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End Effects Influence Short Model Peptide Conformation

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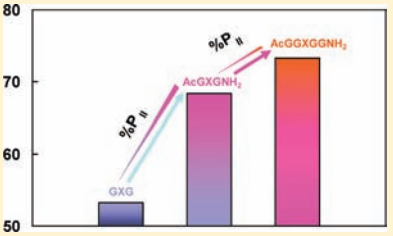
ABSTRACT: Previously, we derived a P_{II} propensity scale using N- and C-terminally blocked host−guest peptide model $AcGGXGGNH_2$ (X ≠ Gly) and concluded that P_{II} represents a dominant conformation in the majority of this series of 19 peptides (Shi et al. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17964−17968). Recently, Schweitzer-Stenner and co-workers examined a series of eight short host−guest tripeptides with the sequence GXG (X = A, V, F, S, E, L, M, and K) in which both N- and C-ends were unblocked and reported major differences in P_{II} content for F, V, and S compared to our scale (Hagarman et al. *J. Am. Chem. Soc.* **2010**, *132*, 540−551). We have investigated four representative amino acids (X = A, V, F, and S) in three series of peptides (GXG, $AcGXGNH_2$, and $AcGGXGGNH_2$) as a function of pH in this study. Our data show that P_{II} content in the GXG series (X = A, V, F, and S) is pH-dependent and that the conformations of each amino acid differ markedly between the GXG and $AcGXGNH_2$/$AcGGXGGNH_2$ series. Our results indicate that P_{II} scales are sequence and context dependent and the presence of proximal charged end groups exerts a strong effect on P_{II} population in short model peptides.



INTRODUCTION

Several recent lines of evidence indicate that the backbone conformation of unfolded peptides is predisposed to the polyproline II (P_{II}) basin, a structure with backbone dihedral angles (ϕ,ψ) centered at (−75°,+145°).[1−13] These new data confirm the original proposal by Tiffany and Krimm,[14,15] that unfolded proteins are statistical chains with significant locally structured P_{II} conformation. It is now generally accepted that P_{II} plays a major role in denatured states of proteins,[16−20] especially in weakly folded or natively unfolded proteins.[21,22] However, there is still no consensus on the extent of conformational preferences for P_{II} of each amino acid as revealed by comparing different P_{II} propensity scales.[23−28]

In 2005, a P_{II} propensity scale was derived using an N- and C-terminally blocked host−guest peptide model $AcGGXGGNH_2$, where X denotes any of 19 natural amino acids apart from Gly.[28] We concluded that P_{II} represents a dominant conformation in the majority of this series of peptides.[28] Recently, Schweitzer-Stenner and co-workers examined a series of eight short host−guest tripeptides with the sequence GXG (X = A, V, F, S, E, L, M, and K) in which both N- and C-ends were unblocked. They used a combination of several spectroscopic techniques: IR, Raman, VCD and NMR in their study and reported major differences in P_{II} content for F, V, and S from our scale.[27] In particular they reported $^3J_{\alpha N}$ values that differ from ours and concluded that our values were inaccurate. However, it seemed likely to us that the proximity of charged end(s) in the GXG tripeptides could account for the discrepancies[29] in scales and in observed $^3J_{\alpha N}$ values. To test this hypothesis, we have investigated four representative amino acids (X = A, V, F, and S) in three series of peptides (GXG, $AcGXGNH_2$ and $AcGGXGGNH_2$) as a function of pH, using a combination of UV CD and NMR. The data show that P_{II} content in the GXG series (X = A, V, F, and S) is in fact pH-dependent and that the conformation of each amino acid differs markedly between the GXG and the $AcGXGNH_2$/$AcGGXGGNH_2$ series, which do not vary with pH. This result shows that P_{II} scales are sequence and context dependent, especially in the presence of proximal charged end groups. We have previously reported that bulky side chains exert a strong next-neighbor effect on P_{II} conformation.[30]

