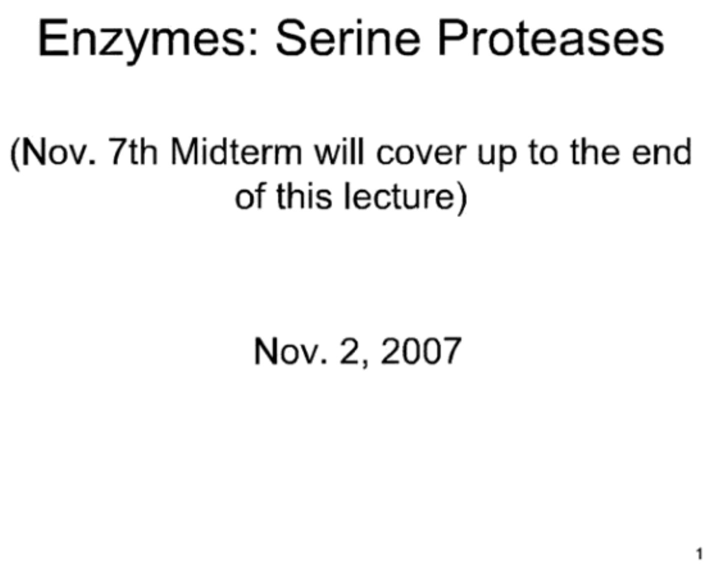
Notes 11/2

Friday, November 02, 2007
9:56 AM

Notes 1112

Audio recording started: 10:02 AM Friday, November 02, 2007

- **Slide 1: Enzymes: Serine Proteases**
    - (Nov 7th Midterm will cover up to the end of this lecture)
    - Q&A session FRANZ 1178 Nov 2nt 5-6pm
    - 

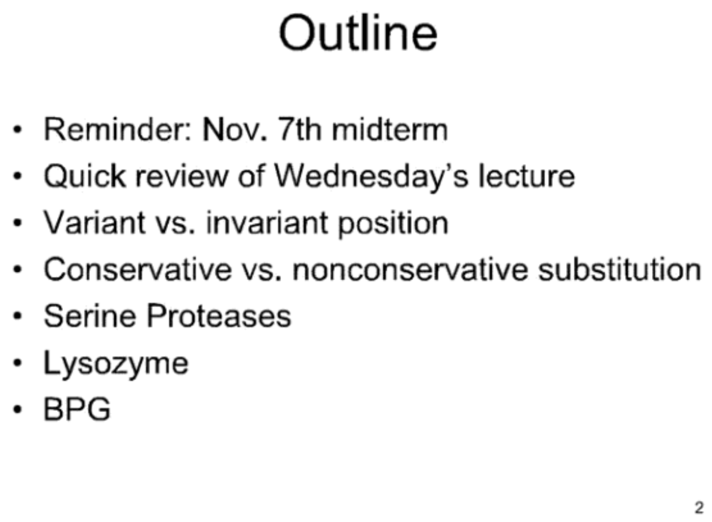
Enzymes: Serine Proteases

(Nov. 7th Midterm will cover up to the end of this lecture)

Nov. 2, 2007

1

- **Slide 2: Outline**
    - Reminder: Nov 7th midterm
    - Quick review of Wedensday's lecture
    - Variant vs. invariant position
    - Conservative vs nonconservative substitution
    - Serine proteases
    - Lysozyme
    - BPG

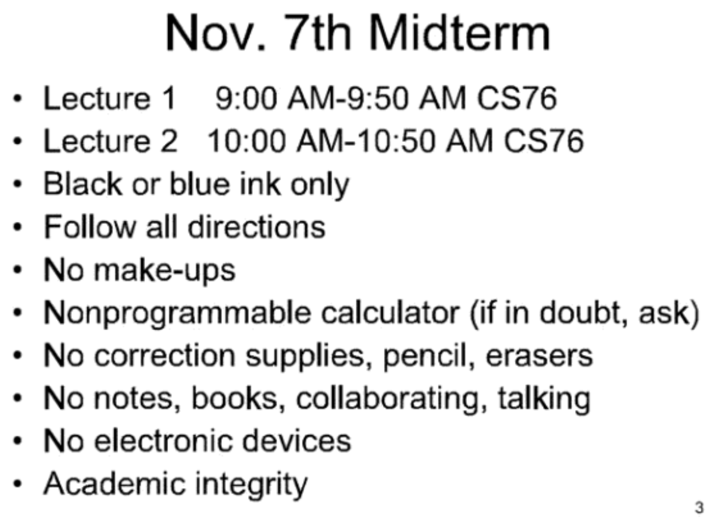


Outline

- Reminder: Nov. 7th midterm
- Quick review of Wednesday’s lecture
- Variant vs. invariant position
- Conservative vs. nonconservative substitution
- Serine Proteases
- Lysozyme
- BPG

- **Slide 3: Nov. 7th Midterm**
  - Lecture 1: 9am-9:50am CS76
  - Lecture 2 10am-10:50Am CS76
  - Black or blue ink only
  - Follow all directions
  - No make-ups
  - Nonprogrammable calculator (if in doubt, ask)
  - No corretion supplies, pencil, erasers
  - No notes, books collaboration, talking
  - No electronic devices
  - Academic integrity

