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Host-Guest Scale of Left-Handed Polyproline II Helix Formation

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ABSTRACT **Despite the clear importance of the left-handed polyproline II (PPII) helical conformation in many physiologically important processes as well as its potential significance in protein unfolded states, little is known about the physical determinants of this conformation. We present here a scale of relative PPII helix-forming propensities measured for all residues, except tyrosine and tryptophan, in a proline-based host peptide system. Proline has the highest measured propensity in this system, a result of strong steric interactions that occur between adjacent prolyl rings. The other measured propensities are consistent with backbone solvation being an important component in PPII helix formation. Side chain to backbone hydrogen bonding may also play a role in stabilizing this conformation. The PPII helix-forming propensity scale will prove useful in future studies of the conformational properties of proline-rich sequences as well as provide insights into the prevalence of PPII helices in protein unfolded states. Proteins 2003;53:68–75.** © 2003 Wiley-Liss, Inc.

Key words: proline-rich sequences; unfolded proteins; protein-protein interactions

INTRODUCTION

Of late it has become increasingly apparent that the left-handed polyproline II (PPII) helix is a secondary structure of great importance. PPII helices play vital roles in processes such as signal transduction, transcription, cell motility, and the immune response.[1–3] They are a major feature of structural proteins such as collagen[4] and plant cell wall proteins.[5] The PPII helix is commonly believed to be the dominant conformation for proline-rich regions of sequence[2,6] as well as being adopted by numerous sequences devoid of proline.[7–11] Although only about 2% of residues in known protein structures are found in PPII helices four residues long or longer,[12,13] as many as 10% of all residues are found in the PPII conformation.[14] Recently, Blanch et al.[15] hypothesized that the PPII helix was the precursor conformation in amyloid formation.

Over three decades ago, Krimm and coworkers[9,10] hypothesized that PPII helices were a significant component of denatured proteins. Over the years a number of groups have published data in support of this hypothesis.[8,16] More recently, Barron and coworkers[16–18] have shown using Raman optical activity that natively disordered proteins contain a significant amount of PPII helix. Pappu et al.[19,20] have shown via computer modeling that a significant fraction of the residues in unfolded proteins occupy the PPII region of (ϕ, ψ)-space. Most recently, Shi et al.[21] found that a seven-residue alanine peptide, too short to form a stable α-helix, is instead a PPII helix in aqueous solution.

Despite the clear importance of the PPII helix, little is known about the physical determinants of this structure, which is characterized by repetitive backbone dihedrals of (ϕ,ψ) = ($-75°$, $+145°$) and precisely three residues per turn. It is known that polymers and peptides consisting solely of proline will adopt this structure as a result of steric interactions between the prolyl rings.[22] In addition, it has been suggested that backbone solvation is an important determinant for nonproline residues adopting this conformation.[7,20,21,23–25] In previous work, we introduced a host-guest system useful for the study of the propensities of residues to adopt the PPII helical structure.[23] This host system consists of a poly(proline)-based peptide known to adopt the PPII conformation, into the center of which guest residues can be introduced. The PPII content can then be determined using circular dichroism (CD) spectroscopy. The data obtained in this earlier work support the hypothesis that backbone solvation is a major determinant of PPII helix formation. Here we present a more complete scale of PPII helix-forming propensities derived using this host-guest system. We have measured propensities for all residues except tryptophan and tyrosine. This more complete scale will provide the foundation for detailed studies into the physical bases of PPII helix formation. Although this scale is most applicable to the study of the conformational properties of proline-rich sequences, the data presented also provide insights into the conformational behavior of sequences devoid of proline.

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TABLE I. Peptides Examined, Positions and Heights of Maxima in Spectra at 5°C and pH 7, and estimated PPII content

