Abstract: UV resonance Raman (UVR) spectroscopy was used to examine the solution conformation of poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) in their non-α-helical states. UVR measurements indicate that PLL (at pH = 2) and PGA (at pH = 9) exist mainly in a mixture of polyproline II (PPII) and a novel left-handed 2.51-helical conformation, which is an extended β-strand-like conformation with $\Psi \approx +170^\circ$ and $\Phi \approx -130^\circ$. Both of these conformations are highly exposed to water. The energies of these conformations are very similar. We see no evidence of any disordered “random coil” states. In addition, we find that a PLL and PGA mixture at neutral pH is $\sim$80% β-sheet and contains PPII and extended 2.51-helix conformations. The β-sheet conformation shows little evidence of amide backbone hydrogen bonding to water. We also developed a method to estimate the distribution of Ramachandran angles for these conformations, which we used to estimate a Ψ Ramachandran angle energy landscape. We believe that these are the first experimental studies to give direct information on protein and peptide energy landscapes.

Introduction


Until recently, protein unfolded states were assumed to consist of random coil conformations, where the polypeptide chains would adopt energetically allowed but randomly distributed $\Phi$ and $\Psi$ dihedral angles. Ideally, these structures were considered to be completely disordered with no correlations between adjacent peptide bonds $\Phi$ and $\Psi$ Ramachandran dihedral angles.21 However, this assumption has recently been seriously challenged.22–28

Numerous theoretical and experimental groups have been working on elucidating protein folding mechanisms over the last 50 years.1–21,23–28 A major challenge in this work is the required development of an energetic understanding of protein folding motifs and the sequence-specific phenomena that determine the folding energy landscape. In this regard, new theoretical paradigms such as the energy landscape theories have significantly aided thinking about protein folding. In addition, new theoretical studies have examined the conformational...
subspace of small and large peptides and have examined conformational energies. Molecular dynamical studies are becoming available which examine the temporal evolution of protein structure in time scales that are relevant for folding into equilibrium structures.29–33

This work has been aided by new experimental studies that characterize peptide conformations.24–49 What is most needed to continue progress is additional experimental insight into protein folding motifs and the energy mountain ranges that surround these structures. We50–52 as well as others,53–58 have been developing UV Raman spectroscopy (UVRS) to probe protein structure and dynamics. We recently examined the first stages in unfolding of α-helices and discovered that a mainly a 21-residue peptide melts from an α-helix conformation into a polyproline II conformation (PPII).34

In this work, we examine peptide conformations of peptides such as poly-L-glutamic acid (PGA) and poly-L-lysine (PLL) under conditions where their side chains are charged. We find that they occur as a mixture of PPII and a novel conformation, which is a subset of extended β-strand conformations, but which is best described as a 2.5-helix. If the side chain charges are neutralized, these peptides form α-helices, whereas if peptides with oppositely charged side chains are mixed, they form β-sheet conformations.59

We have developed insights into peptide secondary structures through examinations of their ~200-nm UV resonance Raman spectra. We obtain the most information from the amide III (AmnIII)56 vibration whose frequency we earlier found was correlated to the peptide conformational Ramachandran Ψ angle.34,60,61 We have recently developed quantitative relationships between peptide bond AmnIII frequencies, the peptide bond Ψ angle, and its hydrogen bonding pattern.62 In this work, these relationships are used to estimate the conformational energy differences between the PPII and 2.5-helix conformations, the Ψ angle energy landscape for the PPII and 2.5-helix conformations, as well as for the β-sheet structure. To our knowledge, these are the first experimental studies to directly give information on the energy landscape of peptide conformations along coordinates involved in conformational evolution.

This work shows clearly that the UVRS spectra of β-sheet conformations significantly differ from those of PPII and 2.5-helix ("single" β-strand) conformations. This ability to discriminate between conformations may prove useful for early detection of amyloid fibril formation in solutions of proteins.62

Experimental Section

Sample Preparation. Poly-L-Lysine HCl (PLL, MW$_{\text{vis}}$ = 28,500, MW$_{\text{alls}}$ = 20 200) and the sodium salt of poly-L-glutamic acid (PGA, MW$_{\text{vis}}$ = 17 000, MW$_{\text{alls}}$ = 8853) were purchased from Sigma Chemical and used as received. Solution spectra of PLL and PGA were measured at pH = 2 and pH = 9, respectively, to ensure the absence of α-helix contributions. The mixed PLL and PGA neutral pH sample solutions contained identical concentrations of lysine and glutamic acid residues. These samples were freshly prepared before the Raman measurements. The total peptide concentrations were kept below 0.3 mg/mL to avoid gel formation.

