Biology 5357

Chemistry & Physics of Biomolecules

Examination #1

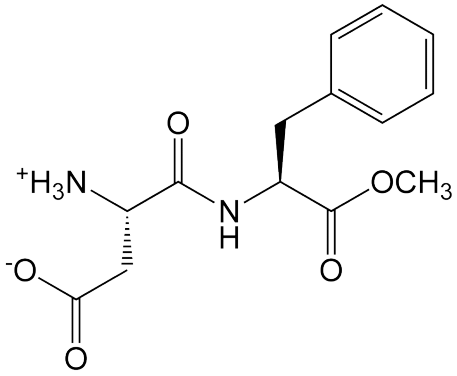
Proteins Module

September 27, 2019

Answer Key

Question 1

(A) (5 points)



3 points for correct amino acid structures/connectivity, 1 point for correct protonation states, and 1 point for having correct chirality at α-carbons

(B) (5 points)

A negative charge 15 Å away from the His site is able to perturb the histidine pKa by 0.4 pK units. This corresponds to an energetic effect of $\Delta G = -RT \ln(10^{+0.4})$ which is about 0.55 kcal/mole at room temperature. From Coulomb's Law the energy of interaction of two unit charges is $E = 332 / (D \bullet r)$ kcal/mol, where D is the dielectric constant and r is the distance between the charges. Since we know E and r, solving for D gives an effective dielectric constant of about 40 for this interaction. This is lower than the standard value of 78 for ions in bulk water, but still quite high since the charges are stated to be on the protein surface.

Question 2

(A) (4 points)

This is a 5-stranded antiparallel β-sheet structure.

(B) (5 points)

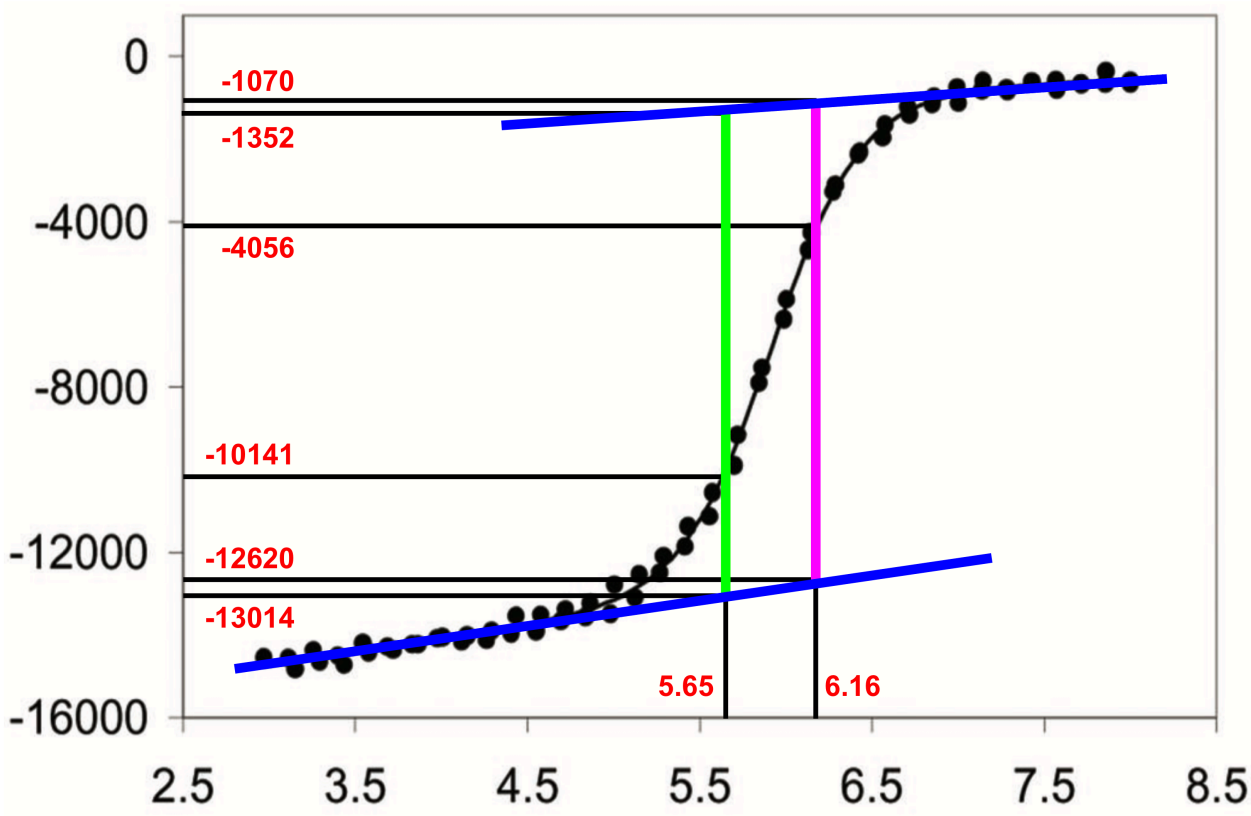
If the β-sheet lies roughly in the plane of the page, then both helices are in front of the page, i.e., coming out toward you.

