Notes 10/30

Tuesday, October 30, 2007
10:00 AM

Notes 1029
& 1030 - 2
Audio recording started: 10:02 AM Tuesday, October 30, 2007

- Read fine print of amino acid table p. 66 (His shown at pH 6)
- Extra credit opportunity - look VOH

Aliphatic vs Aromatic amino acids

- Aromatic
  - Low H:C rations
- Aliphatic

Outline:

- Lineweaver-burk plot
- K_{cat}
- Enzymes are sensiive
- Ihibition
- Catalytic mechanisms

Application and Interpretation of V_{max} and K_m

- K_{cat} turnover number
  - General rate constant that describes the limiting rate of any enzyme -cat reaction at saturation
  - $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$
    - $k_{cat} = k_2$
  - $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \xrightarrow{k_3} E + P$
    - $k_{cat} = k_3$
  - MM eq
    - $k_{cat} = \frac{V_{max}}{[E_t]}$
    - $V_0 = \frac{k_{cat}[E_t][S]}{kM + [S]}$
  - 1st order rate constant s^{-1}
  - The # of S molecules converted to P in a given time on a single enzyme molecule

	Eddie	Ernie
K_{cat}	10,000s^{-1}	10,000s^{-1}
K_m	0.1 mM	100 mM

Specificity constant: K_{cat}/K_m

$[S] << K_m \quad V_0 = \frac{k_{cat}[E_t][S]}{V_0}$ ← 2nd order rate constant

10^8 ... 10^9 M^{-1} -1

$[S]$ K_m $V_0 = \frac{k_{cat}[E_t][S]}{K_m}$

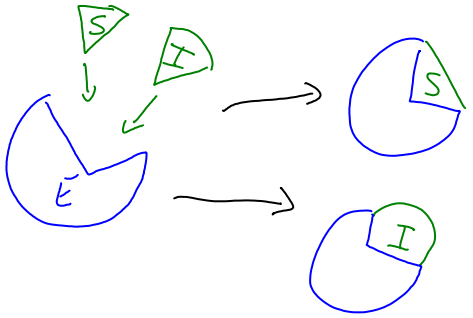
$\frac{V_0}{[E_t][S]}$ ← 2nd order rate constant

10^8 to 10^9 $M^{-1}s^{-1}$ ← "catalytically perfect

- How are enzymes regulated?
  - Availability
    - Rate of synthesis
    - Degradation
    - Availability of location in cell (sub cellular localizaton)
  - Activity
    - Factors effecting activity
      - pH may effect
        - Charges
        - Structure
      - Temperature
        - Increase energy of substrate
        - Protein denaturation
      - Effectors
        - Inhibitors (negative effects)
        - Positive effectors

- Enzymes are subject to inhibition
  - Many inhibitors look a lot like substrate
  - Competitive inhibition - inhibitor goes into substrate so prevents reaction
    - $E + I \rightleftharpoons E\text{-}I$ (E-I: inactive) $\quad K_I = \frac{[E][I]}{[E\text{-}I]}$

$\alpha = \left(1 + \frac{[I]}{K_I}\right) \quad V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$



