
0.60	68.1
0.80	73.5

Use Excel to re-plot these data using a Lineweaver-Burke Plot

83

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-intercept = $1/V_{max}$).

84

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-intercept = $-1/K_M$).

85

Enzyme Kinetics

Summary:

k_{cat}

- It is the cataly
- It is also calle nd tells how often each en bstrate to product per u
- It can be dete al enzyme concentration ($k_{cat} = V_{max}/$

TABLE 5.1 Examples of catalytic constants

Enzyme	$k_{cat}(s^{-1})$*
Pepsin	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.

86

Enzyme Kinetics

Summary:

k_{cat}/K_M

- It is a an enzyme binds rate comp y it conve
- It is u
  - k_{cat} on rate limi

TABLE 5.2 Catalytic proficiencies of some enzymes

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

87

Enzyme Kinetics

Summary:

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

- Is e of h over it

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}	10^9	3×10^{18}
β-Amylase	7×10^{-14}	10^7	10^{20}
Fumarase	10^{-15}	10^9	10^{21}
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}

88

Enzyme Kinetics

Summary:

Question:

Which of the following parameters that characterize an enzyme catalyzed reaction, tells you how many reactions that one enzyme molecule can process per second, on average?

A. K_M
B. k_{cat}
C. V_{max}
D. k_{cat}/K_M
E. v_o

89

Enzyme Kinetics

Summary:

Question:

Which tells you the initial velocity of the reaction when the enzyme is fully saturated with substrate?

A. K_M
B. k_{cat}
C. V_{max}
D. k_{cat}/K_M
E. v_o

90

Enzyme Kinetics

Summary:

Which tells you something about the affinity of the enzyme molecule for its substrate?

A. K_M
B. k_{cat}
C. V_{max}
D. k_{cat}/K_M
E. v_o

91

Enzyme Kinetics

Summary:

Which tells you whether the enzyme is catalytically perfect (and, what does that even mean?)

A. K_M
B. k_{cat}
C. V_{max}
D. k_{cat}/K_M
E. v_o

92

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}
0.015	5.43
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0.10	27.4
0.25	47.9
0.40	59.4
0.60	68.1
0.80	73.5

Use Excel to re-plot these data using a Lineweaver-Burke Plot

93

Enzyme Kinetics

Problem:

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

A. What is the ***turnover number*** in units of s^{-1} for chymotrypsin when it is fully saturated with the substrate?
B. Is chymotrypsin, under the conditions used in this experiment, displaying catalytic perfection?

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8.7 Enzyme Inhibition

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Enzyme Inhibition

Enzyme Inhibition and Activation

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

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Enzyme Inhibition

Reversible Enzyme Inhibition involves reversible binding of an inhibitor to the enzyme

- Competitive inhibition
  - Inhibitor binds only to the free enzyme, E
- Uncompetitive inhibition
  - Inhibitor binds only to the enzyme-substrate complex, ES
- Mixed inhibition
  - An inhibitor can bind to both the free enzyme, E, or the the enzyme/substrate complex, ES.
- The dissociation constant for this binding will then be,

$$E + I \underset{}{\overset{1/K_i}{\rightleftharpoons}} EI$$

$$ES + I \overset{1/K_i}{\rightleftharpoons} ESI$$

$$E + I \overset{1/K_i}{\rightleftharpoons} EI$$

$$ES + I \overset{1/K_i}{\rightleftharpoons} ESI$$

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

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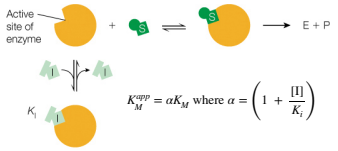
Enzyme Inhibition

Competitive inhibition

- The inhibitor binds to the same site as the substrate and increases the the apparent K_M.



$$K_M^{app} = \alpha K_M \text{ where } \alpha = \left(1 + \frac{[I]}{K_i}\right)$$

- Drugs are often designed to be competitive inhibitors.

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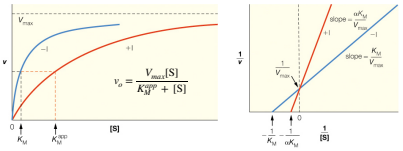
Enzyme Kinetics

Competitive Inhibition

- Has the following affects on the Michaelis-Menten and Lineweaver-Burke plots.