(C) (6 points; 3 for definition & 3 for circled β-turn)

A β-turn is a motif consisting of four consecutive amino acid residues that reverse the direction of the amino acid chain, and with a hydrogen bond between the carbonyl oxygen of the 1st residue and the amide nitrogen & hydrogen of the 4th residue.

There are two classic β-turn structures in TOP7, one connecting the two strands closest to the N-terminus, and other connecting the two strands closest to the C-terminus. Either or both of these could be circled to receive credit.

Question 3



(5 points for calculation setup; 5 points for K_{eq} values; 5 points for final ΔG)

Consider the two data points at 5.65M (green) and 6.16M (magenta) of Gu-HCl. At 5.65M, K_{eq} = [folded] / [unfolded] = (-1352-10141) / (-10141-13014) = 3.06. At 6.16M, K_{eq} = (-1070-4056) / (-4056-12620) = 0.348. From $\Delta G = -RT \ln(K_{eq})$, we get a $\Delta G_{(unfolded->folded)}$ of -0.66 kcal/mol at 5.65M, and +0.62 kcal/mol at 6.16M. Linear extrapolation of these ΔG values to 0M Gu-HCl (*i.e.*, the value in pure water) yields -0.66 – (5.65)(0.62+0.66)/(6.16-5.65) = -14.8 kcal/mol. Note this is only a crude estimate, and full credit is given for any reasonable number obtained from an analogous calculation. Careful analysis by the authors of the original paper (*Science*, ***302***, 1364-1368, 2003) resulted in folded *vs*. unfolded ΔG of -13.2 kcal/mol.

Question 4

(A) (5 points)

The data provides a standard sigmoidal equilibrium protein folding curve as a function of temperature. In this case, the "y-axis" property monitored is the chemical shift of Hα protons. The curve is then analyzed to determine the ΔG between folded and unfolded at temperatures in the transition region. Since the ΔG as a function of temperature is available, the slope of ΔG *vs*. T is -ΔS for the folding process.

(B) (5 points)

In pure water, ΔG = (+7.2)(1000) – (+23)(298) = +346 J/mol = +82.7 cal/mol. Then $K_{eq} = e^{-\Delta G/RT} = e^{-[(82.7)/(1.987)(298)]} = 0.87$. So, the percentage of β-hairpin conformation is 0.87 / (0.87 + 1.0) = 46.5%. For the 50% CH_3OH, we have ΔG = -1082 cal/mol, $K_{eq} = 6.22$, and percentage of β-hairpin = 86.1%.

(C) (5 points)

Upon going from water to 50% CH_3OH the solvent is more hydrophobic and has a lower dielectric constant. This results relatively stronger intra-peptide hydrogen bonds, and a more favorable (ΔH negative) enthalpy of folding. Since the hairpin structure is more folded and has less flexible random coil in 50% CH_3OH, the entropy change on moving from water to 50% CH_3OH is unfavorable (ΔS negative). The large heat capacity change in water is typical of the "hydrophobic effect".

