J|A|C|S
COMMUNICATIONS

Published on Web 06/11/2003

The Pentapeptide GGAGG Has PII Conformation

Liang Ding, Kang Chen, Paul A. Santini, Zhengshuang Shi,† and Neville R. Kallenbach*

Department of Chemistry, New York University, 100 Washington Square East, New York, New York 10003

Received April 9, 2003; E-mail: nrk1@nyu.edu

Many proteins are now thought to lack stable native folded structures upon isolation from cells.[1−3] The question of what structure proteins that are unfolded under normally native conditions have is thus a timely one. Pioneering work by Tanford's group established the paradigm that the overall chain dimensions of a variety of denatured proteins in the denaturing cosolvent GuHCl conform to polymeric random coils.[4] While residual α- or β-type of secondary structure is known to occur in unfolded proteins,[5,6] several recent experimental and theoretical studies suggest that even very short peptides are significantly structured in aqueous solution, contrary to earlier expectations that such models represent a blend of conformations including α-, β-structures and turns.[7] Di- and tripeptide sequences of alanine, for example, show a striking tendency to assume a PII conformation in water; the polyproline II structure is a left-handed 3_1 helix with dihedral angles $\phi = -75°$ and $\psi = +145°$, intermediate between those of the α-helix and β-strand. This conclusion rests on several lines of experimental and theoretical[8] evidence, including NMR dipolar couplings determined in oriented peptides,[9] pump probe IR spectroscopy,[10] polarized Raman spectroscopic analysis,[11] vibrational CD,[12] and Raman optical activity.[13]

An NMR study of an alanine heptamer XAO, an alanine heptamer flanked by short basic side chains,[14] revealed that each alanine residue in the peptide is predominantly PII at low temperatures.[14,15] The $^3J\alpha$N coupling constants and CD spectra of this molecule show clear temperature dependence, inconsistent with any random coil structural model. While analysis of longer peptides is needed to discern the interresidue steric effects that have been predicted by Pappu and Rose,[16,17] we have sought a minimal model system with which to explore host−guest analysis of the amino acid conformational preferences in unfolded states. We show here that a model peptide containing only a single alanine residue flanked on both sides by two glycine residues, $AcGGAGGNH_2$, has PII conformation, determined by NMR measurements and CD analysis (Figure 1).

The coupling constant $^3J\alpha$N of Ala3 in the peptide $AcGGAGGNH_2$ was measured to be 5.9 Hz at 20 °C, which establishes the Φ dihedral angle to be $-73 \pm 10°$ from a revised Karplus equation.[32] The ratio, 4.1, of NOE intensities between Hβ Ala3 and its own HN to that between Hβ Ala3 and the succeeding Gly4 HN determines the Ψ angle to be $+125 \pm 10°$ using the same calculation as before.[14] Thus, PII conformation is dominant in the residue Ala3. The CD spectrum of the peptide $AcGGAGGNH_2$ shows the minimum at 190 nm characteristic of PII conformation, in agreement with the NMR evidence. Taken together, these results indicate a high proportion of PII conformation in $AcGGAGGNH_2$ in water at low temperature, shifting to increasing β-strand structure at high *T*. However, the fractional content of PII is less than that in the XAO peptide in aqueous solution at 4 °C, as is reflected in the ^3J coupling constant values and NOE measurements as well as CD data.