RESULTS AND DISCUSSION

CD spectra for the capped $AcGXGNH_2$/$AcGGXGGNH_2$ and free-end GXG peptides are presented in Figures 1, 2, and 3. All peptides, except for the phenylalanine peptides, show the characteristic CD signature of P_{II} conformation- a strong negative band at ∼195 nm with a weak shoulder at ∼215 nm.[16,31,32] CD spectra for the capped peptides are similar to those reported previously[28] and show negligible changes upon changing pH from 2 to 6. On the other hand the spectra of free-end GXG peptides change significantly upon varying pH from 2 to 4, and less from pH 4 to 6. As expected, the carboxyl pK_a's for different free-end peptides lie in the range between 3 and 3.5, so the charge states of free C-termini of the peptides are affected by the pH and influence the GXG CD spectra. Typically, the CD spectra of GXG peptides show a decrease in intensity of both the 190−195 and 210−215 nm bands with increasing pH.

$^3J_{\alpha N}$ coupling constant values at 20 °C for the three sets of peptides span the range from 5.5 to 7.8 Hz, so that the conformation varies between the series of peptides and among side chains in each series (Figure 4; Tables 1, 2, and 3). If we assume that each peptide favors extended P_{II} or β conformational basins, we can estimate an approximate P_{II} content (%

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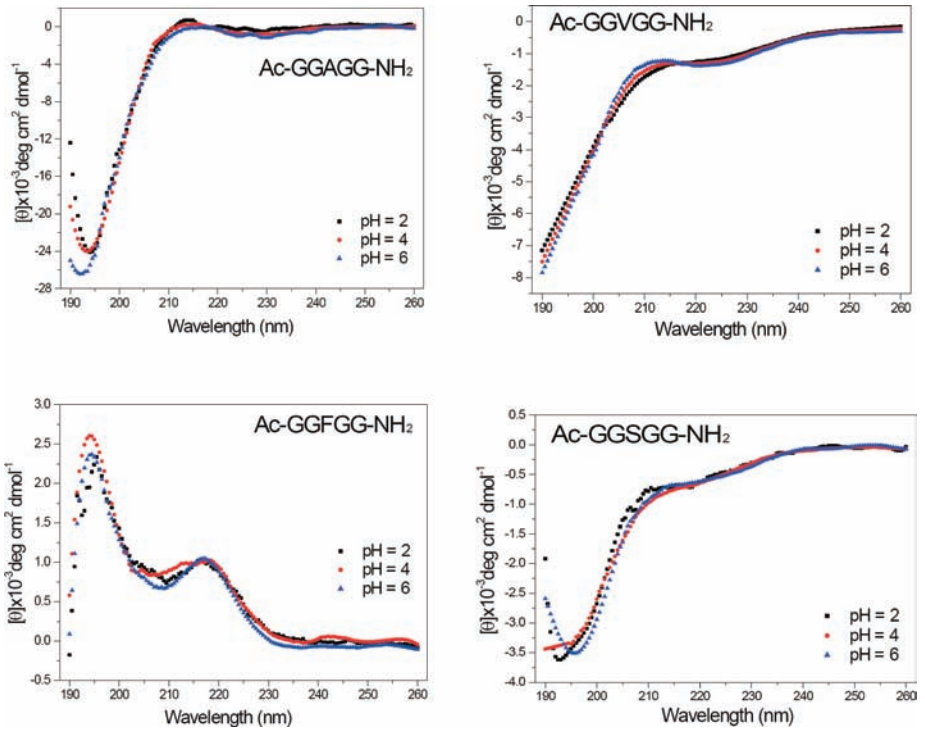

Figure 1. pH dependence of CD spectra for $AcGGXGGNH_2$ (capped peptides).

