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Chapter 16

Mechanisms of Enzyme Action

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Enormous Rate Acceleration

- Rate accelerations by enzymes over uncatalyzed reactions can be very large, as much as 10^{16}. (See Table 16.1 for examples)
- A goal of studying enzyme mechanisms is to understand the factors contributing to this acceleration.

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Stabilization of Transition State

- Enzyme binds transition state better than it binds substrate
- Energy of $EX^{\ddagger}$ lowered more than energy of ES. (See Figure 16.1)
- Some factors "destabilize" the ES complex, bringing it closer in energy to $EX^{\ddagger}$.
  - (See Figures 16.2 and 16.3)

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Some Important Catalytic Mechanisms

- Destabilizing ES complex
  1. Entropy loss in ES formation (Fig. 16.4)
  2. Strain, desolvation, electrostatic effects (Figures 16.5 and 16.6)
- Stabilizing EX‡
  3. Covalent catalysis (Fig. 16.9)
  4. General acid or base catalysis (Fig. 16.11)
  5. Metal ion catalysis (Fig. 16.13)
  6. Proximity and Orientation (Figures 16.14 and 16.15) (same concept as in item 1 above)

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Transition State Analogs

- The affinity of the enzyme for the transition state may be 10^{-15} M!
- Analogs of the transition state are very good inhibitors.
  - Proline racemase reaction (Fig. 16.7)
  - Aldolase and adenosine deaminase (Fig. 16.8)

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Some Example Mechanisms

- Serine proteases
- Aspartic proteases
- Lysozyme

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Serine Proteases

- A mixture of covalent and general acid-base catalysis .
- Catalytic Triad (Figures 16.18 and 16.17)
  - Asp-102 functions only to orient His-57
  - His-57 acts as a general acid and base
  - Ser-195 forms a covalent bond with peptide to be cleaved

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Serine Proteases, con't.

- Stabilization of transition state.
  - Covalent bond formation turns a trigonal C into a tetrahedral C
  - The tetrahedral oxyanion intermediate is stabilized by N-Hs of Gly-193 and Ser-195.
    - (Figure page 519)
- Detailed mechanism (Figure 16.24)
- Burst kinetics (Figures 16.21 and 16.22)

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Serine Proteases, con't.

- Diisopropylfluorophosphate is a general irreversible inhibitor.
  - Binds to the serine residue (Figure 16.23)
- Serine proteases very similar in amino acid sequence. (Figure 16.16)
- Specificity at substrate binding pocket.
  - (Figure 16.19)

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The Aspartic Proteases

Pepsin, chymosin, cathepsin D, renin and HIV-1 protease

- All involve two Asp residues at the active site
- Two Asps work together as general acid-base catalysts, one has a relatively low pK_a, the other has a relatively high pK_a
  - Deprotonated Asp acts as general base, accepting a proton from HOH, forming OH^- in the transition state
  - Protonated Asp (general acid) donates a proton, facilitating formation of tetrahedral intermediate
    - (Mechanism, Fig. 16.27; pH profile, Fig. Page 525)

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Lysozyme

- The first enzyme whose structure was solved by X-ray crystallography (by David Phillips in 1965)
- Lysozyme hydrolyzes polysaccharide chains and ruptures certain bacterial cells by breaking down the cell wall.
  - Hydrolyzes at glycosidic bond of N-acetylmuramic acid residue. (See Figure 16.31)

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Lysozyme
Substrate Analog Studies

- Natural substrates are not stable in the active site for structural studies
- But analogs can be used - like $(NAG)_3$
  - Figure 16.33
- Fitting a NAG into the D site requires a distortion of the sugar.
  - (Figures 16.34 and 16.35)
- This argues for stabilization of a transition state via destabilization (distortion and strain) of the substrate.

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The Lysozyme Mechanism

- Studies with ^{18}O-enriched water show that the C_1-O bond is cleaved on the substrate between the D and E sites.
- This incorporates ^{18}O into C_1
  - Figure 16.36
- Glu^{35} acts as a general acid
  - It is in a hydrophobic environment, causing it to have a much higher pK and to remain protonated.
- Asp^{52} stabilizes a carbonium ion intermediate
  - Figure 16.37