Lecture 12

Enzymes: Inhibition

Reading: Berg, Tymoczko & Stryer, 6th ed., Chapter 8, pp. 225-236

Problems: pp. 238-239, chapter 8, #1, 2, 4a,b, 5a,b, 7, 10


Key Concepts

- $K_m$ and $V_{max}$ can be determined from double reciprocal plots ($1/V_o$ vs. $1/[S]$).
- Enzyme inhibitors: compounds that reduce velocity of enzyme-catalyzed reactions.
- 2 types: reversible and irreversible
  - reversible inhibitors
    - competitive: inhibitor (I) increases $K_m$ but has no effect on $V_{max}$.
    - uncompetitive: I decreases both $K_m$ and $V_{max}$ by same factor.
    - pure noncompetitive: I decreases $V_{max}$ but has no effect on $K_m$.
  - can distinguish different types of reversible inhibitors using double reciprocal plots ($1/V_o$ vs. $1/[S]$) in absence of I and in presence of different concentrations of I.
- Irreversible inhibitors
  - cause irreversible (generally covalent) modification of the enzyme, inactivating it.
  - several types:
    - group-specific chemical modifying reagents that would react with certain types of functional groups on many different enzymes
    - substrate analogs with a reactive group on them (so more specific for one enzyme)
    - "suicide" substrates (mechanism-based inhibitors): not reactive until the specific chemical mechanism of their target enzyme makes them "kill" (covalently modify) the active site they're in.
Key Concepts, continued

- Both reversible and irreversible inhibitors very helpful for:
  - providing information about shape of active site and types of amino acid side chains there
  - working out enzyme mechanisms
  - providing info about control of metabolic pathways
  - design of drugs

Learning Objectives

- Terminology: double reciprocal plot, reversible inhibition (competitive, pure noncompetitive, uncompetitive), irreversible inhibition, affinity label, transition state analog, suicide inhibition (mechanism-based inhibitor)
- Given a hyperbolic $V_o/V_{max}$ vs. $[S]$ plot, explain the meaning of the ratio $V_o/V_{max}$ in terms of occupied active site concentration and total active site concentration, and find $K_m$ directly from the graph, including its units.
- Given a Lineweaver-Burk plot ($1/V_o$ vs. $1/[S]$), find/calculate $V_{max}$ and $K_m$ from the graph.

Learning Objectives, continued

- Explain competitive, uncompetitive, and pure noncompetitive inhibition in terms of a diagram of linked reaction equilibria for formation of ES, EI, and (if it can form) EIS. (See Berg et al., Figs. 8-17, 8-18, 8-19.)
  - Explain how these 3 types of inhibition can be distinguished from each other graphically:
    a) on a $V_o$ vs. $[S]$ plot, and
    b) on a double reciprocal (Lineweaver-Burk) plot.
- What is the effect of a competitive inhibitor on $K_m$ and on $V_{max}$ (compared to the values in absence of inhibitor)?
- What is the effect of an uncompetitive inhibitor on $K_m$ and on $V_{max}$ (compared to the values in absence of inhibitor)?
- What is the effect of a pure noncompetitive inhibitor on $K_m$ and on $V_{max}$ (compared to the values in absence of inhibitor)?
- Why is ethanol used as an antidote for ethylene glycol (antifreeze) poisoning?
- What type of inhibitor is pencillin?
- Give some other specific examples of reversible and irreversible enzyme inhibition.
Graphical Determination of $K_m$ and $V_{max}$

- Enzyme kinetics (experiments measuring $V_o$ as a function of $[S]$ to determine $V_{max}$ and $K_m$)
  - can use computer with programs to fit data to Michaelis-Menten equation, extracting $K_m$ and $V_{max}$ from the data.
  - can't extrapolate "by hand" on Michaelis-Menten $V_o$ vs. $[S]$ hyperbolic plot to get accurate $V_{max}$ and $K_m$ values

\[
V_o = V_{max} \frac{[S]}{K_m + [S]}
\]

- simple solution without a computer: use linear version, double reciprocal plot (Lineweaver-Burk Plot)
  - Take reciprocal of both sides of M-M Equation and rearrange to get Lineweaver-Burk equation:

\[
\frac{1}{V_o} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}
\]

