# *Communications to the Editor*

## Alanine Is an Intrinsic α-Helix Stabilizing Amino Acid

Erik J. Spek, C. Anders Olson, Zhengshuang Shi, and Neville R. Kallenbach*

*Department of Chemistry*
*New York University*
*New York, New York 10003*

*Received January 5, 1999*

Large proteins fold via the formation of intermediate structures.[1] Do these intermediates possess secondary structure such as an α-helix or $\beta$-sheets? A related question is whether specific amino acid side chains have an intrinsic tendency to form incipient or final secondary structure. There is an ongoing debate concerning the helix propensity of alanine. Studies on nucleated peptides and co-polypeptides conclude that alanine is helix-indifferent; other studies on proteins and peptides conclude that alanine is helix-stabilizing.[4−6]

Scheraga et al. synthesized co-polypeptides in which Ala sequences were flanked by extended blocks of charged side chains and concluded that short stretches of alanine (below $N \approx 100$ residues) do not form an α-helix.[2] Introducing natural amino acids as guests into a host matrix of alkylated glutamine residues[3] seemingly confirmed that Ala is helix-indifferent. Still, many alanine-rich short peptide models are found to form α-helical structure,[4−6] and alanine stabilizes helices in proteins.[7] There is then a fundamental contradiction in our understanding of α-helical structure and its role in folding. Either Ala stabilizes α-helices, or it does not.

By using a synthetic nucleating template Kemp's laboratory[8] investigated the helix propensities of amino acids as well as those of natural peptides containing combinations of Ala and Lys.[9] They reached conclusions similar to those of Scheraga, arguing that Ala appears to be helix-stabilizing artifactually:[8,10] the effect arises from neighboring charged side chains that enhance the helix propensity of Ala. In this paper we show that Ala is intrinsically helix-stabilizing and that the helix content of alanine-based peptides is not derived from neighboring amino acids.

We constructed soluble peptides[12] in which Ala and Lys side chains do not alternate (Figure 1). The results show that chains of 13 Ala residues flanked by pairs of Orn or Dpr, charged groups too short to enhance helicity of neighboring Ala residues by direct interaction, are helical. Each chain contains a core of Ala residues, flanked by two Orn or Dpr side chains. A22 consists of alanine only. K22 and O22 have lysine or ornithine substituted for the alanine in position 10. We use two charged flanking residues near the ends because a single basic side chain at the ends favors $\beta$-sheet structure in such models[12] or aggregation of helices.[9] Each peptide is capped by an acetyl group at the N terminus and an amide at the C terminus. Tyrosine facilitates concentration determination. The peptides in solution are monomers, as shown by analytical ultracentrifugation (Figure 2), consistent with the absence of concentration dependence in any peptides over a range of concentrations from 10 $\mu$M to 1 mM. Figure 3 shows CD spectra of the peptides in Figure 1, showing they consist of coil plus α-helix.[13] Substitution of Lys (K22) or Orn (O22) for Ala (A22) at the central position lowers the helicity in low-salt buffer as reported in other peptide models.[4−6] The helicity of the peptide XX22, with Dpr as flanking basic residues, is slightly lower than that of A22.[15]

NMR reveals that the core of K22 (used for spectroscopic reasons) is α-helical; extended NH$i$ − NH$i$ + 1 cross-peak connectivity is seen in the NOESY spectrum together with low values of the $^3J_{NH}$ coupling constants characteristic of α-helix (Figure 4). In addition there are medium range NOEs consistent with α-helical structure. The ends of the chain are frayed, indicating the terminal side chains do not have abnormally high helix propensities. By design, in these peptides the side chains flanking the Ala block cannot directly influence the helicity of the majority of Ala side chains; the flanking Orn or Dpr groups lack the potential to interact with the helix barrel, as Lys can.[11]

Quantitatively, the CD spectra agree closely with the prediction of helix−coil transition theory using a propensity value of 1.4 for Ala or higher, but not 1.1 or less.[14] To reconcile the helicity of these peptides with a propensity of 1.1 or less for alanine the flanking Orn side chains would need very large propensity values, inconsistent with the fraying observed at the ends of the helix in the NMR or the substitution data in Figure 3. The decrease in helix content from A22 to K22 to O22 would be predicted from the lower helix propensities of Lys and Orn however.[15] Recently an increase in helicity was reported upon replacing alanines by lysine in similar peptides, even in low salt.[9] These results as well as the CD data in Figure 3 contradict older results on Ala-rich peptides.[6,15] We believe the discrepancy originates in the limited solubility of peptides containing only a single charge at each end[12]—adding lysines increases the solubility of the chains, hence the apparent helicity.

