Lecture 12: Enzymes: Inhibition

Reading: Berg, Tymoczko & Stryer, Chapter 8, pp. 225-236
Problems: pp. 238-239, chapter 8, #1, 2, 4a,b, 5a,b, 7, 10
Updated on: 2/21/07 at 9:00 pm (deleted problems #4c,d,e and #5c,d)

Key Concepts

- \( K_m \) and \( V_{\text{max}} \) can be determined from double reciprocal plots (\( 1/V_o \) vs. \( 1/[S] \)).
- Enzyme inhibitors are compounds that REDUCE VELOCITY OF ENZYME-CATALYZED REACTIONS.
  - 2 types: reversible and irreversible
    - reversible inhibition:
      - competitive: \( I \) increases \( K_m \); no effect on \( V_{\text{max}} \)
      - pure noncompetitive: \( I \) decreases \( V_{\text{max}} \); "pure" noncompetitive inhibitors have no effect on \( K_m \).
    - The 2 types of reversible inhibitors can be distinguished on the basis of double reciprocal plots (\( 1/v_o \) vs. \( 1/[S] \)) in the absence of \( I \) and in the presence of different concentrations of \( I \).
  - Irreversible inhibitors cause irreversible (generally covalent) modification of the enzyme, inactivating it.
    - Such inhibitors can be group-specific chemical modifying reagents that would react with certain types of functional groups on a variety of enzymes,
    - substrate analogs with a reactive group on them (so more specific for one enzyme), or
    - "suicide" substrates (mechanism-based inhibitors) that aren't reactive until the specific chemical mechanism of their target enzyme makes them "kill" (covalently modify) the active site they're in.
- Both reversible and irreversible inhibitors can be very helpful in
  - 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

- Given a hyperbolic \( V_o/V_{\text{max}} \) vs. \([S]\) plot, explain the meaning of the ratio \( V_o/V_{\text{max}} \) in terms of occupied active site concentration and total active site concentration, and read \( K_M \) directly off the graph, including its units.
- Given a Lineweaver-Burk plot (\( 1/V_o \) vs. \( 1/[S] \)), find/calculate \( V_{\text{max}} \) and \( K_M \) from the graph.
- Explain competitive, uncompetitive, and noncompetitive inhibition in terms of a diagram of linked reaction equilibria for formation of ES, EI, and (if it can form) EIS. (See Berg, Tymoczko & Stryer, 6th ed., Figs. 8.17, 8.18, and 8.19.) Explain how these two 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 \( V_{\text{max}} \) (compared to the values in absence of inhibitor)?
- What is the effect of a noncompetitive inhibitor on \( K_M \) and on \( V_{\text{max}} \) (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 \( V_{\text{max}} \)

- Enzyme kinetics (experiments measuring \( V_o \) as a function of \([S]\) to determine \( V_{\text{max}} \) and \( K_M \)): can use computer with curve-fitting programs to extract \( K_M \) and \( V_{\text{max}} \) from the data.
can’t extrapolate “by hand” on Michaelis-Menten $V_o$ vs. [S] hyperbolic plot to get accurate $V_{\text{max}}$ and $K_M$ values, so use linear version, the double reciprocal plot (Lineweaver-Burk Plot)

- taking reciprocal of both sides of M-M Equation & rearranging:

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

- Equation of a straight line: $y = mx + b$

**LINEWEAVER–BURK PLOT (double reciprocal plot, $1/V_o$ vs. $1/[S]$)**

- 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 $V = 1/2 V_{\text{max}}$).

  There’s no such thing as a negative concentration!

- Y–INTERCEPT = $1/V_{\text{max}}$
- SLOPE = $K_M / V_{\text{max}}$

Berg, Tymoczko & Stryer, 6th ed. Fig. 8:13: A double reciprocal (Lineweaver-Burk) plot

- What if x-intercept = $-2 \times 10^4 \text{M}^{-1}$? (a reciprocal concentration, but extrapolated back to negative values to get intercept)
  
  That means $-1/ K_M = -2 \times 10^4 \text{M}^{-1}$
  
  What is $K_M$?

