Controlling 3_{10}-Helix and α-Helix of Short Peptides in the Solid State

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L-Leu hexapeptide containing α-aminoisobutyric acid (Aib) forms a right-handed (*P*) 3_{10}-helix, whereas that containing cyclic α,α-disubstituted amino acid Ac_5c^{dOM} assumes a right-handed (*P*) α-helix in the solid state.

Key words α,α-disubstituted amino acid; peptide; helix; conformation; secondary structure

α-Aminoisobutyric acid (Aib; α-methylalanine),[1—4] in which the α-hydrogen atom of L-Ala is replaced with a methyl substituent, has strong propensity for helix formation and β-sheet breaker. Thus Aib is widely used to construct helical structures, and to design drug candidates and organocatalysts.[3,4] Although the helical structure in proteins almost always is an α-helix,[5] the tendency of Aib in short peptides is a 3_{10}-helix rather than an α-helix. Furthermore, the Aib is an achiral amino acid, and thus does not have a bias for the helical-screw handedness. Over the last decade, chiral α,α-disubstituted α-amino acids (dAAs) have been widely investigated.[6—9] However, the incorporation of chiral α-methylated dAAs into peptides stabilizes the 3_{10}-helix, but not the α-helix in short peptides. Moreover, it is believed that α-helix formation usually requires a peptide having more than seven amino acid residues,[4,10,11] and the hexapeptide having dAA does not form the α-helix, but assumes the 3_{10}-helix in the crystal state. Herein, we describe chiral cyclic dAA; 1-amino-3,4-dimethoxycyclopentanecarboxylic acid (Ac_5c^{dOM}), which has propensity for α-helix formation, and the right-handed (*P*) α-helix of its short Leu-hexapeptide.

We efficiently synthesized (*S*,*S*)-Ac_5c^{dOM} as previously reported,[12] and also the enantiomeric (*R*,*R*)-Ac_5c^{dOM} starting from dimethyl D-(−)-tartrate. Four L-Leu hexapeptides; Cbz-(L-Leu-L-Leu-dAA)$_2$-OMe [dAA=**1**: Aib; **2**: Ac_5c; **3**: (*S*,*S*)-Ac_5c^{dOM}; **4**: (*R*,*R*)-Ac_5c^{dOM}] were prepared by solution-phase methods.

At first, we studied the preferred conformation of **1**—**4** in $CDCl_3$ solution (1.0 mM) using FT-IR absorption spectroscopy. The IR spectra of **1**—**4** showed a weak band at 3430 cm^{-1} [free (solvated) peptide NH groups], and a strong band at 3340 cm^{-1} [intramolecularly H-bonded peptide NH groups]. These IR spectra are very similar to those of the reported helical Leu-peptides having Aib.[13]

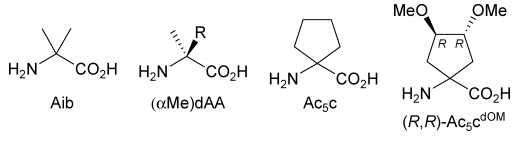

Fig. 1. Achiral and Chiral α,α-Disubstituted α-Amino Acids

The ROESY or NOESY ^{1}H-NMR spectra did not clearly show the complete series of sequential d_{NN} cross-peaks of NOEs, which are characteristic of helical structures. Also, we could not discriminate between 3_{10}- and α-helices of the Ac_5c^{dOM} peptides **3** and **4** because neither the $d_{\alpha N}$ (i, i+2) nor $d_{\alpha N}$ (i, i+4) (i=1 and 2) cross-peaks of NOEs were shown, or the relevant peaks were overlapped, whereas the $d_{\alpha N}$ (i, i+2) (i=1 and 2) cross-peaks of NOEs (typical peaks for the 3_{10}-helix) in the Aib peptide **1** were observed.

Figure 2 shows the CD spectra of **1**—**4** in 2,2,2-trifluoroethanol (TFE) solution, and also in the solid state (KCl disk). All these spectra show negative maxima at 222—228 and 204—208 nm and a positive maximum at 191—193 nm, which are characteristic of a right-handed (*P*) helical structure. The L-Leu residues in the peptides would control the helical-screw direction to the right-handedness.[13]

Judging from the ratio of *R* [maxima: $\theta_{222}/\theta_{208}$] in TFE solution, the Aib, Ac_5c, and (*R*,*R*)-Ac_5c^{dOM} peptides **1** (*R*=0.3), **2** (*R*=0.4), and **4** (*R*=0.4) might form a 3_{10}-helix and the (*S*,*S*)-Ac_5c^{dOM} peptide **3** (*R*=0.6) form a mixture of 3_{10}- and α-helices. The CD spectra of Ac_5c^{dOM} hexapeptides in the solid state are distinct from those of the Aib and Ac_5c hexapeptides. The *R* values of the Ac_5c^{dOM} peptides **3** and **4** were 1.0, while those of the Aib and Ac_5c peptides **1** and **2** were 0.5 (red-shift of the maximum at 222 nm was observed). These *R* values mean that the Ac_5c^{dOM} hexapeptides form (*P*) α-helices and the Aib and Ac_5c hexapeptides form (*P*) 3_{10}-helices.[14,15] The CD spectra of prototype Ac_5c (non-MeO-substituent) peptide are more similar to those of Aib peptides than those of Ac_5c^{dOM} peptides. These results validate the importance of the methoxy substituents, especially in terms of hydrophilicity and stereochemistry, on the cyclopentane ring for the α-helix formation.