The 21-residue alanine-based peptide AAAAAAAAAARAA,$\alpha$ (AP) was prepared (HPLC pure) at the Pittsburgh Peptide Facility by using the solid-state peptide synthesis method. The AP solutions in water contained 1 mg/mL concentrations of AP and 0.2 M concentrations of sodium perchlorate, which was used as an internal intensity and frequency standard. All Raman spectra were normalized to the intensity of the ClO$_4^-$ Raman band (932 cm$^{-1}$).

A$_3$ and A$_2$ peptides were purchased from Bachem Bioscience, Inc. (King of Prussia, PA) and used as received. The A$_3$ – A$_2$ Raman difference spectral measurements utilized identical molar concentrations of A$_2$ and A$_3$ (0.34 and 0.2 mg/mL, respectively) in solutions containing identical sodium perchlorate concentrations (0.2 M). We normalized the Raman spectra to the intensity of the 932 cm$^{-1}$ perchlorate internal standard band. The A$_3$ – A$_2$ difference spectra were calculated by subtracting the normalized A$_3$ spectrum from the normalized A$_2$ spectrum at each temperature.

The undecapeptide XAO (MW = 985) was prepared (HPLC pure) at the Pittsburgh Peptide Facility by using the solid-state peptide synthesis method. The sequence of this peptide is Ac-XXAAAGAAOOAmide, where all amino acids are in their L form, A is ala, X is diaminobutyric acid (side chain CH$_2$CH$_2$NH$_2$), and O is ornithine (side chain CH$_2$NH$_2$+$\alpha$). We used 1 mg/mL solutions of XAO peptide containing 0.15 M sodium perchlorate. The UVRR spectra of XAO were also normalized to the ClO$_4^-$ Raman band intensity.
High-temperature spectra indicate a lack of conformational dependencies (Table 1) of the AmIII bands to those of PPII and the superscript question mark labels assignments that remain uncertain. The temperature dependence of the spectra involves small downshifts for the AmIII and AmII bands and small upshifts for the AmI band (Table 1) as the temperature increases. This temperature dependence is characteristic of peptide backbone conformations where the amide carbonyl and N–H groups are hydrogen bonded to water. The shifts occur because the water–amide hydrogen bond strengths decrease as the temperature increases. This favors a peptide bond resonance form with stronger bonding for the carbonyl and weaker bonding for the C(O)–N linkage, which result in the observed amide band shifts.

AmIII superscript 3 Band (∼1245 cm⁻¹) Signals PPII Conformations. Figure 2 compares the 204-nm UVR spectra of the unfolded PGA and PLL samples to spectra of the three ala-based peptides: XAO, AP, and A₅–₈ under conditions where they are predominantly in PPII conformations. The observed AmIII superscript 3 band frequency closely coincides with the AmIII frequency of the PPII conformations of XAO, AP, and A₅–₈. Since we know that the AmIII frequency strongly depends on the Ψ angle (and to much lesser degree on allowed Φ angles), we conclude that both PLL and PGA have solution conformations with Ψ angles similar to that of the PPII conformation.

The coincidence in frequency and the similar temperature dependencies (Table 1) of the AmIII superscript 3 bands to those of PPII conformations militates for the assignment of this band to PPII conformations of PGA and PLL. This conclusion is consistent with previous studies that also concluded that the unfolded state (PLL and PGA) have significant PPII content. These important observations dramatically simplify the spectral analysis in the AmIII region.

Thus, we enumerate these bands as AmIII superscript 3 (∼1271 cm⁻¹ (PLL) and ∼1272 cm⁻¹ (PGA)), AmIII superscript 3 (∼1245 cm⁻¹ (PLL) and ∼1249 cm⁻¹ (PGA)), AmIII superscript 7 (∼1316 cm⁻¹ (PLL) and ∼1319 cm⁻¹ (PGA)), and AmIII superscript 7 (∼1296 cm⁻¹ (PLL) and ∼1298 cm⁻¹ (PGA)), where the subscripts label different amide III spectral region bands as we recently discussed in detail, and the superscript question mark labels assignments that remain uncertain.