- High substrate concentrations can overcome the effects of the inhibitor

99

Enzyme Inhibition

Uncompetitive inhibition

- The inhibitor binds to a site that forms after the substrate binds.
  - Inhibitor binding decreases both the V_{max}, and K_M.

$$K_M^{app} = \frac{K_M}{\alpha'} \text{ and } V_{max}^{app} = \frac{V_{max}}{\alpha'}$$

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

$$+ \quad \Big\uparrow \downarrow K'_i$$

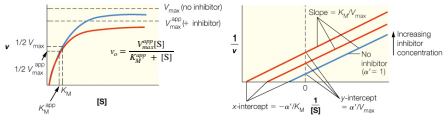
$$EIS \not\rightarrow \text{No reaction}$$

100

Enzyme Kinetics

Uncompetitive Inhibition

- Has the following affects on the Michaelis-Menten and Lineweaver-Burke plots.

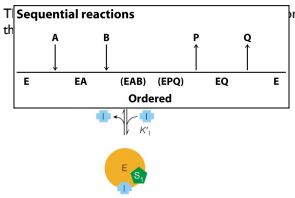


101

Enzyme Inhibition

Uncompetitive Inhibition

- Often the site where the inhibitor binds is a site where a second substrate would bind in a sequentially ordered reaction.
- T[illegible]r for th[illegible]



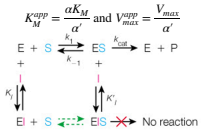
102

Enzyme Inhibition

Mixed inhibition

- The inhibitor can bind to both the free enzyme, E, and to the enzyme-substrate complex, ES.
  - Inhibitor binding decreases the V_{max},
  - But it has a more complex effect on K_M, where binding to E increases K_M, while binding to ES decreases K_M

$$K_M^{app} = \frac{\alpha K_M}{\alpha'} \text{ and } V_{max}^{app} = \frac{V_{max}}{\alpha'}$$

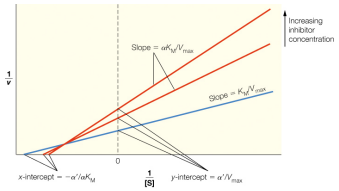


103

Enzyme Kinetics

Mixed Inhibition

- Has the following affects on the Lineweaver-Burke plot.

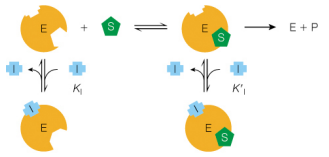


104

Enzyme Inhibition

Mixed Inhibition

- Often the site where the inhibitor binds is a site somewhere removed from the active site where the substrate binds.
  - This type of inhibition is often referred to as ***allosteric inhibition***.

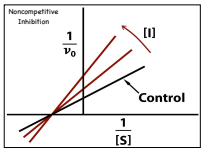


105

Enzyme Inhibition

Mixed Inhibition

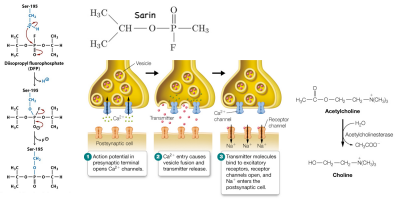
- In, the special case where the binding affinity of the inhibitor to both the free enzyme and the enzyme substrate complex are the same ($\alpha = \alpha'$), then there is no apparent effect on K_M.
- This is called ***noncompetitive inhibition*** and produces a Lineweaver-Burke plot that looks like this.



106

Enzyme Kinetics

Irreversible Enzyme Inhibition occurs through covalent modification, and its a strategy used in designing pesticides and nerve agents.



Inhibits serine proteases and acetylcholinesterase

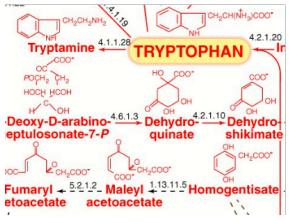
107

8.7 The Regulation of Enzyme Activity

108

Regulation of Enzyme Activity

***Concept**: Regulation of enzyme activity is essential for the efficient and order flow of metabolism.*



109

Regulation of Enzyme Activity

Substrate-Level Control

- When the substrate concentrations are low, [S] << K_M, then the rate of the reaction is first-order with respect to [S].
  - The rate of the reaction will dependent on the substrate concentration.

Product Inhibition

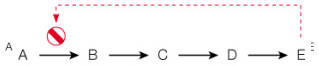
- If the concentration of the product of a reaction build up due to a slowdown in the flow of intermediates through a pathway, then the binding of the product to enzyme will inhibit the further production of that product.

110

Regulation of Enzyme Activity

Enzymes that lie at key points along a metabolic pathway can also be regulated by metabolites that are neither as substrate or product of that enzyme.

- For example, consider a pathway that leads to the synthesis of metabolite E.



- If, in addition to being the product of this pathway, if E also served as an inhibitor of Enzyme 1, then it could serve to shut down the pathway leading to its synthesis when its levels were high.
- This is type of regulation is called ***feedback inhibition***.