The short terminal side chains in our model cannot influence a significant fraction of the Ala groups by a local interaction as could lysine. The spacing of charges is also incompatible with

(1) (a) Kim, P. S.; Baldwin, R. L. *Annu. Rev. Biochem.* **1982**, *51*, 459. (b) Sosnick, T. R.; Shtilerman, M. D.; Mayne, L.; Englander, S. W. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8545. (c) Matagne, A.; Chung, E. W.; Ball, L. J.; Radford, S. E.; Robinson, C. V.; Dobson, C. M. *J. Mol. Biol.* **1998**, *277*, 997.

(2) Ingwall, R. T.; Scheraga, H. A.; Lotan, N.; Berger, A.; Katchalski, E. *Biopolymers* **1968**, *6*, 331.

(3) Wojcik, J.; Altmann, J. H.; Scheraga, H. A. *Biopolymers* **1994**, *30*, 121.

(4) (a) Chakrabartty, A.; Kortemme, T.; Baldwin, R. L. *Protein Sci.* **1994**, *3*, 843. (b) Rohl, C. A.; Chakrabartty, A.; Baldwin, R. L. *Protein Sci.* **1996**, *5*, 2623.

(5) (a) Lyu, P. C.; Liff, M. I.; Marky, L. A.; Kallenbach, N. R. *Science* **1990**, *250*, 669. (b) Yang, J.; Spek, E. J.; Gong, Y.; Zhou, H.; Kallenbach, N. R. *Protein Sci.* **1997**, *6*, 1264.

(6) Park, S. H.; Shalongo, W.; Stellwagen, E. *Biochemistry* **1993**, *32*, 7048.

(7) (a) Blaber, M.; Zhang, X. J.; Matthews, B. W. *Science* **1993**, *260*, 1637. (b) Myers, J. K.; Pace, C. N.; Scholtz, J. M. *Biochemistry* **1997**, *36*, 10923.

(8) (a) Groebke, K.; Renold, P.; Tsang, K. Y.; Allen, T. J.; McClure, K. F.; Kemp, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4025. (b) Renold, P.; Tsang, K. Y.; Shimizu, L. S.; Kemp, D. S. *J. Am. Chem. Soc.* **1996**, *118*, 12234. (c) Kemp, D. S.; Boyd, J. G.; Muendel, C. C. *Nature* **1991**, *352*, 451.

(9) Williams, L.; Kather, K.; Kemp, D. S. *J. Am. Chem. Soc.* **1998**, *120*, 11033.

(10) Vila, J.; Williams, R. L.; Grant, J. A.; Wojcik; J. Scheraga, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7821.

(11) (a) Nemethy, G.; Scheraga, H. A. *J. Phys. Chem.* **1962**, *66*, 1773 (b) Nemethy, G.; Steinberg, I. Z.; Scheraga, H. A. *Biopolymers* **1963**, *1*, 43 (c) Lotan, N.; Yaron, Y.; Berger, A. *Biopolymers* **1966**, *4*, 365.

(12) Blondelle, S. E.; Forood, B.; Houghten, R. A.; Perez-Paya, E. *Biochemistry* **1997**, *36*, 8393.

(13) Cantor, C. R.; Schimmel, P. R. in *Biophysical Chemistry*; WH Freeman: New York, 1980.

(14) Andersen, N. H.; Tong, T. *Protein Sci.* **1997**, *6*, 1290.

(15) (a) Padmanabhan, S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *J. Mol. Biol.* **1996**, *241*, 706. (b) Marqusee, S.; Robbins, V. H.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *85*, 5286−5290.

10.1021/ja990056x CCC: $18.00 
Published on Web 05/29/1999

| | |
|---|---|
| A22 | ac A OO AAAAAA A AAAAAA OO A GGY-$NH_2$ |
| O22 | ac A OO AAAAAA O AAAAAA OO A GGY-$NH_2$ |
| K22 | ac A OO AAAAAA K AAAAAA OO A GGY-$NH_2$ |
| X22 | ac A XX AAAAAA A AAAAAA XX A GGY-$NH_2$ |

**Figure 1.** Peptide sequences of this study. A = alanine, O = ornithine, G = glycine, Y = tyrosine, K = lysine, X = 2,3 diamino propionic acid, ac = acetyl.