Question 5

(A) (4 points)

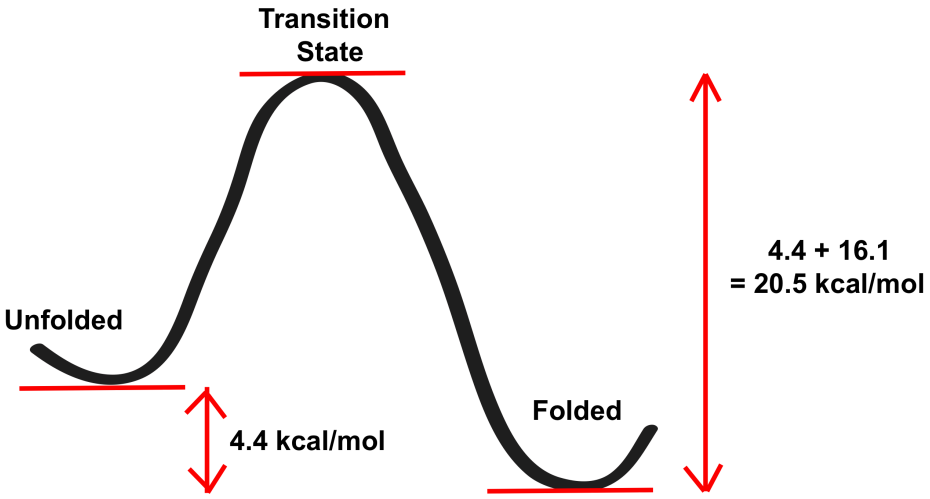
Extrapolated to 0M denaturant, we can use the y-intercepts to estimate $\ln(k_f) = +2.2$ and $\ln(k_u) = -5.3$. Then if $K_{eq} = [F] / [U] = k_f / k_u$, we have $\ln(K_{eq}) = \ln(k_f) - \ln(k_u) = +7.5$, or $K_{eq} = e^{+7.5} = 1800$. From $\Delta G = -RT \ln(K_{eq})$, we obtain ΔG = -(0.0019872) (298) (7.5) = -4.4 kcal/mol. Thus, in the absence of denaturant, the folded form of the protein is roughly 4.4 kcal/mol more stable than the unfolded form.

(B) (6 points; 3 for $\Delta G^{\pm}$ value & 3 for diagram)

Rearranging the given equation to solve for the free energy barrier ($\Delta G^{\pm}$) gives the expression $\Delta G^{\pm} = -RT \ln (h\,k / k_b T) = -RT [\ln(k) + \ln(h / k_b T)]$. To get the barrier from the unfolded state we use $k = k_f$, and then $\ln(k) = +2.2$ from part A. Substituting

in values gives $\Delta G^{\pm} = -(0.0019872)(298)\,[\,2.2 + \ln(6.626\times10^{-34} / 1.38\times10^{-23}\,(298))\,] = -(0.0019872)(298)\,[\,2.2 - 29.46\,] = 16.1$ kcal/mol.

The reaction coordinate diagram for the folding process is shown below:



Question 6

(A) (5 points)

The correct answer is the landscape on the right of the figure.

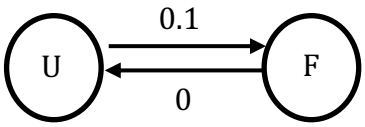
(B) (5 points)

The landscape on the right is flatter, thus it has higher entropy.

Question 7

(A) (5 points)

The second of the two states, shown below, is the correct answer.



(B) (5 points)

The state shown above violates detailed balance since the folded "F" state is a sink state.

Question 8 (5 points)

From detailed balance, the probability is (0.9 / 0.1) x 0.005 = 0.045.

Question 9 (10 points)

The red dashed line is a better coarse graining of the black surface. It captures the low energy (high probability region) well and is less accurate for the higher energy (lower probability region). The blue dasher line gets the high energy part right but is off for the (more important) low energy portion.