Short Sequences of Non-Proline Residues Can Adopt the Polyproline II Helical Conformation†

Brian W. Chellgren and Trevor P. Creamer*

Center for Structural Biology, Department of Molecular and Cellular Biochemistry, University of Kentucky, 800 Rose Street, Lexington, Kentucky 40536-0298

Received January 9, 2004; Revised Manuscript Received March 19, 2004

ABSTRACT: The left-handed polyproline II (P_{II}) helix is a structure that has been given a great deal of attention lately because of its role in a wide variety of physiologically important processes and potential significance in protein unfolded states. Recent work by several authors has shown that residues besides proline can adopt this structure. A scale of relative P_{II}-helix-forming propensities has been generated but only for single guest residues in a proline-based host system. Here, we present multiple guest residues in a proline-based host system. Using circular dichroism spectroscopy, we have shown that not only single residues, but also short sequences of non-proline residues can adopt the P_{II} conformation.

The polyproline II (P_{II})[1] structure is a left-handed 3_1 helix with ideal backbone dihedral angles of $\phi = -75°$ and $\psi = 145°$. Approximately 10% of all protein residues have P_{II} dihedral angles (*1*), with about 2% of the residues in known structures being part of P_{II} helices of four residues or longer (*2*, *3*). On average, each protein possesses one P_{II} helix. A polyproline I (P_I) structure also exists with similar ϕ and ψ dihedrals but with all peptide bonds in the cis conformation. The P_I conformation is energetically unfavorable in aqueous solutions (*4*) and is not considered in the paper presented here.

P_{II} helices are important in numerous biological functions. They are known to play roles in processes such as signal transduction (*5*), cell motility (*6*), immune response (*7*), and perhaps formation of amyloid plaques (*8*, *9*). P_{II} helices are components of structural proteins such as collagen (*10*) and plant cell wall proteins (*11*). The P_{II} helix is believed to be the dominant conformation of proline-rich sequences (*5*, *12*), as well as being adopted by sequences that lack proline (*13*−*18*).

Over 30 years ago, Tiffany and Krimm (*13*) hypothesized that protein unfolded states were composed of short stretches of P_{II} helix interspersed with turns and bends. This was based partially on similarities between the circular dichroism (CD) spectra of denatured proteins and that of homopolymers of proline, which are known to adopt the P_{II} conformation. Various methods of denaturation result in P_{II}-like CD spectra for numerous proteins and peptides, suggesting that the P_{II} conformation is an integral part of the denatured state (*19*). Several groups have explored this hypothesis over the years, including Dukor and Keiderling (*20*), Woody (*15*), Wilson and co-workers (*12*), and Kallenbach and co-workers (*17*), with a recent increase in its interest (*16*, *21*−*23*).

Despite the clear importance of this structure, little is known about the physical determinants of P_{II}-helix formation. Polymers of proline in aqueous solution are known to adopt this conformation as a result of steric interactions between prolyl rings (*24*). The prolyl ring restricts the ϕ angle of the proline to $-63 \pm 15°$ (*25*). Steric interactions between the δ carbon of the ring and the β carbon of the preceding proline restrict the ψ angle of the preceding residue to the β region of (ϕ,ψ) space (*26*). Such steric interactions will result in any non-glycine residue immediately preceding a proline to be restricted primarily to the β region (*25*). These steric restrictions are not present for residues without a following proline. It has been suggested that interactions between the backbone and solvent play a major role in non-proline residues adopting the P_{II} conformation (*16*, *17*, *22*, *23*, *27*−*29*). Samulski and co-workers hypothesized that bridging water molecules are responsible for an alanine dipeptide adopting the P_{II} conformation (*30*). Other work suggests that the P_{II} conformation is preferred for minimizing intrapeptide steric conflicts and that interactions with the solvent compensate for attractive intrapeptide interactions (*31*).

In earlier work (*28*, *32*), our group utilized a host−guest system consisting of peptides of sequence Ac-$(Pro)_3$-X-$(Pro)_3$-Gly-Tyr-NH_2, where X is each of the 20 naturally occurring residues, to determine P_{II}-helix-forming propensities. The guest residue in our host system is immediately followed by a proline. Guests with a β carbon are therefore restricted to the β region but not necessarily the P_{II} conformation because their ϕ angles are not restricted by following prolines. Although the scale of P_{II}-helix-forming propensities derived from these studies is useful for the study of the conformational properties of proline-rich sequences, it is not clear how applicable it is to systems where residues are not sterically restricted by proline. When more than one guest residue is added to this host system, only the C-terminal guest is restricted by the following prolyl ring; the rest are

† This work was supported by a grant to T.P.C. from the National Science Foundation (MCB-0110720). The University of Kentucky Center for Computational Sciences provided support for B.W.C.

