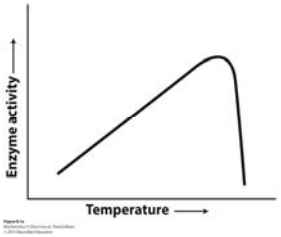
Enzyme Mechanisms and Inhibition

Pratt & Cornely Ch 7

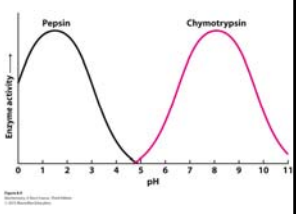
Other Factors

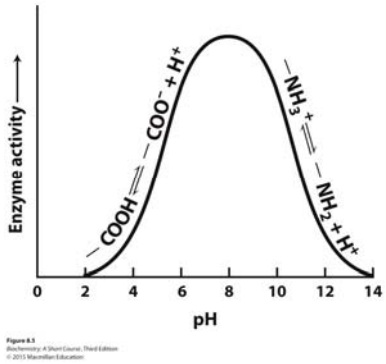
- Other factors affect enzyme activity
  - Temperature
  - pH

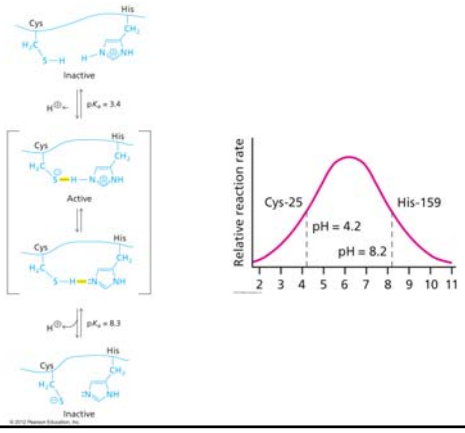


pH Optimum

- Determined by structural stability
  - Compartmentalization
- Determined by active site residues
  - Bases must be deprotonated
  - Acids must be protonated







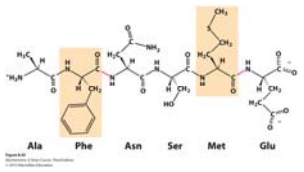
Mechanisms

- Two major mechanisms—any or all may be used in a given enzyme
  - Chemical Mechanisms: Change the pathway
    - Acid-base catalysis
    - Covalent catalysis
    - Metal ion catalysis
  - Binding Mechanisms: Lower Activation energy
    - Proximity/orientation effect
    - Transition State Stabilization

Case Study 1: Chymotrypsin

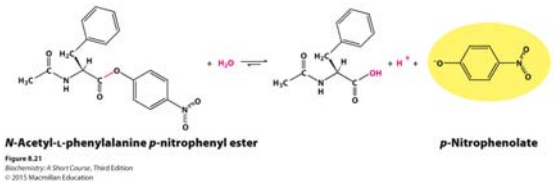
- Well studied enzyme
- Example of how enzyme mechanism studied
- Example of types of mechanism

Catalyzes hydrolysis of amide



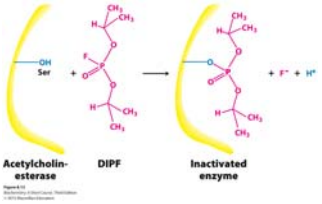
Enzyme Assays

- Make artificial substrate
- Product formation may be followed with UV-vis spectroscopy



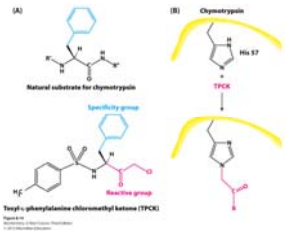
Group Specific Reagents

- Chemical that reacts with a particular residue
- Covalent modification
- Can be used to determine especially reactive serine-195



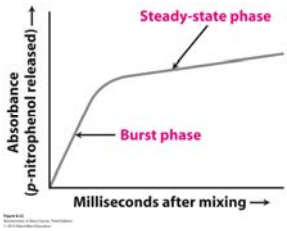
Affinity Label

- Chemical that has affinity for active site
- Discovered active site Histidine



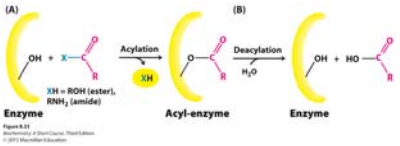
Pre-Steady State Kinetics

- "Burst" kinetics
- Small initial product formation followed by steady rate of product formation
- Suggests a two step mechanism

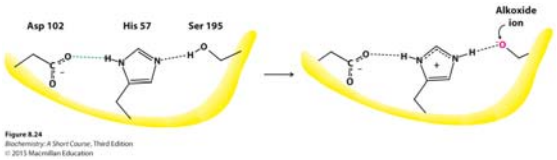


Covalent Catalysis

- Consistent with two phases
- First, enzyme forms bond with substrate to give initial burst of product
- Then the catalytic cycle begins