The temperature dependence of the spectra involves small downshifts for the AmIII and AmII bands and small upshifts for the AmI band (Table 1) as the temperature increases. This temperature dependence is characteristic of peptide backbone conformations where the amide carbonyl and N–H groups are hydrogen bonded to water. The shifts occur because the water–amide hydrogen bond strengths decrease as the temperature increases. This favors a peptide bond resonance form with stronger bonding for the carbonyl and weaker bonding for the C(O)–N linkage, which result in the observed amide band shifts.

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The PPII structure is a commonly observed non-α-helix low-energy conformation because of its stabilization by peptide-water interactions. This open conformation permits the simultaneous hydrogen bonding of water to amide bonds, as well as important bridging hydrogen bonds between water molecules. In addition, Hinderaker and Raines recently proposed an additional PPII stabilization mechanism. They suggested that the PPII conformation is stabilized because of especially favorable n-π* interactions between the carbonyl oxygen of peptide bonds and the carbonyl carbons of adjacent peptide bonds. Whatever the case, investigators now find that the unfolded states of many proteins as well as the unfolded states of moderate and long peptides contain significant fractions of PPII.

AmIII≤φ Band (≈1271 cm−1) Signals the Presence of a β-Strand (2.5-Helix) Conformation. The electrostatic repulsions between the PLL- and PGA-charged side chains prevent formation of α-helical conformations and should force more extended conformations, such as PPII and/or extended β-strand(s). The PPII conformation clearly does not require side chain repulsion since it occurs for polyalanine derivatives such as AP and XAO. Further, the K7 peptide shows significant PPII content at pH 12 in the absence of salt as well as in 4 M NaCl.

The pH = 2 PLL and pH = 9 PGA spectra also show a second AmIII≤φ region band at ≈1271 cm−1 denoted as AmIII≤φ (Figures 1 and 2). This band is absent in mainly PPII ala-based peptides with neutral side chains. Thus, it must result from the additional PLL and PGA electrostatic repulsions between ionized side chains. We expect that these repulsions will induce a more extended conformation with a Ramachandran angle greater than the ψ = 145° of the PPII conformation. Given the dependence of the AmIII≤φ frequency on the ψ angle that we previously demonstrated, we expect a new AmIII≤φ band to occur at a higher frequency, as observed.

Because of the severe overlap with the AmIII≤φ bands, it is not possible to accurately determine the AmIII≤φ temperature dependence. However, the temperature dependence is qualitatively similar to that of fully exposed conformations such as PPII. Thus, we assign the conformation to an extended β-strand-like conformation (2.5-helix, see below) and conclude that these

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Table 1. Temperature Dependence of Amide UV Raman Bands of Non-α-Helical Polypeptides: pH = 2 PLL, pH = 9 PGA, and PPII Peptides: XAO, Ala5-Ala3, AP

<table>
<thead>
<tr>
<th>Peptide</th>
<th>AmIII≤φ Band (&gt;80% PPII), neutral pH</th>
<th>AmIII≤φ Band (essentially PPII), neutral pH</th>
<th>AmIII≤φ Band (essentially PPII), neutral pH</th>
<th>PLL (unfolded (PPII + extended), pH = 2)</th>
<th>PGA (unfolded (PPII + extended), pH = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>0.02 ± 0.01</td>
<td>0.052 ± 0.02</td>
<td>0.052 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>AII</td>
<td>−0.14 ± 0.01</td>
<td>−0.14 ± 0.01</td>
<td>−0.14 ± 0.01</td>
<td>−0.15 ± 1.05</td>
<td>−0.13 ± 1.05</td>
</tr>
<tr>
<td>C6H4(I)</td>
<td>0.008 ± 0.016</td>
<td>−0.015 ± 0.02</td>
<td>−0.015 ± 0.02</td>
<td>−0.01 ± 0.01</td>
<td>−0.01 ± 0.01</td>
</tr>
<tr>
<td>C6H6(B)</td>
<td>0.018 ± 0.017</td>
<td>−0.01 ± 0.02</td>
<td>−0.01 ± 0.02</td>
<td>−0.04 ± 1.01</td>
<td>0.036 ± 1.01</td>
</tr>
<tr>
<td>AII1≤φ</td>
<td>−0.03 ± 0.02</td>
<td>−0.03 ± 0.01</td>
<td>−0.03 ± 0.01</td>
<td>−0.03 ± 1.00</td>
<td>−0.03 ± 1.00</td>
</tr>
<tr>
<td>AII1≤φ (2.5-helix)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>AII1≤φ (PPII)</td>
<td>−0.10 ± 0.02</td>
<td>−0.094 ± 0.018</td>
<td>−0.094 ± 0.018</td>
<td>−0.1 ± 1.02</td>
<td>−0.12 ± 1.02</td>
</tr>
</tbody>
</table>

---

Figure 2. Comparison of 204-nm UVR spectra of unfolded states of PGA (pH = 9) and PLL (pH = 2) in water at 0 °C to the spectra of the PPII states of alanine-rich peptides XAO, AP, and As–As at 0 °C.