  - Mixed inhibition - more complicated... won't focus on this

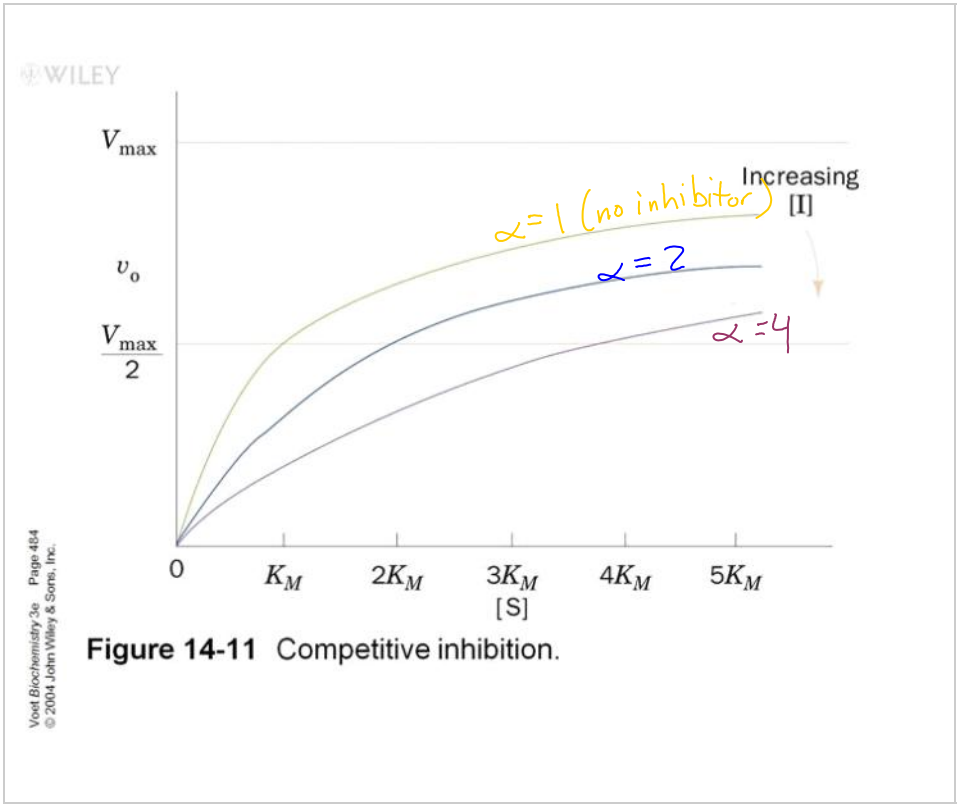


Figure 14-11 Competitive inhibition.

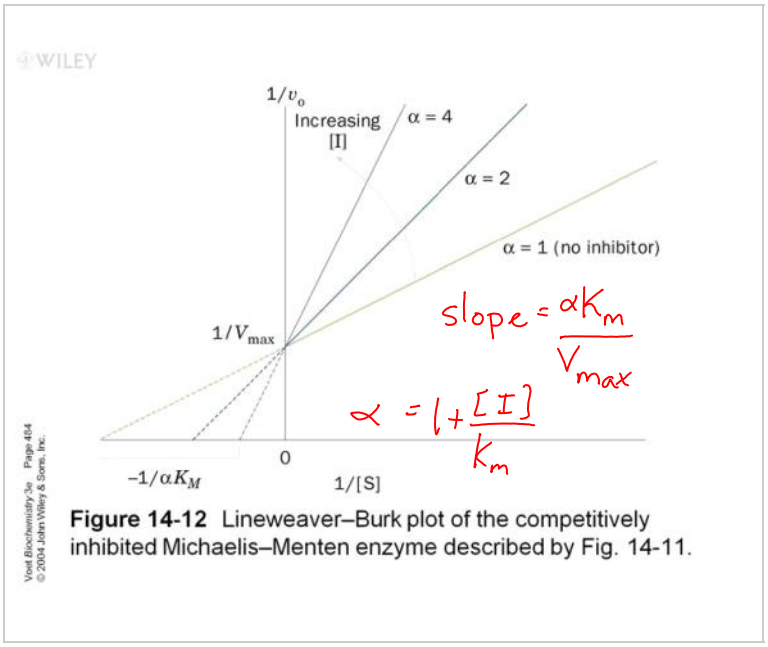


Figure 14-12 Lineweaver–Burk plot of the competitively inhibited Michaelis–Menten enzyme described by Fig. 14-11.

- **Catalytic Mechanisms**
  - Acid-base catalysis
  - Covalent catalysis
  - Metal ion catalysis
    - Metal ion in active site
  - Electrostatic catalysis
  - Catalysis through proximity and orientation effects
    - If you're able to put substrate close to active site and orient it correctly, it will help it go
  - Catalysis by preferential transition state bonding

Keto-enol tautomerization

Transition state can be stabilized with catalyst (see next slide)

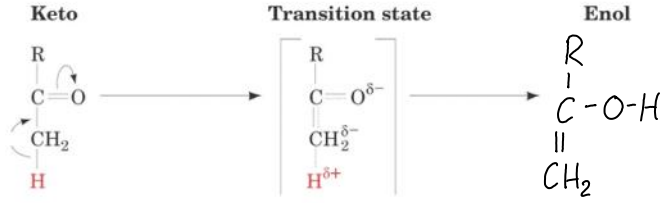


Figure 15-1a Mechanisms of keto–enol tautomerization. (*a*) Uncatalyzed.

Acid-Catalyzed

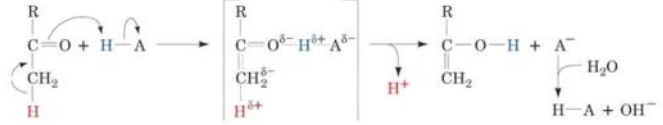


Figure 15-1b Mechanisms of keto–enol tautomerization. (*b*) General acid catalyzed.