* To whom correspondence should be addressed. Telephone: (859) 323-6037. Fax: (859) 323-1037. E-mail: trevor.creamer@uky.edu.

[1] Abbreviations: CD, circular dichroism; HPLC, high-pressure liquid chromatography; NMR, nuclear magnetic resonance; P_{II}, polyproline II; P_I, polyproline I; P7, peptide Ac-$(Pro)_7$-Gly-Tyr-NH_2; PAP, peptide Ac-$(Pro)_3$-Ala-$(Pro)_3$-Gly-Tyr-NH_2; PVP, peptide Ac-$(Pro)_3$-Val-$(Pro)_3$-Gly-Tyr-NH_2.

10.1021/bi049922v CCC: $27.50
Published on Web 04/23/2004

unaffected by local steric interactions with proline. Here, we present data for our proline-based host system containing multiple alanine, glutamine, asparagine, or valine guests. Peptides containing multiple alanine or glutamine residues are found to adopt the P_{II} conformation. Asparagine and valine guests disfavor P_{II}-helix formation. The data presented demonstrate that sequences of multiple non-proline residues are able, and in some cases prefer, to adopt the P_{II} conformation and that residues possess intrinsic P_{II}-helix-forming propensities that are independent of interactions with proline.

MATERIALS AND METHODS

Peptides used in this paper were purchased from Peptidogenic Research and Co. (Livermore, CA). All peptides had their identities confirmed using mass spectrometry and were purified to ≥95% 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, with the buffer pH adjusted to 7. The peptide concentration was determined using the method of Brandts and Kaplan (*33*). Absorbance was measured using a 1.0-cm path-length cuvette in a Beckman−Coulter DU 640B spectrophotometer. The monomeric states of the peptides were determined from CD spectra collected during temperature titrations.

CD spectra were measured using Jasco J-810 and J-710 spectropolarimeters. For far-UV spectra, a 1-mm path-length quartz cuvette was used with solutions containing approximately 100 μM peptide. For some far-UV spectra, as noted in the text, a 1-cm path-length quartz cuvette was used with solutions containing approximately 10 μM peptide. Near-UV spectra were collected using a 1-cm path-length quartz cuvette with approximately 200 μM peptide solutions. Spectra were collected at 5 °C with a 0.5-nm resolution and a scan rate of 200 nm min^{-1}. Reported spectra are averages of 30 scans and are not smoothed. Each spectrum was measured at least three times with individually prepared peptide solutions. Reported molar ellipticities are estimated to have errors of approximately ±3%.

RESULTS AND DISCUSSION

The CD spectrum of a seven proline peptide, Ac-$(Pro)_7$-Gly-Tyr-NH_2 (P7), at 5 °C is shown in Figure 1A. The P7 peptide has been shown previously to be predominantly a P_{II} helix through NMR and CD spectroscopy (*28*). It has a CD spectrum with a maximum at 228 nm and a minimum at 205 nm, which are hallmarks of the P_{II} helix in aqueous solution. The P_{II} helix is the only secondary structure that has a maximum in this region (*34*); therefore, we use the maximum to indicate the presence of the conformation. The maximum moves to lower wavelengths as non-proline residues are added to the host system, a result of the different absorbance properties of primary, secondary, and tertiary amides (*15*). The maximum in a P_{II} CD spectrum can also shift to higher or lower wavelengths because of contributions from other secondary structures at nearby wavelengths.

The effect of adding single nonproline guest residues (Gln, Ala, Asn, or Val) to the host system is shown in Figure 1B. Each peptide still has a clear P_{II} signal, but the effect of the guest residues differs greatly. It should be noted that these