Proposed Mechanism: Catalytic Triad



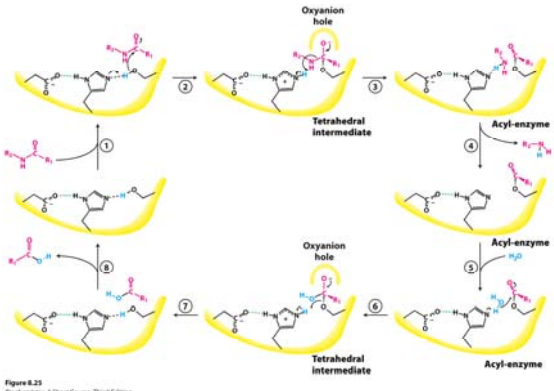
- Roles for serine, histidine, and aspartate

Catalysis

- Uncatalyzed hydrolysis of amide
  - Look for points of instability (high transition states)
- Acid-catalyzed hydrolysis
  - Stabilize places of instability
  - Break one high hurdle into more lower hurdles
- Enzyme-catalyzed hydrolysis

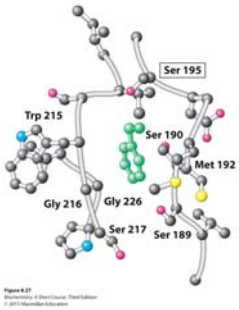
Stabilization of intermediates

- Locate high energy intermediates
- How are they stabilized?
- Oxyanion hole
  - H-bonds
  - Bond angles



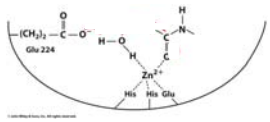
Origin of Substrate Specificity

- Specificity binding pocket
- Also orients amide bond into the right location



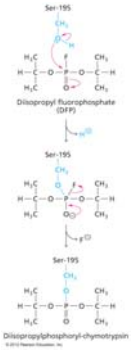
Case Study 2: Metalloprotease

- Propose a mechanism for this protease:



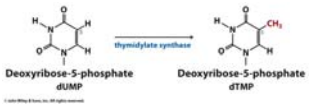
Irreversible Enzyme Inhibition

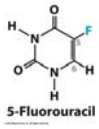
- Some compounds inhibit enzymes irreversibly
  - Covalent linkage
  - Tight binding
- Major Types
  - Group-Specific
  - Affinity labels
  - Mechanism-based
  - Transition State Analog



Mechanism Based Inhibitors

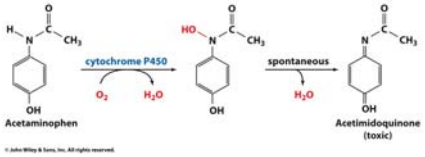
- Suicide inhibitors
- Selectivity
- Targeting fast-growing cells





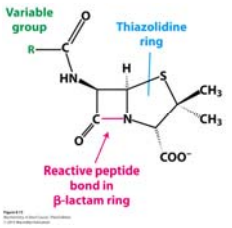
Drug Byproducts

- Oxidation of xenobiotics by P450 enzymes
- Pharmacology
- Liver damage—covalent binding to cysteine



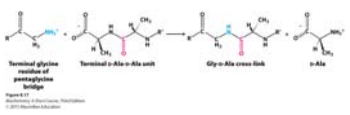
Case Study: Penicillin

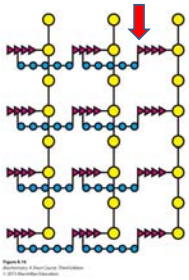
- First antibiotic discovered
- Highly reactive b-lactam bond
- Works against a bacterial target not found in humans



Glycopeptide Transpeptidase

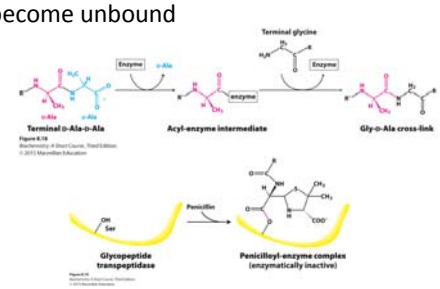
- Peptidoglycan gives *Staph* its structural support
- Crosslinked pentagly to D-Ala



