

Ionizable group in peptides and proteins	<i>Approximate</i> ("generic") pK_a in <u>peptides &</u> <u>proteins</u>
α-amino	8.0
α-carboxyl	3.0
ε-amino	10.0
guanidino	12.0
thiol	8.5
imidazole	6.5
aromatic hydroxyl	10.0
side chain carboxyl	4.0

1. (17 pts) A protein of unknown structure has been purified and is subjected to gel filtration (molecular sieve) chromatography under different conditions, with the following results:
- 1) Chromatography under native conditions suggests a molecular weight of 240,000 from comparison to standards of known molecular weight chromatographed on the same column.
 - 2) Chromatography in the presence of 5 M urea yields a single protein peak corresponding to a M.W. of 60,000 from comparison to standards of known molecular weight chromatographed on the same column in urea.

A. (5 pts) What information do the results of (1) and (2) give about the structure of the protein? Describe the structure as completely as the data permit.

Urea would unfold the protein and dissociate the subunits if they're not held together with disulfide bonds, so if native M.W. is 240,000 and denatured M.W. is 60,000, protein must be a tetramer of identical or similar 60,000-M.W. subunits.

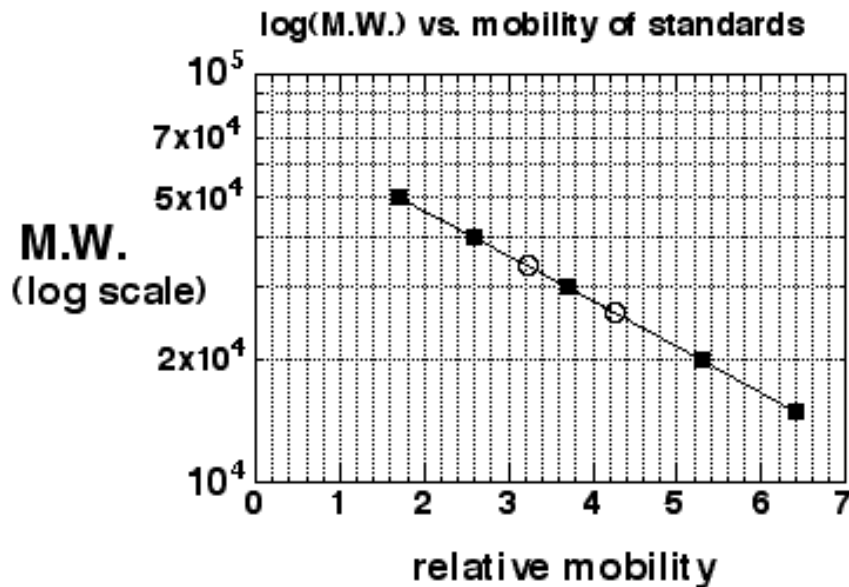
B. (4 pts) What method other than molecular sieve chromatography might you use to confirm the native molecular weight of the protein?

ultracentrifugation

C. (4 pts) What would be the best method for determining the **isoelectric point** (pI) of the protein?

isoelectric focusing

D. (4 pts) On an SDS polyacrylamide gel, the pure protein shows **two stained bands**. The relative mobilities of those 2 "unknown" bands are shown on the plot below as open circles (O), with the mobilities of 5 known polypeptide standards of M.W. 15,000; 20,000; 30,000, 40,000 and 50,000. **Reminder:** SDS gel electrophoresis is run with sodium dodecyl sulfate in the gel, the starting protein sample, and all the buffers, and the protein sample is boiled in the SDS sample buffer with β -mercaptoethanol before being loaded on the gel.



