

Quiz

Class Business

I will have Project 1 graded by the end of the week.

Project 2 is due on 11/15

The discussion groups for Project 2 are cancelled

There is additional reading for classes held on 10/30 and 11/1 – I will post the remaining handouts on Toolkit and hand them out in class next week

I have updated the last two lectures with the mechanisms from the 3rd edition for those of you that have the 2nd edition text.

Review of last class

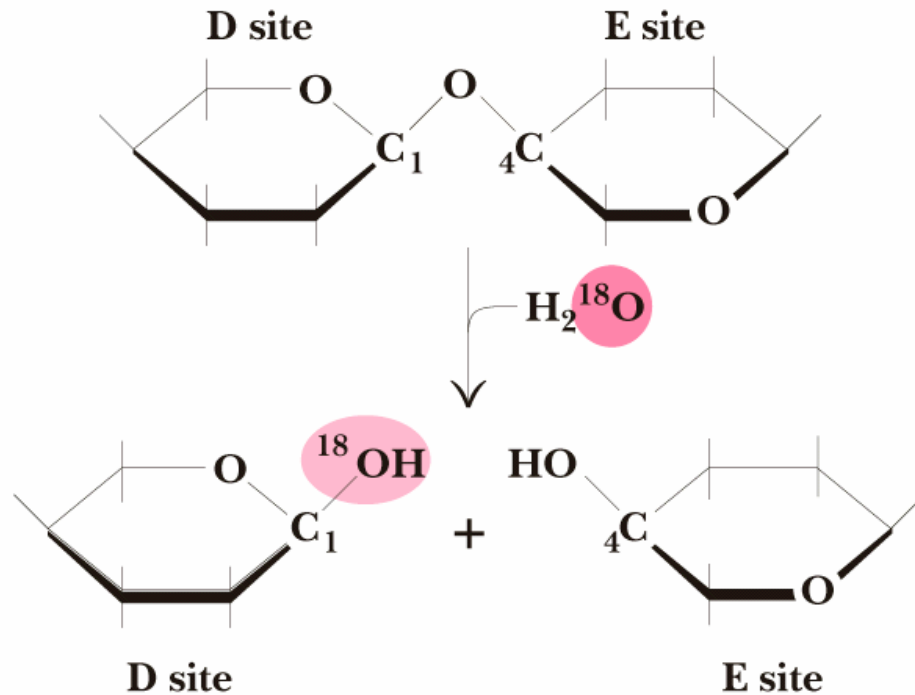
- Detailed mechanisms of serine and aspartic proteases
- General mechanism of old and new lysozyme mechanism
- Know these mechanisms well

How can we test a proposed catalytic mechanism?