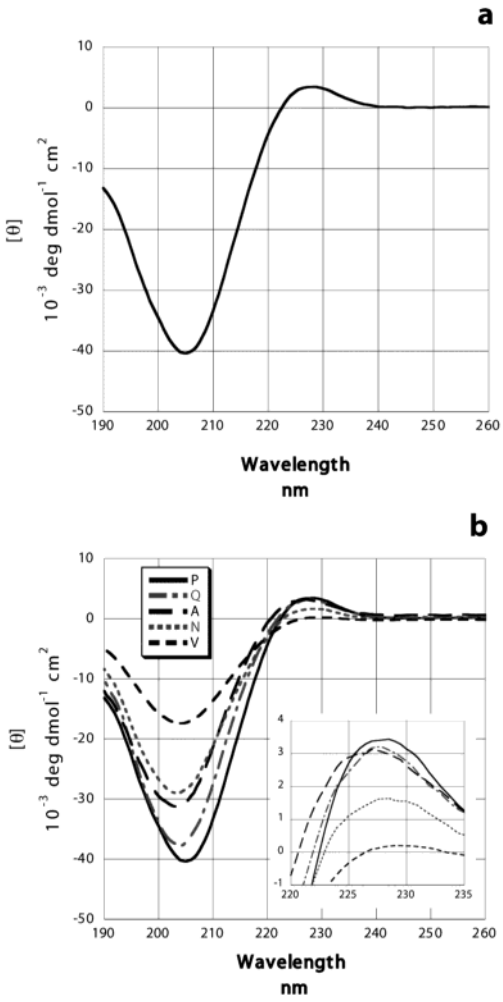

FIGURE 1: CD spectra of peptides at 5 °C. (A) P7 peptide. (B) P7 peptide (solid line) with Gln (long- and short-dashed line), Ala (long-dashed line), Asn (short-dashed line), and Val (medium-dashed line) single guest residue peptides. Inset shows the maxima.

guest residues are added to a proline-based system. Because of the aforementioned steric effects of the prolyl ring, the first, second, fourth, and fifth prolines in the peptide are restricted to the P_{II} region of (ϕ,ψ) space. Because of this, the CD spectra presented in this paper will always have a component of P_{II}, despite the effect of guest residue(s). The propensity of a given residue to adopt a P_{II} conformation relative to proline is demonstrated by any deviation from the P7 peptide model.

The introduction of alanine results in only a slight decrease in the maximum of the CD spectrum. This indicates that the addition of this residue to the host system does not greatly disrupt the P_{II} conformation. It has been demonstrated using NMR and CD spectroscopy that a seven residue alanine peptide prefers the P_{II} conformation in an aqueous environment (*17*). Other experimental work has shown that alanine possesses significant P_{II} character in conditions that preclude α-helix formation (*23*, *35*, *36*). Under these conditions, it is believed that backbone solvation favors P_{II}-helix formation (*17*, *27*, *32*, *35*). The side chain of alanine is small enough to not interfere with solvation. Bulky β-branched residues will partially bury the backbone, preventing optimal solvation and disfavoring the P_{II} conformation (*28*). This could be the

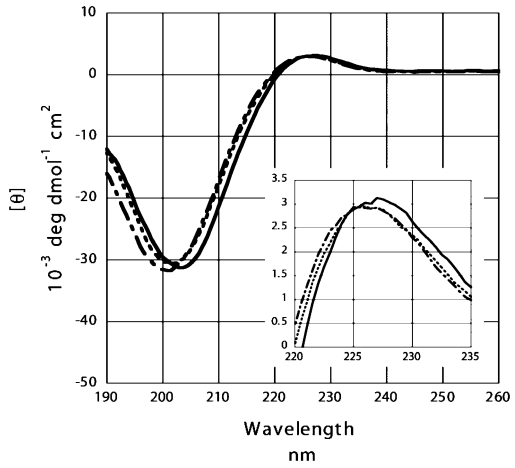

FIGURE 2: CD spectra of alanine guest peptides at 5 °C, with single Ala guest (solid line), two Ala guests (small-dashed line), three Ala guests (long- and short-dashed line). Inset shows the maxima.

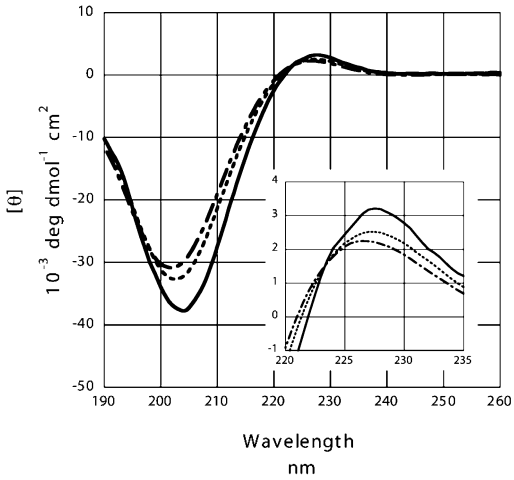

FIGURE 3: CD spectra of glutamine guest peptides at 5 °C, with single Gln guest (solid line), two Gln guests (small-dashed line), three Gln guests (long- and short-dashed line). Inset shows the maxima.

reason for the dramatic decrease in the maximum of the valine peptide as compared to P7. The specific mechanism of backbone solvation is not yet fully understood, but it is thought to be more complex than simple accessibility (*27*, *30*, *31*, Chellgren and Creamer unpublished data).

The introduction of a glutamine guest residue results in a slight decrease in the maximum of the CD spectrum. As noted previously (*28*), glutamine has the second highest P_{II}-forming propensity of all of the residues in the proline-based host system. We hypothesized that this is due to an intermittent side chain to backbone hydrogen bond between the glutamine side chain amide group and the carbonyl oxygen of the following residue (*3*, *28*, *29*, *32*). Such a bond would restrain the ψ angle of the glutamine in addition to both ϕ and ψ angles of the following residue. The proper orientation of atoms for this hydrogen bond occurs if both the glutamine and the following residue are in P_{II}-like conformations. Interestingly, the similar asparagine peptide has a much lower maximum. Asparagine is capable of forming a similar side chain to backbone hydrogen bond, but to obtain the proper orientation, asparagine cannot be in P_{II} (*29*).