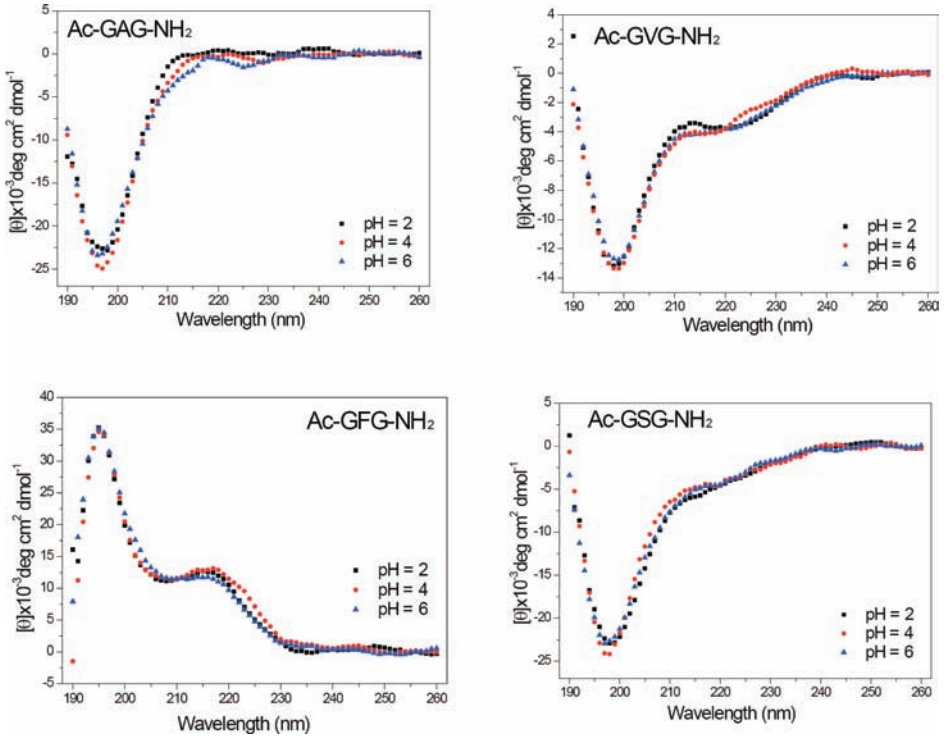

Figure 2. pH dependence of CD spectra for $AcGXGNH_2$ (capped peptides).

P_{II}) for each peptide using eq 1, in which J_{PII} and J_β are reference $^3J_{\alpha N}$ values for P_{II} and β basins for each amino

$$\%P_{II}J_{PII} + (1 - \%P_{II})J_\beta = J_{measured} \quad (1)$$

acid,[28,33] respectively. Following this procedure, $^3J_{\alpha N}$ values of each residue X in both capped and free peptides can then be used to derive a quantitative distribution of major conformations (P_{II} and β).[28] P_{II} content differs among the three series of peptides, as indicated in Tables 1, 2, and 3 and Figure 4.

On average, $^3J_{\alpha N}$ values for $AcGGXGGNH_2$ at pH 4 reported in this study differ from our previous values[28] by 0.16 Hz (Tables 1 and 4), corresponding to a difference of 4% in P_{II} content; $^3J_{\alpha N}$ values for GXG at pH 2 reported in this study differ from values of Schweitzer-Stenner and co-workers by

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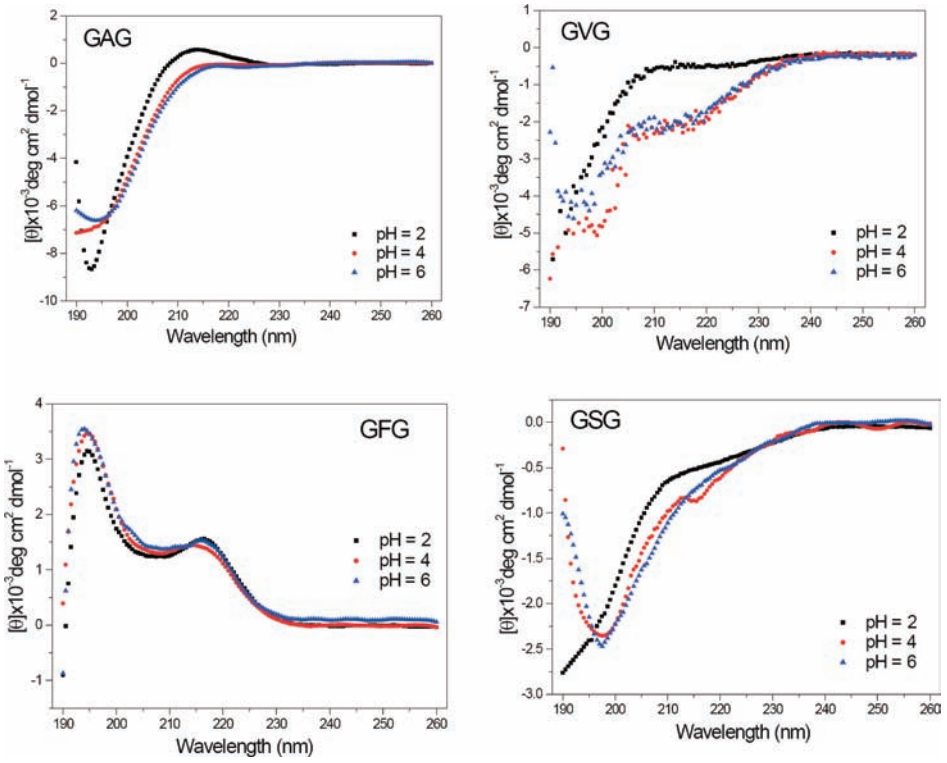