Lineweaver Burk Plot (double reciprocal plot)

- Equation of a straight line: $y = mx + b$
  - $x$–intercept = $-1/K_m$
  - NOTE: $x$-intercept is a negative number, which itself is the negative of the reciprocal of $K_m$
  - $(-) (-) = (+)$
  - $K_m$ is always a positive number (the substrate concentration at which $V = 1/2 V_{max}$).
  - $y$-intercept = $1/V_{max}$
  - slope = $K_m / V_{max}$

Berg et al., Fig. 8-13
Lineweaver Burk Plot (double reciprocal plot)

\[
\frac{1}{V_o} = \frac{K_m}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}}
\]

- Equation of a straight line: \( y = mx + b \)
- \( x\)-intercept = \(-1/K_m\)
- \( y\)-intercept = \(1/V_{\text{max}}\)
- slope = \(K_m / V_{\text{max}}\)
- What if \(x\)-intercept = \(-2 \times 10^4 \text{ M}^{-1}\)? (a reciprocal concentration, extrapolated back to negative values to get intercept)
  \(-1/K_m = -2 \times 10^4 \text{ M}^{-1}\)
- What is \(K_m\)?
  \(K_m = -1/(-2 \times 10^4 \text{ M}^{-1}) = +0.5 \times 10^{-4} \text{ M}\) (or \(5 \times 10^{-5}\) M)
- Note POSITIVE value!

Enzyme Inhibitors

- reversible (rapid binding/release from enzyme in an equilibrium) or
- irreversible (very tightly bound to enzyme, either covalently or noncovalently, but effectively don't come off)

Reversible inhibitors
- type of inhibition diagnosed by effect of inhibitor on \(K_m\) and \(V_{\text{max}}\)
- effects/diagnosis obvious on double reciprocal plot.
- basis for drugs' actions
- research tools in figuring out enzyme chemical catalytic mechanisms

Reversible Inhibition:
- competitive
- uncompetitive, or
- noncompetitive
- defined operationally by their effects on enzyme kinetic parameters, \(K_m\) and/or \(V_{\text{max}}\).
Reversible Enzyme Inhibitors, continued

**ES Complex**
- Decreases $K_m$ and reduces $V_{max}$ by same factor so slope of $1/V_o$ vs. $1/[S]$ doesn't change
- binds only to ES complex

**Competitive inhibitor**
- Prevents $S$ from binding, so increases $K_m$
- Has no effect on $V_{max}$

**“Pure” Noncompetitive inhibitor**
- Has no effect on $K_m$ (no effect on $S$ binding)
- reduces $V_{max}$ (reduces $k_{cat}$)

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**Competitive Inhibition**
- Enzyme can bind either substrate or inhibitor, but **not both**
- Either
  - Inhibitor binds in same site as $S$, or
  - (more rarely) inhibitor binds to different site, causing conformational change in active site so substrate can't bind.
- Enzyme active site can be **free** (neither ligand bound, $E$) or have $S$ **bound** (ES complex) or have $I$ **bound** (EI complex), but **there's no ES$I$ complex**.
- Competitive inhibitor increases apparent $K_m$, but doesn't affect $V_{max}$.
- High $[S]$ overcomes effect of $I$.

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Berg et al. Fig. 8-15

Berg et al., Fig. 8-17
**Competitive Inhibition, continued**

- Competitive inhibitor increases apparent $K_m$, but doesn’t affect $V_{max}$.
- High [S] overcomes effect of I.

\[
\text{Berg et al., Fig. 8-17}
\]

- $K_m$ higher with inhibitor than without inhibitor
- $V_{max}$ not changed

\[
\text{Berg et al., Fig. 8-20}
\]

**Uncompetitive Inhibition**

- Inhibitor binds only to E-S complex.
- Binding site for I is created only upon S binding.
- There’s no detectible EI complex, only E, ES, and ESI, but ESI can’t make product.
- High [S] does NOT overcome effect of I.

