Concomitant Occurrence of Peptide 3_{10}- and α-Helices Probed by NMR

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Oligopeptides based on protein (C^{α}-trisubstituted) α-amino acids are known to undergo α-helix ⇄ unordered conformation transitions under appropriate experimental conditions.[1] On the other hand, oligopeptides rich in C^{α}-tetrasubstituted α-amino acids present a rather peculiar stereochemistry due to significant constraints imposed on their conformational freedom by these residues.[2] Specifically, most of the C^{α}-tetrasubstituted α-amino acids have been extensively documented to possess a very high intrinsic helix-forming capacity. The narrow conformational space accessible includes both the classical α-helix and the 3_{10}-helix.[3] It was shown that among the C^{α}-tetrasubstituted chiral α-amino acids the *β*-branched C^{α}-methyl-L-valine [L-(αMe)Val] is the residue with the most pronounced bias toward the right-handed 3_{10}-helix.[2d]

Several initial experimental evidences have indicated that the terminally blocked −[L-(αMe)Val]$_8$− sequence adopts a fully developed, right-handed 3_{10}-helical conformation both in the crystal state and in structure-supporting solvents.[4] More recently, the interesting property of this peptide to fold in solution *both* in the 3_{10}- and in the α-helix has emerged.[4c] The type of helical conformation adopted was found to depend on experimental conditions, as clearly shown by vibrational and electronic CD techniques. Under appropriate conditions, the conformational transition from 3_{10}- to α-helix is very slow (the time scale is on the order of several days).

Our observations can be rationalized on the basis of a stabilization of the 3_{10}-helical structure by interpeptide interactions. This conclusion is supported by experimental evidence that the extent of peptide self-association is enhanced by increasing concentration and decreasing solvent polarity [e.g., from 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) to 2,2,2-trifluoroethanol (TFE)] as well as temperature. If this assumption is correct, then the conformational transition between the two types of helical structures can be slow because the first step of this process requires disruption of the molecular aggregates. Indeed, this structural helix−helix transition was theoretically predicted to take place very rapidly at the monomeric level.[3b] Different (3_{10}- and α-helical) polymorphic forms have already been found in the crystal state for mixed oligopeptides of protein and C^{α}-tetrasubstituted α-amino acids.[5] However, our −[L-(αMe)Val]$_8$− homooligopeptide system represents a unique tool to fully characterize both kinds of helices in solution and to study their conversion by NMR.

In the present work, we initially performed a CD study (Jasco model J-715 dichrograph) of Ac-[L-(αMe)Val]$_8$-O*t*Bu (Ac, acetyl; O*t*Bu, *tert*-butoxy) at a peptide concentration ($\cong 1 \times 10^{-2}$ M) appropriate for an NMR analysis (results not shown). In TFE, the CD spectrum is typical of a right-handed 3_{10}-helical peptide,[4b] displaying a negative band at 207 nm accompanied by a weak shoulder centered at 222 nm (the ratio $R = [\Theta]_{222}/[\Theta]_{207}$ is $\cong 0.3$). This CD pattern does not change within 7 days. Conversely, in HFIP the CD spectrum changes gradually to having a well-developed band at 222 nm ($R \cong 1$), typical of an α-helical peptide, after a week. On the basis of these CD results, two solutions in TFE were prepared for the NMR investigation. The first one (solution A) was obtained by direct dissolution of the peptide in TFE (concentration $\cong 1 \times 10^{-2}$ M). Alternatively, the peptide was dissolved in HFIP; after 7 days the solvent was evaporated and the compound was redissolved in TFE (solution B; peptide concentration $\cong 1 \times 10^{-2}$ M). The CD spectrum of solution B coincides with that measured in HFIP after 7 days. By means of NMR (Bruker model Avance DMX-600 spectrometer) and distance geometry (DG) and molecular dynamics (MD) calculations,[6] we performed a detailed conformational study of solutions A and B, i.e., two differently treated samples of the same terminally blocked homooctapeptide under the same conditions of solvent (TFE), concentration ($\cong 1 \times 10^{-2}$ M), and temperature (298 K).