Figure 3. pH dependence of CD spectra for GXG (peptides with free ends).

0.15 Hz[27] (Tables 3 and 4), corresponding to a similar difference of 4% in P_{II} content. Among the values obtained in this study, the $^3J_{\alpha N}$ values at pH 4 differ by an average of 0.205 Hz between $AcGXGNH_2$ and $AcGGXGGNH_2$ peptides (Tables 1 and 2), by 0.605 Hz between $AcGXGNH_2$ and GXG peptides (Tables 2 and 3), and by 0.81 Hz between GXG and $AcGGXGGNH_2$ peptides (Tables 1 and 3). Assuming that the reference $^3J_{\alpha N}$ values are constant for three series of peptides, 0.81 Hz translates to a difference of 20% in P_{II} content, 0.605 Hz corresponds to 15%, and 0.205 Hz corresponds to 5% in P_{II} content difference, respectively.

$AcGXGNH_2$ and GXG are identical apart from their termini, so that 15% differences in P_{II} content between $AcGXGNH_2$ and GXG can be attributed to the effects from charged end(s). Comparing the results from $AcGGXGGNH_2$ and $AcGXGNH_2$ suggests that an extra Gly on each end makes a minor contribution, that is, 5% differences in P_{II} content. Thus the charged end(s) in GXG series make major contributions to the observed discrepancies in the $^3J_{\alpha N}$ values as compared to either series of capped peptides, in particular for Ser with a neutral polar side chain. The results also suggest that P_{II} contents in series with different charged end(s) are intrinsically different and hence that the P_{II} scale is sequence and context dependent.

From the results in Table 3, the average of $^3J_{\alpha N}$ values for GXG increases by 0.33 Hz upon changing pH from 2 to 4, and by 0.44 Hz between pH 2 and 6, corresponding to mean decreases in P_{II} content of 8.2% and 10.8%, respectively. For the $AcGXGNH_2$/$AcGGXGGNH_2$ series, there are negligible changes in $^3J_{\alpha N}$ values from pH 2 to 6 (Tables 1 and 2). The $^3J_{\alpha N}$ data thus corroborate the CD results. The results show that P_{II} content in a system with charged end(s) is pH-dependent and that the corresponding P_{II} scale is dependent on solution conditions.

Comparing our previous values[28] for V, F, and S to those from GXG derived by Schweitzer-Stenner and co-workers,[27] we find a difference in P_{II} content of nearly 30% on average (Table 4), as they reported.[27] Part of this difference reflects the data analysis procedures used in the two studies. Following our own procedure[28] and using their published $^3J_{\alpha N}$ values on V, F, and S,[27] we would derive P_{II} content values of 63.3%, 51.8% and 57.4% for V, F and S, respectively (Table 4). On average, these values are 14% smaller than values we reported previously,[28] that is, 57.5% vs 71.9% (Table 4). This suggests that half or more of the 30% difference in P_{II} content (14−20%) can be explained by the combined effects of charged end(s) and extra Gly context as well as experimental error (~4%); the remainder can be attributed to analysis and related issues that we discuss below.