- $K_M = -1/(−2 \times 10^4 \text{M}^{-1}) = +0.5 \times 10^{-4} \text{M}$ (or $5 \times 10^{-5} \text{M}$)
  
  Note POSITIVE value!

- INHIBITORS/TYPE OF INHIBITION diagnosed by effect of inhibitor on $K_M$ AND $V_{\text{max}}$

  effects/diagnosis obvious on double reciprocal plot.

**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)

- 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 $V_{\text{max}}$
  
  - Berg, Tymoczko & Stryer, 6th ed. Fig. 8.15: Schematic diagram of competitive, uncompetitive, and noncompetitive inhibition

- E-S complex

- Uncompetitive inhibitor binds only to E-S complex.
• **Competitive inhibitor** binds at active site and thus prevents substrate from binding.

• **Noncompetitive inhibitor** does not interfere with S binding; it reduces turnover number, $k_{cat}$.

• **Competitive Inhibition**
  - Enzyme can bind either substrate or inhibitor, but NOT BOTH
  - Either
    - Inhibitor resembles substrate chemically/sterically so they compete for binding to same site, or
    - (more rarely) inhibitor binds to different site, causing a conformational change in the 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 EIS complex.
  - Competitive inhibitor increases $K_M$, but doesn't affect $V_{max}$.

• **GRAPHICAL DEPICTION OF COMPETITIVE INHIBITION**
  - Apparent $K_M$ changed (increased), but NO EFFECT on $V_{max}$. (High [S] overcomes effect of I.)
Berg, Tymoczko & Stryer, 6th ed. Fig. 8.17: $V_0$ vs. [S], competitive inhibition

Berg, Tymoczko & Stryer, 6th ed. Fig. 8.20: $1/V_0$ vs. $1/[S]$, competitive inhibition

- **Uncompetitive Inhibition**
  - Inhibitor binds only to E-S complex, because binding site for I is created only upon S binding.
  - Uncompetitive inhibitor decreases $K_M$ (S appears to bind more tightly in presence of I), and decreases $V_{max}$ by the same factor.

- **GRAPHICAL DEPICTION OF UNCOMPETITIVE INHIBITION**
  - Both $K_M$ and $V_{max}$ change, both decreased by same factor. (High [S] does NOT overcome effect of I.)
**Noncompetitive Inhibition**

("pure" noncompetitive inhibition, simplest case)
- Enzyme can bind both substrate and inhibitor simultaneously, but **ESI complex can't make product**, so I must dissociate in order for catalysis to occur. Inhibitor binding decreases $V_{\text{max}}$ (decreases $k_{\text{cat}}$).

**GRAPHICAL DEPICTION OF PURE NONCOMPETITIVE INHIBITION**
- $V_{\text{max}}$ decreased but NO EFFECT on $K_M$ (high $[S]$ does NOT overcome effect of $I$)
Berg, Tymoczko & Stryer, 6th ed. Fig. 8.19: $V_0$ vs. [S], *PURE NON*competitive inhibition

\[
\begin{align*}
E + I & \rightleftharpoons EI \quad K_i \\
E + S & \rightarrow ES \rightarrow E + P \quad [I] = K_i \\
E + I + S & \rightarrow ESI \quad [I] = 10 K_i \\
E + I + S & \rightarrow ESI \quad [I] = 5 K_i
\end{align*}
\]

Berg, Tymoczko & Stryer, 6th ed. Fig. 8.22: $1/V_0$ vs. $1/[S]$, *PURE NON*competitive inhibition

- **Examples of reversible inhibitors (competitive)**
  - dihydrofolate reductase (enzyme in nucleotide biosynthesis):
  - alcohol dehydrogenase (enzyme):

http://www.biochem.arizona.edu/classes/bioc460/spring/460web/lectures/LEC12_EnzInhib/LEC12_EnzInhib.html
Methotrexate used for cancer chemotherapy