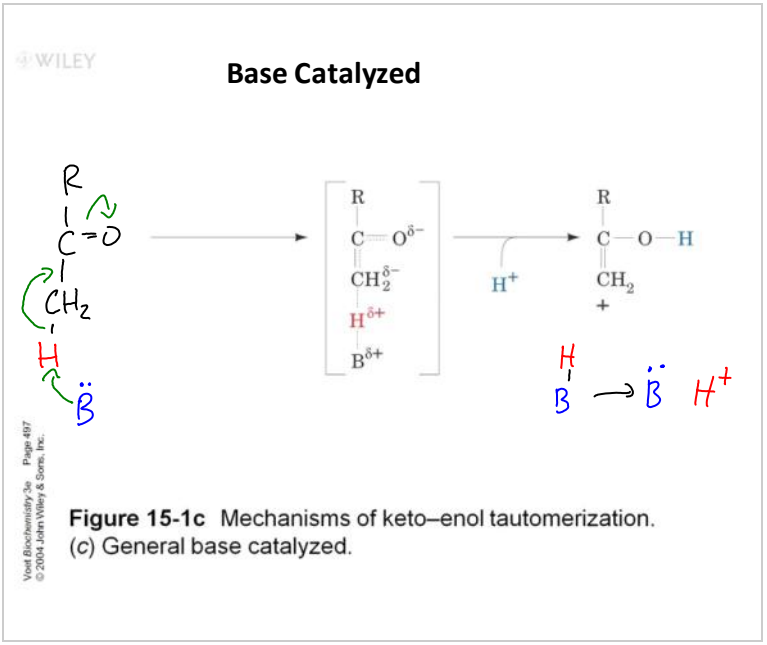


Figure 15-1c Mechanisms of keto–enol tautomerization. (*c*) General base catalyzed.

Concerted general acid-base catalyzed reaction

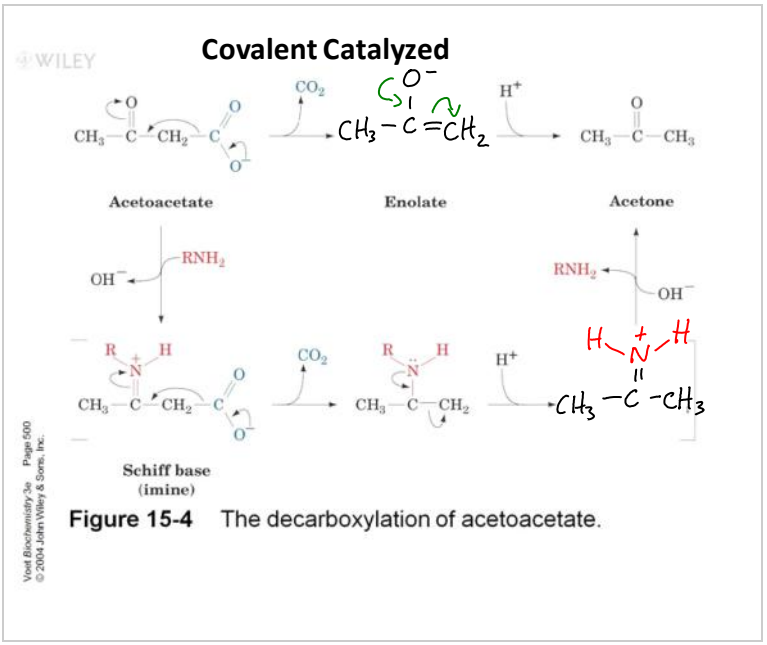


Figure 15-4 The decarboxylation of acetoacetate.