Given the results of the SDS gel, **how would you alter your description of the protein structure from the description you gave in part A?** Be as specific as you can about your revised description of the structure, and explain the reason for the altered description

ANS: There's no 60,000 M.W. polypeptide on the gel for the unknown, but instead there are 2 polypeptide chains, one of ~34,000 M.W. and the

other of ~26,000 M.W. That adds up to 60,000 M.W., and remembering that the SDS gel not only denatures proteins but (with β -mercaptoethanol) also reduces disulfide bonds, it seems that the 60,000 M.W. "subunits" in urea each consist of 2 polypeptide chains (34,000 + 26,000 M.W.) connected by one or more disulfide bonds. Native protein would thus be 4 x 34,000 plus 4 x 26,000, 8 chains total.

/17

2. (13 pts) Two fragments of the polypeptide chain of cytochrome c were purified:

Fragment 1: the amino acid sequence that forms the amino-terminal α -helix in the native structure.

Fragment 2: the amino acid sequence that forms the C-terminal α -helix in the native structure.

Experimental observation: It was discovered that while either fragment by itself in solution appeared to have a "random" secondary structure, a *mixture of the 2 fragments* in solution showed that a majority of all the amino acid residues in the peptide molecules in the solution had adopted an **α -helical** conformation.

A. (4 pts) Use your understanding of protein structures and folding to suggest an explanation of the experimental observation above (random structures for the individual fragments in solution, but helical structures for the mixture of the two).

The fragments must be interacting in the mixture, and the interaction stabilizes the α -helical conformation for both. Maybe a coiled coil structure forms, with the helix-helix interactions between R groups stabilizing the secondary structure.

B. (9 pts) Experimental determination of secondary structure

1) What spectroscopic method would have been used to determine the predominant secondary structures of the individual peptides and the mixture?

circular dichroism (C.D. spectroscopy)

2) In which of the following general wavelength regions would the method have been most useful for examination of secondary structure? (Circle your answer.)

200-230 nm 270-290 nm 565-585 nm

3) What type of chromophore in the peptides would be indicating secondary-structure-sensitive differences in this wavelength range?

peptide bonds

3. (10 pts) Suppose that the *equilibrium dissociation constant* of an F_{ab} -antigen complex is 5×10^{-7} M at 25 °C. (Hint: Just treat F_{ab} -antigen complex like any other protein-ligand complex.) **SHOW YOUR WORK AND STATE UNITS.**

A. (4 pts) What is the **standard free energy change** for the *dissociation* reaction?

$$K_d = \frac{[F_{ab}][\text{antigen}]}{[F_{ab} \cdot \text{antigen}]}$$

$$\Delta G^{\circ}_{\text{dissoc}} = -RT \ln K_d$$

$$\Delta G^{\circ}_{\text{dissoc}} = -2.478 \text{ kJ/mol} \cdot \ln(5 \times 10^{-7} \text{ M}) = -2.478 \text{ kJ/mol}(-14.5)$$

$$\Delta G^{\circ}_{\text{dissoc}} = +35.9 \text{ kJ/mol for the dissociation reaction}$$

B. (3 pts) What is the *equilibrium association constant* for formation of the F_{ab} -antigen complex? (K_a is often referred to by immunologists as the *affinity* of the antibody for the antigen.)

$$K_{\text{assoc}} = \frac{[F_{ab} \cdot \text{antigen}]}{[F_{ab}][\text{antigen}]}$$

$$K_{\text{assoc}} = 1/K_{\text{dissoc}} = (5 \times 10^{-7} \text{ M})^{-1} = \underline{2 \times 10^6 \text{ M}^{-1}}$$

C. (3 pts) What is the **standard free energy change** for the *association* reaction?

Either answer is correct:

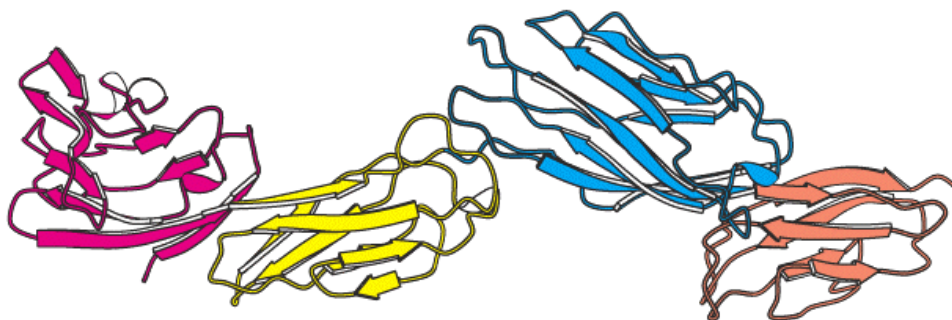
$$\text{easiest way: } \Delta G^{\circ}_{\text{assoc}} = -\Delta G^{\circ}_{\text{dissoc}} = \underline{-35.9 \text{ kJ/mol}}$$

$$\text{or (long way!) calculate } \Delta G^{\circ}_{\text{assoc}} = -RT \ln K_{\text{assoc}}$$

$$\Delta G^{\circ}_{\text{assoc}} = -2.478 \text{ kJ/mol} \cdot \ln(2 \times 10^6 \text{ M}^{-1}) = -2.478 \text{ kJ/mol}(14.5)$$

$$\Delta G^{\circ}_{\text{assoc}} = \underline{-35.9 \text{ kJ/mol}}$$

4. (4 pts) There are 4 immunoglobulin domains apparent in the single polypeptide chain below. What type of tertiary structural motif is exemplified by *all 4* of these domains?
 A. β barrel *C. 2-layer β sandwich E. None of these is correct.
 B. $\alpha\beta$ barrel D. $\alpha\beta\alpha$ 3-layer sandwich



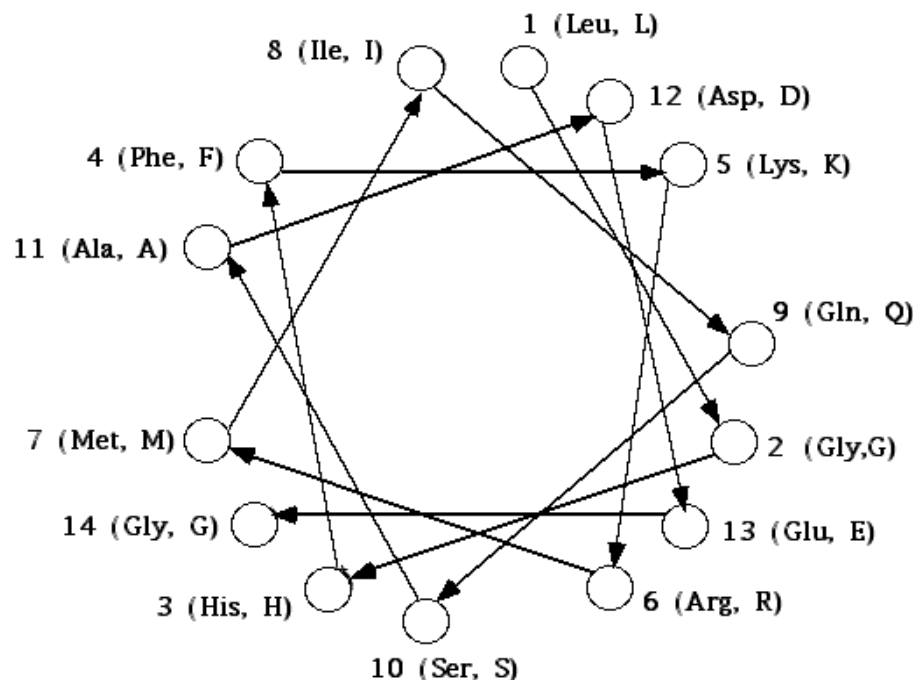
5. (9 pts) The structure of a certain protein includes the following 14-residue amino acid sequence in an α -helical conformation:

- - - - **Leu-Gly-His-Phe-Lys-Arg-Met-Ile-Gln-Ser-Ala-Asp-Glu-Gly** - - - -

- A. (5 pts) Is this α -helix amphipathic? Justify your answer, including explaining how you would recognize an amphipathic helix. Put the sequence on the helical wheels representation below as part of your explanation.