Peptide	Sequence	$[\theta]_{max}$ (10^{-3} deg · dmol^{-1} · cm^2)	Wavelength of maximum (nm)	%PPII[a]
PPP	Ac-$(Pro)_7$-Gly-Tyr-NH_2	3100	228.5	67 ± 2
PQP	Ac-$(Pro)_3$-Gln-$(Pro)_3$-Gly-Tyr-NH_2	2900	227.5	66 ± 2
PDP	Ac-$(Pro)_3$-Asp-$(Pro)_3$-Gly-Tyr-NH_2	2500	227.5	63 ± 2
PAP	Ac-$(Pro)_3$-Ala-$(Pro)_3$-Gly-Tyr-NH_2	2300	226.5	61 ± 2
PRP	Ac-$(Pro)_3$-Arg-$(Pro)_3$-Gly-Tyr-NH_2	2300	228.0	61 ± 2
PEP	Ac-$(Pro)_3$-Glu-$(Pro)_3$-Gly-Tyr-NH_2	2200	227.0	61 ± 2
PKP	Ac-$(Pro)_3$-Lys-$(Pro)_3$-Gly-Tyr-NH_2	2000	227.5	59 ± 2
PGP	Ac-$(Pro)_3$-Gly-$(Pro)_3$-Gly-Tyr-NH_2	1900	227.0	58 ± 2
PLP	Ac-$(Pro)_3$-Leu-$(Pro)_3$-Gly-Tyr-NH_2	1800	227.5	58 ± 2
PFP	Ac-$(Pro)_3$-Phe-$(Pro)_3$-Gly-Tyr-NH_2	1800	228.5	58 ± 2
PSP	Ac-$(Pro)_3$-Ser-$(Pro)_3$-Gly-Tyr-NH_2	1800	228.5	58 ± 2
PMP	Ac-$(Pro)_3$-Met-$(Pro)_3$-Gly-Tyr-NH_2	1500	227.0	55 ± 2
PCP	Ac-$(Pro)_3$-Cys-$(Pro)_3$-Gly-Tyr-NH_2	1500	229.5	55 ± 2
PHP	Ac-$(Pro)_3$-His-$(Pro)_3$-Gly-Tyr-NH_2	1500	228.0	55 ± 2
PNP	Ac-$(Pro)_3$-Asn-$(Pro)_3$-Gly-Tyr-NH_2	1400	228.5	55 ± 2
PTP	Ac-$(Pro)_3$-Thr-$(Pro)_3$-Gly-Tyr-NH_2	1100	228.5	53 ± 1
PIP	Ac-$(Pro)_3$-Ile-$(Pro)_3$-Gly-Tyr-NH_2	700	229.5	50 ± 1
PVP	Ac-$(Pro)_3$-Val-$(Pro)_3$-Gly-Tyr-NH_2	600	229.0	49 ± 1

[a]Errors estimated by taking the error in measured molar ellipticities to be ±3%.

MATERIALS AND METHODS

Peptides used in this study are listed in Table I and were purchased from Peptidogenic Research, and Co. (Livermore, CA). All peptides had their identities confirmed using mass spectrometry and were purified to ≥95 % purity using reverse-phase HPLC. Stock solutions were prepared by dissolving peptides in a buffer containing 5 mM potassium phosphate, 5 mM sodium fluoride, and 0.02 % sodium azide as a preservative. The pH of each stock solution was adjusted to 7 using sodium hydroxide and hydrochloric acid solutions. Peptide concentration was determined using the method of Brandts and Kaplan.[26] Absorbance was measured using a 1.0 cm path length cuvette in a Genesys 5 spectrophotometer.

Circular dichroism (CD) spectra were measured using a Jasco J-710 spectropolarimeter. A water-jacketed, 1 mm path length cylindrical quartz cuvette was used with solutions containing 100–200 μM peptide. Spectra were collected at 0.5 nm resolution and a scan rate of 200 nm · min.$^{-1}$ Reported spectra are averages of 20 or 30 scans and are not smoothed. Each spectrum was measured at least three times using separately prepared peptide solutions. Reported molar ellipticities are estimated to have errors of around ±3%.

PPII helical content of the peptides was estimated from the height of the maximum in each CD spectrum, $[\theta]_{max}$, using

$$\%\text{PPII} = \frac{[\theta]_{max} + 6100}{13700} \times 100. \quad (1)$$

The upper (100%) and lower (0%) limits were determined from CD spectra for the poly(proline) host peptide in an 8.4 M guanidine hydrochloride solution and for a peptide model for completely disordered proteins, respectively.[23] The upper limit is somewhat approximate. The PPII content of the host poly(proline) peptide increased upon titration from 8.0 to 8.4 M guanidine hydrochloride, with the rate of increase as a function of concentration only just appearing to become asymptotic at these levels.[23]

The oligomeric states of the peptides were determined from CD spectra collected during temperature titrations and from dynamic light scattering (DLS). DLS data were collected on a DynaPro-MS/X by Protein Solutions (Charlotte, VA) using solutions with peptide concentrations in the range 7–14 mM. Analysis was performed using software provided with the instrument.