To support our assertion that the extra Gly's exert small effects as revealed by the comparison between $AcGGXGGNH_2$ and $AcGXGNH_2$, we point to the minor differences in $^3J_{\alpha N}$ values for $AcGGXGGNH_2$ and the blocked "dipeptides", AcXNHMe, that are widely used as a benchmark for protein backbone conformation.[18] On average, $^3J_{\alpha N}$ values of 19 amino acids for $AcGGXGGNH_2$[28] differ by 0.26 Hz from those reported for AcXNHMe, corresponding to a difference of less than 7% in P_{II} content by our analysis. However, we expect one extra Gly on each end in an uncapped system (GGXGG vs GXG) to make significant differences, since the distances between the end group charges and the central residue in GXG are significantly shorter than those in GGXGG. Thus differences between GGXGG and $AcGGXGGNH_2$ should be smaller than those between GXG and $AcGXGNH_2$/$AcGGXGGNH_2$. Merutka et al. reported $^3J_{\alpha N}$ values for two uncapped GGXGG peptides, that is, 5.5 Hz for Ala and 7.4 Hz for Asn.[34] Their values are close to those that we reported previously for $AcGGXGGNH_2$,[28] that is, 5.73 Hz for Ala and

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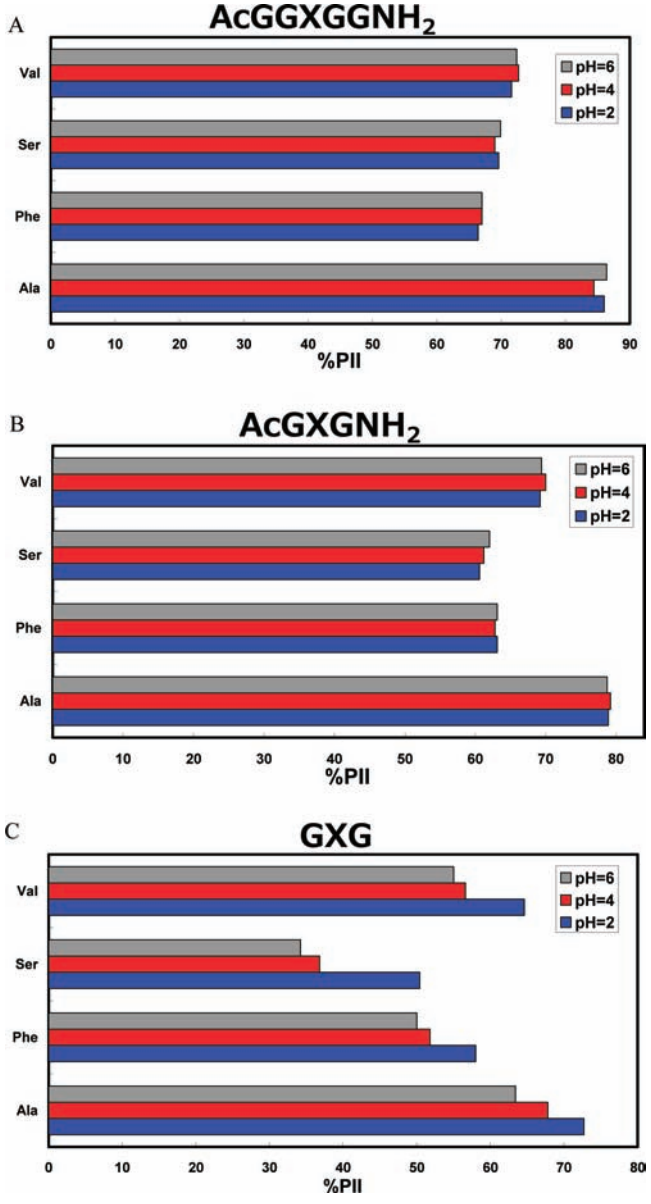

Figure 4. Histogram comparing P_{II} contents at different pH for the capped peptides $AcGGXGGNH_2$ (A) and $AcGXGNH_2$ (B) and GXG peptides (C) with free ends.