As previously mentioned, the addition of multiple guest residues into this host system will demonstrate the intrinsic conformational preferences of the guest, with only the C-terminal guest residue being sterically restricted by proline. The data presented in Figure 2 show that addition of multiple alanine residues has little effect on the P_{II} signal, indicating that alanine favors this conformation. This is in accordance with the work of Kallenbach and co-workers, who found that alanine peptides too short to form α helices prefer the P_{II} conformation (*17*). The data also agree with Schweitzer-Stenner and co-workers, who have demonstrated that a tetraalanine peptide in water adopts the P_{II} conformation (*36*). Alanine is essentially pure backbone, implying that the backbone favors the P_{II} conformation. Scheraga and co-workers performed calculations suggesting that, in water, alanine has a low propensity to adopt the P_{II} conformation (*37*). These calculations were performed using a gas-phase representation of the potential energy function with either a solvent-accessible surface area model or a multigrid boundary element method to represent the solvent interactions. The relevance of these results as compared to the experimental data is not clear.

The glutamine peptides in Figure 3 show a decrease in the P_{II} content as the number of guests is increased. There is a substantial drop in the maximum in the CD spectrum with the introduction of the second glutamine and then a smaller change with the addition of the third guest. This may be due to the nature of our proposed side chain to backbone hydrogen bond. The formation of a hydrogen bond, which locks the glutamine side chain and the backbone of two residues into a single conformation, is entropically costly. For the C-terminal glutamine residue and the following proline, this cost is lowered because the ϕ angle of the proline is restricted in a prolyl ring and the ψ angles of both residues are restricted by following prolines. Because of this, the three dihedral angles, which would be restricted by this hydrogen bond, are already restricted to be in or near the P_{II} conformation by steric interactions. This situation is changed when more than one glutamine is added. Each additional glutamine is not followed by a proline and will thus have two freely rotatable backbone dihedrals. In a two glutamine guest peptide, the N-terminal guest residue is unrestrained and the C-terminal guest residue has a restrained ψ angle because of the following proline. The formation of this hydrogen bond has an entropic cost from restraining two rotatable backbone dihedrals instead of none. In a peptide of three or more glutamine residues, each additional hydrogen bond would restrain three rotatable backbone dihedrals. This may not be a purely additive effect, because restraining two residues with a hydrogen bond would make the entropic cost of forming the preceding or following hydrogen bond lower, suggesting the possibility of cooperativity. Because of this entropic effect, we might expect the P_{II} content of glutamine-containing peptides to begin high and then decrease to some basal level as the differences in hydrogen bond formation entropic effects average out. Even with the decrease in the maxima, however, there is still a clear P_{II} spectrum from each of the three glutamine-containing peptides. This indicates that glutamine has a relatively high intrinsic propensity to adopt the P_{II} conformation.

Figure 4 shows the results of the addition of multiple asparagine residues. The maximum decreases with each

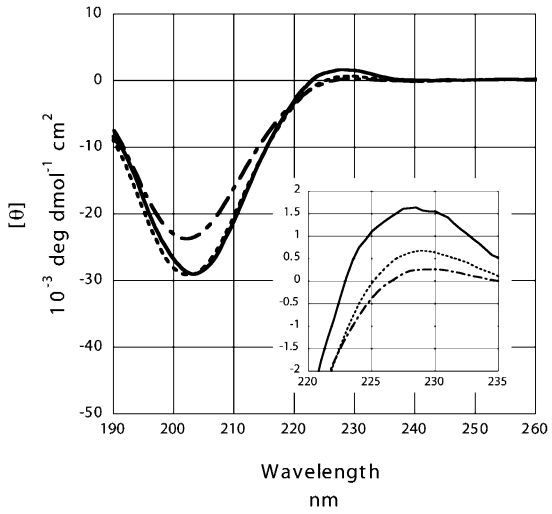

FIGURE 4: CD spectra of asparagine guest peptides at 5 °C, with single Asn guest (solid line), two Asn guests (small-dashed line), three Asn guests (long- and short-dashed line). Inset shows the maxima.