RESULTS AND DISCUSSION

Host-Guest Experiment Design

The host-guest measurements described here were carried out using peptides of the form

Ac-$(Pro)_3$-Xaa-$(Pro)_3$-Gly-Tyr-NH_2

where Xaa is the guest residue (Table I). Each peptide is named PXP where the X corresponds to the one-letter amino acid code for the guest residue. The termini are blocked in order to remove electrostatic interactions. The C-terminal –Gly-Tyr pair are included for concentration determination using tyrosine absorbance.[26]

We have not included data for tyrosine and tryptophan guest residues. As noted by Chakrabartty et al.[27] and later by Krittanai and Johnson,[28] the side chains of the aromatic residues, in particular tyrosine and tryptophan, absorb in the far-UV region and have effects upon CD spectra that are independent of the structures adopted by the polypeptide backbone. Consequently, the CD signals collected for peptides with tyrosine and tryptophan guest residues will contain such effects, and estimated PPII helix contents for these peptides would be inaccurate unless corrected. Such corrections have proven problematic. Following the approach of Chakrabartty et al.,[27] we have attempted to determine correction factors by measur-

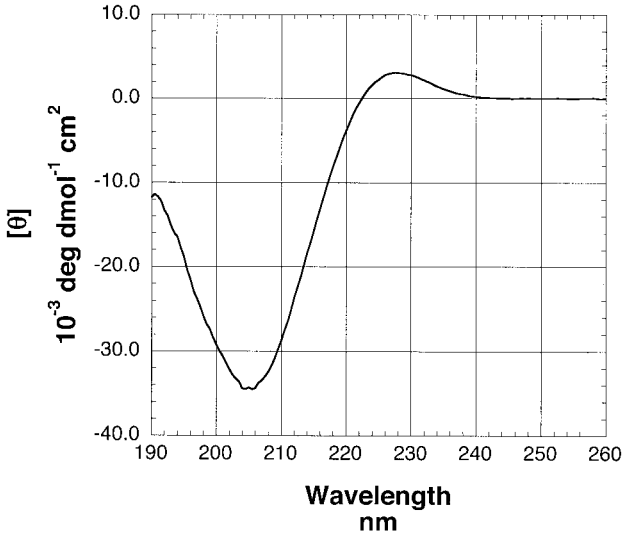

Fig. 1. Far-UV CD spectrum of peptide PPP (Ac-$(Pro)_7$-Gly-Tyr-NH_2) at 5°C and pH 7. This spectrum is characteristic of the PPII helical conformation.

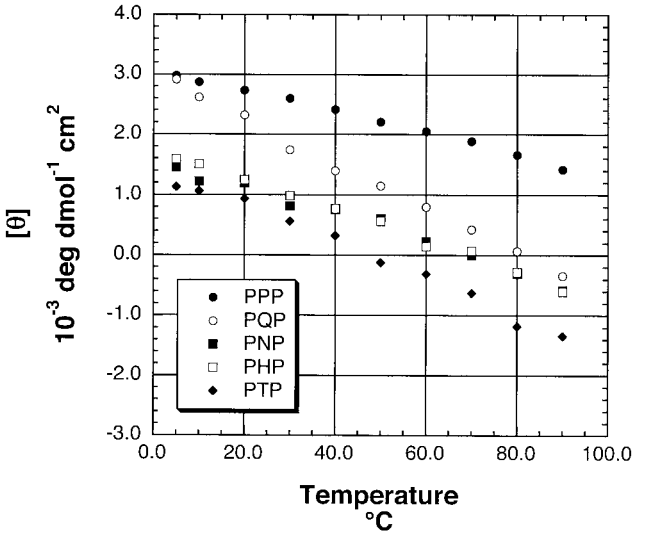

Fig. 2. Temperature dependence of the maxima in the CD spectra for peptides PPP, PQP, PNP, PHP, and PTP.

ing the CD spectra of peptides of sequence Ac-$(Pro)_7$-Yaa-Zaa-NH_2, with the Yaa-Zaa pairs Gly-Tyr, Tyr-Gly, Gly-Trp, and Trp-Gly. Differences in the spectra for the peptides terminated with Gly-Tyr and Tyr-Gly and with Gly-Trp and Trp-Gly could then be assigned to the known electronic effects of tyrosine and tryptophan. However, we have evidence that these residues, particularly tryptophan, interact strongly with the immediately *preceding* prolyl ring in peptides terminated with Tyr-Gly and Trp-Gly pairs (data not shown). Such effects would be an intrinsic part of propensities measured for these residues as guests in the host peptide system used here. Consequently we cannot use this approach to correct for the electronic effects. Moving the Yaa-Zaa pairs to the N-terminus of the peptide would not help. In this case any residues possessing a Cβ, such as tyrosine and tryptophan, would be sterically restricted by the immediately *following* prolyl ring,[22] another effect that would be an intrinsic part of propensities measured in our system. Given these difficulties, we have chosen not to include data on these two residues. We have included data for phenylalanine because its perturbations of far-UV CD spectra are thought to be smaller than the other aromatics.[27] We are exploring the nature of the interactions between tryptophan and tyrosine residues and surrounding prolines in ongoing work.