○



Nov. 7th Midterm

- Lecture 1 9:00 AM-9:50 AM CS76
- Lecture 2 10:00 AM-10:50 AM CS76
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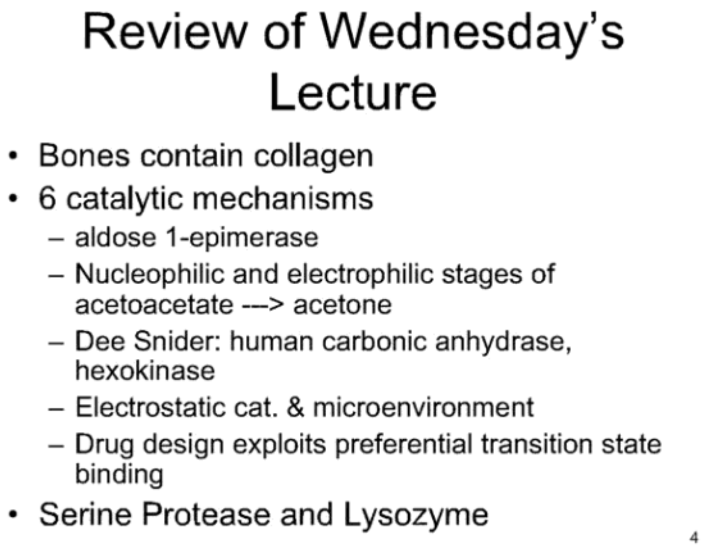
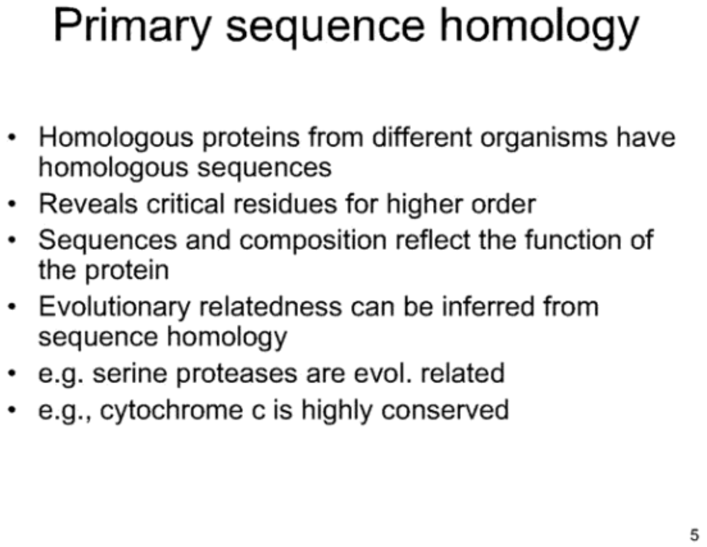
- **Slide 4: Review of Wednesday's Lecture**
  - Bones contain collagen
    - Collagen is connective tissue
    - Gly tripple repeat
    - Tripple hilical structure... 3.3 residues per polypeptide turn (not alpha)
  - 6 catalytic mechanisms
    - Aldose 1-epimerase
      - Uses acid-base concerted mechanism
      - Catalyzes mutarotation
    - Nucleophilic and electrophilic stages of acetoacetate --> acetone
    - Dee Snider: human carbonic anhydrase, hexokinase
    - Electrostatic cat. & microenvironment
      - Electrostatic cat - dielectric constant within microenvironment. Dielectric constant in active site is lower than water.
    - Drug design exploits preferential transition state binding.



Review of Wednesday's Lecture

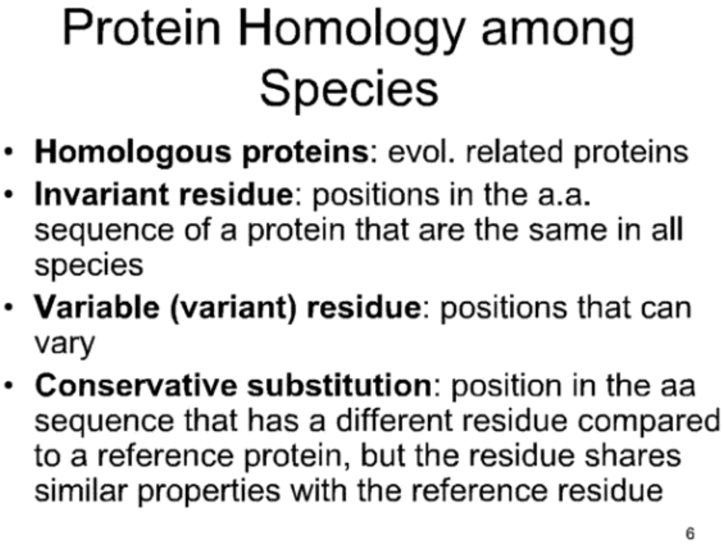
- Bones contain collagen
- 6 catalytic mechanisms
  - aldose 1-epimerase
  - Nucleophilic and electrophilic stages of acetoacetate ---> acetone
  - Dee Snider: human carbonic anhydrase, hexokinase
  - Electrostatic cat. & microenvironment
  - Drug design exploits preferential transition state binding
- Serine Protease and Lysozyme

- **Slide 5: Primary sequence homology (OCT 8 lecture)**
  - Homologous proteins from different organisms have homologous sequences
    - Cytochrome
  - Reveals critical residues for higher order
  - Sequences and composition reflect the function of the protein
  - Evolutionary relatedness can be inferred form sequence homology
  - e.g. serine proteases are evolutionary related
    - Called serine proteases because have serine in active site
  - e.g., cytochrome C



Primary sequence homology

- Homologous proteins from different organisms have homologous sequences
- Reveals critical residues for higher order
- Sequences and composition reflect the function of the protein
- Evolutionary relatedness can be inferred from sequence homology
- e.g. serine proteases are evol. related
- e.g., cytochrome c is highly conserved

- **Side 6: Protein Homology among Species**
    - Homologous proteins: evolutionary related proteins
    - Invariant residue: positions in the amino acid sequence of a protein that are the same in all species
    - Variable (variant) residue: positions that can vary.
    - Conservative substation: position in the amino acid sequence that has a different residue compared to a reference protein, but the residue shares similar properties with the reference residue
        - e.g. if organism A has alanine at position and organism B has valine at position then it is conservative substitution
            - If organism a has alanine and gutamine, it is nonconservative because these a.a. are so different in properties
            - 

Protein Homology among Species

- **Homologous proteins**: evol. related proteins
- **Invariant residue**: positions in the a.a. sequence of a protein that are the same in all species
- **Variable (variant) residue**: positions that can vary
- **Conservative substitution**: position in the aa sequence that has a different residue compared to a reference protein, but the residue shares similar properties with the reference residue

- **Side 7: Amino Acid Sequence of Cytochromes c from 38 species (OCT 8 lecture)**
    - High degree of conservation between species
    - Position 1 - every organism has glycine
        - Invariant position or invariant residue
    - Position 21 - glutamic acid and then aspardic acid
        - Residues vary but similar properties so it is conservative substitution
    - Pisition 3 (at end)
        - Alanine, Valine in animals. Theorine in plants
    - Able to tell how related different species are to another

- ○

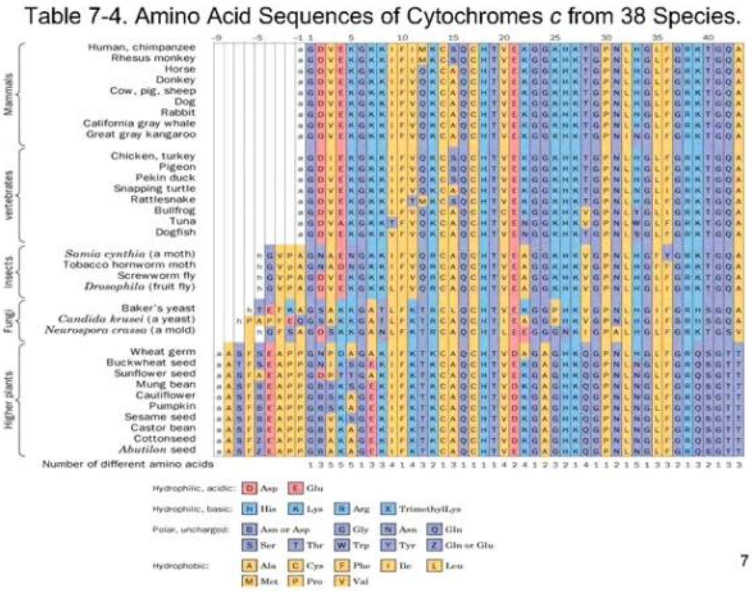


Table 7-4. Amino Acid Sequences of Cytochromes *c* from 38 Species.