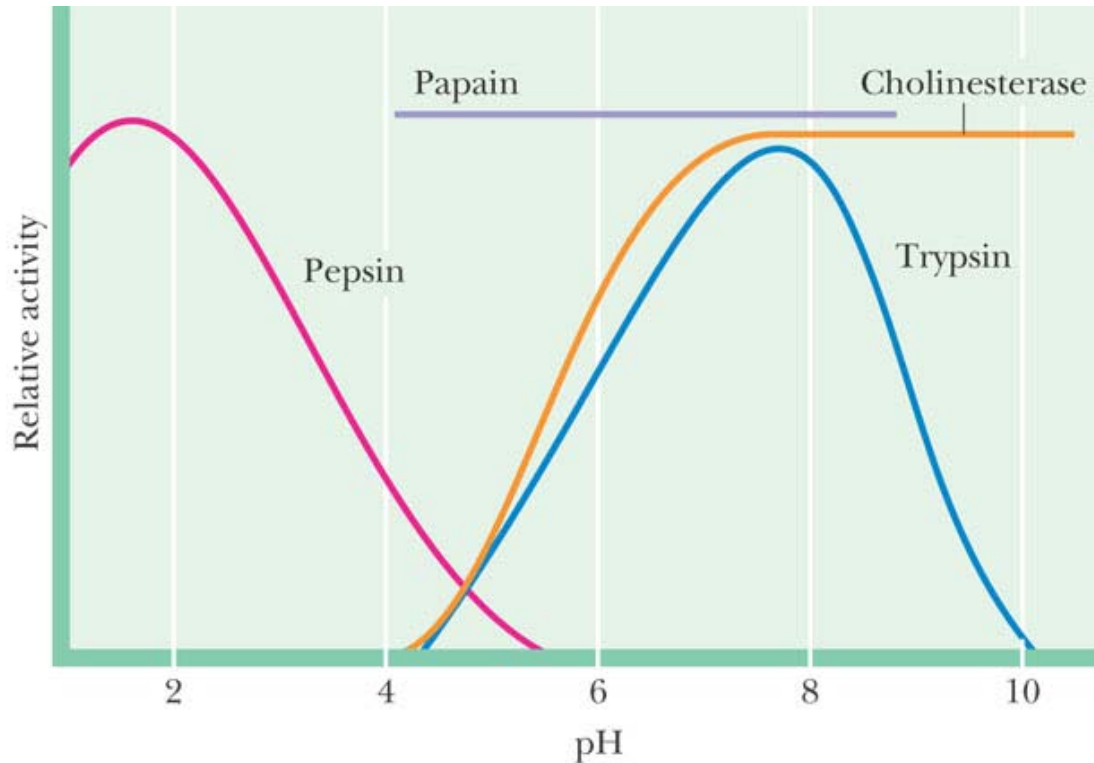
Mutational Analysis

- Single or multiple amino acid substitutions can be made using site-directed mutagenesis and recombinant protein preparation
- For instance, when Asp102 of trypsin was replaced with Asn, the activity was 1/10000 that of wild type.

Isotope labeling or tracing



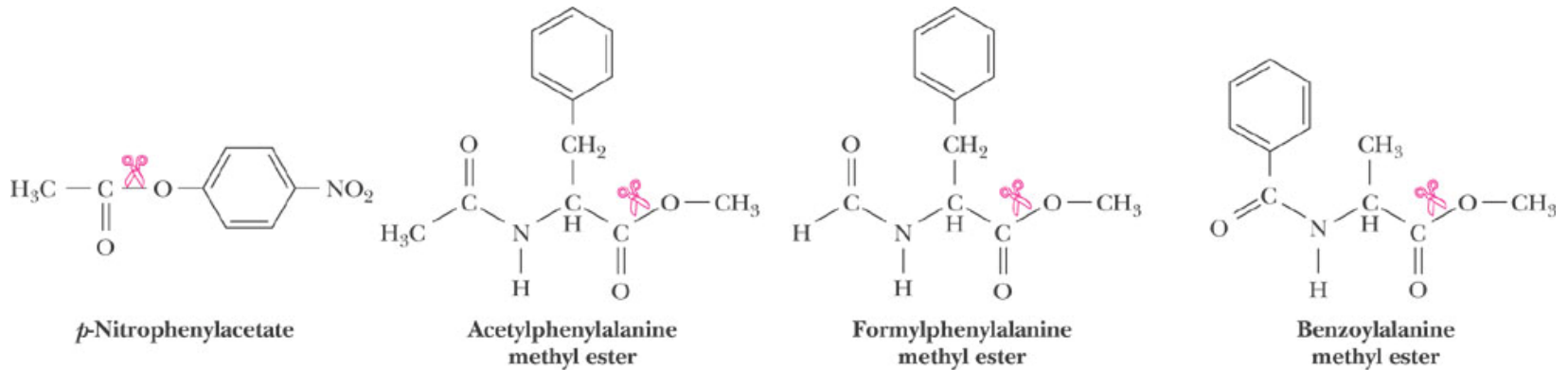
pH dependence



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

The pH activity profiles of four different enzymes. *Trypsin*, an intestinal protease, has slightly alkaline pH optimum, whereas *pepsin*, a gastric protease, acts in the acidic confines of the stomach and has a pH optimum near 2. *Papain*, a protease found in papaya, is relatively insensitive to pHs between 4 and 8. *Cholinesterase* activity is pH sensitive below pH 7 but not between pH 7 and 10. The cholinesterase pH activity profile suggests that an ionizable group with pK' near 6 is essential to its activity. Might it be a histidine residue within the active site?