Host Peptide Structure

Using NMR and CD spectroscopy, we had previously shown that the poly(proline)-based host peptide, PPP, is predominantly a PPII helix in neutral aqueous solution.[23] The far-UV CD spectrum for PPP is shown in Figure 1. The shape of the curve, in particular the maximum at 228 nm, is characteristic of the left-handed PPII helical conformation.[29–33] The minimum at around 205 nm can also be considered characteristic of this conformation, but only when considered in conjunction with the maximum. Disordered peptides and proteins also possess a minimum in this region of the spectrum, making the minimum a poor choice for determining PPII helical content.[8,9,23]

Oligomeric State of the Peptides

In previous work we had shown using sedimentation equilibrium that the PPP, PAP, PGP, and PVP peptides are monomeric in solution.[23] This was particularly important for PGP because its sequence is somewhat similar to that of collagen (Table I). In the current work we have used both temperature titrations and dynamic light scattering (DLS) to show that all of the peptides examined are monomeric. CD spectra were collected at 5°, 10°, 20°, 30°,… and 90°C. The height of the maxima are plotted as a function of temperature for representative peptides in Figure 2. All peptides undergo an almost linear decrease in the height of the maximum, and consequently PPII content, as the temperature is raised. However, there are no large discontinuities in Figure 2 such as would be expected were there a change in oligomeric state. Similar results were obtained for all peptides listed in Table I.

DLS can be used to measure the distribution of molecule sizes in a solution.[34,35] This is done by measuring the distribution of hydrodynamic radii, R_h. The hydrodynamic radius of a molecule is indicative of the apparent size of the dynamic solvated particle. Figure 3 is a series of plots of R_h distributions for peptides PPP, PKP, PFP, and PVP. Clearly each of these peptides possesses a monomodal R_h distribution, suggesting that they each exist as a single species in solution. The widths of the distributions vary in part as a function of the flexibility of each peptide as well as the size of the guest residue. Molecular weights are all estimated to be ~1 kDa, which corresponds to the molecular weight of monomeric peptides. Similar results were obtained for all peptides studied.

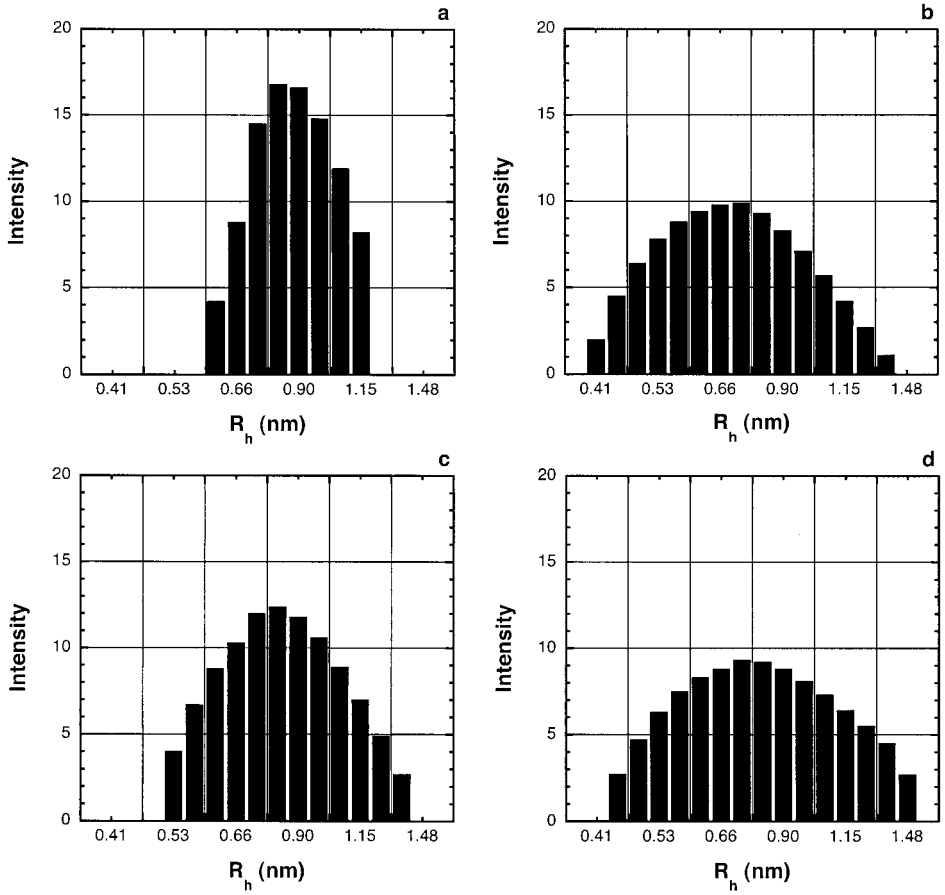

Fig. 3. Dynamic light scattering data for peptides (a) PPP, (b) PKP, (c) PFP, and (d) PVP. Scattering intensity (arbitrary units) is plotted against rheological radius, R_h (nm).