Yes, the α -helix IS amphipathic; the R groups on one side of the helix are hydrophobic, and the R groups on the other side are hydrophilic. (Labels below)

- B. (4 pts) If this helix were part of a *coiled coil* structure in the protein, indicate on the drawing exactly what part of the helix you would expect to be interacting with another helix and **why**.

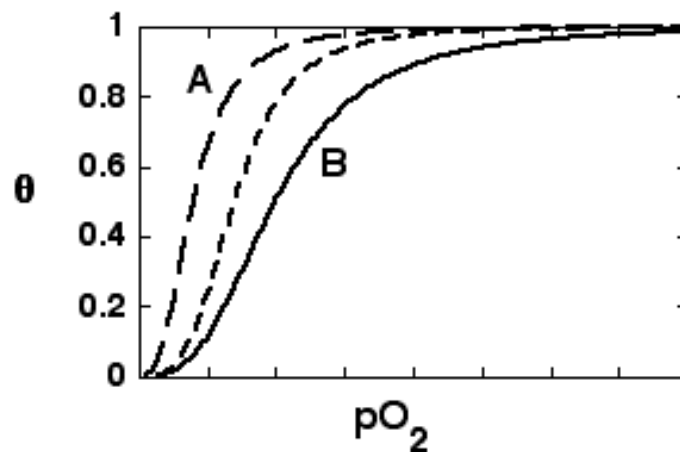


Right-hand side/bottom is polar (from Asp12 to His3 or Gly14). Top/left side (Gly14 or Met7 to Leu1) is hydrophobic, and THIS side of the helix would be expected to interact with another helix in coiled coil, the structure being stabilized by hydrophobic interactions.

6. (10 pts) The figure at right shows the oxygen binding curve for hemoglobin in the red blood cells of normal blood, pH 7.6.

A. (5 pts) Sketch on the plot the oxygen binding curve you would expect if all 2,3-bisphosphoglycerate were removed from the blood, and label your curve "A". **Explain.**

BPG is a negative heterotropic effector (stabilizes T state, so R-T equilibrium shifts toward R state in absence of BPG). Thus hemoglobin binds O_2 more tightly (lower P_{50}) with less 2,3-BPG bound.



B. (5 pts) On the curve above, sketch another oxygen binding curve to show what you would expect for an oxygen binding curve if the pH of the blood were 7.3. Label this curve "B". **Explain.**

H^+ binding stabilizes T state (protons are negative heterotropic effectors), so higher $[H^+]$ (lower pH) shifts R-T equilibrium toward the T state (weaker O_2 binding affinity). Weaker binding - - > higher P_{50} , i.e., hemoglobin binds O_2 more tightly at higher pH, more weakly at lower pH.

7. (10 pts) Suppose you had a mutant hemoglobin in which a crucial hydrogen bond between the α_1 and β_2 (and thus also between the α_2 and β_1) subunits is lost. That hydrogen bond is involved in stabilizing the R state of hemoglobin, so loss of the hydrogen bond results in a shift in the R-T equilibrium toward the T state. Those hydrogen bonds between the protomers also help stabilize the quaternary structure of the normal tetrameric hemoglobin, so loss of the hydrogen bonds shifts the tetramer-protomer equilibrium in the direction of *dissociation* to the $\alpha\beta$ protomers.

A. (5 pts) Would you expect the *Hill coefficient* for this mutant to be *higher* or lower than that of the normal HbA? **Explain.**

The Hill coefficient n_H would be expected to be LOWER because the quaternary structure is shifted toward $\alpha\beta$ protomers, with resultant loss of communication between subunits and thus decrease in slope of Hill plot in the direction of 1 (n_H of 1 would mean no communication at all between binding sites).