The crystal structures of Aib hexapeptide **1** and (*S*,*S*)-Ac_5c^{dOM} hexapeptide **3** were determined by X-ray crystallographic analysis as shown in Fig. 3.[16] As usual, in the crystal structure of the Aib peptide **1**, three consecutive hydrogen bonds of the $i \leftarrow i+3$ type, N(4)H···O=C(1) (N···O 3.20 Å; N–H···O 146.3°), N(5)H···O=C(2) (N···O 2.99 Å; N–H···O 159.5°), and N(6)H···O=C(3) (N···O 3.19 Å; N–H···O 149.4°) were observed, albeit the distance of N(3)H···O=C(0) (N···O 3.43 Å) is long for a hydrogen bond. The average ϕ,ψ torsion angles are −66.1°, −31.3°, meaning the right-handed (*P*) 3_{10}-helix.[17] Contrary to the 3_{10}-helix of Aib peptide **1**, in the crystal structure of (*S*,*S*)-Ac_5c^{dOM} peptide **3**, two crystallographically independent molecules *A* and *B*, which are not 3_{10}-helices but right-handed (*P*) α-helices (3.6_{13}-helices) exist, along with methanol and water molecules. In general, both molecules *A* and *B* are similar in the peptide backbone, but some differences at the side chain, the N-terminus protecting group, and especially at the C-terminal amino acid Ac_5c^{dOM} (6) and the L-Leu (5) were observed. The average ϕ,ψ torsion angles are *A*: ϕ=−63.7°, ψ=−40.4° and *B*: ϕ=−75.8°, ψ=−28.4°, respectively.

Judging from the torsion angles, the molecule *B* seems to

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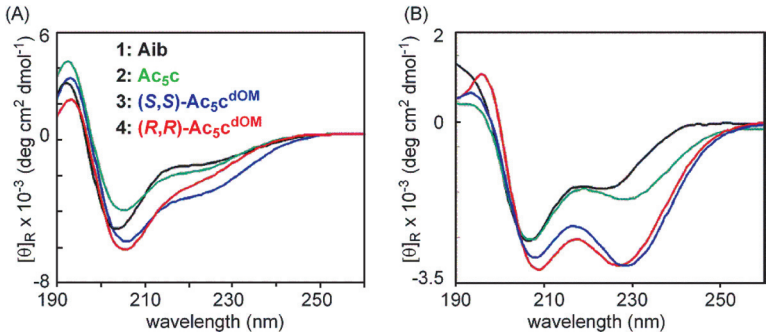

Fig. 2. CD Spectra of Leu-Hexapeptides

(A) Hexapeptides **1**—**4** in TFE solution; (B) **1**—**4** in KCl disk.

be a distorted (*P*) α-helix, especially at the amino acid residues L-Leu (5) ($\phi=-99.6°$, $\psi=-11.4°$) and Ac_5c^{dOM} (6) ($\phi=+64.9°$, $\psi=-167.0°$). In the crystal state, two consecutive intramolecular hydrogen bonds of the $i\leftarrow i+4$ type, N(5)H···O=C(1) (N···O 2.99 Å; N–H···O 150.4°) and N(6)H···O=C(2) (N···O 3.03 Å; N–H···O 151.1°) in the molecule *A*, and N(5)H···O=C(1) (N···O 2.91 Å; N–H···O 145.2°) and N(6)H···O=C(2) (N···O 2.98 Å; N–H···O 136.6°) in the molecule *B*, are found, respectively. The N···O distances (3.49, 3.76 Å) of N(4)H···O=C(0) in the molecules *A* and *B* are too long for a hydrogen bond. Interestingly, the (*S*,*S*)-Ac_5c^{dOM} peptide **3** crystallized to give two shapes of crystals: plates and needles. The latter seem to have different lattice parameters.