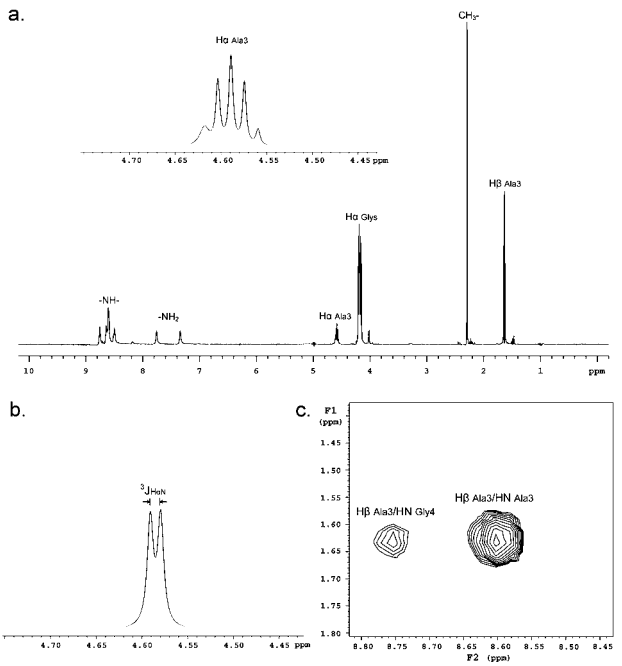

Figure 1. (a) The 1D NMR spectrum of $AcGGAGGNH_2$ is at the bottom. The upper left inset is the enlarged Hα Ala3 peak split by HN and Hβ of Ala3. (b) The Hα Ala3 peak is split by HN Ala3 while decoupling Hβ, allowing measurement of the $^3J\alpha$N coupling constant. (c) The NOE cross-peaks of Hβ Ala3 (1.63 ppm) to HN of Ala3 (8.59 ppm) and Gly4 (8.75 ppm) show the integrated volume ratio to be 4.05. Assignments of the HN region were accomplished by analysis of 2D TOCSY and NOESY spectra. NMR experiments were carried out with 2 mM GGAGG in 20 mM sodium acetate buffer pH 4.6, 20 °C on a Varian Unity 500 spectrometer.

Both $AcGGAGGNH_2$ and XAO exhibit thermal transition behavior inconsistent with a coil-like ensemble of interconverting conformations. Relative to the longer chain, the transition in $AcGGAGGNH_2$ is completed at a lower temperature and appears sharper. One possible explanation is that despite its conformational flexibility Gly itself has a high propensity for PII (see ref 15). A second is that longer range interactions are present in XAO which stabilize PII yet reduce the apparent enthalpy. Because the initial and final points of the thermal profiles are not clearly defined, estimation of the transition enthalpies from the data in Figures 2 and 3 can only be crude.

What accounts for the propensity of Ala for the PII conformation? Steric factors,[16,17] dipole−dipole interactions, and solvation of the backbone may all play a role. On the basis of the observation of a predominant PII conformation in $AcGGAGGNH_2$, it seems unlikely that steric factors alone are responsible for maintaining this structure in the absence of prolines. However, sterics possibly contribute, because the percentage of PII in $AcGGAGGNH_2$ is lower than that

† Current address: Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104.

10.1021/ja035551e CCC: $25.00

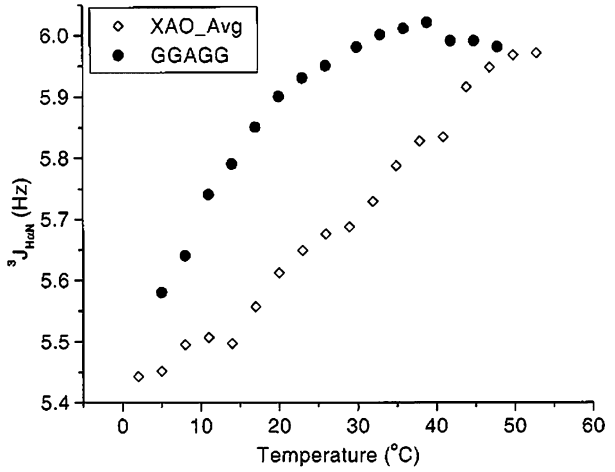

Figure 2. The $^3J\alpha$N coupling constants of Ala3 of AcGGAGGNH$_2$ were measured at different temperatures. For reference in the figure, the thermal profile (the average ^3J value of six Ala residues is used) of XAO was superimposed.

