

Biology 5357

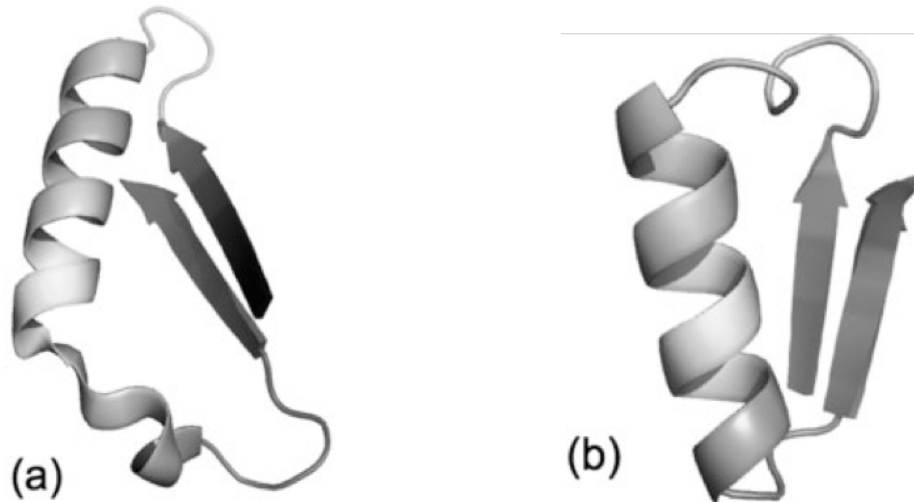
Chemistry & Physics of Biological Molecules

Examination #1

Protein Structure, Folding & Design
X-Ray Crystallography
NMR Spectroscopy

October 20, 2009

1. (10 points) The structures below are (a) residues 123-165 of SurE, a metal ion-dependent phosphatase (PDB: 1J9L), and (b) residues 57-87 of a phospholipase D family member (PDB: 1BYS).



(A) Characterize each of these β - α - β motifs as having either left- or right-handed crossover connections. One of the above structures occurs about 65 times more often than the other in a recent study of the Protein Data Bank. Which is the more common structure and why?

(B) In a set of 431 three-helix bundles contained in all- α proteins, 61.5% were found have right-handed connections and 38.5% left-handed. Compare this to the results for the β - α - β motif, and suggest an explanation for the milder structural preference found in helical bundles.

2. (5 points) In the protease subtilisin, removal of a negative charge via mutagenesis changes the pK_a of a His residue located some 15 Å away by about 0.4 units. Use this information to estimate an “effective dielectric constant” which measures the shielding between these charges located at the protein surface.

3. (5 points) The heat of sublimation of ice is 12.2 kcal/mol at the freezing point. Based on your knowledge of ice and water structure, estimate the energy associated with a single water-water hydrogen bond.

4. (10 points) Reduced glutathione is a “tripeptide” with the sequence Glu-Cys-Gly, but where the peptide bond between Glu and Cys utilizes the side chain carboxylate instead of the backbone carboxylate.

(A) Draw the structure of reduced glutathione at pH 7, paying attention to chirality and protonation state.

(B) Indicate how the structure changes upon going from the reduced form of glutathione to the oxidized form?

(C) Explain why glutathione is often a component of *in vitro* protein refolding buffers.

5. (15 points) A synthetic 16-residue peptide capped with *N*-acetyl at the N-terminus, Ac-KKYTVSINGKKITVSI, has been shown to reversibly fold into a β -hairpin structure. NMR experiments monitored the chemical shift of the H_{α} protons as a function of temperature in both pure water and 50% methanol. Analysis of the data gives the thermodynamic values shown below for the folding process. All values are corrected to 298K, and are given in kJ/mol for ΔH , and J/mol/K for ΔS and ΔC_p (1 cal = 4.184 J).

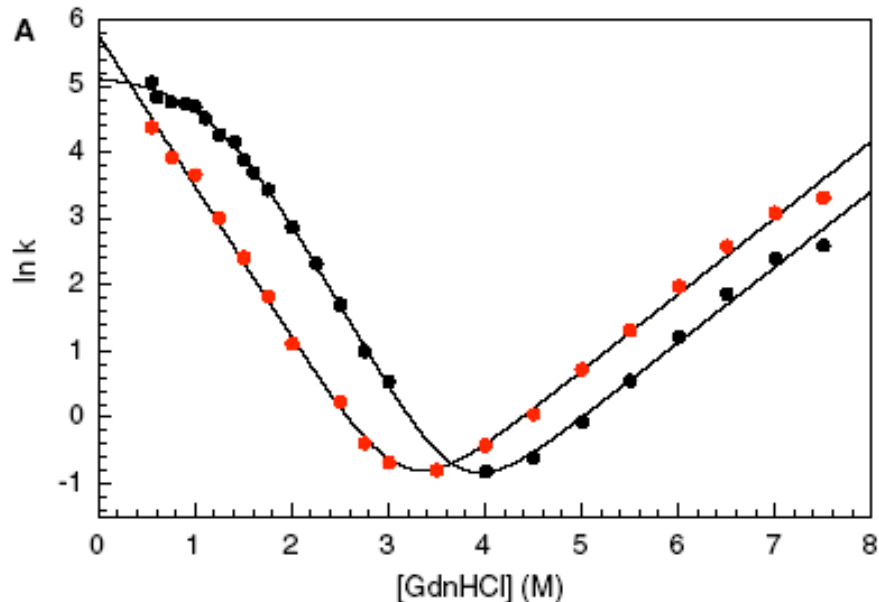
Solvent	ΔH°	ΔS°	ΔC_p°
Pure H ₂ O	+7.2	+23	-1400
50% CH ₃ OH	-38.5	-114	-11

