Chem 452 - Lecture 4
Enzymes
111012

Enzymes are biological catalysts. Nearly every reaction that takes place in a living cell is catalyzed by an enzyme. Most enzymes are proteins. Beside their role in speeding up the rates of chemical reactions, enzymes also play an important role in controlling the flow of material through the myriad of metabolic pathways required to sustain a living cell.

Thermodynamics

"I think that enzymes are molecules that are complementary in structure to the activated complexes of the reactions that they catalyze, that is, to the molecular configuration that is intermediate between the reacting substance and the products of reaction for these catalyzed processes. the attraction of the enzyme molecule for the activated complex would thus lead to a decrease in its energy and hence to the decrease in the energy of activation of the reaction and to the increase in the rate of the reaction."

- Linus Pauling (Nature 161 (1948):707-709)

Enzyme-Substrate Complex

- The reaction rate profile, as a function of substrate concentration, provides evidence for the formation of an enzyme-substrate complex.

Enzyme-Substrate Complex

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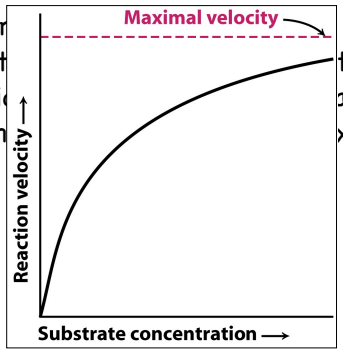
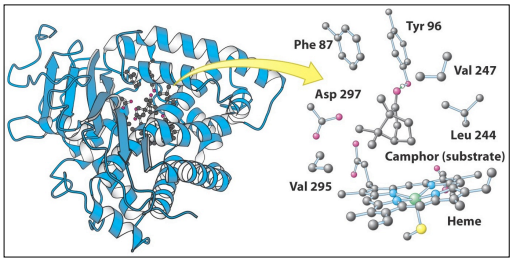


Enzyme-Substrate Complex

- X-ray crystal structures confirm this:



Cytochrome P450

The Enzyme Active Site

- The **active site** of an enzyme is the location where substrates bind and reactions take place.
  - The interactions between the enzyme and the substrate promote the formation of the transition state, $X^{\ddagger}$.

The Enzyme Active Site

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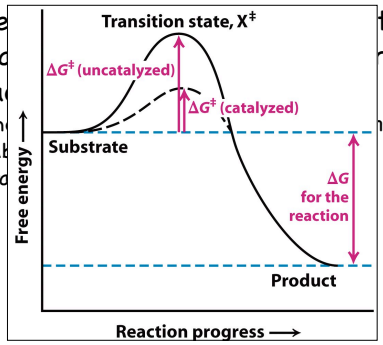
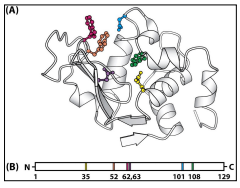


The Enzyme Active Site

- Characteristics of the active site include:
  - Active site is 3-dimensional crevice that brings together residues from distant locations on the polypeptide chain



Lysozyme

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The Enzyme Active Site

- Characteristics of the active site include:
  - Active site is 3-dimensional crevice that brings together residues from distant locations on the polypeptide chain
  - Provides a unique microenvironment for the catalytic groups and the substrate.
  - Substrates are bound by multiple weak interactions.

The Enzyme Active Site

- Characteristics of the active site includ
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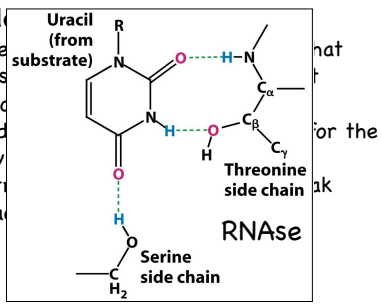
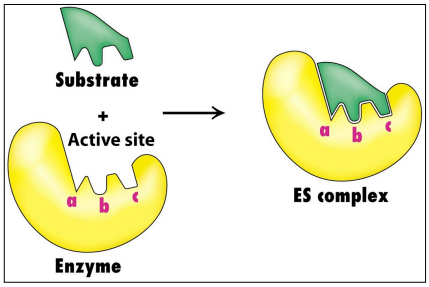
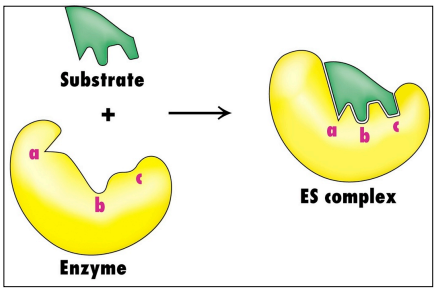


The Enzyme Active Site



Emil Fischer's (1890)
Lock and Key Model

The Enzyme Active Site



Daniel Koshland's (1958)
Induced-Fit Model

The Enzyme Active Site

- Enzyme bind preferentially to the transition state
  - There by lowering $\Delta G^{\ddagger}$

Hexokinase

Enzyme Kinetics

- In 1913, Leonor Michaelis and Maud Menten proposed a simple model to explain the substrate dependence for enzyme catalyzed reactions.

