Working with Ramachandran Plots and secondary structure

1 This problem and the following one introduce you to an elegant tool for the analysis of polypeptide structure called the **Ramachandran diagram.**

The possible structures of a polypeptide are limited by the geometry of the peptide bond and amino acid side chains. The six atoms of a peptide (amide) bond all lie in the same plane (Figure 4.15). With C_{α_1} as a reference point, amide plane 1 can rotate only around the C_α–N axis and amide plane 2 can rotate only around the C_α–C axis. Verify for yourself, using models if necessary, that because of the tetrahedral orientation of the bonds to C_{α_1}, the two planes can be coplanar only when both are perpendicular to a third plane, defined by H_1 and the C_{α_1}–R_1 bond (reference orientation). The angles ϕ and ψ are defined as the degree of clockwise rotation of planes 1 and 2, respectively, away from the reference orientation. When defined for each pair of adjacent residues, the values of ϕ and ψ completely determine the conformation of a polypeptide. Some ϕ and ψ angles are favorable because there is no crowding of atoms, and some are unfavorable because they bring atoms too close together. For example, at $\phi = 180°$ and $\psi = 0°$, the two carbonyl oxygens are crowded into an energetically unfavorable configuration (imagine amide plane 1 rotated 180° from the position shown in Figure 4.15).

When a polypeptide assumes a repeating structure, for example an α helix, every peptide bond in the chain will have the same ϕ and ψ angles. As a consequence, any repeating structure can be defined by characteristic ϕ and ψ angles. These angles are presented in the Ramachandran or ϕ, ψ diagram shown in Figure 4.16.

a. What are the Ramachandran angles for a collagen helix?
b. How does an α helix differ from a collagen helix in terms of Ramachandran angles?
c. How does an antiparallel β sheet differ from a collagen helix?
d. What are the Ramachandran angles for an antiparallel β sheet?
e. What will be the conformation of a random coil in terms of ϕ and ψ? What limitations are imposed?
f. All the common favorable secondary structures fall within the range $\phi = 20°$ to $\phi = 140°$. What advantage does this range have for every amino acid except Gly?

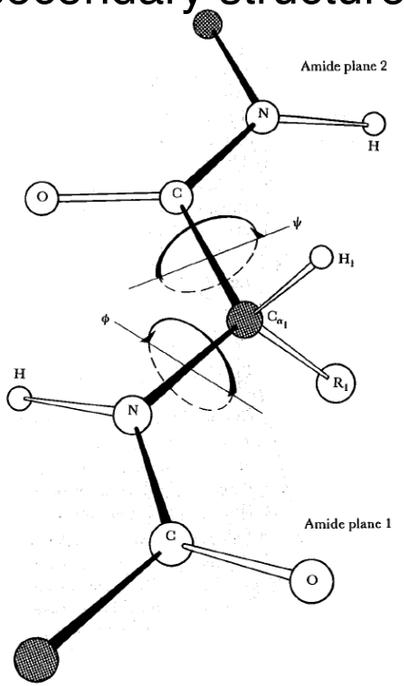

Figure 4.15
Rotation of adjacent amide planes in a polypeptide (Problem 4.19). (From R. E. Dickerson and I. Geis, *The Structure and Action of Proteins,* Benjamin/Cummings, Menlo Park, Calif. © 1969 Dickerson and Geis.)

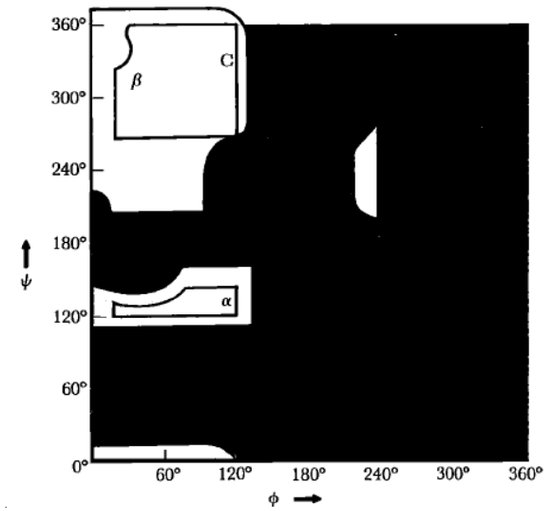

Figure 4.16
Ramachandran diagram showing favored rotational angles (ϕ, ψ) for the amide planes in polypeptides (Problem 4.19). White areas enclosed by solid lines represent the most favorable ϕ, ψ angles; light gray areas represent less favorable angles, and dark gray areas represent highly unfavorable angles. The ϕ, ψ angles for antiparallel β sheet (β), collagen triple helix (C), and right-handed α helix (α) fall within favorable regions as shown by circled symbols. (Adapted from R. E. Dickerson and I. Geis, *The Structure and Action of Proteins,* Benjamin/Cummings, Menlo Park, Calif. © 1969 Dickerson and Geis.)