Mammals
Human, chimpanzee
Rhesus monkey
Horse
Donkey
Cow, pig, sheep
Dog
Rabbit
California gray whale
Great gray kangaroo

Other vertebrates
Chicken, turkey
Pigeon
Pekin duck
Snapping turtle
Rattlesnake
Bullfrog
Tuna
Dogfish

Insects
Samia cynthia (a moth)
Tobacco hornworm moth
Screwworm fly
Drosophila (fruit fly)

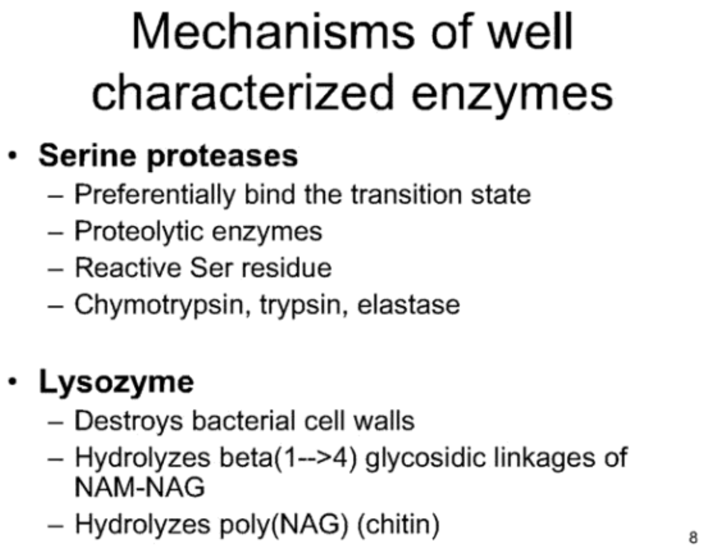
Fungi
Baker's yeast
Candida krusei (a yeast)
Neurospora crassa (a mold)

Higher plants
Wheat germ
Buckwheat seed
Sunflower seed
Mung bean
Cauliflower
Pumpkin
Sesame seed
Castor bean
Cottonseed
Abutilon seed

Number of different amino acids 1 3 5 5 5 1 3 3 4 1 4 3 2 1 3 1 1 1 1 4 2 4 1 2 3 2 1 4 1 1 2 1 5 1 3 3 2 1 3 2 1 3 3

Hydrophilic, acidic: D Asp E Glu
Hydrophilic, basic: H His K Lys R Arg X Trimethyllys
Polar, uncharged: B Asn or Asp G Gly N Asn Q Gln S Ser T Thr W Trp Y Tyr Z Gln or Glu
Hydrophobic: A Ala C Cys F Phe I Ile L Leu M Met P Pro V Val

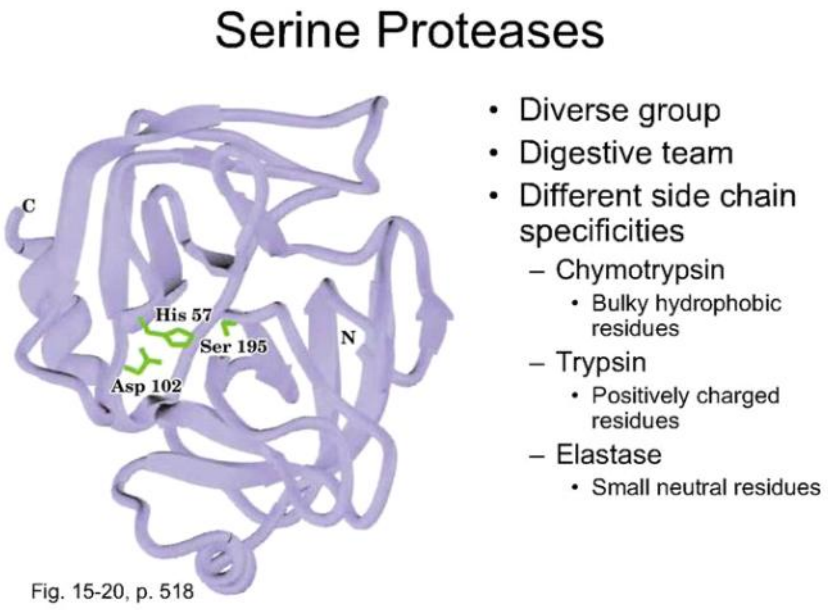
- **Slide 8: mechanims of well characterized enzymes**
  - Serine proteases
    - Preferentially bind the transition state
    - Proteolytic enzymes
      - Hydrolyze peptide bonds
    - Reactive Ser residue
    - Chymotrypsin, trypsin, electase
  - Lysozyme
    - Destroys bacterial cell walls
      - Found in secretion of vertebrates (found in our tears)
    - Hydroyzes beta (100>4) glycosidic linkages of NAM-NAG
      - Be able to draw NAM-NAG **
    - Hydrolyzes poly(NAG)
      - Poly(NAG) is chitin



Mechanisms of well characterized enzymes

- **Serine proteases**
  - Preferentially bind the transition state
  - Proteolytic enzymes
  - Reactive Ser residue
  - Chymotrypsin, trypsin, elastase
- **Lysozyme**
  - Destroys bacterial cell walls
  - Hydrolyzes beta(1-->4) glycosidic linkages of NAM-NAG
  - Hydrolyzes poly(NAG) (chitin)

- **Slide 9: Serine Proteases**
  - Diverse group
  - Digestive team
    - Made in pancreas
    - Get secreted into intestines
  - Different side chain specificities
    - Chymotrypsin
      - Bulky hydrophobic residues
    - Trypsin
      - Positively charged residues
    - Elastase
      - Small neutral Residues
  - Active site has catalytic triad meaning has 3 different residues that catalyze reaction
    - His 57, Ser 195, Asp 102
    - All serine proteases have this catylitic triad