Artificial substrates

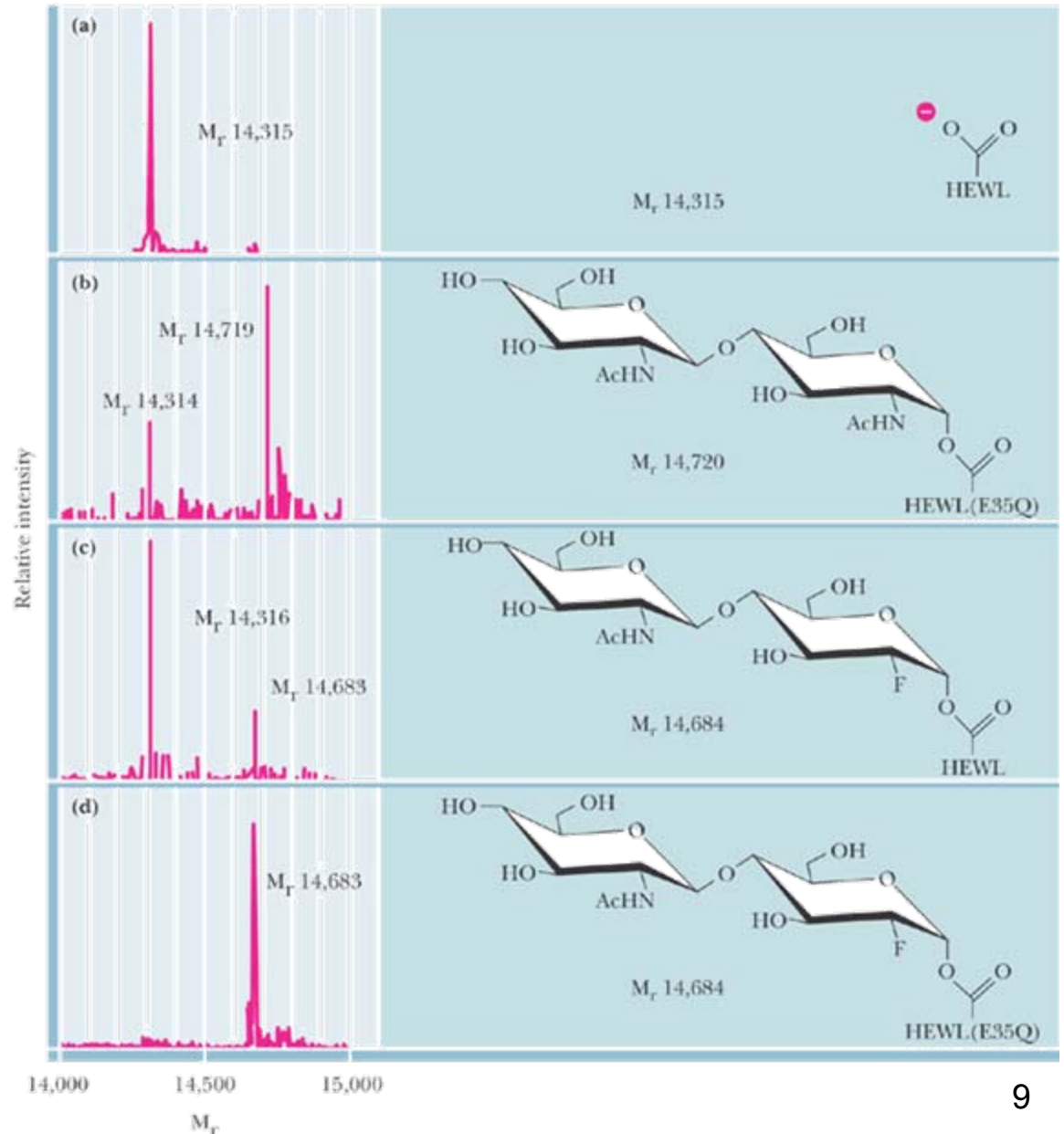


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Artificial Substrates and Mass Spectrometry