PGA and PLA samples contain a mixture of PPII and extended β-strand-like conformations.

Assuming similar Raman cross sections for these conformations, we roughly estimate that “unfolded” PLL and PGA both consist of ~60% PPII and ~40% β-strand. In contrast, we only detect very small contributions from β-strand-like conformations that significantly differ spectrally from those of β-sheet conformations (compare Figures 2, 7, and 8).

The lack of a temperature dependence of the relative intensity ratios suggests that these conformations have similar energies (Table 4).

It should be noted that Raman optical activity studies of “unfolded” peptides and proteins show positive features between ~1314 to ~1325 cm⁻¹, which are thought to signal the PPII conformation.63–66 The large frequency spread for these positive bands may indicate the existence of a variety of PPII-like left-handed helical conformations with significantly differing Ψ and Φ angles.

Table 2. Temperature Dependencies of Amide UV Raman Bands of PLL–PGA Mixture

<table>
<thead>
<tr>
<th>PLL–PGA mixture, ~60% β-sheet, ~40% unfolded, a</th>
<th>PLL–PGA mixture, pure PPII, 2.5i conformations b</th>
<th>PLL–PGA mixture, pure β-sheet spectrum c</th>
</tr>
</thead>
<tbody>
<tr>
<td>dν/dT</td>
<td>ν(νC, cm⁻¹)</td>
<td>dν/dT</td>
</tr>
<tr>
<td>AI</td>
<td>0.022</td>
<td>1668</td>
</tr>
<tr>
<td>AII</td>
<td>-0.071</td>
<td>1548</td>
</tr>
<tr>
<td>CH₂(1)</td>
<td>-0.007</td>
<td>1402</td>
</tr>
<tr>
<td>CH₂(2)</td>
<td>0</td>
<td>1381</td>
</tr>
<tr>
<td>AIII2</td>
<td>0</td>
<td>1290</td>
</tr>
<tr>
<td>AIII3</td>
<td>-0.062</td>
<td>1310</td>
</tr>
</tbody>
</table>

a Experimental spectra, neutral pH (~50% β-sheet, ~50% unfolded). b See text for details.

Table 3. Distances between Ionized Side Chain Charges in PLL and PGA for Ψ and Φ Angles of PPII and 2.5i-Helix Conformations

<table>
<thead>
<tr>
<th>distance between side chain charges</th>
<th>Ψ = +145°</th>
<th>Ψ = +170°</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLL</td>
<td>11.245</td>
<td>11.67</td>
</tr>
<tr>
<td>i ↔ i + 1</td>
<td>12.389</td>
<td>10.146</td>
</tr>
<tr>
<td>i ↔ i + 2</td>
<td>9.722</td>
<td>12.266</td>
</tr>
<tr>
<td>i ↔ i + 3</td>
<td>16.427</td>
<td>18.130</td>
</tr>
<tr>
<td>PGA</td>
<td>8.328</td>
<td>8.421</td>
</tr>
<tr>
<td>i ↔ i + 1</td>
<td>9.852</td>
<td>8.639</td>
</tr>
<tr>
<td>i ↔ i + 2</td>
<td>9.232</td>
<td>11.381</td>
</tr>
<tr>
<td>i ↔ i + 3</td>
<td>14.563</td>
<td>16.101</td>
</tr>
</tbody>
</table>

The table lists the standard deviation of the Ψ angle, σ, the ratio R of amplitudes of the 2.5i-helix relative to that of the PPII conformation, the Gibbs free energy difference between the 2.5i-helix and the PPII conformations, and the torsional constant K for Ψ angle deformations.

Whatever the case, we detect only PPII and extended β-strand-like conformations that significantly differ spectrally from those of β-sheet conformations (compare Figures 2, 7, and 8).