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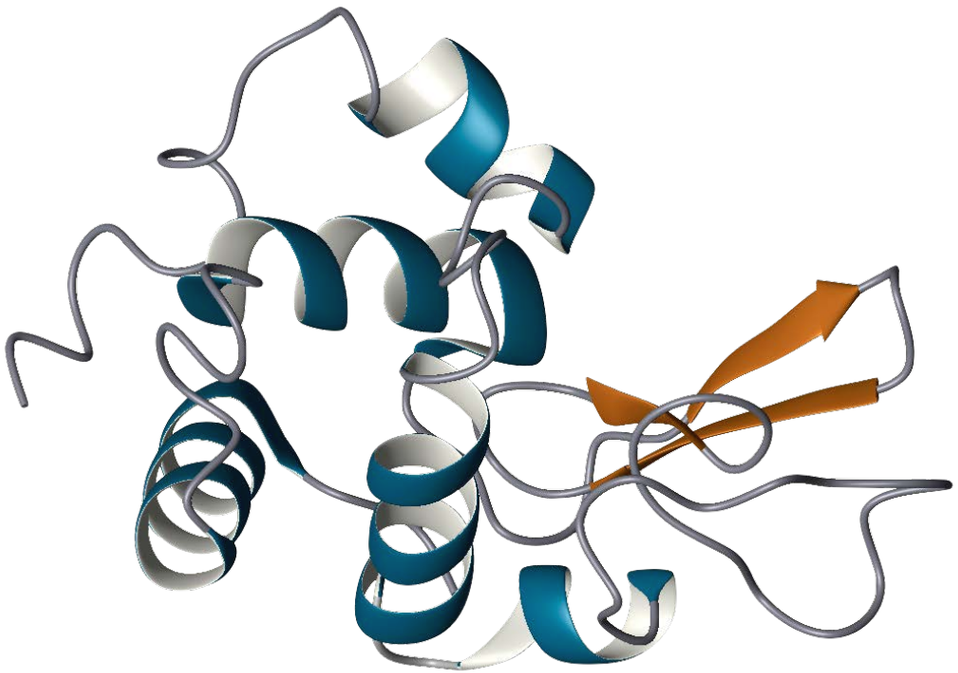
2

Examine the Ramachandran diagram of the peptide bonds in lysozyme shown in Figure 4.17.

a. What is the predominant secondary structure?
b. Are any other ordered structures represented by enough peptide bonds to be identifiable?
c. What can you say about the majority of bonds whose ϕ, ψ angles are in highly unfavorable regions?
d. What can you say about the composition of the helical regions of lysozyme?
e. What can you say about the composition of the β-sheet regions of lysozyme?
f. Compare your answers with the actual structure of lysozyme shown in Figure



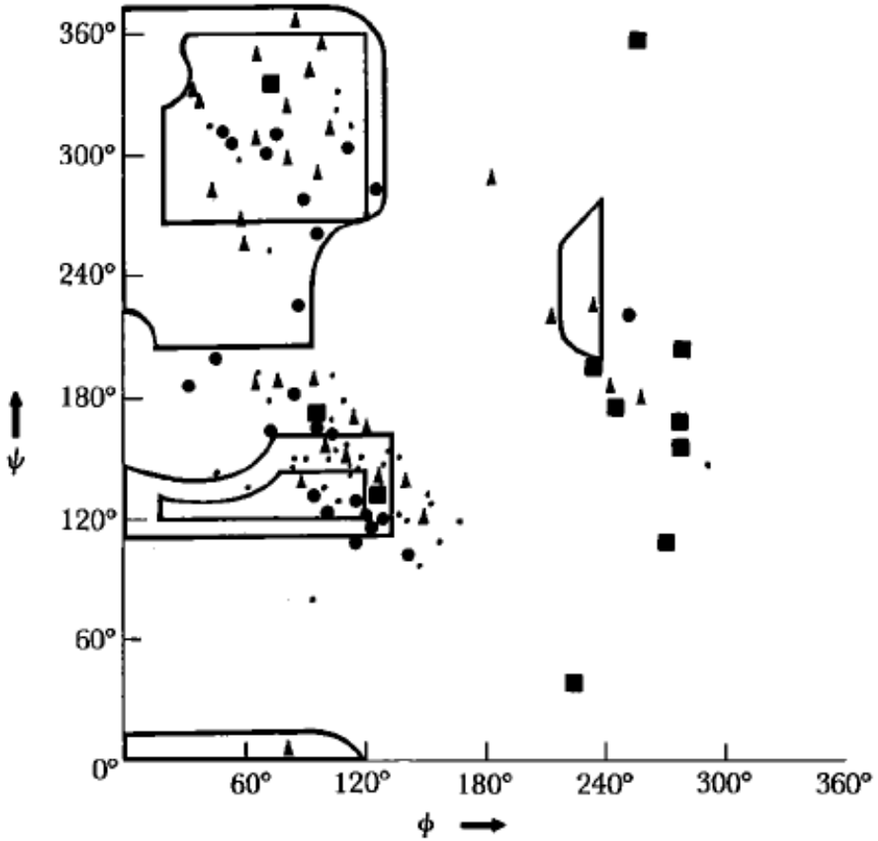
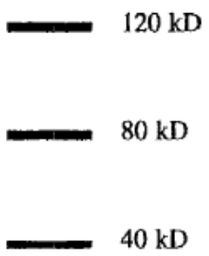


Figure 4.17
Ramachandran diagram of lysozyme (Problem 2). The following symbols are used to represent four classes of residues: ·, nonpolar residues; •, charged polar residues; ▲, neutral polar residues; ■, glycine residues. (Adapted from R. E. Dickerson and I. Geis, *The Structure and Action of Proteins,* Benjamin/Cummings, Menlo Park, Calif. © 1969 Dickerson and Geis.)