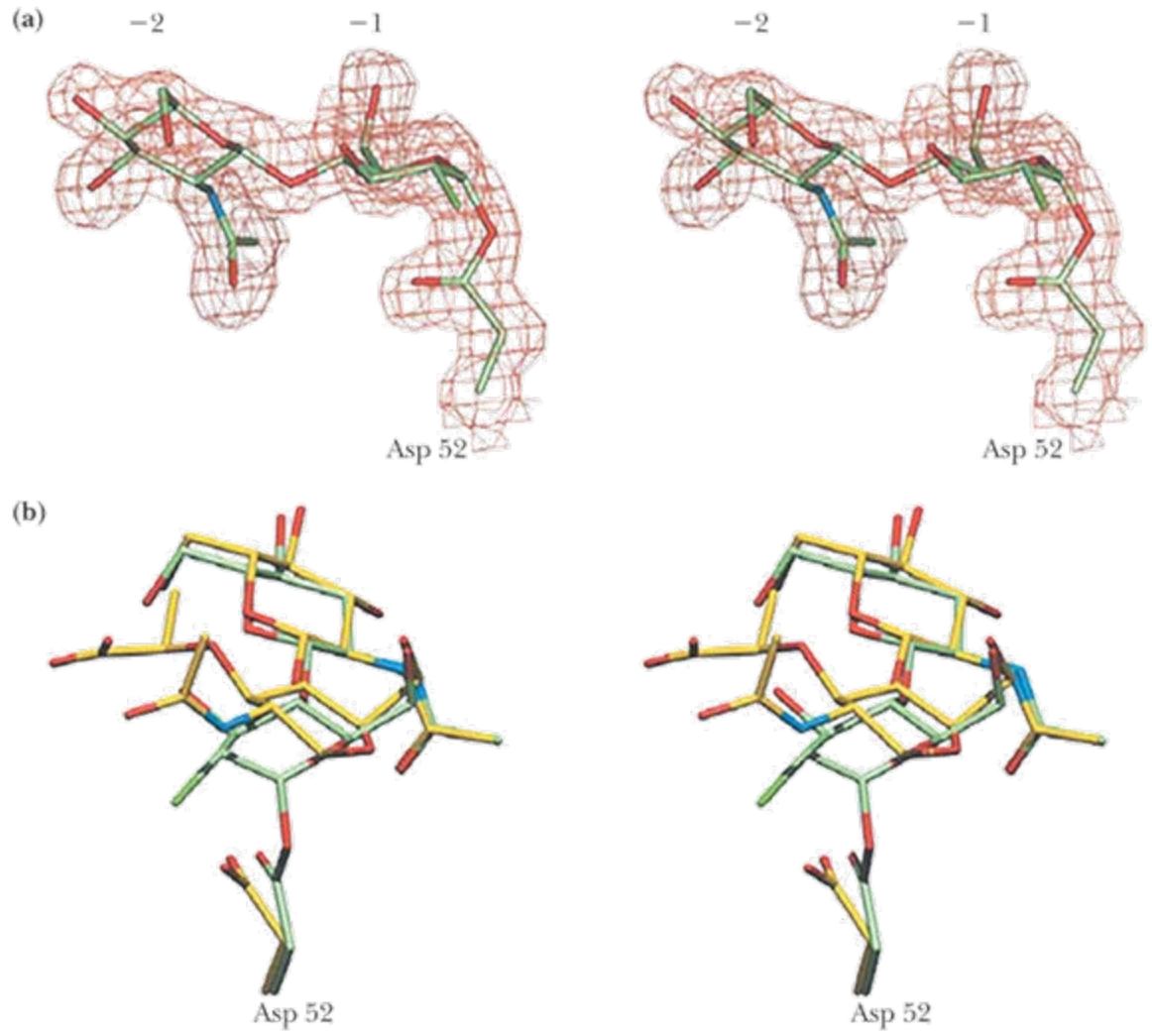
Figure 14.40

Mass spectra of lysozyme complexes. **(a)** Wild-type hen egg white lysozyme (HEWL). **(b)** Mutant lysozyme with Glu³⁵ replaced with glutamine [HEWL(E35Q)], incubated with chitobiosyl fluoride (NAG2F). **(c)** Wild-type HEWL, incubated with 2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 4)-2-deoxy-2-fluoro- β -D-glucopyranosyl fluoride (NAG2FGlcF). **(d)** HEWL(E35Q), incubated with NAG2FGlcF. Structures of the species corresponding to each peak observed in the mass spectra are shown to the right, with their expected relative molecular mass.



Artificial Substrates and X-ray Crystallography

Stereo view of the covalent NAG2FGlcF intermediate of the lysozyme reaction.