As discussed below (eq 2), the ~1271 cm⁻¹ AmIII band frequency results in a calculated β-strand-like Ψ angle of ~170°, if we neglect any Φ angle frequency dependence. This neglect of the Φ angle dependence is justified in view of the known small Φ angle amide III frequency dependence60,67 and the fact that only modest changes in the Φ angle are likely to occur between the relevant conformations with different Ψ angles. Further, we and others recently estimated that the Ψ angle dependence can result in up to an ~110 cm⁻¹ shift,61 while the Φ angle results in no more than a 20 cm⁻¹ AmIII frequency shift.67,68

The β-Strand-like Conformation Is a 2.5i-Helix. We developed insight into this new conformation by examining the dependence of the electrostatic repulsion energies on the Φ angle for a fixed Ψ angle of +170° (Figure 3). We utilized the HyperChem amino acid database to construct approximate structures to estimate the distances between charges located on PLL and PGA side chains. Figure 3 shows that the total

electrostatic repulsion energy has a minimum near $\Phi \approx -130^\circ$ for PGA. The situation for PLL has the same trend (Table 3).

Figure 4 indicates a rough structure for our PGA minimum repulsion energy conformation, which utilizes the determined $\Psi$ and $\Phi$ angles of $+170^\circ$ and $-130^\circ$, respectively. The resulting extended $\beta$-strand occurs as a $2.5_1$-helix conformation.

Krimm and Mark’s previous theoretical study of conformations of polypeptides with ionized side chains also proposed that the charged side chains of PLL and PGA stabilize a helical conformation with approximately 2.5 residues per helical turn. They also showed that the number of residues per turn was essentially independent of side chain length for side chains equal to or longer than that of glutamic acid. However, for a 64-residue PGA they proposed a minimum energy conformation with $\Psi = -170^\circ$ and $\Phi = -155^\circ$ (in their original article they used an older definition for the $\Psi$ and $\Phi$ angles). Future work will be required to discriminate between these very similar structures to determine the actual $\Phi$ angles.

Our study here is the first, to our knowledge, to experimentally detect a stable $2.5_1$-helix conformation in peptides and proteins. We also compared the distances between charges in our putative $2.5_1$-helix to those in a PPII helix. Table 3 shows that the larger separation distances occur in the $2.5_1$-helix compared to the PPII helix. This lowers the $2.5_1$-helix total energy such that it is very close to that of the PPII conformation (Table 4).

Our spectral data and the lack of a significant temperature dependence of the relative Raman intensities clearly demonstrate that these conformations are close in energy. Given our present inability to accurately curve resolve the PPII peak from the $2.5_1$ AmIII peaks, our incomplete understanding of the degeneracies of these two conformations, the unknown dependence of the Raman cross sections on conformation, and the measured modest temperature dependence, on the basis of the relative intensity ratios, we can only visually roughly estimate from the relative Raman intensities that the $2.5_1$ conformations of PLL and PGA are $<300$ cal/mol higher in energy than the PPII conformation at room temperature (however, see below). We are in the process of modeling this $2.5_1$-helix conformation to better determine its detailed geometry.

Our observations of the $2.5_1$-helix was, in fact, partially presupposed by Tiffany and Krimm who originally proposed that aqueous solution denatured states of PLL and PGA would contain some local order and would not be in a completely “disordered” form. The structure was suggested to involve an extended $3_1$-helix or a PPII helix, which is also a left-handed helix, with three amino acid residues per turn, with $\Phi$- and $\Psi$-Ramachandran angles of $-75^\circ$ and $145^\circ$, respectively. In addition, more recent studies report evidence for PPII content in “unfolded” PLL and PGA.

2. PLL—PGA $\beta$-Sheet Conformation. Equimolar mixtures of PLL and PGA at neutral pH are known to form antiparallel $\beta$-sheets. This is clearly demonstrated in the Figure 5 comparison of the CD spectrum of a PLL—PGA mixture to the CD spectra of $\alpha$-helical, $\beta$-sheet, and “unordered” peptides. The PLL—PGA mixture spectrum, especially the $\sim$217-nm negative feature, clearly demonstrates a significant fraction of $\beta$-sheet. In addition, the CD spectra of the PLL—PGA mixture shows an increasing 217-nm trough as the temperature increases, which indicates that the $\beta$-sheet content slightly increases with temperature (see inset to Figure 5).
Figure 6 shows the UVR spectra of the PLL–PGA mixture at 0 and +70 °C. The entire AmIII3 band profile of the PLL–PGA mixture is red-shifted compared to unfolded PLL and PGA (Figures 1, 2, and 7, Tables 1 and 2) due to formation of the antiparallel β-sheet structure. Figure 6 shows that overall spectra of the PLL–PGA mixture are almost independent of temperature, indicating that the β-sheet conformation does not melt significantly over this temperature range. Further, the temperature dependence of the AmI, II, and III band frequencies in the PLL–PGA mixture (∼60% β-sheet) is significantly decreased (∼2-fold) compared to those in the PPII and β-strand (2.51-helix) conformations due to the decreased peptide-water hydrogen bonding of the β-sheet structure.