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

1) group specific covalent modifying agents
- react with specific type of enzyme functional group (e.g., with Ser-OH, or with Cys-SH, or with His imidazole)
  - Berg, Tymoczko & Stryer, 6th ed. Fig. 8.23: *Diisopropylphosphofluoridate (DIPF), a potent nerve gas (poison) reacts with specific, reactive Ser-OH on enzymes*
  - example: reaction with the reactive, catalytic OH group of the enzyme *acetylcholinesterase* at synaptic junctions (modified enzyme inactive)
2) **affinity labels**
- structural similarity to substrate "guides" reagent to active site
- reaction at active site covalently inactivates enzyme
- Berg, Tymoczko & Stryer, 6th ed. Fig. 8.25, *Tosyl phenylalanyl chloromethylketone (TPCK)* has a *phenyl group* that binds in substrate specificity site of chymotrypsin.

![Diagram of Natural substrate for chymotrypsin](image)

3) **suicide substrates** (mechanism-based inhibitors)
- structural similarity to substrate "guides" reagent to active site, and
- enzyme TREATS IT AS A SUBSTRATE, starting chemical catalytic process with the inhibitor.
- However, 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.

4) **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, Tymoczko & Stryer, 6th ed. Fig. 8.28 for an example
- transition state analogs useful for:
  - understanding catalytic mechanisms (clues about structure of transition state)
  - very specific inhibitors of enzymes (pharmaceutical applications)
  - 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")

**JUST A FEW OF THOUSANDS OF EXAMPLES OF PHARMACOLOGICALLY IMPORTANT INHIBITORS:**

1) **Penicillin** (an antibiotic)
- example of an inhibitor that is 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 enzyme catalytic mechanism:
  - nucleophilic attack of enzyme Ser–OH on substrate, making a covalent acyl-enzyme intermediate
  - covalent intermediate continues in the 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 inactive -- it committed suicide!
  (See Berg, Tymoczko & Stryer, 6th ed. pp. 232-234.)

- **2) Aspirin (acetylsalicylate)**
  - covalently (irreversibly) inactivates enzyme (PGH₂ synthase, cyclooxygenase activity, COX 1 and COX 2) involved in prostaglandin biosynthesis
    - covalently modifies (acetylates) a Ser-OH group, blocking access of substrates to active site
    - *Jmol structure of COX 2* with various inhibitors/drugs
  - Anti-inflammatory action of aspirin is due to blocking of prostaglandin synthesis.
  - Aspirin also antithrombotic (reduces blood clotting) because same enzyme is also needed for synthesis of thromboxane A₂ (TXA₂), which is involved in blood platelet aggregation in clotting.
  - See below for discussion of reversible inhibition of same enzyme by non-aspirin nonsteroidal antiinflammatory drugs (NSAIDs).

- **3) 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)

- **4) HIV enzymes (anti-AIDS drugs)**
  - AZT: metabolized to AZT-triphosphate, which terminates the growing DNA chains in the 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)

- **5) inhibitors of dihydrofolate reductase (DHFR)**
  - enzyme in both prokaryotic and eukaryotic cells essential for biosynthesis of thymidine monophosphate, precursor for the triphosphate needed for DNA synthesis
  - vertebrate DHFR - inhibited by methotrexate (selectively kills rapidly dividing cells)
  - prokaryotic DHFR - inhibited by trimethoprim (has little effect on human DHFR, so a good antibiotic)

- **6) statins**
  - HMG-CoA reductase (enzyme): 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 (mevalonate)
  - e.g., Mevacor (lovastatin), Pravachol (pravastatin), and Zocor (simvastatin)

- **7) non-aspirin NSAIDs (nonsteroidal anti-inflammatory agents)**
  - 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
  - block access to enzyme active site, so they 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.