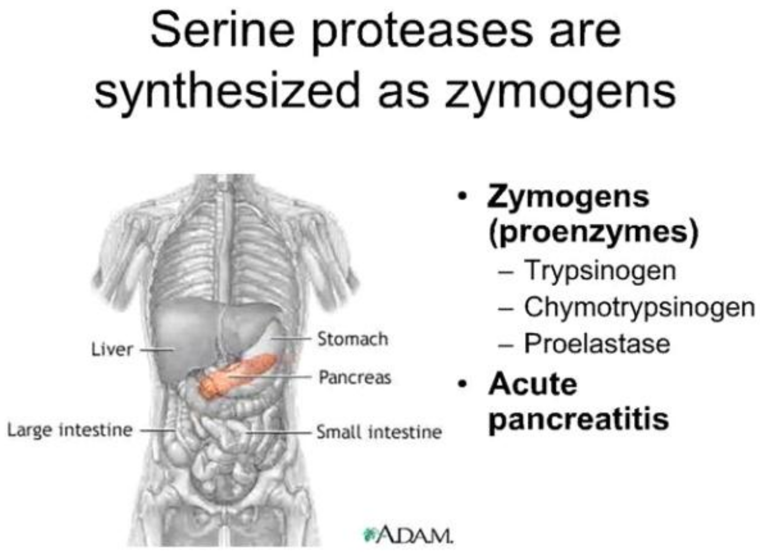


Serine Proteases

- Diverse group
- Digestive team
- Different side chain specificities
  - Chymotrypsin
    - Bulky hydrophobic residues
  - Trypsin
    - Positively charged residues
  - Elastase
    - Small neutral residues

Fig. 15-20, p. 518

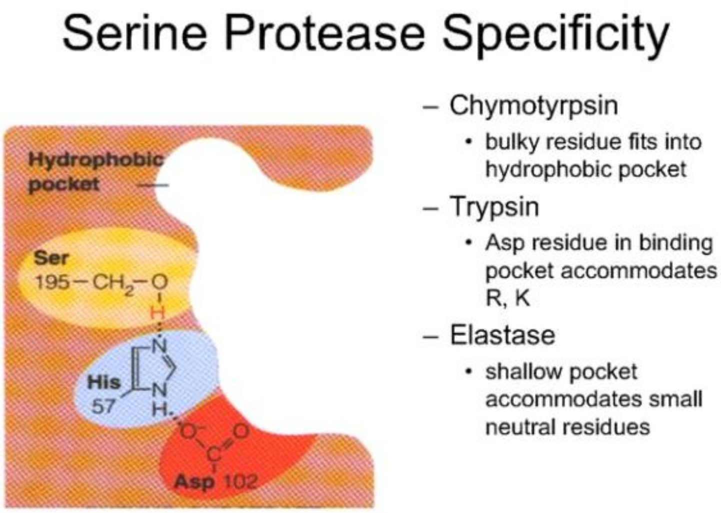
- **Slide 10: Serine proteases are synthesized as zymogens**
  - Zymogen (proenzymes)
    - Larger inactive precursor to active enzyme
    - Activated through protealysis
    - By cleaving part of a.a. sequence of zymogen to active enzyme, the conformation changes allowing it to be active
  - Zymogens (proenzymes)
    - ☐ Trypsigonen
      - Proenzyme for trypsin
    - ☐ Chymotrypsogen
      - Proenzyme for chymotrypsin
    - ☐ Proelastase
      - Proenzyme for elastase
  - Acute pancreatitis
    - If you cannot regulate zymogen correctly



Serine proteases are synthesized as zymogens

- **Zymogens (proenzymes)**
  - Trypsinogen
  - Chymotrypsinogen
  - Proelastase
- **Acute pancreatitis**

- **Slide 11: Serine Protease Secificity**
  - Missed everything… go back
  - Chymotrypsin
    - Bulky residue that fits into hydrphobic pocket

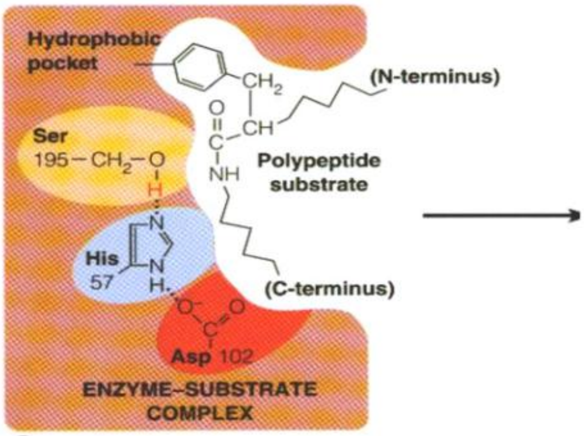


Serine Protease Specificity

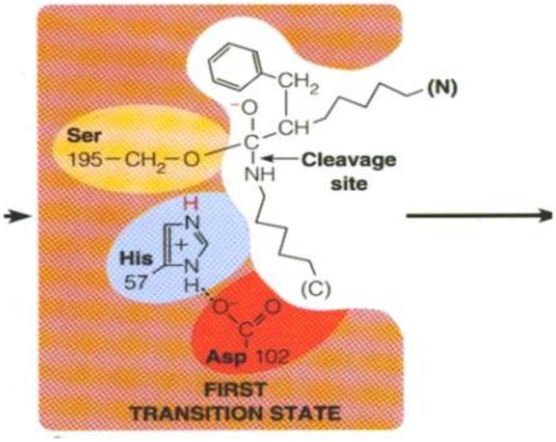
- Chymotyrpsin
  - bulky residue fits into hydrophobic pocket
- Trypsin
  - Asp residue in binding pocket accommodates R, K
- Elastase
  - shallow pocket accommodates small neutral residues

- **Slide 12: Chymotrypsin and Catalytic Triad**
  - Enzyme Substrate complex
  - H-bond between Ser and His