(A) Briefly explain the data analysis procedures used to derive these thermodynamic values from the experimental data.

(B) What percentage of the time is the peptide in the β -hairpin conformation in each of these two solvents?

(C) All three of the thermodynamic parameters undergo a change with solvent composition. Provide a physical rationalization for the observed shift in each value.

6. (10 points) Shown below are chevron plots for two variants of the 76-residue protein ubiquitin at pH 5.0. The black circles are data for the F45W mutant of ubiquitin, where the mutation was made to increase fluorescence and aid kinetic measurements. The red circles are for ubiquitin with the F45W mutation and a few additional residues of an affinity tag sequence (GLVPRGS-) attached at the N-terminus. The tag happens to significantly increase the solubility of the protein.



(A) Estimate the stability of the folded form of the affinity tagged protein relative to its unfolded form.

(B) Nonlinearity of chevron plots at low denaturant concentration has been used to argue for non-2-state folding behavior, *i.e.*, for folding intermediates. Explain. Suggest another possible reason for nonlinearity in this particular case, and propose an experimental test of your suggestion.

7. (10 points) Naturally occurring L-plectasin is a 40-residue protein with antimicrobial activity against several species of gram-positive bacteria, including various strains of *S. pneumonia* that are resistant to conventional antibiotics.

(A) Recently, the x-ray crystal structure of a racemic mixture of D- and L-plectasin was determined (PDB: 3E7R). The racemate crystallizes in the centrosymmetric space group P1(-). What symmetry element(s) are present in P1(-)? Can pure L-plectasin crystallize in this space group?

(B) Show that for a centrosymmetric space group the phase value of each reflection must be exactly equal to 0 or π . Remember each structure factor F_h for reflection (h,k,l) contains a contribution f_h from each atom. If an atom is located at the position (x,y,z), then there will be an atomic contribution of the form:

$$f_h = f_j \exp [2\pi i(hx+ky+lz)] = f_j [\cos 2\pi(hx+ky+lz) + i \sin 2\pi(hx+ky+lz)]$$

Hint: If a centrosymmetric unit cell has an atom at (x, y, z), what other position must also have a symmetry-related atom, and what is its structure factor contribution?

8. (10 points) The 2009 Nobel Prize in Chemistry was awarded to Venki Ramakrishnan, Tom Steitz and Ada Yonath for determination of x-ray structures of the ribosome. In a 2000 publication (*Science*, **289**, 905 '00), Steitz and coworkers reported the structure of the ribosomal large subunit from *H. marismortui* containing 2833 nucleotides and 27 separate proteins. The crystals formed in space group C222₁ and with unit cell parameters of $a = 211.66 \text{ \AA}$, $b = 299.67 \text{ \AA}$, $c = 573.77 \text{ \AA}$, and $\alpha = \beta = \gamma = 90^\circ$. Data was collected for 665,928 unique reflections to 2.4 \AA resolution using x-rays of wavelength 1.0 \AA from the Advanced Photon Source synchrotron at Argonne National Lab.

(A) Both isomorphous replacement and anomalous scattering phase information was collected from four separate heavy metal derivatives obtained by soaking crystals with complexes of iridium, uranium, osmium and tantalum. The final refined structural model had $R_{cryst} = 25.2\%$ and $R_{free} = 26.1\%$. Briefly explain the need for several heavy metal derivatives, and the meaning of the R -factor values.

(B) What is the “resolution” associated with the (0,100,100) reflection, and at what scattering angle relative to the incident beam would this reflection be detected?

9. (8 points) What spin system is observed in an HNCACO experiment? In an HNCA experiment? Identify these systems in a dipeptide.

10. (10 points) What is scalar coupling? What is dipolar coupling? Explain how each is used in the structure determination of a protein by NMR?

11. (7 points) In the typical protein $^1\text{H}/^{15}\text{N}$ -HSQC experiment, what spin systems are seen in the spectrum?