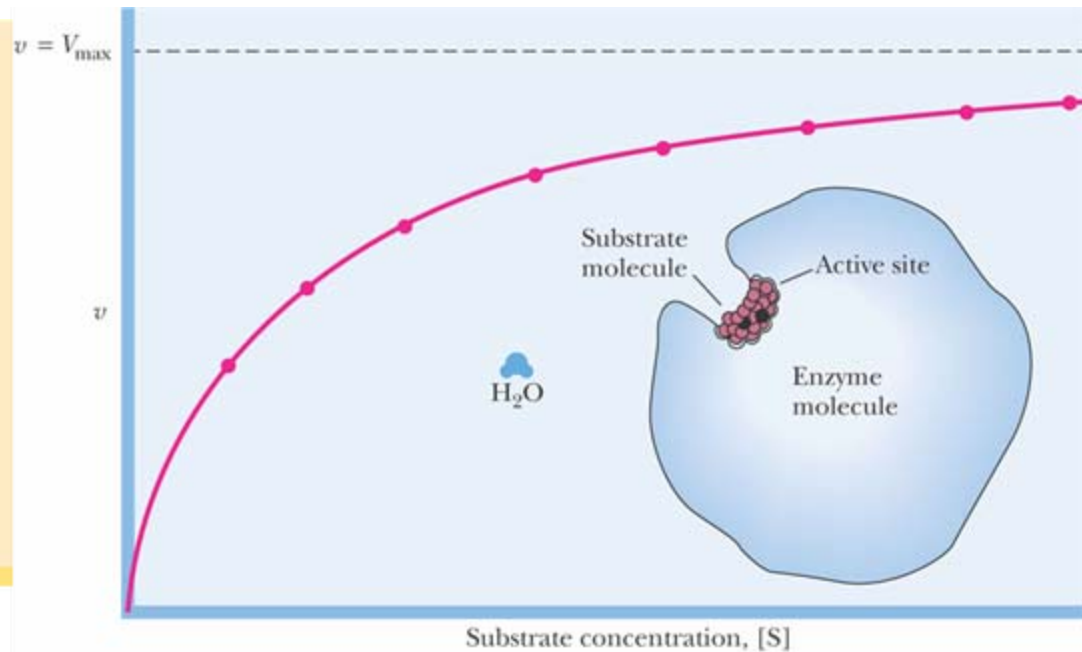
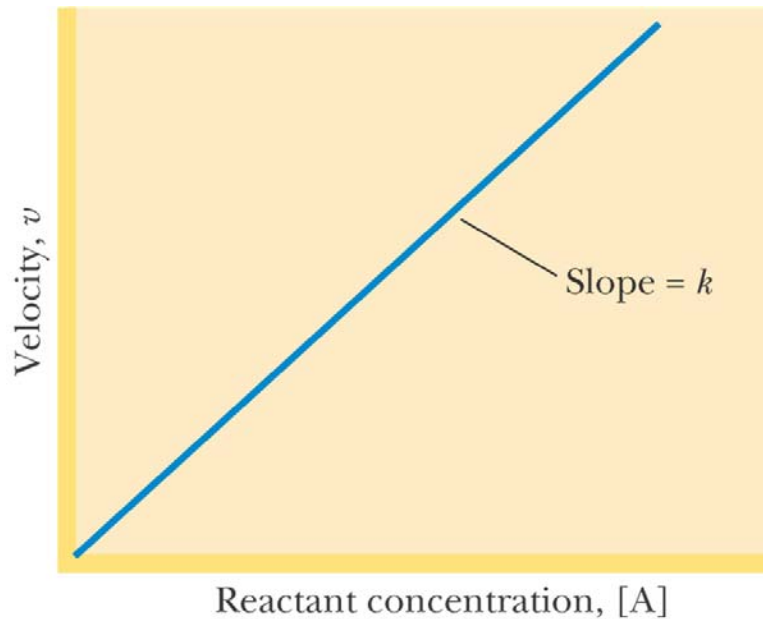


Can the Rate of an Enzyme-Catalyzed Reaction Be Defined in a Mathematical Way?

- Enzymes can accelerate reactions as much as 10^{16} over uncatalyzed rates!
- Urease is a good example:
 - Catalyzed rate: 3×10^4 /sec
 - Uncatalyzed rate: 3×10^{-10} /sec
 - Ratio is 1×10^{14} !

Enzyme Kinetics

What Equations Define the Kinetics of Enzyme-Catalyzed Reactions?