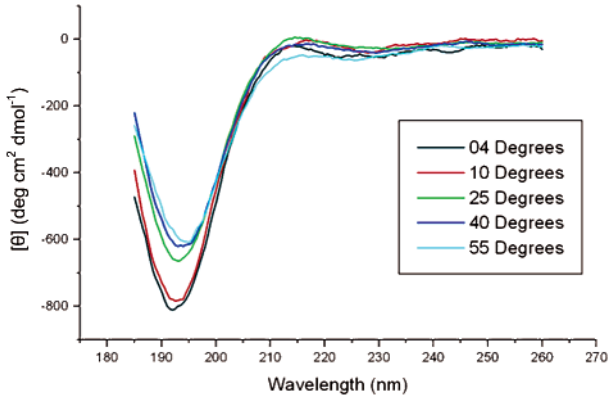

Figure 3. Far UV CD spectra of AcGGAGGNH$_2$ show the temperature dependence of PII conformation. CD experiments were carried out with 1 mM GGAGG in 10 mM phosphate buffer, pH 7.0.

in XAO at the same temperature. The role of water seems crucial, as is indicated by several recent studies.[8,9,33] PII appears to be effectively hydrated, as can be seen in the structures of collagen related peptides,[18] which show bridged waters surrounding the helix. Simulations of the water environment of different conformations suggest that PII is better hydrated than the β-structure.[19] PII residues in native structures of folded proteins also tend to occur in hydrated regions.[20,21] It would then be predicted that the PII structure is sensitive to solvation effects,[33] and neutral peptides such as AcGGAGGNH$_2$ could provide additional useful models with which to test this hypothesis.

A second question concerns the generality of the presence of PII conformation in oligo-Ala model peptides, including AcGGAGGNH$_2$ and XAO: Is this perhaps a characteristic of Ala? Mounting evidence suggests many peptide sequences adopt PII conformation.[22,23] Thus, oligo (Lys)[24] and (Glu)[25] both favor PII. Many short peptides exhibit the characteristic PII CD spectrum as well, which is that assigned as random coil, but which is incompatible with any random coil CD spectrum;[26] for example, the negative band at 190 nm is as intense (but opposite in sense) in many unfolded proteins as that of the α-helix at 222 nm.[27]

Several studies have sought to calibrate values for "random coil" NMR parameters by using peptide models related to the sequence we have studied.[28,29] This work implies that series of model peptides that have been accepted for many years as standards for the purpose might in fact contain differing degrees of PII conformation rather than the expected coillike blend. The fact that PII conformation is a significant contributor in unfolded peptide structure points to a simplification in ideas about folding: the unfolded ensemble of chains may be substantially less disordered and hence may have much less backbone entropy than was supposed in earlier models.[30,31]

Acknowledgment. This work was supported by a grant from the ONR.