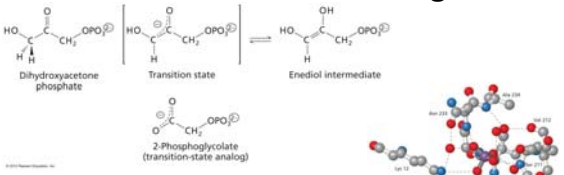


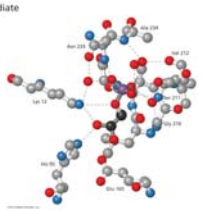
Trojan Horse

- Penicillin mimics substrate, but does not become unbound

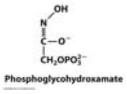


Transition State Analog

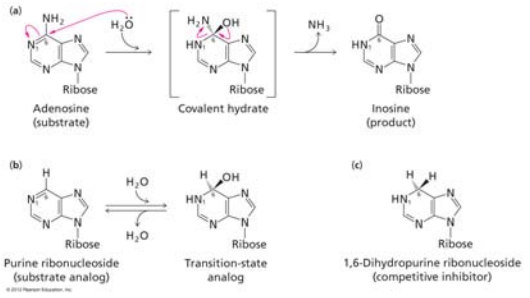




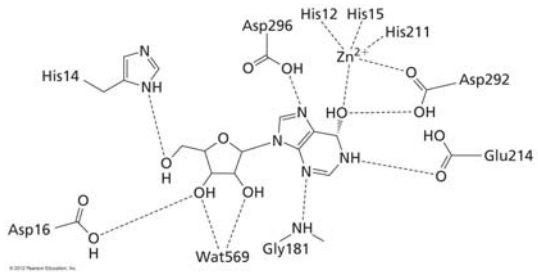
- Your book presents high energy intermediate analog



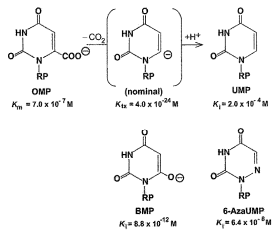
Designing a Transition State Analog



Binding of Transition State Analog



Case Study: Orotidine Decarboxylase



Mechanism of Catalysis

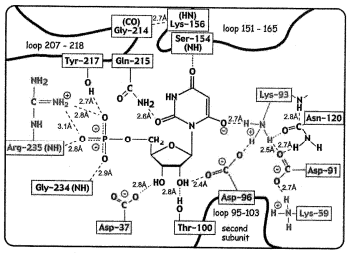
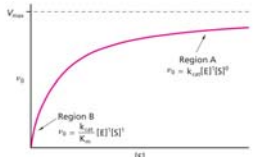


Figure 9 Schematic view of the active site of yeast ODCase (71), showing the active site residues that contact the inhibitor 6-hydroxyUMP (BMP). Positively charged residues are shown in red, negatively charged residues in green.

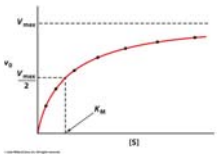
Reversible Inhibition Kinetics

- Know types of Reversible Inhibition
- Know effect on kinetic parameters
- Understand why
- Interpret MM and L-B plots



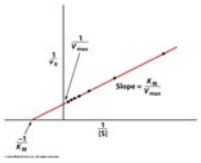
Review: Lineweaver Burke Plot

- Analyze hyperbola
- Construct linear plot
- Double reciprocal



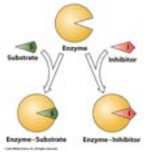
Lineweaver–Burk equation:

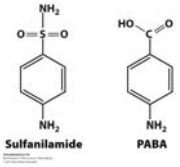
$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



Competitive Inhibition

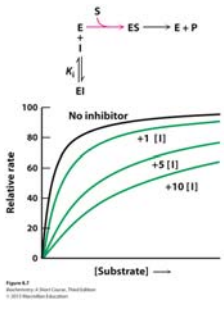
- Binds to same site as substrate-both cannon be bound at same time
- EI complex is inactive
- Usually structurally resembles substrate
- Sulfanilamide
- Draw altered MM plot