Host-Guest Measurements

Here we extend our previous study[23] to include all potential guest residues except tyrosine and tryptophan. This expanded set of data provides clues as to the origins of PPII helix formation and will form the foundation for future studies into the physical factors governing this secondary structure. The CD spectra for all peptides listed in Table I were collected in the wavelength range 190–260 nm. The maxima from spectra collected at 5°C for all peptides are shown in Figure 4. The spectrum for PPP is shown in all three panels of Figure 4 as a reference. All of these peptides possess significant PPII content, although there are clear differences in the heights of these maxima and therefore in the relative PPII content. The estimated PPII contents for these peptides are listed in Table I. Inspection of the rank order in Table I reveals that the relative PPII helix-forming propensities undoubtedly have complex origins. However, careful consideration of the data, coupled with the work of others, reveals common themes in the ordering, as will be discussed.

The peptide containing a proline guest residue, PPP (the host peptide), has the highest PPII helix content, indicating that proline is the best PPII helix former. This is to be expected because PPP is essentially a poly(proline) peptide, which is known to form a strong PPII helix as a result of steric interactions between prolyl rings.[22] These steric interactions are so strong that the structure of the PPP peptide cannot be disrupted even by temperatures as high as 90°C; (Fig. 2).[23] This remarkable property of our short seven-residue proline peptide also precludes the use of thermodynamics in the analysis of PPII helix-forming propensities measured in this system.

The guest site in our host peptide is bounded by two sets of three proline residues. The C-terminal proline of each of these sets will sterically hinder the preceding proline, restricting it to the PPII region of (ϕ, ψ) space. This restricted proline will in turn restrict the proline preceding it to the PPII region. Thus, no matter which guest residue is inserted, we will always have a minimum of four prolines in the PPII conformation, which cannot be melted out (see Fig. 2), and will result in a minimum PPII content estimated from Equation 1 of ~40% at 5°C. Furthermore, the guest residues, other than glycine, will be mostly restricted to the β-region of (ϕ, ψ)-space by steric interactions with the Cδ of the following proline in sequence.[22,36]

CD spectra for the peptides containing apolar guest residues, plus PPP, PGP, and PFP, are shown in Figure 4(a). The guest residues in these peptides have PPII