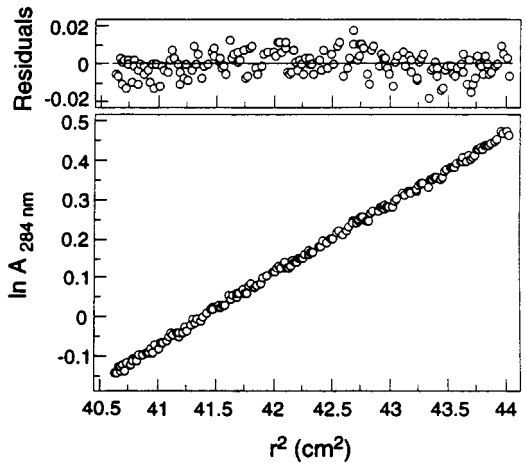


**Figure 2.** Analytical ultracentrifugation data (39 krpm) for A22 (350 $\mu$M) collected at 4 °C and pH 7. The natural logarithm of the absorbance at 284 nm is plotted against the square of the radial position. Deviations from the values calculated for a single species of MW 1930 D are plotted as residuals above the absorbance data.

the solvation effect postulated by Vila et al.[10] Thus, alanine stabilizes α-helix. If so, why does Ala fail to stabilize helix in Kemp's models? One explanation is that instead of the postulated helix-barrel interaction between Lys and neighboring Ala side chains, Lys at position 6 in the pre-nucleated chains interacts with the template itself.[8] A minor rearrangement of the template geometry allows its C═O groups to form a cap that interacts with the Lys $\epsilon$-$NH_3$ group, perhaps artificially stabilizing the helix structure between these sites.[8a] Those Ala's whose effect on helix is weaker than in natural sequences lie between the capping side chains; residues distal to the cap show higher helix propensity.[8a] However Kemp's data on artificially nucleated helices are rationalized, our results demonstrate that Ala side chains stabilize α-helix structure intrinsically, and not by enhancing effects extending from neighboring side chains.

**Acknowledgment.** This research was supported by grant GM 40746 from the NIH and a grant from the Human Frontiers of Science Program. We thank Drs. Baldwin and Rohl for communicating their manuscript to us prior to publication, Dr. Min Lu for sedimentation experiments, and Dr. Alex Vologodskii for CD calculations.

**Supporting Information Available:** This material is available free of charge via the Internet at http://pubs.acs.org.

JA990056X

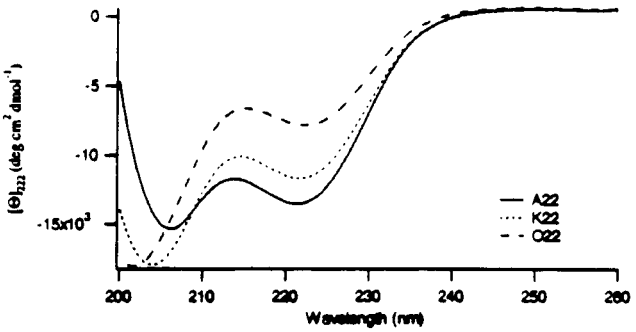


**Figure 3.** (A) Circular dichroism spectra of A22, O22, and K22 in 10 mM KF, 1 mM phosphate pH 7 at 4 °C. The concentrations are 50 $\mu$M as determined by tyrosine absorbance.

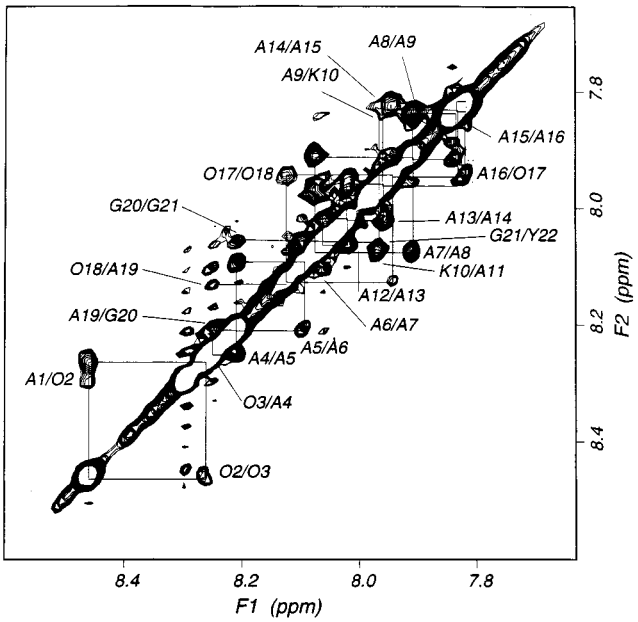


**Figure 4.** Section of 2D NOESY spectrum of K22 at ca. 8.0 mM at 5 °C with a 500 ms mixing time, indicating cross-peaks between backbone amide protons. Sequential cross-peaks are indicated by the numbers of the residues in Figure 1.[5b]