\[
\text{Berg et al., Fig. 8-18}
\]
Uncompetitive Inhibition, continued

- Inhibitor binds only to E-S complex.
- Binding site for I is created only upon S binding.
- There’s no EI complex, only E, ES, and ESI, but ESI can’t make product.
- \( K_m \) lower than without inhibitor
- \( V_{max} \) lower by exactly same factor
- Slope of \( 1/V_o \) vs. \( 1/[S] \) unchanged

Pure Noncompetitive Inhibition

- Enzyme can bind both substrate and inhibitor simultaneously, but ESI complex can’t make product.
- Inhibitor must dissociate in order for catalysis to occur.
- Inhibitor binding decreases \( V_{max} \) (decreases \( k_{cat} \)), but has no effect on \( K_m \).
Pure Noncompetitive Inhibition, continued

- Enzyme can bind both substrate and inhibitor simultaneously.
- ESI complex can't make product, so I must dissociate in order for catalysis to occur.
- Inhibitor binding decreases $V_{max}$ (decreases $k_{cat}$).

![Diagram showing the effect of noncompetitive inhibition](Berg et al., Fig. 8-19)

- $V_{max}$ decreased
- no effect on $K_m$
- High [S] does NOT overcome effect of I.

Examples of reversible inhibitors (competitive)

- Inhibitors of dihydrofolate reductase
- enzyme in nucleotide biosynthesis (new nucleic acids required for cell division)
- vertebrate DHFR: inhibited by methotrexate
  - selectively kills rapidly dividing cells (cancer chemo)
- prokaryotic DHFR - inhibited by trimethoprim (has little effect on human DHFR, so a good antibiotic)

![Diagram showing examples of inhibitors](Berg et al., Fig. 8-16)
Another example of a reversible inhibitor (competitive)

Succinate dehydrogenase (enzyme in citrate cycle)

\[
\text{succinate (substrate)} \quad \text{malonate (competitive inhibitor)}
\]

(more examples at the end of notes, discussion of some examples of pharmaceutically important enzyme inhibitors)

Irreversible Inhibition

1) group-specific covalent modifying agents
2) affinity labels
3) transition state analogs
4) suicide inhibitors (mechanism-based inhibitors)

1) group specific covalent modifying agents: react with specific type of enzyme functional group (e.g., Ser-OH, or Cys-SH, or His imidazole) on any enzyme/protein

Diisopropylphosphofluoridate (DIPF), potent nerve gas (poison)

- reacts with specific, reactive Ser-OH on many enzymes
- example: reaction with reactive, catalytic OH group of acetylcholinesterase at synaptic junctions
- modified enzyme inactive

Berg et al., Fig. 8-23
2) **affinity labels**: structural similarity to substrate "guides" reagent to active site
   *reaction at active site covalently inactivates enzyme
   - Example: **Tosyl phenylalanyl chloromethylketone (TPCK)**
   - phenyl group
     binds in substrate specificity site of chymotrypsin

3) **transition state analogs**
   - structurally similar to transition state, which binds even more tightly to enzyme than substrate binds, so very high affinity for active site
   - See Berg et al., Fig. 8.28 for an example
   - Transition state analogs useful for:
     1. understanding catalytic mechanisms (clues about structure of transition state)
     2. very specific inhibitors of enzymes (pharmaceutical applications)
     3. antigens for immunizing lab animals to generate antibodies with binding sites complementary to the transition state such that the antibodies themselves have catalytic activity ("abzymes")

4) **suicide substrates** (mechanism-based inhibitors)
   - Structural similarity to substrate "guides" reagent to active site.
   - Enzyme **treats it as a substrate**, starting chemical catalytic process with inhibitor.
   - **Chemical mechanism itself** leads enzyme to react covalently with inhibitor, thus "committing suicide".
   - Mechanism-based inhibition depends on **chemical mechanism of catalysis**.
     Example: penicillin (inhibits an enzyme, a transpeptidase, required for bacterial cell wall synthesis) -- see below, and text pp. 232-234.
Just a few of thousands of pharmaceutically important enzyme inhibitors