Human Carbonic Anhydrase
Takes carbonate and makes into water
Zn^{2+}

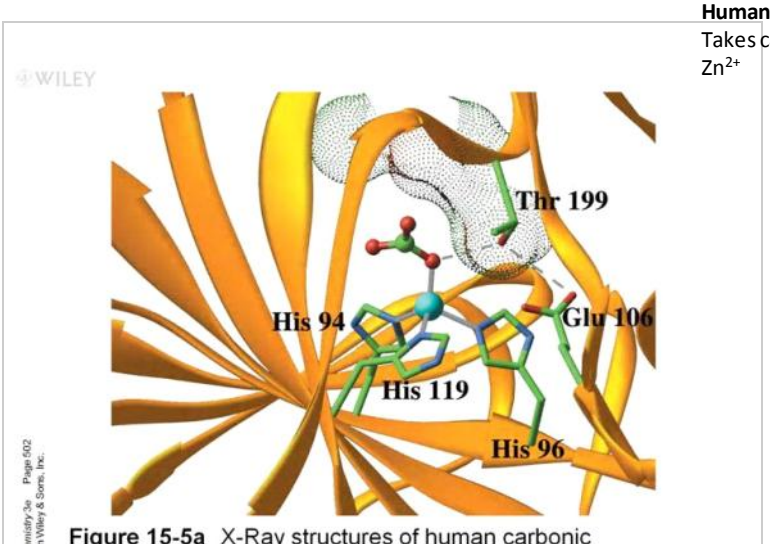


Figure 15-5a X-Ray structures of human carbonic

Takes carbonate and makes into water

Zn^{2+}

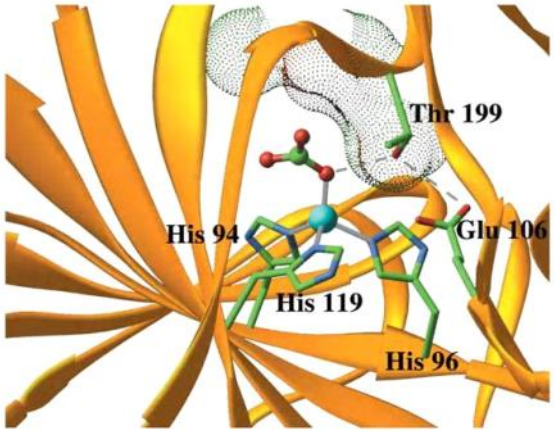


Figure 15-5a X-Ray structures of human carbonic anhydrase. (*a*) Its active site in complex with bicarbonate ion.

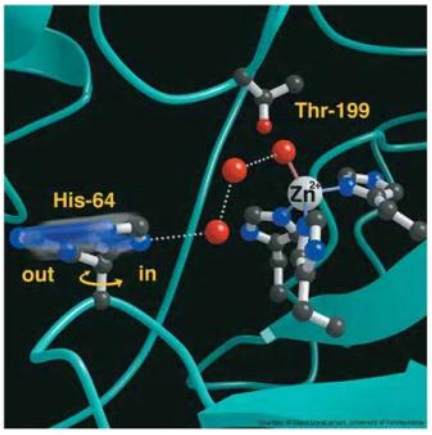
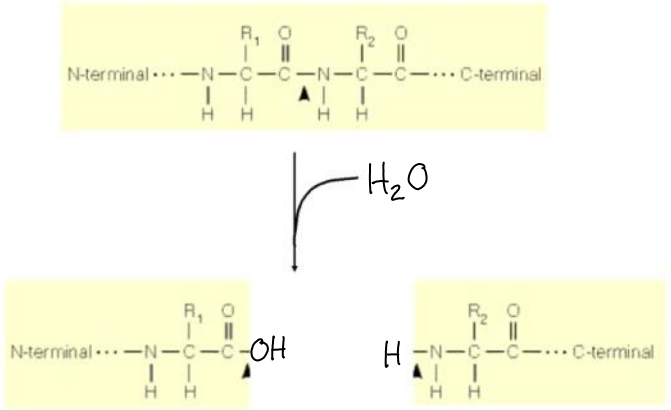


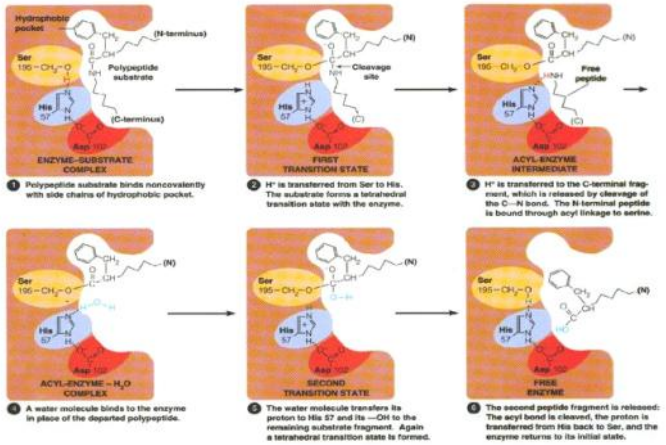
Figure 15-5b X-Ray structures of human carbonic anhydrase. (*b*) The active site showing the proton shuttle.

Serine Proteases



Serine Proteases and the Catalytic Triad

Not much time to go over this… will continue 10/31



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.