Calculation of Pure β-Sheet Spectrum from PLL–PGA Mixture UV Raman Spectra. The PLL–PGA mixture UV spectra in Figures 6 and 7 are broad and show high frequency shoulders. This contrasts with the AmIII symmetric band shape found by Chi et al.52 for the β-sheet conformation of a library of proteins (Figure 7). Thus, the PLL–PGA mixture sample appears to contain additional peptide conformations. Since the PLL and PGA side chains are highly ionized at this neutral pH, it is likely that these other conformations are the extended 2.51-helix and the PPII conformations discussed above.

We can calculate the pure β-sheet PGA–PLL Raman spectrum by subtracting off the spectra of these other conformations. We assume that the spectra of these other conformations are the sum of the individual PLL and PGA PPII and β-strand (2.51-helix) spectra. The criteria for the amounts subtracted are that the resultant spectra (Figure 7) best fit the β-sheet spectrum of Chi et al.52 (except that we do not include the amide I region in the fit due to the potential residual contribution of the water bending band). We find non-β-sheet conformation fractions of 42% at 0 °C and 35% at 70 °C. Thus, the β-sheet content slightly increases with temperature.

Figure 8 shows these calculated pure β-sheet spectra at 0 and 70 °C. The β-sheet AmIII peak is symmetric without any shoulders and is similar to that found by Chi et al.52 (compare Figures 7 and 8). As also shown in Figure 8, the pure PLL–PGA mixture β-sheet spectrum shows a ∼10-fold decreased temperature frequency dependence for the AmII and III bands and a ∼3-fold decreased AmI band frequency dependence than occurs for the PPII and 2.51-helix conformations. This is expected due to the decreased water–amide bond hydrogen bonding of the β-sheet since the β-sheet satisfies its hydrogen bonding mainly through interpeptide hydrogen bonds.

The large UVRS spectral differences between the PLL–PGA mixture pure β-sheet conformation and that of PLL and PGA in their unfolded states (compare Figures 8 and 7) offer opportunities for characterizing subtle issues of β-sheet con-
formation. This could be valuable in kinetic and steady-state investigations of systems such as amyloid fibrils.62

Ψ Ramachandran Angular Distribution for PLL and PGA β-Sheet, PPII, and 2.51-Helix. The β-sheet, PPII, and 2.51-helix amide bands are significantly broadened from their estimated 7.5 cm\(^{-1}\) homogeneous line width determined in crystals.34 This broadening probably results from the distribution of Ψ angles that occurs for these conformations in solution. We developed a deconvolution method for the AmIII\(_3\) band frequencies that determines the inhomogeneous distribution of AmIII\(_3\) band frequencies.34 We then developed a method to use this frequency distribution to calculate the Ψ angle distribution from the measured AmIII\(_3\) band shapes.34,61 Originally we estimated 7.5 cm\(^{-1}\) for the 2.51-helix with ψ

\[ υ_{\text{III}3}(Ψ) = 1265 \text{ cm}^{-1} - 46.8 \text{ cm}^{-1} \sin(Ψ + 5.2°) \] (1)

which allows us to roughly estimate the relationship between the AmIII\(_3\) frequency and Ψ Ramachandran angle for amides and proteins in solution. The sinusoidal nature of eq 1 was theoretically predicted by Asher et al.60 and explained in terms of different degrees of coupling between the N−H and C\(\alpha\)−H bending motions at different Ψ angles.

In our most recent study,61 we examined the dependence of the AmIII\(_3\) frequencies of peptide bonds upon the peptide bond hydrogen bonding. This study also elucidated the temperature dependence of the AmIII\(_3\) frequencies that result from the temperature dependence of its hydrogen bonding. We quantified this hydrogen bonding-induced frequency dependencies for all major peptide/protein secondary structure conformations61 and were able to propose a family of equations to relate the AmIII\(_3\) frequency directly to the Ψ Ramachandran angle for both the PPII and the 2.51-helix forms.34 Originally we utilized the following equation:

\[ v_{\text{III}3}(Ψ) = 1256 \text{ cm}^{-1} - 54 \text{ cm}^{-1} \sin(Ψ + 26°) \] − 0.11 \text{ cm}^{-1}C(T − T_0) \] (2)

where \( T_0 = 0 \text{ °C} \).