B. (5 pts) Would you expect the P_{50} for the mutant hemoglobin to be *higher* or *lower* than P_{50} for the normal hemoglobin? **Explain.**

The mutant's R-T equilibrium is shifted toward the T state, *weaker* O_2 binding, so P_{50} of mutant is HIGHER than that of normal HbA.

8. (6 pts) One important stabilizing interaction in the T state (a contributor to the Bohr effect) is a salt link involving the α -amino group of the α subunits of hemoglobin; that salt link is not present in the R state. Would you expect the pK_a of the α -amino group to be *higher* in the R state or in the T state, or would the pK_a be *unaffected* by the conformational change between R and T? **Explain.**

The pK_a of the α -amino group would be higher in the T STATE, because it can be involved in a salt link (ionic bond) in the T state IF it retains its positive charge, so conjugate acid (protonated) form is favored in the T state -- it binds its proton more tightly (higher pK_a) in T state.

9. (12 pts) Answer the following questions about the structure of collagen:

A. (3 pts) What amino acids are particularly frequently found in the sequence of collagen? (Be specific.)

- 1) Gly (Glycine, G)
- 2) Pro (Proline, P)
- 3) HyP (Hydroxyproline)

B. (3 pts) 3 individual collagen helices are supercoiled into a supersecondary structure called tropocollagen. What type of amino acid residues are found in the central contact interfaces among the 3 collagen helices in this structure, and why is this important for the protein structure?

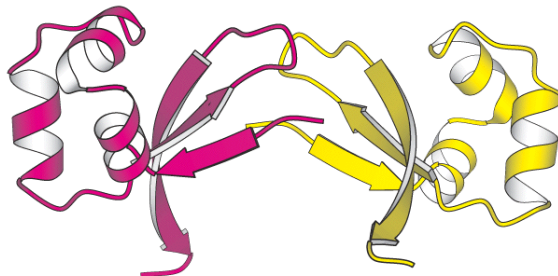
Gly residues are found in the central contact interfaces among the 3 collagen helices because the small R group of glycine (just a hydrogen atom) is the only R group that will fit in the tightly packed center of the triple helix -- no room for larger R groups.

C. (6 pts) Compare the collagen helix with the α helix in the following ways (circle your choice of answer in each question):

- 1) Does collagen have *more* or *fewer* residues per turn than the α helix? (3.0 vs. 3.6)
- 2) Is the pitch of the collagen helix (distance per turn along helix axis) *smaller (shorter distance)* or *greater (longer distance)* than the pitch of the α helix? (collagen 1.0 nm/turn, α -helix 0.54 nm/turn)
- 3) Do the properties of the collagen helix make it *more rigid* or *more stretchy* than the α helix?

10. (4 pts) What type of rotational axis of symmetry is apparent in the structure below?

2-fold (There are 2 identical subunits, 1 at left and 1 at right), so rotation by 180° around a vertical 2-fold axis superimposes one on the other.)



11. (5 pts) The enzyme ribonuclease A has a total of 4 disulfide bonds. Christian Anfinsen showed that if all the disulfide bonds in ribonuclease were reduced and the enzyme unfolded in urea, then upon removal of both the urea and the reducing agent at the same time, the enzyme spontaneously refolded to its native active tertiary structure with the correct disulfide bonds. What would happen to the structure of ribonuclease if the enzyme were permitted to reoxidize in the presence of urea, and the urea were not removed to initiate refolding until after oxidation was complete?

If the reoxidation occurs while the protein is unfolded, RANDOM combinations of Cys residues in disulfide bonds will occur instead of the CORRECT combinations that form when the protein refolds before the oxidation events, orienting the correct pairs of Cys residues close to each other. Random disulfides "stapling" parts of the chain to each other would prevent successful refolding when the urea is subsequently removed.