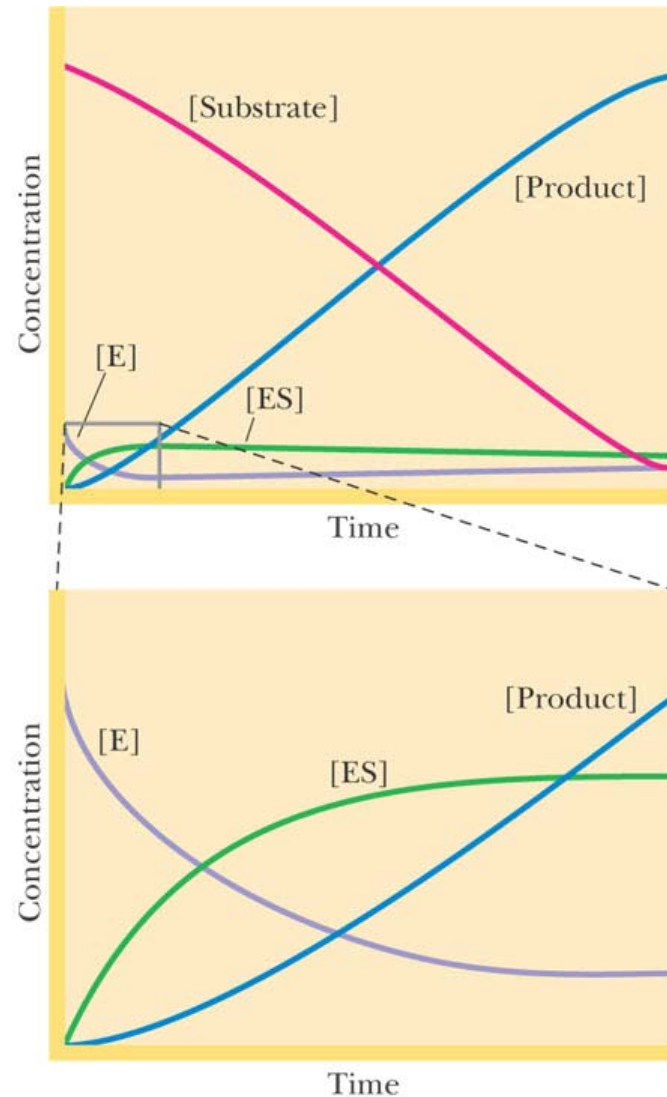
A plot of v versus $[A]$ for the unimolecular chemical reaction, $A \rightarrow P$, yields a straight line having a slope equal to k .

Substrate saturation curve for an enzyme-catalyzed reaction. The amount of enzyme is constant, and the velocity of the reaction is determined at various substrate concentrations. The reaction rate, v , as a function of $[S]$ is described by a rectangular hyperbola. At very high $[S]$, $v = V_{max}$. That is, the velocity is limited only by conditions (temperature, pH, ionic strength) and by the amount of enzyme present; v becomes independent of $[S]$.

The Michaelis-Menten Equation

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

Time course for the consumption of substrate, the formation of product, and the establishment of a steady-state level of the enzyme-substrate [ES] complex for a typical enzyme obeying the Michaelis-Menten, Briggs-Haldane models for enzyme kinetics.



The dual nature of the Michaelis-Menten equation

Combination of 0-order and 1st-order kinetics

- When S is low, the equation for rate is 1st order in S
- When S is high, the equation for rate is 0-order in S
- The Michaelis-Menten equation describes a rectangular hyperbolic dependence of v on S !

MM Eqn Assumptions

1. The reaction involves only one substrate or only one substrate is varied while the others are held constant
2. The reaction $ES \rightarrow E + P$ is irreversible or the experiment is limited to observing only initial velocities where $[P] = 0$
3. $[S] > [E_T]$ and $[E_T]$ is constant
4. Steady state $d[ES]/dt = 0$

Understanding K_m

The "kinetic activator constant"

- K_m is a constant
- K_m is a constant derived from rate constants
- K_m is, under true Michaelis-Menten conditions, an estimate of the dissociation constant of E from S

Understanding V_{\max}

The theoretical maximal velocity

- V_{\max} is a constant
- V_{\max} is the theoretical maximal rate of the reaction - but it is NEVER achieved in reality
- To reach V_{\max} would require that ALL enzyme molecules are tightly bound with substrate
- V_{\max} is asymptotically approached as substrate is increased

The turnover number

A measure of catalytic activity

- k_{cat} , the turnover number, is the number of substrate molecules converted to product per enzyme molecule per unit of time, when E is saturated with substrate.
- If the M-M model fits, $k_2 = k_{\text{cat}} = V_{\text{max}}/E_t$
- Values of k_{cat} range from less than 1/sec to many millions per sec