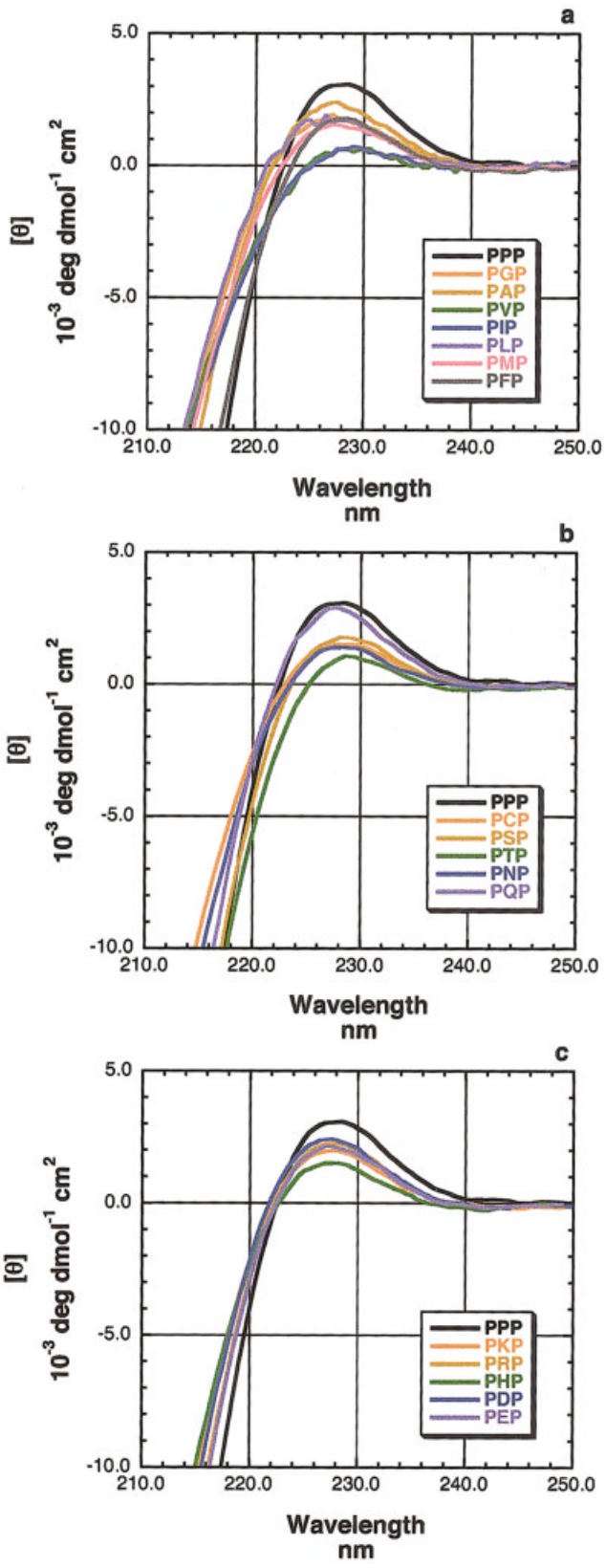

Fig. 4. CD spectra from around the characteristic PPII maximum for all eighteen PXP peptides at 5°C and pH 7. (a) The apolar guest residues, plus proline, glycine and phenylalanine. (b) Polar guest residues. (c) Charged guest residues. All three panels include the spectrum from PPP for reference.

helix-forming propensities of rank order: Pro > Ala > Gly, Leu, Phe > Met > Ile > Val (Table I). It is notable that alanine has a relatively high PPII propensity (Table I). Shi et al.[21] recently demonstrated using both NMR and CD spectroscopy that a seven-residue alanine peptide is predominantly PPII in aqueous solution. Ferreon and Hilser[37] have also used an experimental system to demonstrate that alanine possesses significant PPII character under conditions where peptide formation of α-helices is precluded or unfavorable. It is believed that under these conditions, backbone solvation favors PPII helix formation, with the side chain of alanine being small enough that it does not interfere.[7,21,23–25] The detailed nature of this backbone solvation is not as yet fully understood but is thought to be related to the ordering of solvent rather than to simple accessibility.[24] Given that alanine consists solely of backbone, this solvation effect would be expected to play a role in the rank ordering of all residues. Of course solvation will not be the only determinant of the PPII helix-forming propensities, perhaps not even the dominant one.

That glycine is more favorable in PPII helices than many residues is at first surprising [Table I and Fig. 4(a)]. Glycine is generally considered to be the most energetically unfavorable residue in regular secondary structures because of the large entropic loss associated with restricting its backbone. Furthermore, because it lacks a Cβ, glycine is not restricted to the β-region of (ϕ, ψ)-space through steric interactions with the following proline.[22] Much like alanine, however, backbone solvation would appear to favor formation of PPII helices by glycine. In fact, polyglycine has long been known to adopt PPII-like conformations in concentrated solutions.[8] Finally, Ferreon and Hilser[37] demonstrated directly that glycine possesses reasonable PPII character in peptides.

The remaining apolars have PPII helix-forming propensities that presumably reflect in part the extent to which they interfere with the ordering of solvent around the backbone in the PPII conformation. Leucine and methionine both have relatively flexible side chains that will at times occlude backbone from solvent, disrupting the solvation shell, whereas at other times will be oriented in ways that do not occlude the backbone.[23] Phenylalanine, despite its bulky aromatic ring, has a propensity similar to leucine, suggesting that the ring does not significantly disrupt backbone solvation. Isoleucine and valine, which possess the lowest PPII helix-forming propensities of all the residues, have bulky β-branched side chains that will partially bury backbone in the PPII conformation, leading to partial desolvation. Because desolvation is unfavorable, this will favor these residues occupying other extended conformations.[23]

The CD spectra for the uncharged polar residues are given in Figure 4(b). As we had noted previously,[23] the glutamine peptide, PQP, possesses the second highest PPII helix-forming propensity of all the residues [Table I and Fig. 4(b)]. We have hypothesized that this is due to a side chain to backbone hydrogen bond between the glutamine side chain and the carbonyl oxygen of the next