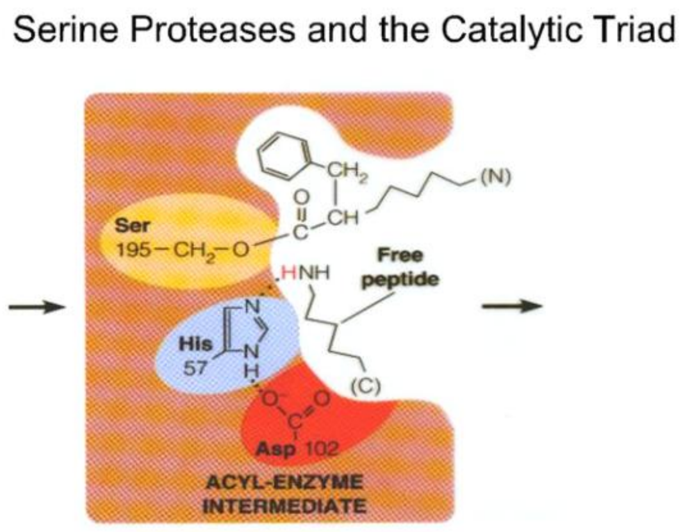
Chymoptrypsin and the Catalytic Triad



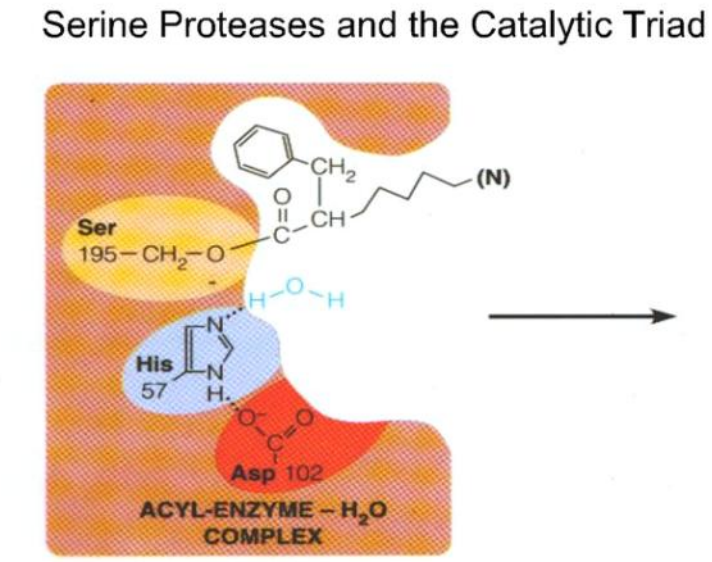
- **Slide 13: Serine Proteases and the Catalytic Triad**
  - From slide 12, Ser bonds to carbonyl and Ser His h-bond is broken
  - Transition state
  - Carbon is in tetrahedral
  - Aspartic acid forms h-bond with histidine
  - Serine Proteases and the Catalytic Triad



- **Slide 14: Serine proteases and the catalytic triad**
  - Acyl-enzyme intermediate
  - H from his forms on NH and free peptide forms with breakage of tetrahedral carbon

- ○ 
Serine Proteases and the Catalytic Triad

- **Slide 15: Serine proteases and the Catalytic Triad**
  - ○ Acyl-Enzyme - H_2O Complex
  - ○ Water attached to His

- ○ 
Serine Proteases and the Catalytic Triad

- **Slide 16: Serine Proteases and the Catalytic Triad**
  - ○ Second Transition State
  - ○ OH leaves from water and attaches to make tetrahedral carbon again

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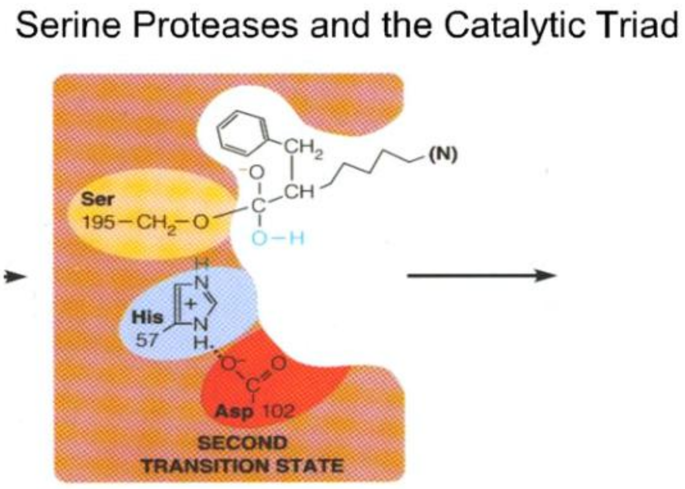

Serine Proteases and the Catalytic Triad

- **Slide 17: Serine Proteases and the Catalytic Triad**
    - Free Enzyme
    - His and Ser H bond

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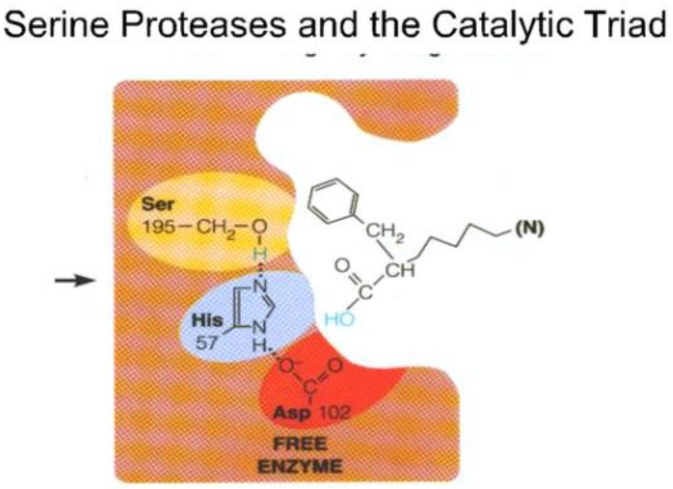
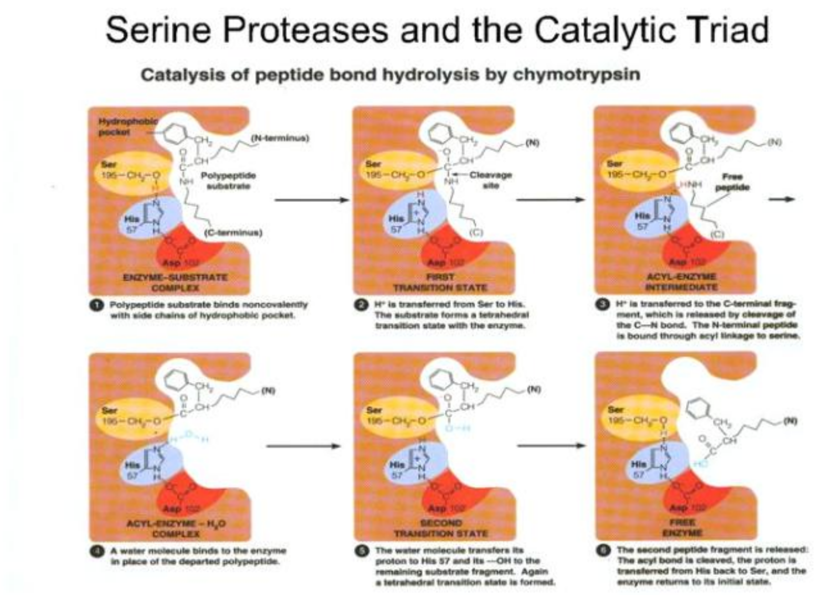