111

Regulation of Enzyme Activity

Enzymes that lie at key points along a metabolic pathway can also be regulated by metabolites that are neither as substrate or product of that enzyme.

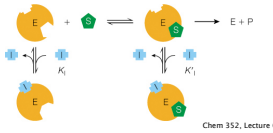
- There are other scenarios where the the buildup of a metabolite may signal the need to activate another pathway.
- The binding of metabolites to regulatory enzymes can also serve to activate them

112

Regulation of Enzyme Activity

Allosteric Enzymes

- Are enzymes that can be inhibited or activated by metabolites that are neither their substrates or products.
- Typically the binding of these allosteric effectors is at a site the is removed from the active site, and their binding leads to a conformational changes in the enzyme that affects its activity.
- This is what we saw with the mixed inhibitor model of reversible enzyme inhibition.

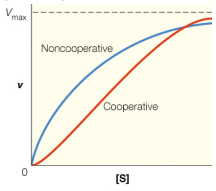


113

Regulation of Enzyme Activity

Allosteric Enzymes

- The enzymes typically have multiple subunits with multiple active sites, which display cooperative substrate binding, similar what we saw with O_2 binding to hemoglobin.
- The binding of substrate to one active site can increase the activity at another site.
  - This is called ***homoallostery.***
- As with Hb, this shifts the steepest part of the activity curve away from the y-axis.

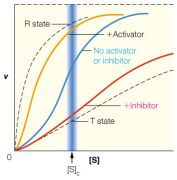


114

Regulation of Enzyme Activity

Allosteric Enzymes

- The binding of inhibitors and activators can then shift this curve to the right (inhibition) or left (activation).
- This is called ***heteroallostery***.

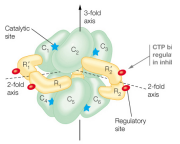


115

Regulation of Enzyme Activity

Allosteric Enzymes

- The enzyme, aspartate carbamoyltransferase (ATCase) is a classic example.
- ATCase is a multisubunit enzyme comprising 6 catalytic subunits and 6 regulatory subunits arranged as two catalytic trimers plus three regulatory dimers.

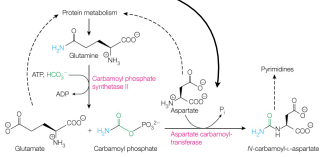


116

Regulation of Enzyme Activity

Allosteric Enzymes

- ATCase catalyses the first reaction in the pathway leading to the synthesis of the pyrimidine ribonucleotide, cytidine triphosphate (CTP)

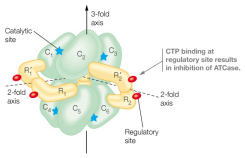


117

Regulation of Enzyme Activity

Allosteric Enzymes

- When CTP levels rise, it binds to the regulatory subunits and stabilize the tense (T) state and thereby inhibits the synthesis of additional pyrimidine bases.

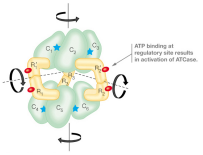


118

Regulation of Enzyme Activity

Allosteric Enzymes

- On the other hand, when purine nucleotide concentrations rise it signals an inadequate supply of pyrimidines for RNA synthesis, as a purine, ATP serves as an activator by bind to the regulatory subunits and switching to switch ATCase to its relaxed (R) state.

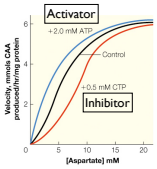


119

Regulation of Enzyme Activity

Allosteric Enzymes

- The regulatory effects of the CTP and ATP are reflected in the Michaelis-Menten plots for ATCase in the presence and absence of these effectors.

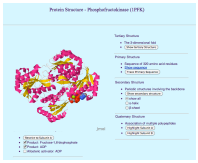


120

Enzyme Kinetics

Allosteric Enzymes

- The enzyme phosphofructokinase, PFK, which is found in the glycolytic pathway, is another good example.
- The enzyme phosphorylates fructose-6-phosphate to fructose-1,6-bisphosphate. Using ATP.
- ADP is a product of this reaction, but it is also an activator of PFK, because one of the overall purposes of the glycolytic pathway is to produce ATP from ADP and inorganic phosphate, P_i.
  - Hi ADP levels signals a cell's need for ATP.



121

8.8 Covalent Modification Used to Regulate Enzyme Activity

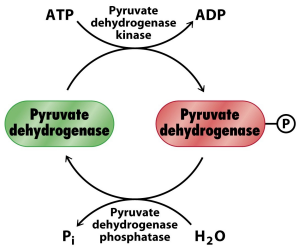
122

Regulation by Covalent Modifications

Covalent modifi
irreversible.

Reversible phos
of enzymes is an

- It provides a lo
of regulation th
regulation.
- Is often linked
control of met
systemic level.