Enzyme Kinetics

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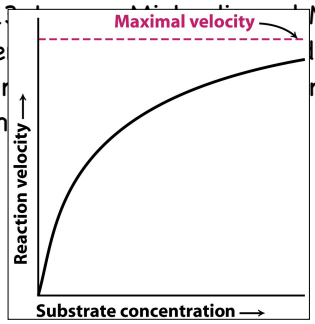

Enzyme Kinetics

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Enzyme Kinetics

- It is usually the initial rate of a reaction, v_o, that is measured. Where there is
  - no back reaction, and
  - [S] is known

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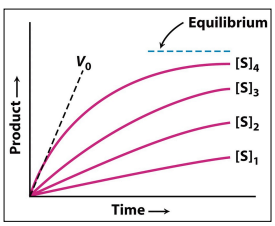


Enzyme Kinetics

- By looking at how the initial rate of a reaction varies with substrate concentration you can gain insight into the mechanism of a reaction.

Enzyme Kinetics

- First order

$$v_o = k_1[\mathrm{S}]^1$$

Enzyme Kinetics

- Second order

$$v_o = k_2[\mathrm{S}][\mathrm{S}]$$
$$= k_2[\mathrm{S}]^2$$
or
$$v_o = k_2[\mathrm{A}][\mathrm{B}]$$

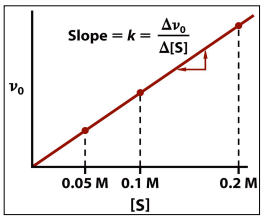
Enzyme Kinetics

- Zero order

$$v_o = k_0[\mathrm{S}]^0$$
$$v_o = k_0$$

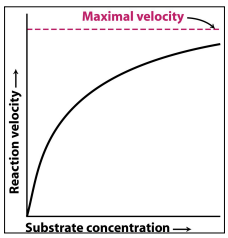
Enzyme Kinetics

- For enzyme-catalyzed reactions:
  - See first-order **substrate** dependence at low [S].



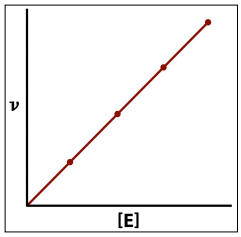
Enzyme Kinetics

- For enzyme-catalyzed reactions:
  - See zero-order **substrate** concentration dependence at high [S].



Enzyme Kinetics

- For enzyme-catalyzed reactions:
  - See first-order **enzyme** concentration dependence at high [S].



Enzyme Kinetics

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

Maud Menten
(1879-1960)

Leonor Michaelis
(1875-1949)

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].

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:

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

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

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

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 in the expression for v_o, the variable [E] needs to be eliminated.

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

  - [E] is the free enzyme concentration, which is equal to the total enzyme concentration, $[E]_T$ minus the enzyme-substrate concentration

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

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]:

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

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

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

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

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

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

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

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

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}

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

Enzyme Kinetics

- The meaning of the catalytic rate constant, k_{cat} (= k_2).
  - Represented by the first order rate constant at high [S]
  - Is determined from Vmax (kcat = Vmax/[E]total)
  - Has units of frequency 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**

Enzyme Kinetics

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Table 8.5 Turnover numbers of some enzymes

Enzyme	Turnover number (per second)
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Enzyme Kinetics

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Enzyme Kinetics

- The meaning of 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.

Enzyme Kinetics

- The meaning of K_M.

TABLE 8.4 K_M values of some enzymes

Enzyme	Substrate	K_M (μM)
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

Enzyme Kinetics

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Enzyme Kinetics

- Combining k_{cat} and K_M gives a measure of the catalytic efficiency

$$\text{catalytic efficiency} = \frac{k_{cat}}{K_M}$$

Enzyme Kinetics

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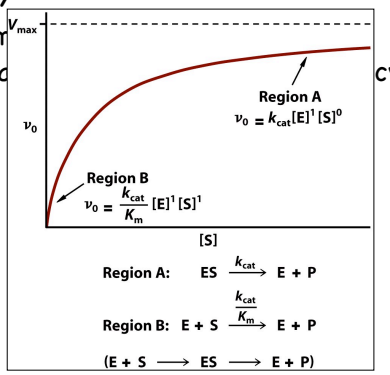

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Enzyme Kinetics

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TABLE 8.7 Enzymes for which k_{cat}/K_M is close to the diffusion-controlled rate of encounter

Enzyme	k_{cat}/K_M ($s^{-1}\,M^{-1}$)
Acetylcholinesterase	1.6×10^8
Carbonic anhydrase	8.3×10^7
Catalase	4×10^7
Crotonase	2.8×10^8
Fumarase	1.6×10^8
Triose phosphate isomerase	2.4×10^8
β-Lactamase	1×10^8
Superoxide dismutase	7×10^9

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 4.5.