In contrast, for PLL−PGA mixture antiparallel β-sheet, which is dominated by two end-on peptide bond−peptide bond hydrogen bondings,61 we would use eq 2:

\[ v_{\text{III}3}^{αβ}(Ψ, T, \text{HB}) = [1244 \text{ cm}^{-1} - 54 \text{ cm}^{-1} \sin(Ψ + 26°)] \] (3)

Figure 9 shows the Ψ angle distribution calculated for PLL (pH = 2) and PGA (pH = 9) and for the PLL−PGA mixture β-sheet. Figure 9 displays the existence of the two different conformations of unfolded PLL and PGA, with two distinct maxima near Ψ ≈ 145° (PPII) and Ψ ≈ 170° (2.51-helix).

As shown in Figure 9, these calculated distributions are well fit by Gaussian line shapes. The estimated standard deviation for PLL at pH = 2 is σ ≈ 11° for both the PPII and the 2.51-helix conformations, while PGA at pH = 9 shows a broader distribution for PPII with σ ≈ 22° and a sharper distribution for the 2.51-helix with σ ≈ 5° (Table 4). Both of these distributions are consistent with an electrostatic repulsive interaction that destabilizes the PPII conformation relative to the 2.51-helix. The electrostatic interactions are larger for the shorter side chain PGA relative to PLL, which should lead to a sharper distribution of angles for the 2.51-helix. In fact, the PGA PPII conformation minimum Ψ angle is shifted toward that of the 2.51-helix conformation.

The calculated β-sheet spectrum of the PLL−PGA mixture shows the broadest distribution of Ψ angles with σ ≈ 23°. This indicates that the β-sheet conformations show the smallest energy penalty for changes in their Ψ angles. This is expected since a significant flexibility would exist for interpeptide bond hydrogen bond linkages; the angular dependence of hydrogen bond energies should be small around the equilibrium configuration.

Determination of Gibbs Free Energy Landscape for PPII ↔ 2.51-Helix along the Ψ Angle Reaction Coordinate. We can estimate the Gibbs free energy landscape of the PPII and 2.51-helix conformations along the Ψ angle reaction coordinate from the calculated Ψ angle distributions. We assume that the distributions have identical degeneracies and that the probability of each conformation to occur at a particular Ψ angle is given by a simple Boltzmann distribution:

\[ \frac{n(Ψ)}{n(Ψ_0)} = \exp\left\{-\frac{[G(Ψ) − G(Ψ_0)]}{Nk_BT}\right\} \] (4)

where \( n(Ψ)/n(Ψ_0) \) is the ratio of populations with Ramachandran angles Ψ, and \( G(Ψ_0) \) is the Gibbs free energy of the conformations with angle Ψ, \( G(Ψ) \) is the Gibbs free energy at
The angle distributions are well modeled by Gaussians (Figure 9) results from the fact that the potential energy of a conformation about its equilibrium is given by

\[ \Delta E = G(\Psi) = 0 \] (see text for details).

The lowest conformational energy, \( N_\Lambda \) is Avogadro’s number, \( k_B \) is the Boltzmann constant, and \( T \) is temperature.

Thus, the Gibbs free energy difference between the PPII and 2.51-helix conformations can be estimated as:

\[ \Delta G = G_{\text{PPII}} - G_{2.51} = -N_A k_B T \ln(n_{\text{PPPI}}/n_{2.51}) = -N_A k_B T \ln R \] (5)

where \( R \) is the fractional distribution ratio at the energy minimum of the two conformations in Figure 9.

Using eq 5, we obtain \( \Delta G \) values of \(-152 \) and \(-74 \) cal/mol for PGA and PLL, respectively, for \( \Psi \) angles at the minimum of these energy landscapes (see Table 4). The fact that these \( \Psi \) angle distributions are well modeled by Gaussians (Figure 9) results from the fact that the potential energy of a conformation about its equilibrium is given by \( E = E_o + kA\Psi^2 \), where \( E_o \) is the energy at the minimum \( \Psi \) of a conformation and \( K \) is the torsional force constant. Table 4 shows that the torsional constant is the smallest for the \( \beta \)-sheet conformation while it is the largest for the PGA 2.51, conformation. The 2.51-helix \( \rightarrow \) PPII crossing barriers for PLL and PGA have similar values of \(-170 \) and \(-200 \) cal/mol, respectively. In contrast, PPII \( \rightarrow \) 2.51-helix crossing barriers for PLL and PGA have values of \(-250 \) and \(-70 \) cal/mol, respectively. These observations are expected, since the stronger electrostatic repulsion between charges located on shorter side chains of PGA (with respect to that of PLL) should stabilize the 2.51-helix and destabilize the PPII conformations.