Molecular-mechanics calculation of the Ac_5c^{dOM} hexapeptides **3** and **4** with MacroModel produced right-handed (*P*) 3_{10}-helices as a global minimum-energy conformation, but not α-helices.[18)]

In conclusion, we have disclosed that the propensity of Ac_5c^{dOM} is an α-helix formation, whereas that of Aib is a 3_{10}-helix formation. Although it is generally believed that the α-helix formation usually needs a peptide composed of more than seven amino acid residues,[4,10,11)] the L-Leu-hexapeptides containing Ac_5c^{dOM} assumed the right-handed (*P*) α-helices in the crystal state. These peptides might be one of the shortest (*P*) α-helical ones, albeit the $3_{10}/\alpha$-helical pentapeptide containing Aib has been reported.[19)] The helicogenic property of (*S*,*S*)-Ac_5c^{dOM} is left-handed and that of enantiomeric (*R*,*R*)-Ac_5c^{dOM} is right-handed,[12)] though their properties of helical handedness are weaker than that of L-Leu. The bulkiness, flexibility, and hydrophilicity of substituents at the cyclopentane ring would affect the secondary structures of their peptides, not only helical-screw handedness but also helical pitches (α-helix or 3_{10}-helix).[12,20–22)] Study of the detailed effect of substituents at the cyclopentane rings on the secondary structures is currently underway.

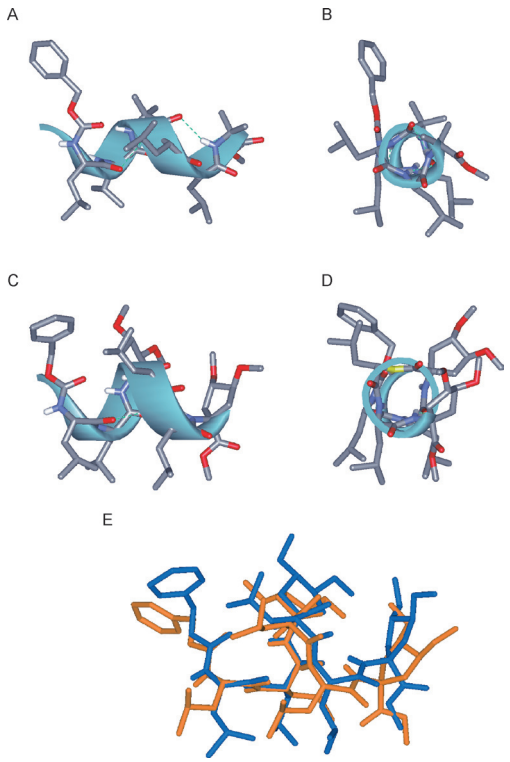

Fig. 3. Illustrative Structures Determined by X-Ray Crystallographic Analysis

(A), (B) Right-handed (*P*) 3_{10}-helix of Cbz-[L-Leu-L-Leu-Aib]$_2$-OMe **1**. (C), (D) Right-handed (*P*) α-helix of Cbz-[L-Leu-L-Leu-(*S*,*S*)-Ac_5c^{dOM}]$_2$-OMe **3** (molecule *A*). (E) Overlay of molecules *A* and *B* of **3**.

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16) CCDC-602473, and 602475 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.htcm3 (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk). Crystal data: **1**: $C_{41}H_{68}N_6O_9$, Mr=789.0, space group $P1$, a=10.411, b=11.003, c=11.330 Å, α=106.31°, β=94.75°, γ=103.83°, V=1193.8 Å^3, Z=1, T=302 K, μ(Mo$K\alpha$)=0.77 cm^{-1}, 3417 reflections measured, 2950 unique reflections (R_{int}=0.0252) R_1 ($I>2\sigma$)=0.0434, wR_2 ($I>2\sigma$)=0.0870, GOF=1.576. **3**: $2(C_{49}H_{80}N_6O_{13})\cdot CH_4O\cdot H_2O$, Mr=1972.4, space group $P1$, a=12.903, b=14.824, c=16.669 Å, α=104.88°, β=93.48°, γ=112.92°, V=2791.8 Å^3, Z=2, T=200 K, μ(Mo$K\alpha$)=0.86 cm^{-1}, 12192 reflections measured, 10971 unique reflections (R_{int}=0.0248) R_1 ($I>2\sigma$)=0.0522, wR_2 ($I>2\sigma$)=0.1427, GOF=1.010.
17) In both peptides **1** and **3**, ϕ and ψ sign inversions at the C-terminus are observed. Thus for mean value calculations, the C-terminal residues were omitted.
18) Conformational search calculations were performed with the package of *MacroModel* ver. 8.1 (Schrodinger, Inc.) on SGI workstation. Monte Carlo Multiple Minimum (MCMM) method and AMBER* force field were used for finding the global minimum energy conformation and local ones. As initial structures, extended structure, 3_{10}-helix and α-helix structures were used. More than 50000 conformers were optimized. The right-handed (P) α-helix of **3** produced by the restricted calculation was a local minimum-energy conformation, which was less stable than the 3_{10}-helical conformation by +9.24 kcal/mol.
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