Serine Proteases and the Catalytic Triad

- **Slide 18: Serine Proteases and Catalytic Triad**
    - Catalysis of peptide obond hydrolysis by chymotripsin
        1. Polypeptide substrate binds noncovanently with side chains of hydrophobic pocket
        2. H^+ is transferred from Ser to Hos. The substrate forms a tetrahedral transition state with enzyme
        3. H^+ is transferred to the C-terminal fragment, which is released by cleavage of C-N bond.

The….
4. See slides
5. See slides
6. See slides

- 

  Serine Proteases and the Catalytic Triad

  Catalysis of peptide bond hydrolysis by chymotrypsin

  ① Polypeptide substrate binds noncovalently with side chains of hydrophobic pocket.

  ② H⁺ is transferred from Ser to His. The substrate forms a tetrahedral transition state with the enzyme.

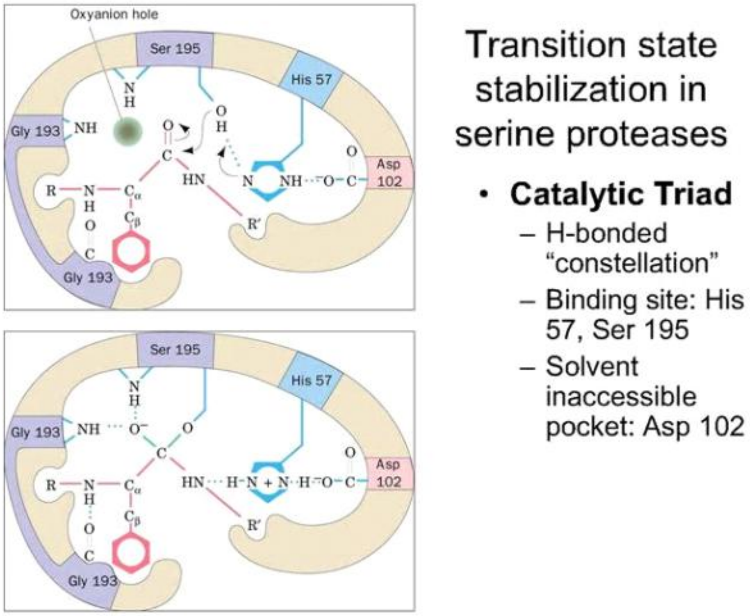
  ③ H⁺ is transferred to the C-terminal fragment, which is released by cleavage of the C—N bond. The N-terminal peptide is bound through acyl linkage to serine.

  ④ A water molecule binds to the enzyme in place of the departed polypeptide.

  ⑤ The water molecule transfers its proton to His 57 and its —OH to the remaining substrate fragment. Again a tetrahedral transition state is formed.

  ⑥ The second peptide fragment is released: The acyl bond is cleaved, the proton is transferred from His back to Ser, and the enzyme returns to its initial state.

- **Slide 19: Transition state stabilization in serine proteases**
  - Catalytic Triad
    - H-bonded "constellation"
    - Binding site: His 57, Ser 195
    - Solvent inaccessible pocket: Asp 102
      - Water cannot get into it
  - Chymotripsin is through preferential binding of transition site

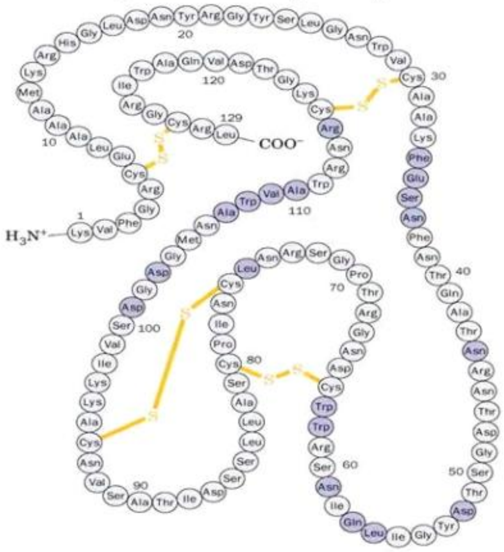


Transition state stabilization in serine proteases

- **Catalytic Triad**
  - H-bonded "constellation"
  - Binding site: His 57, Ser 195
  - Solvent inaccessible pocket: Asp 102

- **Slide 20: Fig. 15-9 Lysozyme**
  - Disulfide linkages from cystines
    - Also called cystine
  - Disrupt disulfide linkages through reducing agent such as beta-mercapto ethanol
  - Picture for Hen egg white

Fig. 15-9. Lysozyme



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- **Slide 21: Figure 15-8**
  - Lysozyme is breaking b(1-->4) glycosodic linkage