7.57 Hz for Asn, corresponding to differences in P_{II} content of 5% and 6%, respectively.

Our analysis relies on a simplified model in which we assume that each peptide samples extended P_{II} or β basins exclusively;[28] while this might be appropriate for amino acids with a low tendency to sample turn conformations, for example, it is not necessarily the case. Schweitzer-Stenner and co-workers used a distribution model, which includes more than the major P_{II} and β populations and is thus more robust.[27] Given that the major population is either P_{II} or β, there are certainly minor (<10%) turn and/or α populations as recently reported for a series of blocked dipeptides by Grdadolnik el al.[18] Since $^3J_{\alpha N}$ values for turn or α conformations are closer to those for P_{II} than those for β, our derived P_{II} content is thus likely to overestimate P_{II} by 0−10% (an average of 5.5% α conformation based on the published data[18]). In addition, the derived results are sensitive to the reference $^3J_{\alpha N}$ values for P_{II} and β in our analysis. There are still some uncertainties regarding the proper reference values.

Published data on unblocked $(Ala)_3$ reveal that the middle Ala amide has a $^3J_{\alpha N}$ value (5.70 Hz) about 0.85 Hz smaller than the C-terminal Ala amide $^3J_{\alpha N}$ value (6.55 Hz),[35,36] which corresponds to a change of 17% in P_{II} content according to our deconvolution. This reinforces our conclusion that P_{II} scales are sequence and context dependent. Additional evidence that P_{II} contents are context sensitive comes from the observation by Graf et al.,[35] that for unblocked $(Ala)_n$ (n = 3−7) peptides, the $^3J_{\alpha N}$ value of Ala increases along the peptide chain from N- to C-terminus, representing a small shift of conformation from P_{II} toward β. For this reason, in our design of XAO and $AcGGXGGNH_2$ peptides, we chose to neutralize all peptide end charges by blocking both N- and C-ends;[6,28,37] as a result, the observed $^3J_{\alpha N}$ coupling constants (at each of the temperatures below room temperature) for A2 through A7 in XAO are identical within ±0.1 Hz.[6]

We have previously noted that next-neighbor residue effects can influence P_{II} conformation in Ala peptides.[30] Compared to the central Ala in $AcGGAAAGGNH_2$, the central Ala in $AcGGIAAGGNH_2$ and $AcGGIAIGGNH_2$ peptides samples more β and less P_{II} conformation. This effect is likely due to steric occlusion[38] and/or hydration differences.[33] Schweitzer-Stenner and co-workers observed that Phe in AFA samples more P_{II} and less β conformation than in GFG.[39] Both results support our conclusion that P_{II} contents are sequence and context dependent.

Moreover Graf et al. designed two short peptides, HEWL-9mer and HEWL-19mer, that include $(Ala)_3$ sequences in two different sequence contexts from the protein hen egg white lysozyme (HEWL).[35] They found a major change in the conformational distribution of the central $(Ala)_3$: the one in HEWL-9mer is predominantly P_{II}, while $(Ala)_3$ in the HEWL-19mer samples both P_{II} and α conformations.[35]

The accuracy of the derived results is sensitive to the parameters used in Karplus equations[40] for all procedures that rely heavily on the observed $^3J_{\alpha N}$ and other coupling constants.[6,28,35] In most applications, substitution-effects on Karplus equation parameters tend to be ignored. In principle each residue should have a particular set of Karplus equation parameters due to individual side-chain character; similarly, N-

Table 1. Reference $^3J_{\alpha N}$ Values for P_{II}/β of Each Amino Acid and the Experimentally Determined $^3J_{\alpha N}$ as Well as Derived $\%P_{II}$ at 20 °C in the Capped $AcGGXGGNH_2$ Peptides at Three Different pH Values

	ref		pH 2.0		pH 4.0		pH 6.0	
amino acid	$^3J_{\alpha N}(P_{II})$, Hz	$^3J_{\alpha N}(\beta)$, Hz	$^3J_{\alpha N}$, Hz	$\%P_{II}$	$^3J_{\alpha N}$, Hz	$\%P_{II}$	$^3J_{\alpha N}$, Hz	$\%P_{II}$
Ala	4.81	9.87	5.52	86.0	5.60	84.4	5.50	86.4
Phe	5.34	9.86	6.86	66.4	6.83	67.0	6.83	67.0
Ser	5.52	8.97	6.57	69.6	6.59	69.0	6.56	69.9
Val	6.09	9.82	7.15	71.6	7.11	72.7	7.12	72.4