We can use this information to calculate the Gibbs free energy landscape of these peptides along the Ramachandran \( \Psi \) angle coordinate (Figure 10). We do not know the absolute energy scale to relate the energy landscapes of these different peptides. However, shown here are the relative Gibbs energies, \( G-G_0 \). For each peptide, we assign the Gibbs energy value at the lowest conformational energy \( G_0 \) to be zero. We find similar energy landscapes for PGA and PLL when their side chains are ionized.

The side chain electrostatic repulsions lower the 2.51-helix conformation energy relative to that of the PPII conformation. This effect is more significant for the shorter side chain PGA peptides, where the electrostatic repulsions exert a larger energetic penalty for deviations from the minimum energy \( \Psi \) angle conformation geometry of the 2.51-helix. The barriers between these two conformations are slightly less than \( N_A k_B T \) (\( \sim 540 \) cal/mol at \( 0 ^\circ C \)). Thus, it is likely in solution that these conformations rapidly interconvert. The major changes in geometry would involve mainly the \( \Psi \) and \( \Phi \) coordinates. These changes would merely change the number of residues per turn.

The \( \alpha \)-helix conformation is the most stable PGA and PLL conformation at \( 0 ^\circ C \) if the side chains are neutralized by changing pH.\(^{90,99} \) In this case, the \( \alpha \)-helix conformations melt mainly to the PPII conformations upon temperature increases to room temperature.

The molecular mechanism of this conformational change is difficult to envision since not only do the interpeptide hydrogen bonds have to rupture, but the helix must also unwind and then rewind to reverse its handedness. Previous considerations of \( \alpha \)-helix melting imagined that it was induced by the stepwise \( \alpha \)-helix hydration which stabilized intermediates such as \( \beta \)-turns and reverse turns.\(^{100–102} \) However, the set of steps that would lead to a reverse helix are harder to visualize.

Conclusions

Our study of unfolded states of PLL and PGA indicates that they exist as a mixture of PPII and the extended 2.51-helix (\( \beta \)-strand-like) conformations. The charged side chains of pH = 2 PLL and pH = 9 PGA force the PLL and PGA chains in water to adopt more extended conformations to minimize interchain repulsions. The \( \beta \)-sheet structure of the PLL–PGA mixture showed little evidence for hydrogen bonding between the polypeptide backbone and water. We also utilized a new algorithm that allowed us to estimate the \( \Psi \) Ramachandran angle from the AmIH$_3$ frequency. This analysis demonstrates that each conformation has a distribution of \( \Psi \) angles about the minimum \( \Psi \) conformational energy. The \( \Psi \) angle distribution of the PGA 2.51-helix, which is sharper than that of PLL 2.51-helix, as well as the absence of the 2.51-helix conformation in alanine-based peptides are consistent with the hypothesized electrostatic mechanism of stabilization of the 2.51-helix. We were able to calculate the \( \Psi \) angle energy landscape of these observed conformations. This is an important advance since the \( \Psi \) angle coordinate is the most important coordinate for protein and peptide secondary structure changes.

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Figure 10. Estimated Gibbs free energy landscapes \( G-G_0 \) for pH = 2 PLL, pH = 9 PGA, and PLL–PGA pure \( \beta \)-sheet along the \( \Psi \) angle coordinate. The side chain electrostatic repulsions lower the 2.51-helix conformation energy relative to that of the PPII conformation. This effect is more significant for the shorter side chain PGA peptides, where the electrostatic repulsions exert a larger energetic penalty for deviations from the minimum energy \( \Psi \) angle conformation geometry of the 2.51-helix. The barriers between these two conformations are slightly less than \( N_A k_B T \) (\( \sim 540 \) cal/mol at \( 0 ^\circ C \)). Thus, it is likely in solution that these conformations rapidly interconvert. The major changes in geometry would involve mainly the \( \Psi \) and \( \Phi \) coordinates. These changes would merely change the number of residues per turn.

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