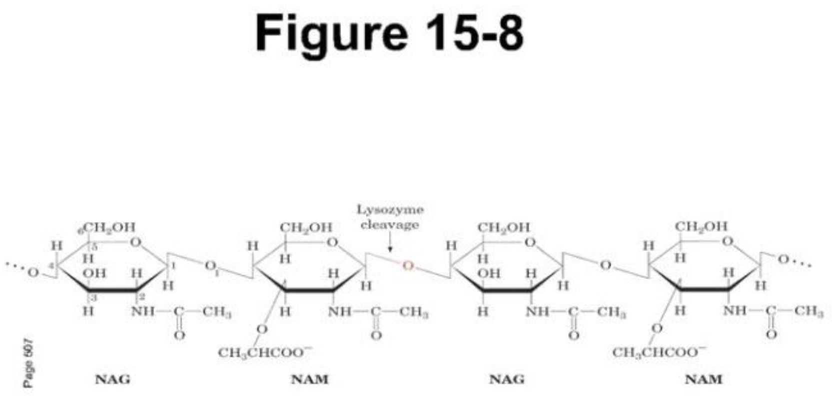
  - 

Figure 15-8

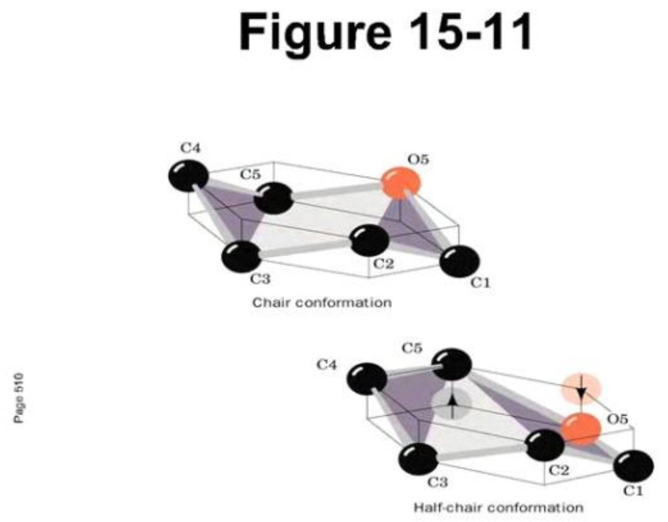
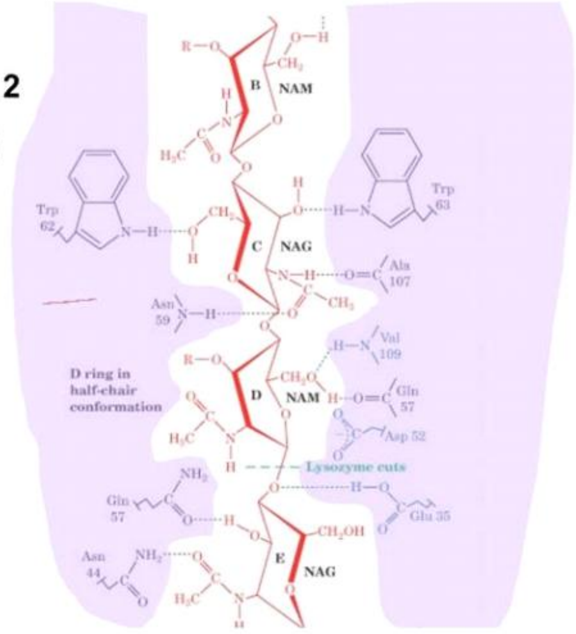
- **Slide 22: Figure 15-11**
  - Lysozyme works by straining by hexose ring
  - In chair conformation there is little sterric strain
  - In 1/2 chair there is steric strain. C1, C2, C5, and O5 become planar
  - 

Figure 15-11

- **Slide 23: figure 15-12 (modified from book)**
  - Diagram of active site of lysozyme
  - Lysozyme is shaded purple
  - NAM-NAG is in red
  - On D ring

        - In 1/2 chair conformation through binding with lysozyme residues
        - Figure 15-12 (modified)



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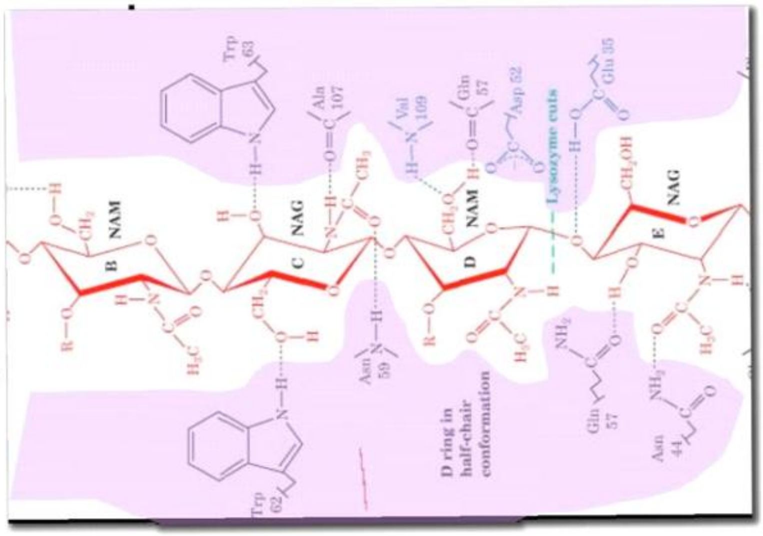
- **Slide 24: figure 15-12 (flipped 90^0)**
    - 1/2 chair confirmation is clear
    - Slipped because we are more used to seeing NAM-NAG horizontally
    - Transition state is electrostatically stabilized

Figure 15-12



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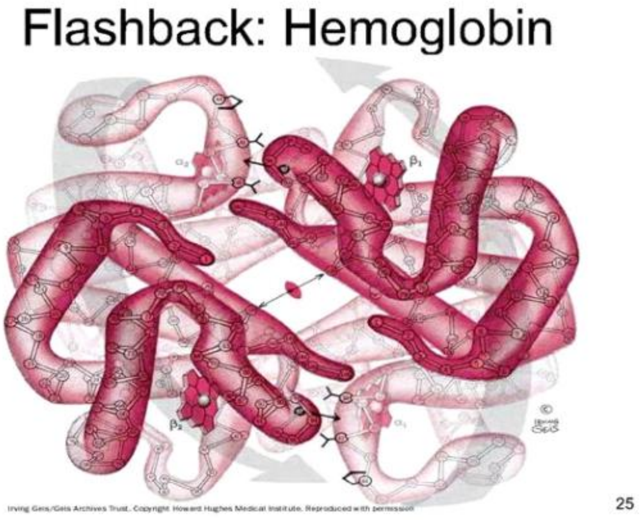
- **Slide 25: Flashback: Hemoglobin**
    - Figure 10-13
    - Oxegnated hemoglobin