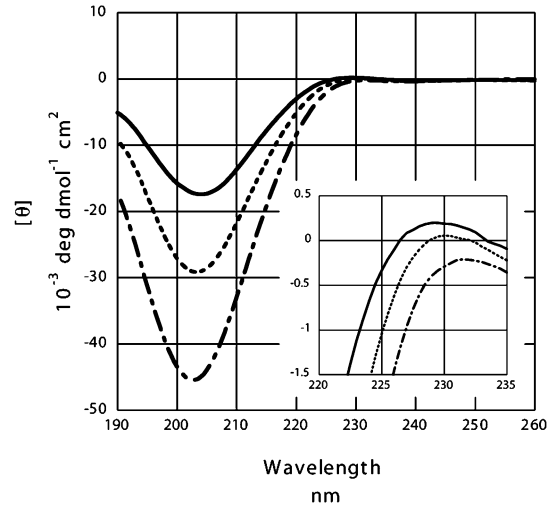

FIGURE 5: CD spectra of valine guest peptides at 5 °C, with single Val guest (solid line), two Val guests (small-dashed line), three Val guests (long- and short-dashed line). Inset shows the maxima.

additional guest residue. The small maximum remaining in the three asparagine guest peptide is likely due almost entirely to the prolines in the host system. As previously mentioned, four of the prolines in the host peptide are restricted to the P_{II} conformation, so there will always be an element of P_{II} in the CD spectra. The negative maxima of the single and double guest peptides are similar but decrease significantly for the three asparagine peptide. It is not currently known why the addition of the third asparagine guest specifically causes this.

The data shown in Figure 1B, as well as earlier work (*18*, *28*, *32*), suggest that valine strongly disfavors the P_{II} conformation. Peptides containing additional valine residues, shown in Figure 5, confirm this finding. There is a clear decrease in the maximum with each additional guest residue. There is a significant *decrease* in the minimum (strengthening of the negative band), which will be discussed later. The decrease in the maximum follows the results of our P_{II} propensity scale, which indicate β-branched residues, especially valine, strongly disfavor the P_{II} conformation (*32*). As

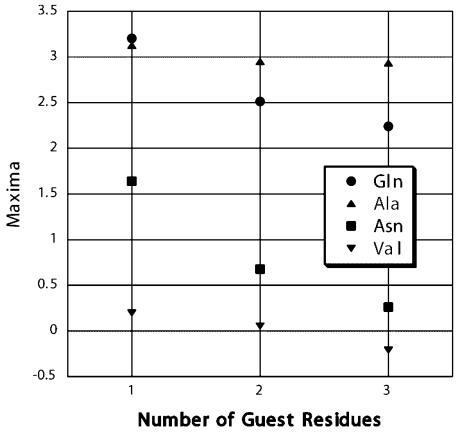

FIGURE 6: Plot of CD spectra maxima versus the number of guest residues for the peptides containing a guest single, double, or triple (Gln, Ala, Asn, or Val) sequence.

with asparagine, we believe the broad, low peaks seen in the CD spectra of the valine peptides are predominantly the result of the prolines in the host system.

The results of adding multiple guest residues to a proline-based host system are summarized in Figure 6, which is a plot of the maxima from the CD spectra as a function of the number of guest residues. Peptides with one, two, or three alanine guest residues have roughly equivalent maxima in their CD spectra. This supports Kallenbach and co-workers (*17*), who assert that an alanine oligomer in aqueous solution will adopt a P_{II} conformation if it is too short to form an α helix. Glutamine-containing peptides show a decrease in the P_{II} content with each additional guest residue, though still retaining a clear P_{II} CD spectrum with three guests. This indicates that glutamine has a relatively high propensity to adopt the P_{II} conformation. The high propensity for glutamine to adopt the P_{II} conformation has been proposed to be due to a side chain to backbone hydrogen bond, although more work is needed to confirm this. Asparagine-containing peptides show a decrease in the maxima with each additional guest residue and possess a low P_{II} content. Valine guests strongly disfavor the P_{II} conformation. The maxima in the CD spectra (Figure 5) do not rapidly decrease with additional guest residues because these peptides likely already possess the lowest P_{II} content possible for our proline-based host system.

A possible method of analyzing our CD data is through the use of difference plots. Difference plots can be generated by subtracting the CD spectrum of peptide Ac-$(Pro)_3$-X_Y-$(Pro)_3$-Gly-Tyr-NH_2 from that of Ac-$(Pro)_3$-X_{Y+1}-$(Pro)_3$-Gly-Tyr-NH_2. Such plots might indicate if an increasing propensity for another specific secondary structure is responsible for the changes in the CD spectra with an increasing number of guests. As previously noted, the maximum of a CD spectrum moves to a lower wavelength, referred to as a blue shift, as the ratio of secondary to tertiary amines increases. A difference plot between the residues with different numbers of guest residues would also display this blue shift. To model this effect, we took the data from the single alanine guest peptide CD experiment and shifted the spectrum to shorter wavelengths by 0.5 or 1 nm. Figure 7 depicts the difference plot between the double and single alanine guest peptides in comparison to the difference plots between the single alanine guest peptide and the artificially blue-shifted data.