The catalytic efficiency

Name for k_{cat}/K_m

An estimate of "how perfect" the enzyme is

- k_{cat}/K_m is an apparent second-order rate constant
- It measures how the enzyme performs when S is low
- The upper limit for k_{cat}/K_m is the diffusion limit - the rate at which E and S diffuse together

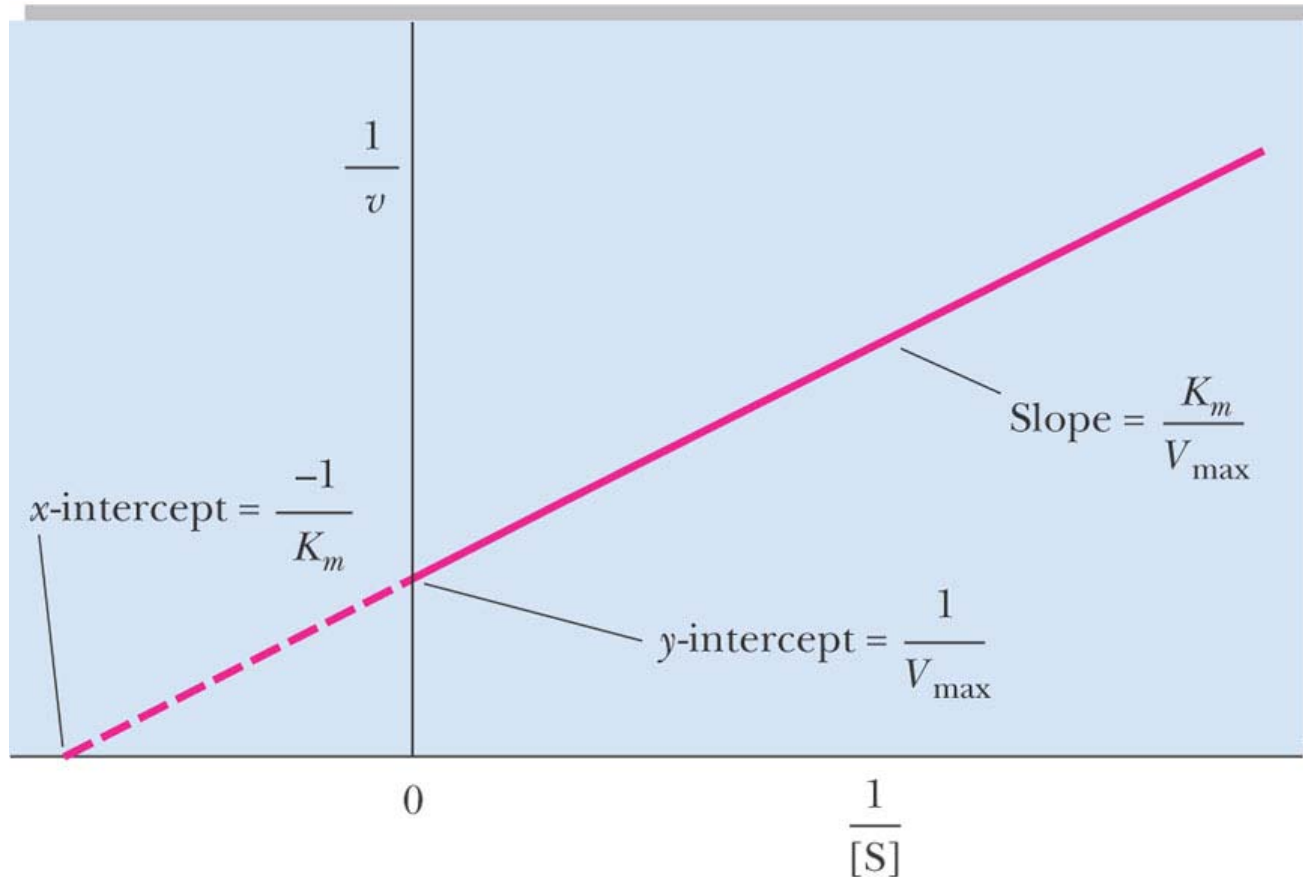
Linear Plots of the Michaelis-Menten Equation

Linear plots allow K_m and V_{max} to be estimated by extrapolation of lines rather than asymptotes

