TABLE 2 Experimentally determined and calculated distances between labelled side chains

Distance between β-carbons for two helix geometries

Helix	$d_{\beta\beta}(4,6)$ (Å)	$d_{\beta\beta}(4,7)$ (Å)	$d_{\beta\beta}(4,8)$ (Å)
α-helix	7.68	6.56	6.79
3_{10}-helix	7.32	6.59	10.0

ESR results for the doubly labelled peptides

Reciprocal linewidth ($M_I=0$)	3K-(4, 6)	3K-(4, 7)	3K-(4, 8)
$\delta^{-1}(G^{-1})$	0.410	0.353	0.621

Distance between nitroxide N atoms from molecular dynamics

Helix	$\langle d(4,6)\rangle$ (Å)	$\langle d(4,7)\rangle$ (Å)	$\langle d(4,8)\rangle$ (Å)
α-helix	14.0±0.9	9.3±2.8	9.1±1.7
3_{10}-helix	11.7±1.8	8.8±1.6	12.0±1.2

The distances between side-chain β-carbons (Å) for the α-helix geometry ($\phi=-65°$, $\Psi=-40°$) and the 3_{10}-helix geometry ($\phi=-74°$, $\Psi=-4°$) as calculated using the program Insight II (BioSym) running on a personal IRIS computer (Silicon Graphics). For the ESR spectra of the doubly labelled peptides, δ^{-1} (G^{-1}) is the reciprocal of the peak-to-peak width of the centre ($M_I=0$) nitroxide hyperfine line. This parameter is proportional to the distance between the side chains. Constrained molecular dynamics results for helices in the two helical geometries with labels at the indicated positions. The calculated average distance (±s.d.) between nitroxide N atoms on each peptide is $\langle d(i,j)\rangle$. The 3K helix analogues with spin labels were built using InsightII version 1.1.0 (BioSym). Molecular dynamics calculations were performed using Discover v. 2.7 (BioSym) with a dielectric constant of 80. Side-chain atoms were unconstrained but helix backbone atoms were constrained using a strong restoring potential to maintain the helical structure. All of the structures were subjected to an initial 10 ps of molecular dynamics a 300 K followed by energy minimization using the steepest descent method. The structures were then heated to 900 K and molecular dynamics run for 20 ps. The temperature was then lowered to 300 K and molecular dynamics run for 20 ps to equilibrate the structures. After this annealing[21], the calculations were run for 100 ps and analysed. This entire procedure was repeated, and yielded the same results to within experimental error.

line nitroxide spectrum (~25% of the integrated area) and a very broad spectrum (~75%). The broad component shows that the average $d(4,7)$ decreases with increasing helicity and further supports the assignment of a 3_{10}-helix structure. This superposition of two spectra may indicate the coexistence of the two helical forms, with α-helices giving rise to the narrow-line component.

We find that there is significant evidence that α-helix is not the correct structure for these Ala-based peptides. The data in this report can be readily explained by the presence of 3_{10}-helix geometry. Recent work with aminoisobutyric acid rich peptides provides a reference point for these findings. The amino acid is disubstituted at the α-carbon and this restricts its conformation to the helical region of the conformational energy map[22,23]. In general, shorter peptides (eight residues or less) with a high aminoisobutyric acid content favour the formation of 3_{10}-helices. NMR investigations of 8-mers rich in aminoisobutyric acid in organic solvents have demonstrated that the $\alpha \rightleftharpoons 3_{10}$ equilibrium is readily controlled by the choice of solvent[24]. Our finding is surprising as these Ala-based peptides are 16-mers that contain no aminoisobutyric acid. But we have studied aqueous solutions, in which the nature of the $\alpha \rightleftharpoons 3_{10}$ equilibrium is not well understood.