In homooligopeptides based on C^{α}-tetrasubstituted α-amino acids, the lack of scalar coupling between protons precludes the use of homonuclear scalar correlation spectra for resonance assignment. Also, the very narrow range of resonances of nonexchangeable protons causes severe overlap problems in any homonuclear two-dimensional spectrum. To overcome these problems, HMBC (Heteronuclear Multiple Bond Coherence) experiments with Gaussian shaped pulse for selective excitation in the carbon dimension were utilized. The amide proton and the protons of the two side-chain groups are correlated through heteronuclear scalar coupling with an intermediate carbon atom (Figure 1), typically the carbonyl carbon in a CO-selective HMBC experiment. With this experiment, sequential assignment can also be achieved using interactions of the type $NH_i \rightrightarrows CO_i \rightrightarrows NH_{i+1}$. Owing to the greater dispersion of the carbon frequencies, all of the 16 peaks arising from the *γ*-methyl groups were resolved in an HMQC spectrum (deposited) and were assigned. Dipolar interactions from ROESY spectra confirmed the assignment of proton resonances where possible.

From the ROESY spectra of the two solutions, structural conclusions were derived which are summarized in Table 1 and Figure 2. For the peptide, in both solution A and B, the nOe connectivities (deposited) show an uninterrupted NN($i, i + 1$) pattern, indicating the presence of a helical conformation extending over the entire sequence. The type of helix, however, is distinctly

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(1) For leading references, see: (a) Goodman, M.; Toniolo, C.; Pallai, P. In *Forum Peptides*; Castro, B., Martinez, J., Eds.; Dohr: Nancy, 1985; pp 146−174. (b) Brown, J. E.; Klee, W. A. *Biochemistry* **1971**, *10*, 470−476. (c) Marqusee, S.; Robbins, V. H.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5286−5290.

(2) (a) Marshall, G. R. In *Intrascience Chemistry Reports*; Kharasch, N., Ed.; Gordon and Breach: New York, 1971; Vol. 5, pp 305−316. (b) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747−6756. (c) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004−4009. (d) Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, *33*, 1061−1072.

(3) For review articles on the peptide 3_{10}-helix, see: (a) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350−353. (b) Bolin, K. A.; Millhauser, G. L. *Acc. Chem. Res.* **1999**, *32*, 1027−1033. (c) Pal, L.; Basu, G. *Protein Eng.* **1999**, *12*, 811−814.

(4) (a) Polese, A.; Formaggio, F.; Crisma, M.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. *Chem. Eur. J.* **1996**, *2*, 1104−1111. (b) Toniolo, C.; Polese, A.; Formaggio, F.; Crisma, M.; Kamphuis, J. *J. Am. Chem. Soc.* **1996**, *118*, 2744−2745. (c) Yoder, G.; Polese, A.; Silva, R. A. G. D.; Formaggio, F.; Crisma, M.; Broxterman, Q. B.; Kamphuis, J.; Toniolo, C.; Keiderling, T. A. *J. Am. Chem. Soc.* **1997**, *119*, 10278−10285.

(5) Vijayalakshmi, S.; Balaji Rao, R.; Karle, I. L.; Balaram, P. *Biopolymers* **2000**, *53*, 84−98.

(6) DG and MD simulations were performed using the program XPLOR 3.1. A total of 49 (solution A) and 55 (solution B) interproton distance constraints, obtained by integration of ROESY spectra, were used. Addition and subtraction of 10% to calculated distances yielded upper and lower bounds utilized in the restrained MD. For each sample, 150 structures were generated and followed for 100 ps of MD. At the end of the MD run, the 86 (solution A) and 116 (solution B) structures with violations of the experimentally derived constraints lower than 0.4 Å were accepted.