- Lineweaver-Burk
- Hanes-Woolf
 - Preferred because there isn't an overemphasis of the data obtained at low $[S]$

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

Lineweaver-Burk



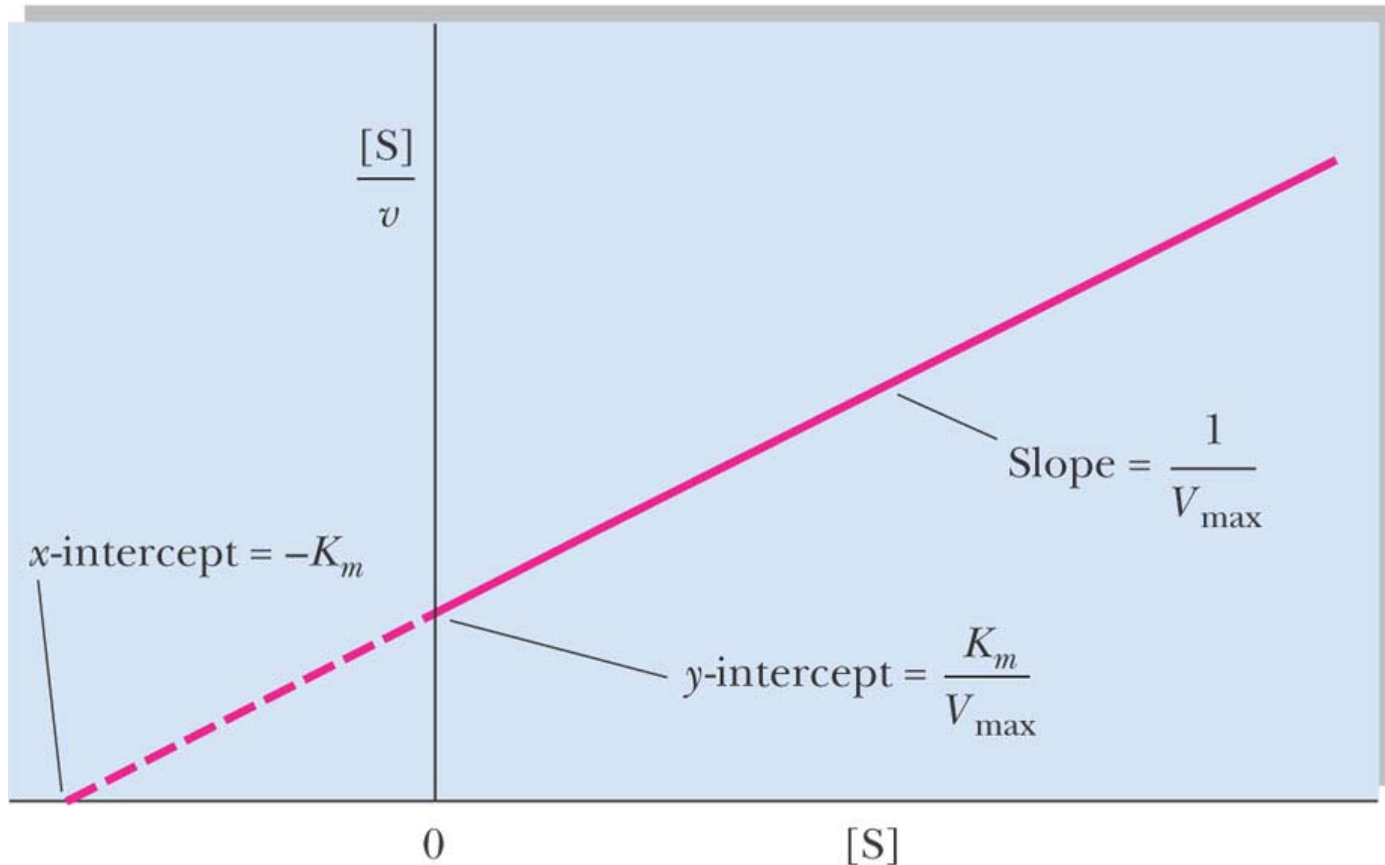
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Figure 13.9

The Lineweaver-Burk double-reciprocal plot, depicting extrapolations that allow the determination of the x- and y-intercepts and slope.

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

Hanes-Woolf



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Figure 13.10

A Hanes-Woolf plot of $[S]/v$ versus $[S]$, another straight-line rearrangement of the Michaelis-Menten equation.

Example