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Table 2. Reference $^3J_{\alpha N}$ Values for P_{II}/β of Each Amino Acid and the Experimentally Determined $^3J_{\alpha N}$ as Well as Derived $\%P_{II}$ at 20 °C in the Capped $AcGXGNH_2$ Peptides at Three Different pH Values

	ref		pH 2.0		pH 4.0		pH 6.0	
amino acid	$^3J_{\alpha N}(P_{II})$, Hz	$^3J_{\alpha N}(\beta)$, Hz	$^3J_{\alpha N}$, Hz	$\%P_{II}$	$^3J_{\alpha N}$, Hz	$\%P_{II}$	$^3J_{\alpha N}$, Hz	$\%P_{II}$
Ala	4.81	9.87	5.88	78.9	5.86	79.2	5.89	78.7
Phe	5.34	9.86	7.01	63.1	7.02	62.8	7.01	63.1
Ser	5.52	8.97	6.88	60.6	6.86	61.2	6.82	62.0
Val	6.09	9.82	7.24	69.2	7.20	70.0	7.23	69.4

Table 3. Reference $^3J_{\alpha N}$ Values for P_{II}/β of Each Amino Acid and the Experimentally Determined $^3J_{\alpha N}$ as Well as $\%P_{II}$ at 20 °C in GXG Peptides with Free Ends at Three Different pH Values

	ref		pH 2.0		pH 4.0		pH 6.0	
amino acid	$^3J_{\alpha N}$ (P_{II}) Hz	$^3J_{\alpha N}$ (β) Hz	$^3J_{\alpha N}$ Hz	$\%P_{II}$	$^3J_{\alpha N}$ Hz	$\%P_{II}$	$^3J_{\alpha N}$ Hz	$\%P_{II}$
Ala	4.81	9.87	6.19	72.7	6.44	67.8	6.66	63.4
Phe	5.34	9.86	7.24	58.0	7.52	51.8	7.60	50.0
Ser	5.52	8.97	7.23	50.4	7.70	36.8	7.79	34.2
Val	6.09	9.82	7.41	64.6	7.71	56.6	7.77	55.0

Table 4. Reference $^3J_{\alpha N}$ Values for P_{II}/β of Each Amino Acid and Measured $^3J_{\alpha N}$ as Well as Derived $\%P_{II}$ Values for $AcGGXGGNH_2$ by Shi et al.[28] and GXG by Hagarman et al.[27] for Comparison

	ref		Shi et al., peptides $AcGGXGGNH_2$		Hagarman et al., peptides GXG		
amino acid	$^3J_{\alpha N}(P_{II})$, Hz	$^3J_{\alpha N}(\beta)$, Hz	$^3J_{\alpha N}$, Hz	$\%P_{II}$	$^3J_{\alpha N}$, Hz	$\%P_{II}{}^a$	$\%P_{II}{}^b$
Ala	4.81	9.87	5.73	81.8	6.11	74.3	79.0
Phe	5.34	9.86	6.97	63.9	7.45	51.8	42.0
Ser	5.52	8.97	6.30	77.4	6.99	57.4	45.0
Val	6.09	9.82	7.05	74.3	7.46	63.3	40.0

[a] P_{II} contents were derived from NMR $^3J_{\alpha N}$ values, in which two representative structures were assumed according to our previous procedure.[28] [b] P_{II} contents were taken from Hagarman et al.,[27] that were derived from combined spectroscopic techniques and fit to a distribution.

and C-terminal end residues should also have different sets of Karplus equation parameters relative to those in the middle of peptide chains. This might help explain the observed differences between the middle Ala amide and the C-terminal Ala amide $^3J_{\alpha N}$ values in the unblocked $(Ala)_3$ peptide.[41]