We consider two additional points. First, the $i+5$ placement of the lysine residues in the original 3K(I) was to ensure that these peptides would not be amphiphilic in the α-helix conformation. Coincidentally, this lack of amphilicity is preserved in the 3_{10}-helix conformation. Second, a recent molecular dynamics study of an α-helical fragment of ribonuclease A suggested the unfolding sequence followed: α-helix → 3_{10}-helix → no hydrogen-bond[25]. We propose that short monomeric Ala-based peptides in aqueous solution actually coexist in both helical forms and that the population of the 3_{10}-helix conformation is much greater than previously expected. It may be that the 3_{10} conformation is mainly a long-lived intermediate between the two terminal states of α-helix and random coil. In contrast, peptides in aqueous solution may prefer the 3_{10}-helix conformation, whereas the α-conformation is favoured in folded proteins. □

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1. Marqusee, S., Robbins, V. H. & Baldwin, R. L. *Proc. natn. Acad. Sci. U.S.A.* **86,** 5286–5290 (1989).
2. Merutka, G., Lipton, W., Shalongo, W., Park, S. H. & Stellwagen, E. *Biochemistry* **29,** 7511–7515 (1990).
3. Chakrabartty, A., Schellman, J. A. & Baldwin, R. L. *Nature* **351,** 586–588 (1991).
4. Padmanabhan, S., Marqusee, S., Ridgeway, T., Laue, T. M. & Baldwin, R. L. *Nature* **344,** 268–270 (1990).
5. Manning, M. C. & Woody, R. W. *Biopolymers* **31,** 569–586 (1991).
6. Osterhout, J. J. *et al. Biochemistry* **28,** 7059–7064 (1989).
7. Bradley, E. K., Thomason, J. F., Cohen, F. E., Kosen, P. A. & Kuntz, I. D. *J. molec. Biol.* **215,** 607–622 (1990).
8. Würthrich, K. *NMR of Proteins and Nucleic Acids* (Wiley, New York, 1986).
9. Voet, D. & Voet, J. G. *Biochemistry* (Wiley, New York, 1990).
10. Barlow, D. J. & Thornton, J. M. *J. molec. Biol.* **201,** 601–619 (1988).
11. Todd, A. P. & Millhauser, G. L. *Biochemistry* **30,** 5515–5523 (1991).
12. Miick, S. M., Rodd, A. P. & Millhauser, G. L. *Biochemistry* **30,** 9498–9503 (1991).
13. Byler, D. M. & Susi, H. *Biopolymers* **25,** 469–487 (1986).
14. Kennedy, D. F., Crisma, M., Toniolo, C. & Chapman, D. *Biochemistry* **30,** 6541–6548 (1991).
15. Malcolm, B. R. *Biopolymers* **22,** 319–321 (1983).
16. Dwivedi, A. M., Krimm, S. & Malcolm, B. R. *Biopolymers* **23,** 2025–2065 (1984).
17. Prestrelski, S. J., Byler, D. M. & Thompson, M. P. *Int. J. Peptide Protein Res.* **37,** 508–512 (1991).
18. Luckhurst, G. R. in *Spin labeling: Theory and Applications* (ed. Berliner, L. J.) Ch. 4 (Academic, New York, 1976).
19. Falle, H. R. *et al. Molec. Phys.* **11,** 49–56 (1966).
20. Lemaire, H., Rassat, A., Rey, P. & Luckhurst, G. R. *Molec. Phys.* **14,** 441–447 (1968).
21. Krystek, S. R. *et al. FEBS Lett.* **299,** 255–261 (1992).
22. Toniolo, C. & Benedetti, E. *Trends. biochem. Sci.* **16,** 350–353 (1991).
23. Karle, I. L. & Balaram, P. *Biochemistry* **29,** 6747–6756 (1990).
24. Gautam, B., Bagchi, K. & Kuki, A. *Biopolymers* **31,** 1763–1774 (1991).
25. Tirado-Rives, J. & Jorgensen, W. L. *Biochemistry* **30,** 3864–3871 (1991).

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ERRATUM

Direct measurement of the optical depth in a spiral galaxy

Raymond E. White III & William C. Keel

Nature **359,** 129–131 (1992)

IN this letter in the 10 September issue, the third sentence in the bold first paragraph should be replaced with the following: "This statistically-derived result (concluding that spiral galaxies are opaque) is however vulnerable to several selection effects, and seems to contradict the fact that the (extragalactic) survey was successfully performed (since we live in a spiral galaxy which must then be partially transparent) as well as anecdotal examples of galaxies visible through other galaxies."