References

(1) Uversky, V. N.; Gillespie, J. R.; Fink, A. L. *Proteins* **2000**, *41*, 415−427.
(2) Iakoucheva, L. M.; Brown, C. J.; Lawson, J. D.; Obradovic, Z.; Dunker, A. K. *J. Mol. Biol.* **2002**, *323*, 573−584.
(3) Dunker, A. K.; Brown, C. J.; Obradovic, Z. *Adv. Protein Chem.* **2002**, *62*, 25−49.
(4) Tanford, C. *Adv. Protein Chem.* **1968**, *23*, 121−282.
(5) Shortle, D.; Ackerman, M. S. *Science* **2001**, *293*, 487−489.
(6) Dyson, H. J.; Wright, P. E. *Curr. Opin. Struct. Biol.* **2002**, *12*, 54−60.
(7) Mattice, W. L.; Lo, J. T.; Mandelkern, L. *Macromolecules* **1972**, *5*, 729−734.
(8) Han, W. G.; Jalkanen, K. J.; Elstner, M.; Suhai, S. *J. Phys. Chem. B* **1998**, *102*, 2587−2602.
(9) Poon, C. D.; Samulski, E. T.; Weise, C. F.; Weisshaar, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 5642−5643.
(10) Woutersen, S.; Hamm, P. *J. Phys. Chem. B* **2000**, *104*, 11316−11320. Woutersen, S.; Hamm, P. *J. Chem. Phys.* **2001**, *114*, 2727−2737.
(11) Eker, F.; Cao, X. L.; Nafie, L.; Schweitzer-Stenner, R. *J. Am. Chem. Soc.* **2002**, *124*, 14330−14341.
(12) Keiderling, T. A.; Xu, Q. *Adv. Protein Chem.* **2002**, *62*, 111−161.
(13) Barron, L. D.; Blanch, E. W.; Hecht, L. *Adv. Protein Chem.* **2002**, *62*, 51−90.
(14) Shi, Z.; Olson, C. A.; Rose, G. D.; Baldwin, R. L.; Kallenbach, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9190−9195.
(15) Shi, Z.; Woody, R. W.; Kallenbach, N. R. *Adv. Protein Chem.* **2002**, *62*, 163−240.
(16) Pappu, R. V.; Rose, G. D. *Protein Sci.* **2002**, *11*, 2437−2455.
(17) Pappu, R. V.; Srinivasan, R.; Rose, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12565−12570.
(18) Bella, J.; Brodsky, B.; Berman, H. M. *Structure* **1995**, *3*, 893−906.
(19) Sreerama, N.; Woody, R. W. *Proteins* **1999**, *36*, 400−406.
(20) Adzhubei, A. A.; Sternberg, M. J. *Protein Sci.* **1994**, *3*, 2395−2410.
(21) Creamer, T. P.; Campbell, M. N. *Adv. Protein Chem.* **2002**, *62*, 263−282.
(22) Ma, K.; Kan, L.; Wang, K. *Biochemistry* **2001**, *40*, 3427−3438.
(23) Shi, Z.; Ph D. Thesis; New York University: New York, 2002.
(24) Rucker, A. L.; Creamer, T. P. *Protein Sci.* **2002**, *11*, 980−985.
(25) Tiffany, M. L.; Krimm, S. *Biopolymers* **1968**, *6*, 1379.
(26) Tiffany, M. L.; Krimm, S. *Biopolymers* **1969**, *8*, 347.
(27) Adler, A. J.; Greenfield, N. J.; Fasman, G. D. *Methods Enzymol.* **1973**, *27*, 675−735.
(28) Braun, D.; Wider, G.; Wuthrich, K. *J. Am. Chem. Soc.* **1994**, *116*, 8466−8469.
(29) Plaxco, K. W.; Morton, C. J.; Grimshaw, S. B.; Jones, J. A.; Pitkeathly, M.; Campbell, I. D.; Dobson, C. M. *J. Biomol. NMR* **1997**, *10*, 221−230. Merutka, G.; Dyson, H. J.; Wright, P. E. *J. Biomol. NMR* **1995**, *5*, 14−24. Wishart, D. S.; Bigam, C. G.; Holm, A.; Hodges, R. S.; Sykes B. D. *J. Biomol. NMR* **1995**, *5*, 67−81.
(30) Baldwin, R. L. *Adv. Protein Chem.* **2002**, *62*, 361−367.
(31) Ferreon, J. C.; Hilser, V. J. *Protein Sci.* **2003**, *12*, 447−457.
(32) Vuister, G. W.; Bax, A. *J. Am. Chem. Soc.* **1993**, *115*, 7772−7777.
(33) Eker, F.; Cao, X.; Nafie, L.; Huang, Q.; Schweitzer-Stenner, R. *J. Phys. Chem. B* **2003**, *107*, 358−365. Weise, C. F.; Weisshaar, J. C. *J. Phys. Chem. B* **2003**, *107*, 3265−3277.

JA035551E