Enzyme Kinetics

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Enzyme Kinetics

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TABLE 8.6 Substrate preferences of chymotrypsin

Amino acid in ester	Amino acid side chain	k_{cat}/K_M ($s^{-1} M^{-1}$)
Glycine	—H	1.3×10^{-1}
Valine	—CH(CH$_3$)$_2$	2.0
Norvaline	—$CH_2CH_2CH_3$	3.6×10^2
Norleucine	—$CH_2CH_2CH_2CH_3$	3.0×10^3
Phenylalanine	—CH_2—C$_6$H$_5$	1.0×10^5

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 7.3.

Enzyme Kinetics

- Combining k_{cat} and K_M gives a measure of the catalytic efficiency

$$\text{catalytic efficiency} = \frac{k_{cat}}{K_M}$$

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, $v_o = V_{max}/2$

$$\text{when } v_o = \frac{V_{max}}{2},\ [S] = [S]_{1/2}$$

$$\frac{V_{max}}{2} = \frac{V_{max}[S]_{1/2}}{K_M + [S]_{1/2}}$$

$$\frac{1}{2} = \frac{[S]_{1/2}}{K_M + [S]_{1/2}}$$

$$K_M + [S]_{1/2} = 2[S]_{1/2}$$

$$K_M = [S]_{1/2}$$

Enzyme Kinetics

- Determining K_M and V_{max}.
  - From
    - V_m
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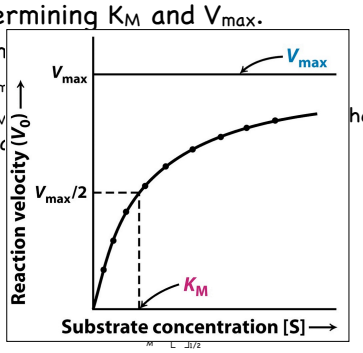

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Enzyme Kinetics

- 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

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

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

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

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

Enzyme Kinetics

- Determining K_M and V_{max}.
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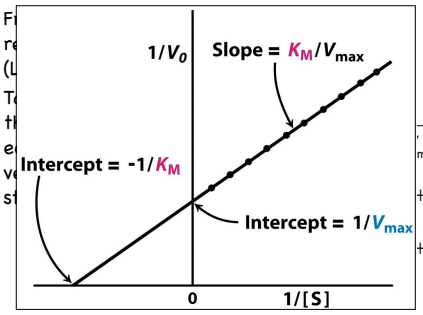

$$\frac{[S]}{V_{max}[S]} + \frac{1}{V_{max}} + b$$

Enzyme Kinetics

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

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 determined from the y-intercept in a Lineweaver-Burk plot (y-intercept = $1/V_{max}$).

Enzyme Kinetics

- Summary:

K_M

- It is a measure of how strongly an enzyme is able to bind to the substrate.
  - The higher the K_M the weaker the binding
- 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 the x-intercept in a Lineweaver-Burk plot (x-intercept = $-1/K_M$).

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

Enzyme Kinetics

- Summary:

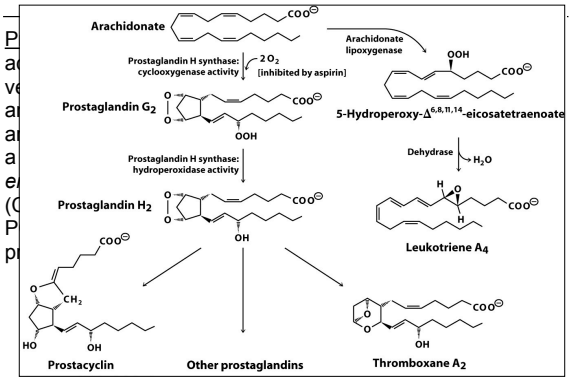
k_{cat}/K_M

  - 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}).

Problem

Prostaglandins are a class of eicosanoid (20-carbon), fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. They are responsible for producing fever and inflammation and the associated pain. Prostaglandins are derived from the 20-carbon fatty acid, arachidonic acid, in a reaction catalyzed by the enzyme *prostaglandin endoperoxide synthetase*. This enzyme is a cycoloxygenase (COX) and uses dioxygen (O_2) to convert arachidonic acid to PGG_2, the immediate precursor to many different prostaglandins.

Problem



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Problem

A) The kinetic data given below are for the reaction catalyzed by *prostaglandin endoperoxide synthetase*. Determine the V_{max} and K_M of the enzyme.

[S] {mM}	v_o {mM/min}
0.5	23.5
1	32.2
1.5	36.9
2.5	41.8
3.5	44

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Problem

B) If the enzyme concentration used in this reaction is 14 nM, what is the **turnover number** for this reaction?

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Next up

- Enzymes (Chapter 8)
  - Enzyme inhibitors
- Catalytic Strategies (Chapter 9)