123

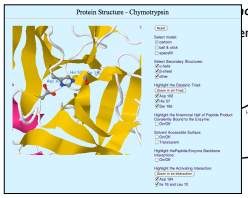
Regulation by Covalent Modifications

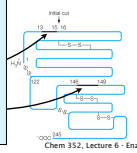
Irreversible modifications are used to activate enzymes in a specific location

- For example, a number of proteolytic enzymes are synthesized in an inactive form in the pancreases.

ogens.

n convert the zymogens





124

Enzyme Inhibition

Problem 8.14:

The steady-state kinetics of an enzyme are studied in the absence and presence of an inhibitor (inhibitor A). The initial rate is give as a function of substrate concentration in the following table.

	v_o [(mmol/L)min^{-1}]	
[S] (mmol/L)	No Inhibitor	Inhibitor A
1.25	1.72	0.98
1.67	2.04	1.17
2.50	2.63	1.47
5.00	3.33	1.96
10.00	4.17	2.38

A. What kind of inhibition (competitive, uncompetitive, or mixed) is involved.

B. Determine V_{max} and K_M in the absence and presence of the the inhibitor.

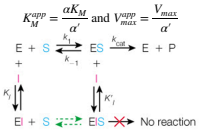
125

Enzyme Inhibition

Mixed inhibition

- The inhibitor can bind to both the free enzyme, E, and to the enzyme-substrate complex, ES.
  - Inhibitor binding decreases the V_{max},
  - But it has a more complex effect on K_M, where binding to E increases K_M, while binding to ES decreases K_M

$$K_M^{app} = \frac{\alpha K_M}{\alpha'} \text{ and } V_{max}^{app} = \frac{V_{max}}{\alpha'}$$

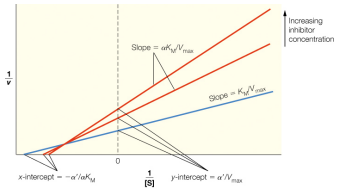


126

Enzyme Kinetics

Mixed Inhibition

- Has the following affects on the Lineweaver-Burke plot.

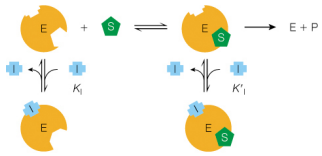


127

Enzyme Inhibition

Mixed Inhibition

- Often the site where the inhibitor binds is a site somewhere removed from the active site where the substrate binds.
  - This type of inhibition is often referred to as ***allosteric inhibition***.

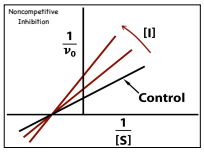


128

Enzyme Inhibition

Mixed Inhibition

- In, the special case where the binding affinity of the inhibitor to both the free enzyme and the enzyme substrate complex are the same ($\alpha = \alpha'$), then there is no apparent effect on K_M.
- This is called ***noncompetitive inhibition*** and produces a Lineweaver-Burke plot that looks like this.



129

Enzyme Inhibition

Problem 8.15:

The same enzyme as in Problem 14 is studied in th epresence of a different inhibitor (inhibitor B).

	v_o [(mmol/L)min^{-1}]			
[S] (mmol/L)	No Inhibitor	Inhibitor A	3mM Inhibitor B	5 mM Inhibitor B
1.25	1.72	0.98	1.25	1.01
1.67	2.04	1.17	1.54	1.26
2.50	2.63	1.47	2.00	1.72
5.00	3.33	1.96	2.86	2.56
10.00	4.17	2.38	3.70	3.49

A. What kind of inhibitor is inhibitor B?

B. Determine the apparent V_{max} and K_M at each inhibitor concentration.

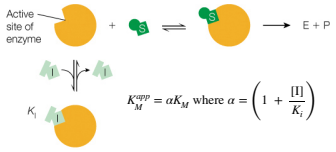
C. Estimate K_i from these data.

130

Enzyme Inhibition

Competitive inhibition

- The inhibitor binds to the same site as the substrate and increases the the apparent K_M.



$$K_M^{app} = \alpha K_M \text{ where } \alpha = \left(1 + \frac{[I]}{K_i}\right)$$

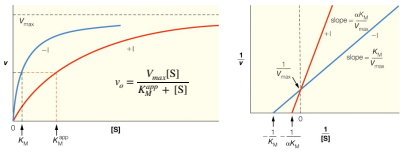
- Drugs are often designed to be competitive inhibitors.

131

Enzyme Kinetics

Competitive Inhibition

- Has the following affects on the Michaelis-Menten and Lineweaver-Burke plots.



- High substrate concentrations can overcome the effects of the inhibitor

132