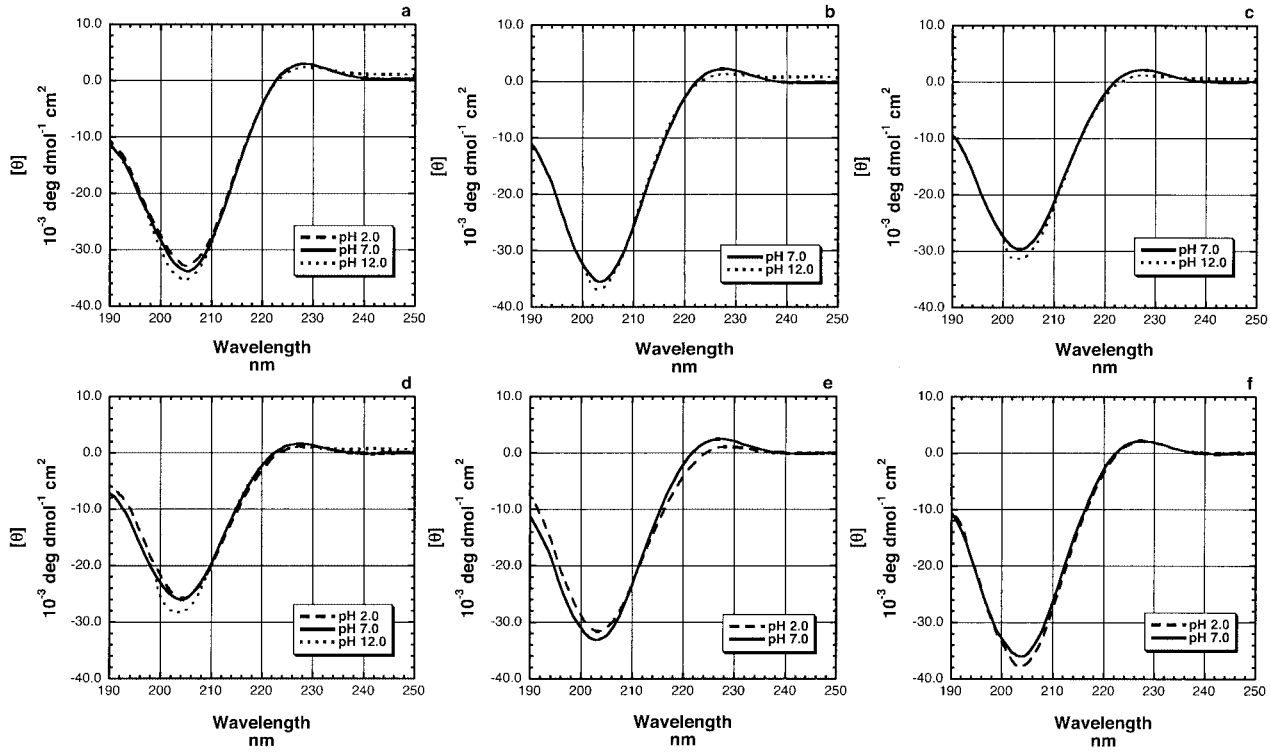

Fig. 5. pH dependence of the CD spectra for (a) PPP, (b) PRP, (c) PKP, (d) PHP, (e) PDP, and (f) PEP at 5°C.

residue in sequence.[13,23,25] Notably, the similar asparagine (peptide PNP) has a significantly lower PPII propensity [Table I and Fig. 4(b)]. The potential role of hydrogen bonds in PPII helices are discussed in more detail below. Serine possesses an intermediate propensity, similar to that of leucine (Table I). This suggests that backbone solvation and solvation of the side chain hydroxyl group are compatible when serine is in the PPII conformation.

Another possibility is that serine forms some kind of side chain to backbone hydrogen bond that helps to stabilize this conformation. The isosterically similar cysteine has a lower PPII helix-forming propensity than serine (Table I). This may be due to the larger radius of the sulfur as compared to the hydroxyl oxygen of the serine. The larger radius could effect PPII helix formation by either directly occluding backbone or through adverse interactions between the hydration shell of the sulfhydryl group and that of the backbone. Not surprisingly, threonine has a significantly lower PPII helix-forming propensity than serine [Fig. 4(b) and Table I]. As with valine and isoleucine, the β-branched threonine will bury backbone, preventing optimal solvation in the PPII conformation. Threonine is a somewhat better PPII helix-former than the other β-branched residues presumably because its hydroxyl group is smaller than a methyl group and will consequently bury less backbone or because of its hydrogen bonding capabilities.