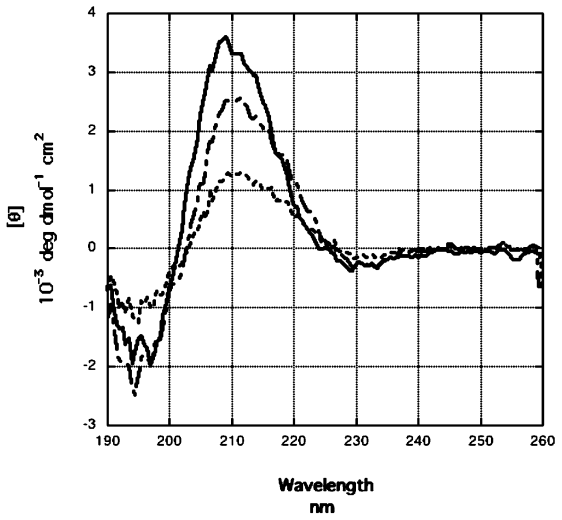

FIGURE 7: Plot of CD difference spectra of the single alanine guest peptide (PAP) subtracted from the double alanine guest peptide (solid line), PAP subtracted from PAP blue-shifted 0.5 nm (small-dashed line), or PAP subtracted from PAP blue-shifted 1.0 nm (long- and small-dashed line).

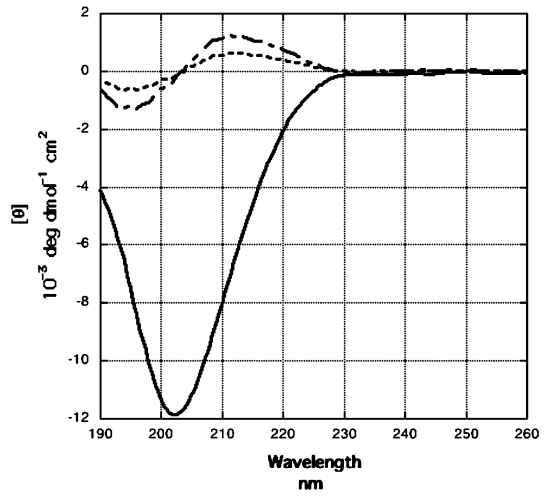

FIGURE 8: Plot of CD difference spectra of the single valine guest peptide (PVP) subtracted from the double valine guest peptide (solid line), PVP subtracted from PVP blue-shifted 0.5 nm (small-dashed line), or PVP subtracted from PVP blue-shifted 1.0 nm (long- and small-dashed line).

The similarity between the genuine difference plot and those created with artificially blue-shifted data indicates that the experimentally derived difference plot results primarily from the changing amide content. Difference plots for the remaining alanine-containing peptides plus all of the glutamine- and asparagine-containing peptides give similar results (data not shown). We therefore cannot conclude that the loss of the P_{II} content observed with an increasing number of guests in these peptides is due to an increasing preference for some other particular secondary structure.

Unlike the other peptides, the difference plots for the valine peptides indicate interactions in addition to the blue shift (Figure 8). As additional valines are added, the CD spectra indicate that the minima deepen, while the maxima decrease (Figure 5). The change in the maxima is due to the decreasing P_{II} content of the peptide. The reason for the deepening of the minima is not understood. It is possible that an inter- or intrapeptide interaction could cause this result. An interpeptide interaction would be dependent upon the concentration of the sample. To explore this possibility, the CD spectra for valine-containing peptides were retaken using a 1-cm cuvette and a 10-fold lower sample concentration. These spectra (data not shown) were identical, within error, to the 1-mm cuvette spectra in Figure 5. To confirm the absence of interpeptide interactions, we performed a dynamic light scattering experiment on the three valine guest peptide (data not shown). A single peak with a calculated molecular weight equivalent to that of the monomer accounted for 100% of the mass of the sample.

An intrapeptide interaction specific for the valine-containing peptides could occur between guest valine(s) and the C-terminal tyrosine residue. We consider this to be unlikely because of the four residues (Pro-Pro-Pro-Gly) between valine and tyrosine. The two N-terminal prolines in this set are restrained in the P_{II} conformation. This forms a fairly rigid shaft between valine and tyrosine and should preclude any hydrophobic interactions between the two residues. To confirm the lack of interaction between tyrosine and valine, we performed near-UV CD. If tyrosine is in a chiral environment, which would occur if it interacted with the guest valine residues, there will be a relatively strong band at approximately 280 nm. Near-UV spectra were collected at 5 °C for the P7 peptide and peptides containing one, two, or three valine guest residues (data not shown). None of the spectra contained bands near 280 nm, and the four spectra were identical within error. These data suggest that the tyrosine is either not interacting specifically with the remainder of the peptide or it is interacting with prolines, not valines. The near-UV CD spectrum of the three valine guest peptide was also collected at 25 °C and was identical within error to that taken at 5 °C, another indication of the lack of interactions between the tyrosine and valine residues (data not shown). Although intrapeptide interactions involving valine appear unlikely, something is clearly happening with the addition of the valine residues to the host peptide besides the decrease in the P_{II} content. Schweitzer-Stenner and co-workers have presented data suggesting that short valine peptides adopt predominantly a single extended conformation in water that is more extended than P_{II} (*23*).

