Biochemistry, Spring 2002 – Exam 2 Solutions

Section A:

1. The correct answer is c, the enthalpy of binding is not zero. This comes from the fact that $\ln K_{eq} = -\Delta H/R\ (1/T)$. You were given one point for a) because it contained the term enthalpy, you were also given one point for d) because of the phrase 'not zero'.

2. The correct answer is d), the K_I values of each drug, because these are directly related to the binding strength. 2 pts were given for K_M, since a comparison of the observed K_M values in the presence of an inhibitor provides information to calculate K_I.

3. Both K_D and K_I can be measured with equilibrium dialysis. K_I is no different than a ligand binding to a protein. K_I' *cannot* be measured in this way because substrate must be present. This substrate would be converted to product. Answers a and b were given 2 pts.

4. c) is the only correct answer.

5. b) is the only correct answer.

6. a) was given full credit. 1 pt was given if you answered b), however substrate would have to be present at all times as well.

7. c) is the only correct answer.

8. a) is the only correct answer.

B1. You could have answered this in two ways:

Method A: the overall efficiency at very low substrate depends on k_{cat}/K_M. Since the ratio of these two parameters are equal for both enzymes, both are equally effective.

Method B: [S] is only 10 fold lower than the lowest K_M so many students calculated the actual rates:

$v_a = 50\ (0.1)/(1 + 0.1) = 4.545$

$v_b = 500(0.1)/(10+0.1) = 4.95$

So, company B's product is slightly better.

B2.

i) Simple calculation gives: $\Delta G^o = -RT \ln K_{EQ} = -2.5 \ln 10^6 = -34.5$ kJ/mol.

ii) This problem can be done in two stages:

First, the complete removal of one of the Trp residues would expose two non-polar surfaces. One where the Trp used to be and one side of the DNP ligand. This surface area would be equivalent to the surface area of one Trp residue. Exposing the non-polar surface to water is unfavorable since the water would become ordered. Therefore ΔG would decrease from –34.5 to –24.5 (add +10 kJ/mol).

However, the addition of an Alanine side chain would bury some of the non-polar surface in the complex, leading to an increase in stability, lowering ΔG by 2 kJ/mol, to –26.5 kJ/mol.

B3. Part A (Enzyme Kinetics)

i) The velocity is just dP/dt = 100 uM/sec

ii) Since the velocity is already at V_{max} the [S] must be very high, but all that can be said is $[S] >> K_M$. It was acceptable to state that you need to know K_M before you could do this calculation.

iii) Since [S]>>K_M any effects of the inhibitor on the K_M can be ignored. For a non-competitive inhibitor $V_{MAX}^{OBS} = V_{MAX}/\alpha'$. When [I]=$K_I$, α'=2, so the slope would decrease by a factor of 2.

Part B (Ligand Binding)

i) The concentration of free ligand inside the bag must be the same as the free ligand outside the bag because the system is at *equilibrium*.

ii) The *excess* ligand inside the dialysis bag must be bound to the protein:

$[ML]=[L]_{IN} - [L]_{Free}$ = 40 uM – 30 uM = 10 uM.

iii) Y = [ML]/([M]+[ML]). Since [ML] = the total concentration of protein, [M]=0.

Y= 10/(0+10) = 1

iv) Since the protein is saturated with ligand [L]>>K_D we have no information about K_D because we need some data for ligand concentrations that do not completely saturate the protein.

v). The fractional saturation would drop by a factor of 2, since only 50% of the binding sites would be occupied. In other words, the concentration of binding sites has increased by 2, from 10 uM to 20 uM.

B4:

i) The K_D is 1 uM. You could have obtained this by using the concentration of ligand that gives Y=0.5. Alternatively you could have used the fact that the logK_D is equal to log[L] when Y=1/2, or when the line crosses the log(Y/(1-Y)) axis.

ii) The cooperativity is positive. You could have justified this by either showing that the slope of the Hill plot was >1 (1.7 to be exact) or by noting that Y was >0.9 when [L]=10 K_D.

This hemoglobin is *more* cooperative than human because 1.7 is closer to 2 (# sites) than 3 is to 4.0

iii) Myoglobin binds O_2 non-cooperatively. Therefore the slope of the Hill plot will be 1. The affinity is 10 fold higher, so K_D is 10 fold lower. Therefore logK_D is decreased by one. You should have drawn a straight line with a slope of 1 that intersected the x-axis at –1.

B5:

i) The peptide bond is cleaved, that is what proteases do.

ii) Both of these are competitive inhibitors because:

- both look like the normal substrate
- double reciprocal plots for both have the same Y-intecept. *Many students did not extrapolate to 1/[S] = 0 to find the Y-intercept.*

iii) Drug A is the better inhibitor. You could have reached this conclusion based on the following three arguments:

- at any given [S], drug A has a slower velocity
- the slope of the line for drug A is higher than that for drug B, giving a higher α, a lower K_I (K_I=[I]/(α-1)).
- actually calculate K_I values, which were 1 nM and 10 nM, respectively.

iv) The answer to iii is consistent with the structure. Drug A can form a favorable electrostatic interaction with the altered HIV protease.

B6.

Part A: BPG binds to the Tense (T) form of Hb at a different site than oxygen. By stabilizing the T form it reduces the affinity for oxygen. However, the change in the shape of the binding curve actually leads to more efficient *delivery* of oxygen to the tissue at high altitudes.

Part B : Acid is produced by active muscles, by the production of lactic acid as well as CO_2. The latter reacts with water to become carbonic acid (H_3CO_3), which releases a proton. Protons bind to hemoglobin, stabilizing the T state, and lowering the affinity for oxygen in the muscle, enhancing delivery.

Part C: Chymotrypsin has a large non-polar pocket that would accommodate the phenyl ring however the positive charge would become buried, this would be unfavorable.

The positive charge on the phenyl ring would interact more favorable with Asp189 which is used by Trypsin to bind Lys and Arg.

B7:

Part A:

1. Both cleave peptide bonds
2. Serine proteases use His to activate nucleophile, while HIV protease uses Asp
3. Acyl intermediate is formed in serine proteases, but not formed in HIV protease
4. Serine and water are the nucleophiles in serine proteases, water is the nucleophile in HIV proteases

Part B:

1. His57 and Asp102 work together to remove proton from Ser.
2. Activated Serine attacks C=O carbon, forming transition state
3. Transition state decays, to release 1st product and form acyl enzyme
4. Water is activated by His57 and Asp102 to form OH ion.
5. OH ion attacks ester linkage between 2nd product and Ser, forming transition state
6. Transition state decays, releasing product and returning enzyme to initial configuration.

Part C:

1. Asp25 activates water by removing proton
2. Nucleophilic attack of OH on C=O, forming transition state
3. Transition state decays, giving two products.
4. Newly formed amide group extracts proton from 2nd Asp.
5. Proton transfer between Asp residues restores enzyme to its original state.

Transition State Stabilization (4 pts):

Both enzymes use proximity of active site residues to the substrate to lower the transition state. The is lowering the activation free energy via a change in the entropy. It would be very unfavorable to bring together the peptide and the catalytic groups if they were free in solution. However, because the protein is folded the way it is, these groups are all in the same place.

Serine proteases also use *direct* interactions to stabilize the transition state. There are hydrogen bonds formed between the tetrahydral intermediate and two amide groups on the main-chain of the serine protease.