

Exam 2
BC 367
November 19, 2009
Answer Key

1. D
2. D
3. B
4. A
5. A
6. A
7. B
8. E
9. B
10. D

11. a) No, the rate plots are sigmoidal.

b) Inhibitor.

c) The enzyme must be an allosteric enzyme with a regulatory site for ATP in addition to its substrate-binding site.

12. a)

Candidate	Hemoglobin Variant
Solomon	Normal (HbA)
Jack	Hb Rahere, in which Lys 82 β → Thr
Klaudia	Novel Hb, with an enhanced Bohr effect
Elaura	Hb Johnstown, with $p_{50} < 5$ mm Hg

b) This Lys residue is involved in BPG binding. This mutant will bind BPG less readily, resulting in stabilization of the R state and shifting of the binding curve to the left.

c) A mutation that leads to a new salt bridge that stabilizes the T state, such as a new His residue that binds to an Asp/Glu in another subunit. The new amino acid should have a pK_a relatively close to neutrality.

d) The R state is stabilized. There could be a new interaction between subunits that stabilizes the R state.

e) Klaudia, because she can deliver much more O_2 at altitude than the other candidates.

13. a) y axis has units of 1/rate (sec/nM); x axis has units of 1/[S] (nM⁻¹).

b) indinavir is a competitive inhibitor.

c) saquinavir is an uncompetitive inhibitor.

d) For a competitive inhibitor: $K_{Mapp} = \alpha K_M$ where $\alpha = 1 + [I]/K_I$

Can obtain the K_M values from the equations of the line (x intercept = $-1/K_M$).

For the inhibited rxn, $K_{Mapp} = 0.18$ nM; for the control reaction, $K_M = 0.082$ nM.

$$0.18 \text{ nM} = \{1 + [I]/K_I\} 0.082 \text{ nM}$$

$$K_I = 13 \text{ nM}$$

e) For an uncompetitive inhibitor: $V_{Maxapp} = V_{Max}/\alpha'$ where $\alpha' = 1 + [I]/K_d$

Can obtain the V_{Max} values from the equations of the line (y intercept = $1/V_{Max}$).

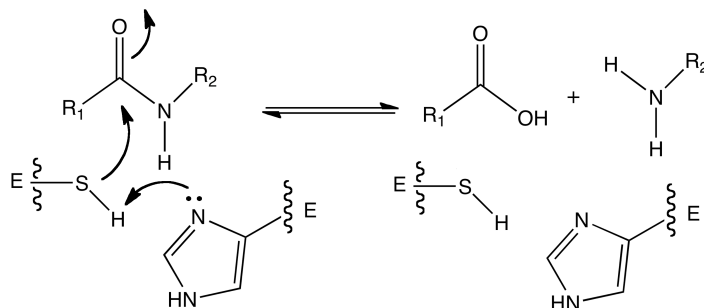
For the inhibited rxn, $V_{Maxapp} = 0.036$ nM/sec; for the control reaction, $V_{Max} = 0.22$ nM/sec.

$$0.036 \text{ nM/sec} = 0.22 \text{ nM/sec} / \{1 + [I]/K_d\}$$

$$K_d = 2.9 \text{ nM}$$

f) In general, competitive inhibitors are less effective because their effects can be swamped out with more substrate. Moreover, saquinavir binds better than indinavir. Even if you didn't calculate the K_I values correctly, you can see that it is a better inhibitor because its double-reciprocal line is higher. Thus, **saquinavir** is the better inhibitor.

14. a)



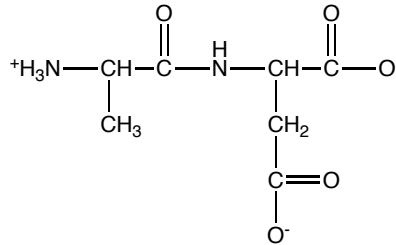
b) covalent

c) general base

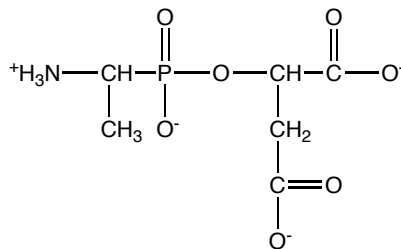
d) pH 6-8

His must be deprotonated so the pH must be $> pK_a$ of His. Cys must be protonated so the pH must be $< pK_a$ of Cys.

e) A good substrate would be this:



But we want an analogue to the transition state (the tetrahedral intermediate formed in part a), so how about this:



f) Not really. TPCK has a Phe side chain that fits into the specificity pocket of chymotrypsin. This enzyme is not specific for Phe, so TPCK may not even bind to the active site.