- ○ 
  Flashback: Hemoglobin

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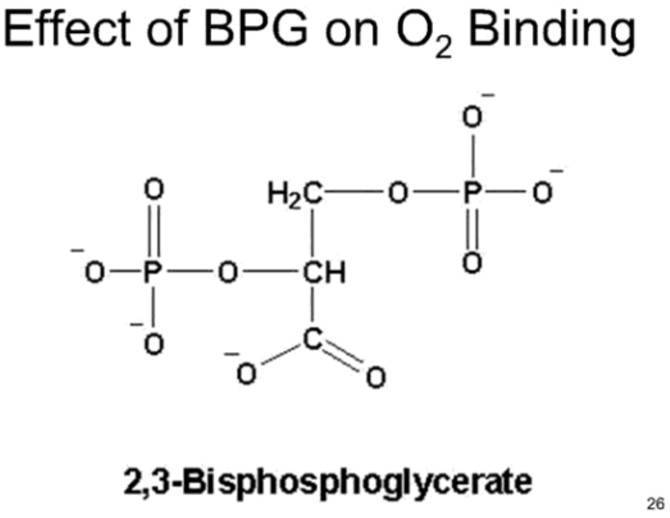
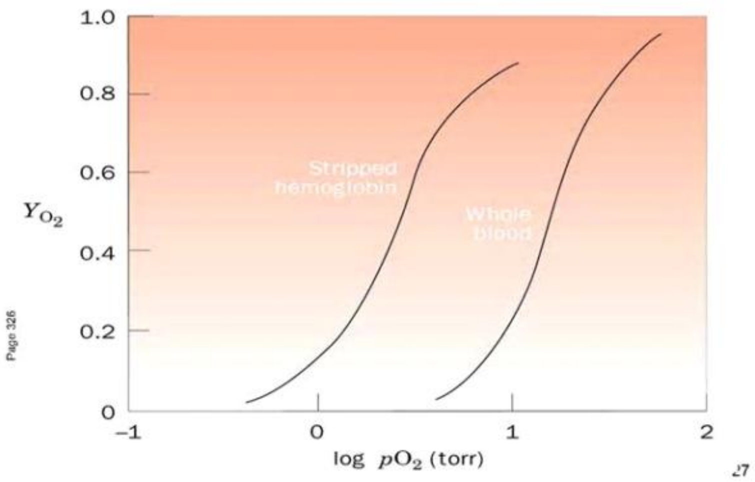
- **Slide 26: Effect of BPG on O_2 Binding**
  - BPG: 2,3-Bisphosphoglycerate
    - Effects oxygen binding
    - Found in blood
    - Hemogobin is able to disassociate from oxygen easier with BPG
    - 
      Effect of BPG on O_2 Binding

      2,3-Bisphosphoglycerate
      26

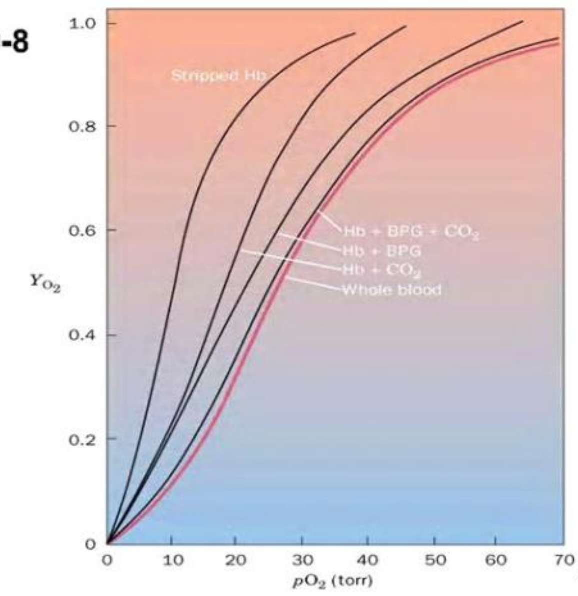
- **Side 27: figure 10-7**
  - Dissociation curve of hemoglobin (log scale)
    - Stripped hemoglobin (not in whole blood)
      - ☐ Binds onto oxygen tighter than whole blood (due to BPG)

- Whole blood
    - Binds onto oxygen looser than whole blood
- Drawn at pH 7
- Y_{O_2} is fractional saturation
    - Hemoglobin can hold 4 Oxygen molecules
- P_{50} occurs when 1/2 of oxygen is disassociated from hemoglobin
- Figure 10-7



- **Slide 28: Figure 10-8**
    - Disassociation curve (linear scale)
        - Arteries partial pressure is 100
        - Veins partial pressure is about 30
    - Whole Blood
    - Hb + CO_2
        - Bohr effect
        - Carbonic anhydrase
            - Lowers pH
        - At lower pH hemoglobin disassociates form oxygen than at a higher pH
    - Hb + BPG
    - Hb + BPG + CO_2
    - Stripped Hb
        - Tightest hold

- ▪ **Figure 10-8**



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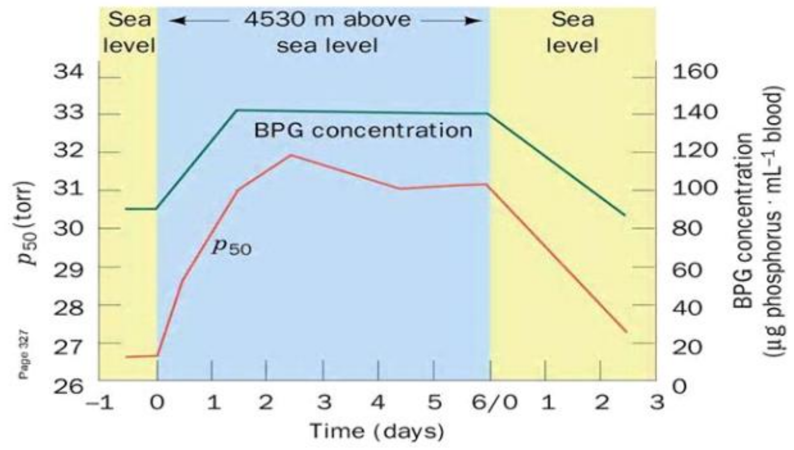
- **Slide 29: Figure 10-9**
  - At different altitudes and BPG
  - At higher altitudes, BPG concentration is higher in blood

  - **Figure 10-9**



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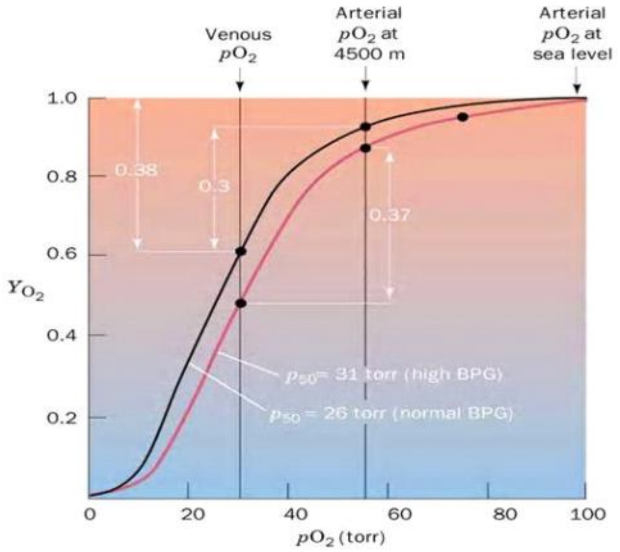
- **Slide 30: Figure 10-10**
  - High BPG releases oxygen more than low BPG

Figure 10-10



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