The results of this paper generally agree with our P_{II}-forming propensity scale (*32*). In that scale, glutamine had the second highest P_{II}-forming propensity following only proline. Alanine ranked in the top quarter of the amino acids examined, while asparagine was in the bottom quarter. Valine ranked last on the scale, with the lowest measured P_{II}-forming propensity. With the inclusion of additional guest residues as shown in Figure 6, three of the four peptides behave in a manner similar to that predicted by our scale. Glutamine, asparagine, and valine each have a decrease in the maxima of their CD spectra as the number of guests increase, but their relative order stays the same. Alanine does not follow this trend and does not show a significant decrease in the maximum of its CD spectra. This suggests that, although single-residue guest peptides do not capture all of the intricacies of P_{II}-helix-forming propensities in a proline-based host system, our previous scale may have some relevance to systems devoid of proline.

ACKNOWLEDGMENT

The authors thank Dr. Rick McCann for use of his spectrophotometer. We also thank Dr. Rohit Pappu for advice and Shelly Whittington for assistance.

REFERENCES

1. Sreerama, N., and Woody, R. W. (1994) Poly(Pro)II helices in globular proteins: identification and circular dichroism analysis, *Biochemistry 33*, 10022−10025.
2. Adzhubei, A. A., and Sternberg, M. J. E. (1993) Left-handed polyproline II helices commonly occur in globular proteins, *J. Mol. Biol. 229*, 472−493.
3. Stapley, B. J., and Creamer, T. P. (1999) A survey of left-handed polyproline II helices, *Protein Sci. 8*, 587−595.
4. Mutter, M., Wohr, T., Gioria, S., and Keller, M. (1999) Pseudo-prolines: induction of cis/trans-conformational interconversion by decreased transition state barriers, *Biopolymers 51*, 121−128.
5. Kay, B. K., Williamson, M. P., and Sudol, M. (2000) The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains, *FASEB J. 14*, 231−241.
6. Mahoney, N. M., Janmey, P. A., and Almo, S. C. (1997) Structure of the profilin-poly-L-proline complex involved in morphogenesis and cytoskeletal regulation, *Nat. Struct. Biol. 4*, 953−960.
7. Jardetzky, T. S., Brown, J. H., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1996) Crystallographic analysis of endogenous peptides associated with HLA-DR1 suggests a common polyproline II-like conformation for bound peptides, *Proc. Natl. Acad. Sci. U.S.A. 93*, 734−738.
8. Blanch, E. W., Morozova-Roche, L. A., Cochran, D. A. E., Doig, A. J., Hecht, L., and Barron, L. D. (2000) Is polyproline II helix the killer conformation? A Raman optical activity study of the amyloidogenic prefibrillar intermediate of human lysozyme, *J. Mol. Biol. 301*, 553−563.
9. Jarvet, J., Damberg, P., Danielsson, J., Johansson, I., Eriksson, L. E. G., and Gräslund, A. (2003) A left-handed 3_1 helical conformation in the Alzheimer Aβ(12−28) peptide, *FEBS Lett. 555*, 371−374.
10. Pauling, L., and Corey, R. B. (1951) The structure of fibrous proteins of the collagen-gelatin group, *Proc. Nat. Acad. Sci. U.S.A. 37*, 272−281.
11. Ferris, P. J., Woessner, J. P., Waffenschmidt, S., Kilz, S., Drees, J., and Goodenough, U. E. (2001) Glycoslyated polyproline II rods with kinks as a structural motif in plant hydroxyproline-rich glycoproteins, *Biochemistry 40*, 2978−2987.
12. Wilson, G., Hecht, L., and Barron, L. D. (1996) Residual structure in unfolded proteins revealed by Raman optical activity, *Biochemistry 35*, 12518−12525.
13. Tiffany, M. L., and Krimm, S. (1968) New chain conformations of poly(glutamic acid) and poly(lysine), *Biopolymers 6*, 1379−1382.
14. Tiffany, M. L., and Krimm, S. (1972) Effect of temperature on the circular dichroism spectra of polypeptides in the extended states, *Biopolymers 11*, 2309−2316.
15. Woody, R. W. (1992) Circular dichroism and conformation of unordered polypeptides, *Adv. Biophys. Chem. 2*, 37−79.