15. a) #1

It is likely that any substrate will bind “backwards” to avoid the enzyme’s $-NH_3$ clashing with the amino terminus of the peptide.

- #1 has the carboxy terminus of the peptide interacting with the NH_3 of the enzyme, a Phe side chain fitting into the hydrophobic pocket, and potential for a H-bond between Asp (and/or the N-terminus) and the $-OH$ group of the enzyme. Thus, #1 should bind well.
- #2 differs from #1 most significantly in the replacement of Phe with Asn, which would not bind in the nonpolar pocket as well.
- #3 has an Ala, which probably won’t form as many stabilizing interactions in the nonpolar pocket because of its small size; also the His could be destabilizing with the $-NH_3$ group.

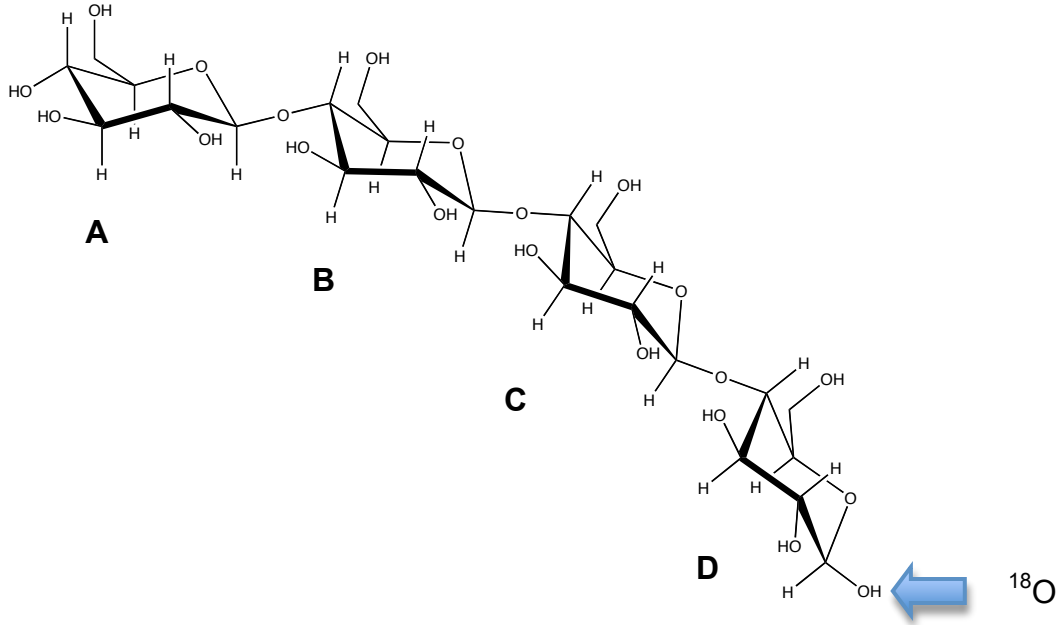
b) #2 (has the largest k_{cat}/K_M)

c) Catalysis, rather than substrate binding. Perhaps substrate #2 is destabilized the most upon binding to make it more reactive.

d) "Burst" phase followed by slower "steady state" phase suggests that $k_2 > k_3$. Enzyme slowly releases second product before catalytic cycle can begin again.

16. a) The active site can accommodate six sugar residues, with binding of any fewer significantly decreasing catalysis. This decrease could occur because binding of the 5th and 6th residues promote binding of the first four residues by perturbing the active-site conformation, or because the binding energy of the 5th and 6th residues is necessary to distort the D ring for catalysis.

b) Yes! The cleavage products will be ABCD-¹⁸O and HO-EF.



c) Residue D becomes distorted to the energetically unfavorable half chair during binding, which promotes catalysis.