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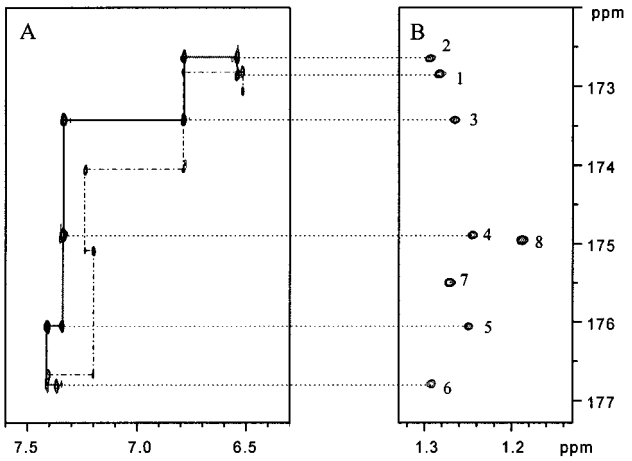

Figure 1. NH_i (NH_{i+1}) ⇒ CO_i (spectrum A) and CO_i ⇒ $(CH_3)_i$ (spectrum B) correlations in a CO-selective HMBC experiment of Ac-[L-(αMe)Val]$_8$-O*t*Bu at 298 K (solution A, after 10 days). The dashed lines indicate the presence of a second, α-helical conformer.

Table 1. Backbone Torsion Angles (deg) and Order Parameters of the Calculated Structures of Ac-[L-(αMe)Val]$_8$-O*t*Bu

	solution A		solution B	
torsion angle/ residue no.	av of 86 structures[c]	order parameter	av of 116 structures[c]	order parameter
ϕ_1	-49.9 ± 5.0	0.996	-41.2 ± 1.2,[a] 83.3 ± 0.5[b]	0.574
ψ_1	-30.3 ± 2.8	0.999	-32.3 ± 0.8	1.000
ϕ_2	-40.3 ± 2.2	0.999	-59.5 ± 4.9	0.996
ψ_2	-46.0 ± 1.6	1.000	-20.6 ± 0.7	1.000
ϕ_3	-38.6 ± 2.7	0.999	-60.9 ± 5.7	0.995
ψ_3	-47.3 ± 3.1	0.999	-39.3 ± 2.4	0.999
ϕ_4	-39.5 ± 2.2	0.999	-55.3 ± 1.7	1.000
ψ_4	-41.8 ± 0.9	1.000	-39.8 ± 1.4	1.000
ϕ_5	-52.2 ± 1.7	1.000	-68.7 ± 2.4	0.999
ψ_5	-22.7 ± 1.5	1.000	-57.0 ± 3.1	0.999
ϕ_6	-66.6 ± 5.1	0.996	-43.3 ± 1.5	1.000
ψ_6	-49.1 ± 14.8	0.967	-39.4 ± 1.5	1.000
ϕ_7	-74.0 ± 32.0	0.873	-67.3 ± 2.4	0.999
ψ_7	90.1 ± 74.4	0.383	-74.1 ± 2.6	0.999
ϕ_8	-136.9 ± 45.5	0.714	-63.2 ± 13.0	0.976

[a] 80 structures were found to adopt this value for ϕ_1. [b] 36 structures were found to adopt this value for ϕ_1. [c] ± standard deviation.

different for the peptide in the two solutions, as shown by the observation of $\beta'N(i, i + 2)$ connectivities in solution A only and by the presence of $\beta'N(i, i + 4)$ connectivities in solution B only. More specifically, all possible $\beta'N(i, i + 2)$ connectivities except the last one were detected in solution A, and all possible $\beta'N(i, i + 4)$ connectivities except the first two were detected in solution B. These results are interpreted with the occurrence of a 3_{10}-helical structure extending over the entire sequence for the peptide dissolved directly in TFE (solution A),[7] while the peptide in solution B adopts an α-helix starting from residue 2 or 3 (Figure 2).