1) Penicillin (an antibiotic)
   - *both* a transition-state analog and a suicide substrate
   - covalently inhibits a transpeptidase (enzyme) involved in bacterial cell wall synthesis (eukaryotic cells don't have this enzyme)
   - Normal transpeptidase catalytic mechanism: nucleophilic attack of enzyme Ser–OH on substrate, making a covalent acyl-enzyme intermediate
   - Covalent intermediate continues in enzyme-catalyzed reaction to form peptide cross-link in peptidoglycan structure of cell wall, regenerating free enzyme for another round of catalysis.
   - Penicillin resembles transition state in structure, so penicillin
     a) binds very tightly and
     b) is very reactive.
   - Normal catalytic mechanism makes covalent intermediate with penicillin, but **enzyme-penicillin derivative can't continue**.
   - Inhibitor is "stuck" on enzyme (covalently attached), and modified enzyme is now inactive because of its own catalytic activity -- it committed suicide!

more pharmaceutically important enzyme inhibitors

2) Aspirin (acetylsalicylate), a non-steroidal anti-inflammatory drug (NSAID) (See also Berg et al., 6th ed., Fig. 12.25, p. 339.)
   - covalently (irreversibly) inactivates enzyme (PGH$_2$ synthase, cyclooxygenase activity, also known in its two forms as COX 1 and COX 2) involved in prostaglandin biosynthesis
   - anti-inflammatory action due to blocking of prostaglandin synthesis
   - covalently modifies (acetylates) specific Ser-OH group in channel through which substrate (arachidonic acid, a 20-C fatty acid) must pass to reach active site; NSAIDs block active site access, inhibiting enzyme, preventing prostaglandin synthesis, reducing inflammation.
   - Jmol structure of COX 2 with various inhibitors/drugs: http://www.biochem.arizona.edu/classes/bioc462/462a/jmol/cox12/cox121.htm

Aspirin also reduces blood clotting because same enzyme is also needed for synthesis of thromboxane A$_2$ (TXA$_2$), involved in blood platelet aggregation in clotting.
more pharmaceutically important enzyme inhibitors

3) other NSAIDs (nonsteroidal anti-inflammatory agents) besides aspirin: e.g., ibuprofen (=Motrin, Advil), acetaminophen (=Tylenol), indomethacin, naproxen (=Aleve)
- competitive (reversible) inhibitors of cyclooxygenase activity of PGH₂ synthase
- block prostaglandin synthesis and thus act as anti-inflammatory agents
  reversibly bind in channel through which substrate must access enzyme active site, so act as competitive inhibitors by preventing substrate binding, even though they don't bind in the active site.
- (Aspirin inhibits same enzyme irreversibly, by acetylating Ser-OH group in "entrance" channel to active site, but not actually in active site.)
- Jmol structure of COX 2 with various inhibitors/drugs: http://www.biochem.arizona.edu/classes/bioc462/462a/jmol/cox12/cox121.htm

4) statins
  - Inhibitors of HMG-CoA reductase, the rate-limiting, control enzyme in cholesterol biosynthesis
    - Competitive inhibitors of HMG-CoA reductase are cholesterol-lowering drugs (decrease rate of cellular cholesterol biosynthesis).
    - structures similar to substrate for HMG-CoA reductase (mevalonate)
      - e.g., Mevacor (lovastatin), Pravachol (pravastatin), and Zocor (simvastatin)

more pharmaceutically important enzyme inhibitors

5) Ethanol
- antidote for ethylene glycol (antifreeze) or methanol (wood alcohol) poisoning
- Toxic effects of ethylene glycol and of methanol depend on their -OH groups being oxidized to aldehyde (by alcohol dehydrogenase in body) and then to carboxylic acids
- Ethanol (another substrate with less toxic oxidation products) competes for binding to alcohol dehydrogenase
- If alcohol dehydrogenase molecules are all occupied with ethanol as a substrate, ethylene glycol (or methanol) passes through body without being oxidized and is excreted (kidneys)

6) anti-HIV drugs (anti-AIDS)
- AZT: metabolized to AZT-triphosphate, which terminates growing DNA chains in reaction catalyzed by HIV viral reverse transcriptase; much higher affinity for HIV reverse transcriptase than for cellular DNA polymerases
- Saquinavir and Ritonavir: VERY tight-binding inhibitors (transition state analogs) of HIV protease (enzyme needed to process large HIV polyprotein precursors to release viral proteins)