All of the peptides containing charged residues, with the exception of PHP, have relatively high PPII helix-forming propensities [Table I and Fig. 4(c)]. These propensities are largely insensitive to changes in pH. CD spectra for the PDP and PEP peptides at pH 2 and 7, PHP at pH 2, 7, and 12, and PRP and PKP at pH 12 and 7 are shown in Figure 5. The CD spectra for PPP at pH 2, 7, and 12 are shown in Figure 5(a) for comparison. Raising the pH to 12 for peptides PRP and PKP appears to slightly lower the PPII helical content [Fig. 5(b) and 5(c), respectively]. However, PPP also appears to have a lower PPII content at pH 12 [Fig. 5(a)], suggesting that this is may be due to the chaotropic effects of the sodium hydroxide used to raise the pH. PHP appears to be remarkably insensitive to changes in pH [Fig. 5(d)]. The peptide that shows the largest change in PPII content with change in pH is PDP [Fig. 5(e)]. This peptide has a markedly lower maximum at pH 2, where the side chain carboxyl group would be predominantly protonated. By contrast, PEP has essentially the same PPII helix content at pH 2 and 7 [Fig. 5(f)].

Physical Determinants of PPII Helix Formation

It has been suggested previously that ordering of the solvent around the backbone is a major driving force in PPII helix formation.[7,20,21,23–25] The PPII helix is a very extended conformation, with backbone carbonyl oxygens and amide hydrogens pointed out from the helical axis into solvent, allowing for favorable interactions with water molecules. That solvation is important is perhaps most clearly demonstrated by the work of Shi et al.,[21] who have shown that a seven-residue alanine peptide is predominantly PPII helix in aqueous solution. This is consistent with the small side chain of alanine not interfering with backbone solvation. The relative PPII helix-forming propensities measured for the aliphatic residues support this

hypothesis, with alanine having the highest propensity of these residues (Table I). The bulky β-branched valine and isoleucine have low propensities presumably as a result of their side chains occluding backbone from solvent when in the PPII conformation.[23,25] The longer, more flexible leucine and methionine side chains result in intermediate propensities.

Inspection of the relative propensities in Table I indicates however that backbone solvation is unlikely to be the only factor determining the propensity for each residue to be in the PPII conformation. Other potential factors could include hydrogen bonds, side chain entropy, side chain-side chain interactions, and perhaps other factors. Examples of side chain-side chain interactions include those between aromatic side chains and the preceding prolyl rings noted above.

In previous work we had suggested that the high PPII helix-forming propensity of glutamine is due to a side chain to backbone hydrogen bond between the glutamine and the carbonyl oxygen of the next residue. Modeling studies indicate that such a hydrogen bond would constrain both the glutamine and the following residue to an area of the β-region of (ϕ, ψ)-space around the PPII conformation.[25] In the same work, it was demonstrated that asparagine was too short to form such a hydrogen bond while in the PPII conformation,[25] explaining the significantly lower PPII helix-forming propensity as compared to glutamine. Indirect evidence for a glutamine side chain to backbone hydrogen bond is presented in Figure 2. The maxima in the CD spectra for PQP, and consequently its PPII helix content, decreases more rapidly than that of PNP with temperature. At high temperature the CD maximum of PQP converges with that of PNP. A highly exposed side chain to backbone hydrogen bond such as that hypothesized would not be expected to be particularly stable except at lower temperatures. Raising the temperature will melt out the hydrogen bond and result in a rapid drop in PPII content. One should note that glutamate does not appear to form a PPII helix-stabilizing hydrogen bond at low pH where the side chain should be protonated and perhaps capable of forming such a bond [Fig. 5(f)]. A hydrogen bond between the glutamine side chain carbonyl oxygen and the residues own backbone amide hydrogen cannot be ruled out as another explanation. This alternative would be consistent with the pH behavior of glutamate.

The relatively high PPII helix-forming propensities of the charged residues, other than histidine, are consistent with backbone solvation being an important component (Table I). These residues possess long, flexible side chains that can point out, away from the peptide, minimizing disruption of backbone solvation. The relatively high propensity measured for lysine (Table I) is in keeping with the finding that a seven-residue lysine peptide has a significant PPII helix content at high pH, where the side chain charges are neutralized.[7] The insensitivity of the propensities to changes in pH (Fig. 5) is an indication that backbone solvation rather than charge could be an important factor. The charged residue with the shortest side chain, aspartate, has a markedly higher propensity than the stereochemically similar leucine. This may be an indication that leucine can interact with surrounding prolyl rings, burying hydrophobic surface area and partially occluding backbone.

SUMMARY

We have presented a scale of relative PPII helix-forming propensities measured in a proline-based host peptide system. This scale includes all residues except tyrosine and tryptophan (Table I). As might be predicted, proline has the highest measured propensity in this system, a result of strong steric interactions that occur between adjacent prolyl rings. The propensities measured for the other residues are consistent with backbone solvation being an important component in PPII helix formation, although it is clearly not the only determinant. The scale presented will be useful in future studies of the conformational properties of proline-rich sequences and should also provide insights into the prevalence of PPII helices in protein unfolded states.

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