The $CO(i)-NH(i + 3)$ ($i = 0-4$) intramolecular H-bonds in the structures generated for the peptide in solution A (deposited) confirm the presence of a 3_{10}-helix spanning from residue 1 to 7 (Figure 2). The high dispersion of backbone torsion angles observed for ψ_7 and ϕ_8,ψ_8 (Table 1) indicates a substantial degree of flexibility at the C-terminus. High-order parameter values

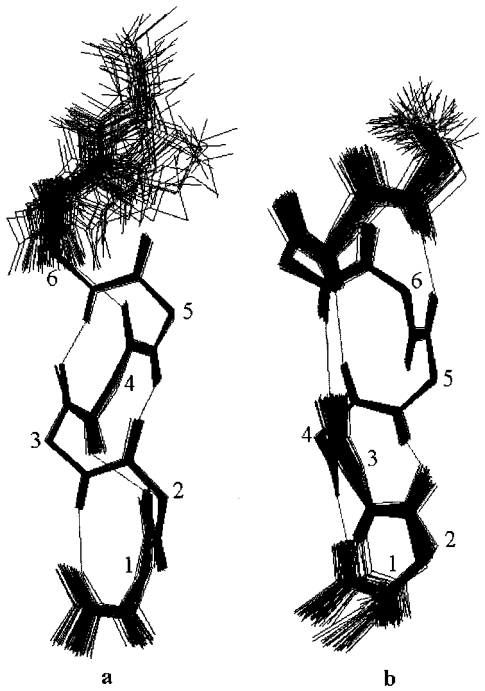

Figure 2. Backbone representation of the structures obtained for Ac-[L-(αMe)Val]$_8$-O*t*Bu from MD calculations and with backbone atoms of residues 1−6 superimposed: (a) overlap of 86 structures of the peptide from solution A; (b) overlap of 116 structures of the peptide from solution B.

(Table 1) for ϕ and ψ are a clear evidence of the stability of a helical conformation from N-terminus to residue 7.

The structures generated for the peptide in solution B display one turn of α-helix at the C-terminus (Figure 2), as evidenced by the presence of $CO(i)-NH(i + 4)$ H-bonds ($i = 2-4$), found with high occurrence in the calculated structures (deposited). The helix shrinks toward the N-terminus where two well-defined 3_{10}-helix type H-bonds are detected. High-order parameters were obtained for the entire sequence (Table 1).

In our view, however, the most interesting phenomenon in the NMR spectra is the very slow conformational transition taking place in solution A. Right after dissolution in TFE, only the set of signals associated with the 3_{10}-helix was observed, but after 10 days a second set of resonances appeared (Figure 1). This set of signals was the one present in solution B from the very beginning and was observed even after several days, indicating that the predominantly α-helical structure is stable under those experimental conditions.

In summary, we confirmed that the unique property of this homooctapeptide to fold in both a 3_{10}- and an α-helical conformation in solution under the same experimental conditions and in a stable manner for at least a week makes this system very interesting in itself. Moreover, by using the NMR technique, for the first time we were able to monitor the coexistence of the 3_{10}- and the α-helical structures at the residue level in the same peptide. Finally, the structural parameters characterizing these two strictly related, ordered conformations were precisely determined. We believe that this work provides a new insight into an important aspect of peptide 3D-structure with potential benefits for future investigations on peptide-based foldamers and the mechanism of protein folding.

Supporting Information Available: Details of the physicochemical techniques used, CD and two-dimensional NMR spectral data, and occurrence of intramolecular H-bonds in the calculated structures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) For NMR studies of 3_{10}-helical peptides, see: (a) Gratias, R.; Konat, R.; Kessler, H.; Crisma, M.; Valle, G.; Polese, A.; Formaggio, F.; Toniolo, C.; Broxterman, Q. B.; Kamphuis, J. *J. Am. Chem. Soc.* **1998**, *120*, 4763−4770. (b) Millhauser, G. L.; Stenland, C. J.; Hanson, P.; Bolin, K. A.; van de Ven, F. J. M. *J. Mol. Biol.* **1997**, *267*, 963−974. (c) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1985; pp 162−174. (d) Basu, G.; Kuki, A. *Biopolymers* **1993**, *33*, 995−1000.