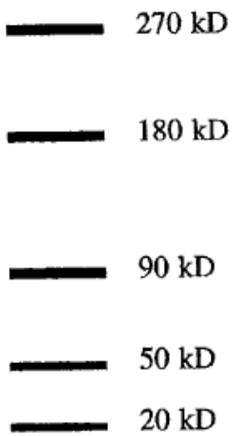
3 The subunit composition of an oligomeric protein can be determined by treating the protein with a cross-linking agent (a bifunctional molecule that reacts with and links groups in two different portions of the polypeptide), denaturing the protein, and analyzing the products by SDS-PAGE (Section 5-2D).

(a) An oligomer analyzed by SDS-PAGE shows a single 40 kD polypeptide. Brief treatment with a cross-linking agent yields the SDS-PAGE banding pattern shown below. What is the protein's probable subunit composition?



(b) Not shown in the electrophoretogram above are faint bands at 240 kD and 360 kD. What do these bands represent?

(c) Another protein analyzed by SDS-PAGE shows two polypeptides at 20 kD and 50 kD. Chemical cross-linking of the native protein yields the results shown below in SDS-PAGE. Not shown is a very faint band at 540 kD. What is the subunit composition of this protein?



Working with quaternary structure

4 Which amino acid pairs might exhibit identical Electron densities at 2.0-Å resolution?
Hint: The electron density of a hydrogen atom is too small to be apparent at this resolution.

5 The X-ray crystallographic analysis of a protein often fails to reveal the positions of the first few and/or the last few residues of a polypeptide chain. Explain.

6 A resolution of ~3.5 Å is necessary to clearly reveal the course of the polypeptide backbone in X-ray crystallography. Can any useful information about protein structure be obtained at 6 Å resolution?

7 What advantages does NMR provide over X-ray crystallography in characterizing protein structure? What is the major limitation of NMR analysis?

8 What specific role does knowledge of the primary amino acid sequence play in the determination of tertiary structure by X-ray crystallography?

ANSWERS Working with Ramachandran Plots and secondary structure

1
a. $\phi = 120°$; $\psi = 320°$.
b. ϕ is identical; ψ for an α helix is about 200° less.
c. ψ is nearly identical; ϕ is about 80° less.
d. $\phi = 40°$; $\psi = 310°$.
e. The ϕ and ψ angles will be random within the ranges $\phi \simeq 20$–$110°$ and $\psi \simeq 110$–$130°$ and 270–360°.
f. The carbonyl group of the peptide bond is rotated away from the side chain of the α carbon.

2
a. α Helix
b. Yes. There are about 20 residues in the β-sheet region.
c. Bonds in the highly unfavorable region involve Gly, which has no side chain and thus no ϕ rotation restriction.
d. The helical regions are mainly hydrophobic.
e. The β-sheet regions are mainly hydrophilic.
f. The features deduced from the Ramachandran diagram all are confirmed by the actual structure of lysozyme.

3
(a) The protein is probably a trimer of 40 kD subunits. The gel shows cross-linked dimers and trimers in addition to free monomers.
(b) The apparent masses of the faint bands are multiples of 120 kD and therefore probably represent protein trimers that have been chemically cross-linked to other trimers.
(c) The protein is a trimer of two 20 kD subunits and one 50 kD subunit. Some of the 90-kD oligomers have been cross-linked to form larger structures of 180 kD (a dimer), 270 kD (a trimer), and 540 kD (a hexamer).

4
Several pairs of amino acids give indistinguishable electron density maps: Asp and Asn, Glu and Gln, Thr and Val. Other amino acids that may have similar shapes are Ser and Cys, although the S atom of Cys has a much greater electron density than the O atom of Ser.

5
These parts of the protein are like tails and have several different Conformations, so they are what is termed "disordered" and will Not diffract xrays sufficient to reveal their multiple locations.

6
At 6 Å resolution, larger elements of protein structure can be identified, most notably helices, which have a diameter of several Å and are visible as rodlike shapes.

7
The advantages of NMR include (a) structural information from proteins that fail to crystallize and (2) information about protein folding and dynamics since protein movements can be traced over relatively long time scales. The primary disadvantage of NMR is that the protein must be no larger than ~40 D.

8
When interpreting an electron density map, knowledge of the primary structure allows the identification of specific amino acids along the polypeptide chain. This would otherwise be quite difficult if not impossible for R groups with similar shapes (see Question 4.)