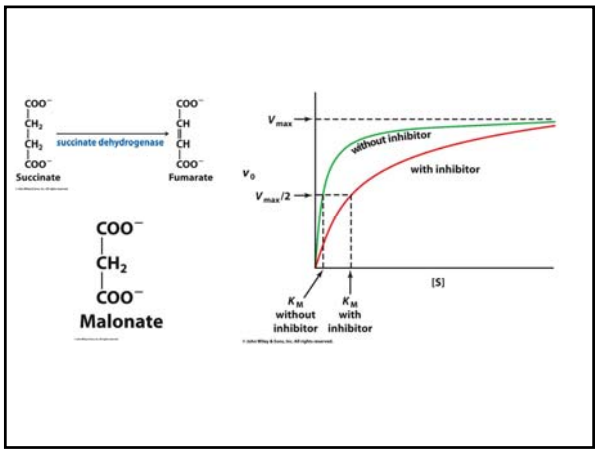


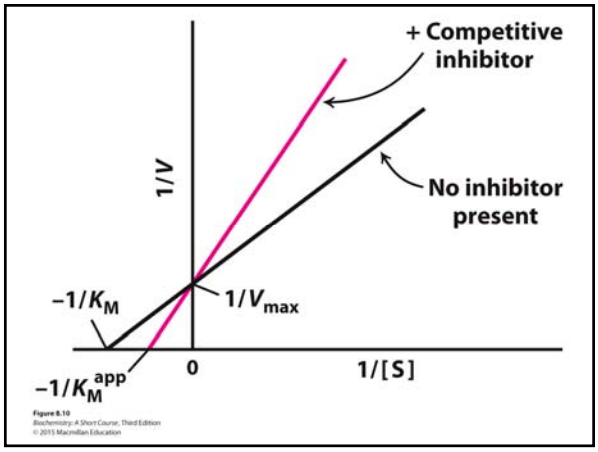


Competitive Inhibition

- Enough added substrate can outcompete inhibitor
- "Feels like…"
  - Same amount of Enzyme at high [S]
    - Vmax unchanged
  - Needs more S to bind (lowers affinity)
    - Km raised

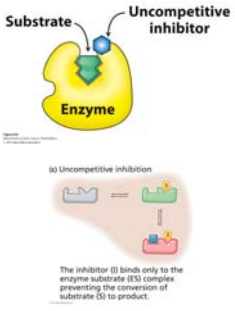






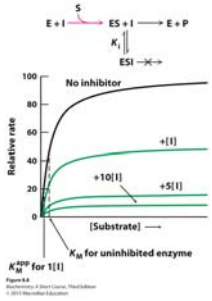
Uncompetitive Inhibition

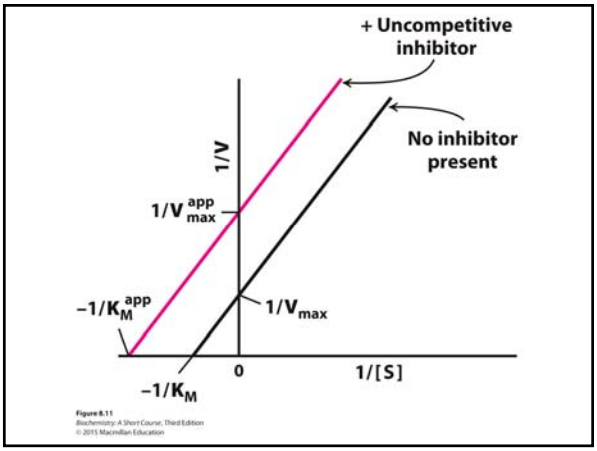
- Inhibitor binds only to [ES], not to free [E]
- [ESI] is inactive
- Added substrate increases inhibitor effect
- Glyphosate (Roundup)
- Draw altered MM plot



Uncompetitive Inhibition

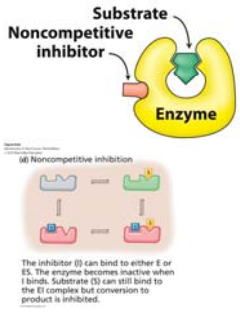
- Increased substrate increases inhibitor effect
- "Feels like…"
  - Less enzyme at high [S]
    - Decrease Vmax
  - Enzyme has greater affinity for substrate
    - Decrease Km





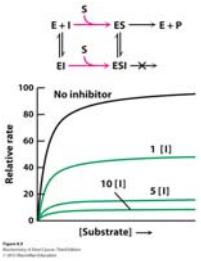
Noncompetitive Inhibition

- Assumes simple case of inhibitor binding equally to E and ES
- [ESI] inactive
- Only in case where inhibitor binding affects chemical rate (kcat) but not binding (Km)
- Very rare
- Draw altered MM plot



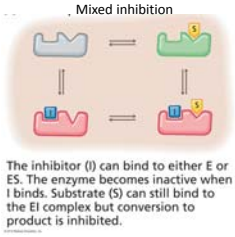
Noncompetitive Inhibition

- "Feels like…"
  - Less enzyme at all [S]
    - Decrease Vmax
  - No effect on substrate affinity (no net shift in equilibrim)
    - $Km_{(app)}$ same as Km



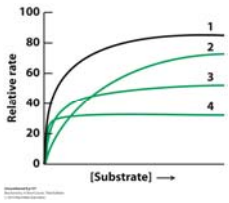
Mixed Inhibition

- Like noncompetitve, but not the simple case
  - Inhibitor may bind E or ES better
- "Feels like…"
  - Less enzyme at all [S]
  - Overall lowering OR raising of affinity for substrate



Fill in the Chart

Inhibition	Effect on K_M	Effect on V_{max}	Effect on V_{max}/K_M
Competitive			
Uncompetitive			
Noncompetitive			
Mixed			



Problem

- A cysteine protease was discovered that has an inhibitor called P56. Shutting down this enzyme could be a strategy for treating malaria. What type of inhibitor is P56?