16. Rucker, A. L., and Creamer, T. P. (2002) Polyproline II helical structure in protein unfolded states: Lysine peptides revisited, *Protein Sci. 11*, 980−985.
17. Shi, Z., Olson, C. A., Rose, G. D., Baldwin, R. L., and Kallenbach, N. R. (2002) Polyproline II structure in a sequence of seven alanine residues, *Proc. Nat. Acad. Sci. U.S.A. 99*, 9190−9195.
18. Eker, F., Cao, X., Nafie, L., and Schweitzer-Stenner, R. (2002) Tripeptides adopt stable structures in water: A combined polarized visible Raman, FTIR, and VCD spectroscopy study, *J. Am. Chem. Soc. 124*, 14330−14341.
19. Tiffany, M. L., and Krimm, S. (1973) Extended conformations of polypeptides and proteins in urea and guanidine hydrochloride, *Biopolymers 12*, 575−587.
20. Dukor, R. K., and Keiderling, T. A. (1991) Reassessment of the random coil conformation: Vibrational CD study of proline oligopeptides and related polypeptides, *Biopolymers 31*, 1747−1761.
21. Ding, L., Chen, K., Santini, P. A., Shi, Z., and Kallenbach, N. R. (2003) The pentapeptide GGAGG has PII conformation, *J. Am. Chem. Soc. 125*, 8092−8093.
22. Pappu, R. V., and Rose, G. D. (2002) A simple model for polyproline II structure in unfolded states of alanine-based peptides, *Protein Sci. 11*, 2437−2455.
23. Eker, F., Griebenow. K., and Schweitzer-Stenner, R. (2003) Stable conformations of tripeptides in aqueous solution studied by UV circular dichroism spectroscopy, *J. Am. Chem. Soc. 125*, 8178−8185.
24. Creamer, T. P. (1998) Left-handed polyproline II helix formation is (very) locally driven, *Proteins 33*, 218−226.
25. MacArthur, M. W., and Thornton, J. M. (1991) Influence of proline residues on protein conformation, *J. Mol. Biol. 218*, 397−412.
26. Schimmel, P. R, and Flory, P. J. (1968) Conformational energies and configurational statistics of copolypeptides containing L-proline, *J. Mol. Biol. 34*, 105−120.
27. Sreerama, N., and Woody, R. W. (1999) Molecular dynamics simulations of polypeptide conformations in water: a comparison of α,β, and poly(Pro)II conformations, *Proteins 36*, 400−406.
28. Kelly, M. A., Chellgren, B. W., Rucker, A. L., Troutman, J. M., Fried, M. G., Miller, A.-F., and Creamer, T. P. (2001) Host−guest study of left-handed polyproline II helix formation, *Biochemistry 40*, 14376−14383.
29. Creamer, T. P., and Campbell, M. N. (2002) Determinants of the polyproline II helix from modeling studies, *Adv. Protein Chem. 62*, 263−282.
30. Poon, C.-D., Samulski, E. T, Weise, C. F., and Weisshaar, J. C. (2000) Do bridging water molecules dictate the structure of a model dipeptide in aqueous solution? *J. Am. Chem. Soc. 122*, 5642−5643.
31. Drozdov, A. N., Grossfield, A. M., and Pappu, R. V. (2004) The role of solvent in determining conformational preferences of alanine dipeptide in water, *J. Am. Chem. Soc. 126*, 2574−2581.
32. Rucker, A. L., Pager, C. T., Campbell, M. N., Qualls, J. E., and Creamer, T. P. (2003) Host−guest scale of left-handed polyproline II helix formation, *Proteins 52*, 68−75.
33. Brandts, J. F., and Kaplan, L. J. (1973) Derivative spectroscopy applied to tyrosyl chromophores. Studies on ribonuclease, lima bean inhibitors, insulin, and pancreatic trypsin inhibitor, *Biochemistry 12*, 2011−2024.
34. Brahms, S., and Brahms, J. (1980) Determination of protein secondary structure in solution by vacuum ultraviolet circular dichroism, *J. Mol. Biol. 138*, 149−178.
35. Ferreon, J. C., and Hilser, V. J. (2003) The effect of the polyproline II (PPII) conformation on the denatured state entropy, *Protein Sci. 12*, 447−457.
36. Schweitzer-Stenner, R., Eker, F., Griebenow, K., Cao, X., and Nafie, L. A. (2004) The conformation of tetraalanine in water determined by polarized Raman, FTIR, and VCD spectroscopy, *J. Am. Chem. Soc. 126*, 2768−2776.
37. Vila, J. A., Baldoni, H. A., Ripoll, D. R., Ghosh, A., and Scheraga, H. A. (2004) Polyproline II helix conformation in a proline-rich environment: a theoretical study, *Biophys J. 86*, 731−742.

BI049922V