CONCLUSIONS

In summary, for the differences of ~30% in P_{II} content values of V, F, and S between GXG and $AcGGXGGNH_2$ peptide series, the effects from charged end(s) account for ~15% (GXG vs $AcGXGNH_2$), and effects from an extra Gly on each end account for ~5% ($AcGXGNH_2$ vs $AcGGXGGNH_2$), while another ~5% can be accounted for by α or turn conformations detected as differences in data-analysis procedures—one set derived from NMR $^3J_{\alpha N}$ values, assuming two representative structures, vs the other derived from combining spectroscopic techniques and fitting to a more complete distribution. In addition to experimental error, minor discrepancies can also be explained by uncertainties in Karplus equation parameters[40] and reference $^3J_{\alpha N}$ values for P_{II} and β.[28,33] Given the above uncertainties, recent experimental data on the backbone conformational distribution in model dipeptides[18] may yield more precise benchmarks for developing force fields[41−43] applicable in simulations and protein structure prediction.[44]

EXPERIMENTAL SECTION

Capped peptides used in this study were synthesized using an automated Liberty Microwave Peptide Synthesizer (CEM) in solid phase. The amidated peptides are assembled on Rink amide resin (Advance ChemTech) using 9-fluorenylmethyloxylcarbonyl (Fmoc) chemistry. Fmoc-amino acids, 2-(1*H*-benzotriazol-1-yl) 1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU) and *N*-hydroxybenzotriazole (HOBT) were purchased from Nova Biochem. The N terminus of each peptide is capped with acetic anhydride after assembly on the solid matrix. Cleavage of peptides from the resin was routinely performed using 95% trifluoroacetic acid (TFA) in the presence of the scavenger 2.5% triisopropylsilane (TIS) and 2.5% H_2O. Free N and C termini peptides were also synthesized through solid-phase peptide synthesis (SPPS). However, they were manually synthesized due to the small size of the peptides. The peptides were assembled in a preloaded Fmoc-Gly-Wang resin. Wang resin was used this time, since a free carboxyl group is desired at the C terminus end. Once the peptide elongation was finished, Fmoc deprotection was performed without acetylation. Cleavage was carried out using the cocktail (95%TFA/2.5%TIS/2.5%H_2O). The products were precipitated with cold ether. Water-soluble peptides were lyophilized overnight and directly purified on a reverse-phase HPLC C-18 preparative column (2.2 × 25 cm, 300 Å, Grace Vydac, Hesperia, CA) with water and acetonitrile in 0.1% TFA in gradients. Fractions containing the product were pooled and lyophilized. The identity and molecular weight of each peptide were confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

Circular dichroism (CD) spectra were recorded on an AVIV 410 spectrometer (Lakewood, NJ) using 0.1-cm path-length quartz CD cuvettes (Hellma QS, Hellma, Forest Hill, NY). The corresponding solvent CD background was measured and subtracted from the sample spectrum at a given temperature, salt concentration and pH. Spectra were collected with a 0.5 or 1 nm resolution and a scan rate of 1 nm min^{-1}. Temperature was maintained and controlled with a refrigerated recirculator (Neslab, CFT-25). Reported spectra are averages of 12 or more scans. Each spectrum was measured at least 3 times with individually prepared peptide solutions and expressed as molar ellipticity. The concentration of peptide was determined from a combination of UV absorbance and NMR peak integration. In this

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procedure, a CD sample solution of unknown concentration was mixed with a small but known amount of tryptophan stock solution. A 1D proton NMR spectrum with water suppression was then recorded on the mixture. The nonexchangeable α protons of Trp (around 3.2 ppm) and those of the corresponding protons of the acetyl group on the N-terminus of each peptide (around 2.0 ppm) or the Hβ from the side chain of the non glycine residue, were then integrated to give a quantitative ratio, from which the peptide concentration could be calculated from the concentration of tryptophan as determined from its UV absorbance at 279 nm.

NMR measurements were carried out on a Bruker AVANCE 400/500/600 MHz spectrometer. $^3J_{\alpha N}$ coupling constants were determined from high resolution 1D spectra recorded with 64 scans using an acquisition time of 4.4 s and a sweep width of 7200 Hz. Water suppression was achieved using a 3919 Watergate sequence.[45] The original free induction decays were zero-filled to 128K data points and Fourier transformed without any weighting function applied. The $^3J_{\alpha N}$ constants were measured directly by the extent of amide proton splitting.

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