

Controlling 3_{10} -helix and α -helix of short L-leucine-peptides by α,α -disubstituted amino acid

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Introduction

α -Aminoisobutyric acid (Aib; α -methylalanine) [1-4], in which the α -hydrogen atom of L-Ala is replaced with a methyl substituent, is an achiral α,α -disubstituted amino acid (dAA). The introduction of Aib into oligopeptides strongly induces helical secondary structures. The helical structure in proteins is almost always an α -helix, but there is a tendency of Aib in short peptides toward 3_{10} -helix formation rather than α -helix. Furthermore, the incorporation of chiral α -methylated dAAs into peptides stabilizes 3_{10} -helix but not the α -helix in short peptides. We have designed and synthesized chiral cyclic α,α -disubstituted amino acid (S,S)-Ac₅c^{dOM}, in which the α -carbon is not a chiral center but chiral centers exist at the side chain. Then we found that the helical-screw sense of its homopeptides could be controlled into the left-handedness by chiral centers at the side chain, and the (S,S)-Ac₅c^{dOM} octapeptide formed α -helix both in solution and the solid state[5] (Fig. 1).

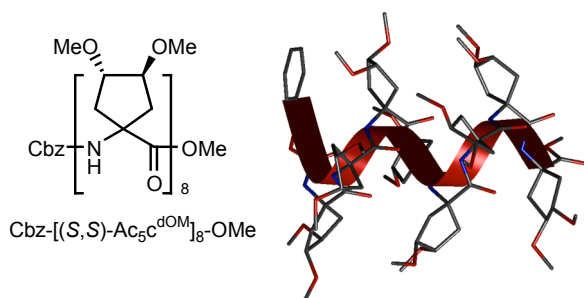


Fig. 1. X-ray structure of (S,S)-Ac₅c^{dOM} octapeptide.

Herein, we designed and synthesized four kinds of heteropeptides containing different dAAs in L-Leu sequence: two peptides have achiral Aib and Ac₅c, respectively, and the other two peptides have chiral (S,S)- and (R,R)-Ac₅c^{dOM}, respectively (Fig. 2). We have interested in the preferred secondary structure of these short peptides. Also, we thought that comparison of these peptides would reveal the effect of side-chain chiral centers on the secondary structure of peptides.

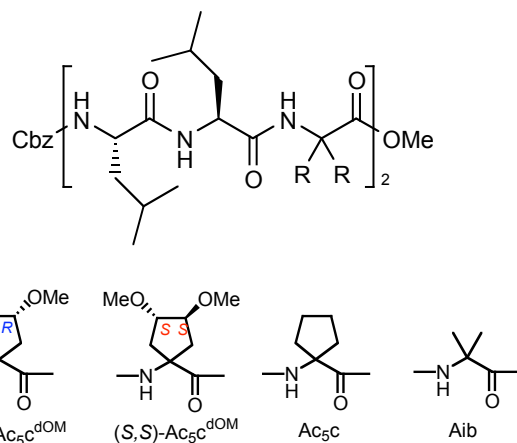


Fig. 2. L-Leu heteropeptides containing dAAs.

Results and Discussion

Both enantiomers of chiral cyclic α,α -disubstituted amino acids Ac₅c^{dOM} were synthesized starting from dimethyl L-(+)- and D-(-)-tartrate, according to our previous report⁵. Four L-Leu hexapeptides; Cbz-(L-Leu-L-Leu-dAA)₂-OMe [dAA = 1: Aib; 2: Ac₅c; 3: (S,S)-Ac₅c^{dOM}; 4: (R,R)-Ac₅c^{dOM}] were prepared by solution-phase methods.

Conformational analysis of these peptides in solution was performed by CD, FT-IR, and ¹H NMR spectra. Figure 3 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.

Judging from the ratio of R [maxima: $\theta_{222}/\theta_{208}$] in TFE solution, the Aib, Ac₅c, and (R,R)-Ac₅c^{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)-Ac₅c^{dOM} peptide 3 ($R=0.6$) form a mixture of 3_{10} - and α -helices. The CD spectra of Ac₅c^{dOM} hexapeptides in the solid state are distinct from those of the Aib and Ac₅c hexapeptides. The R values of the Ac₅c^{dOM}

peptides **3** and **4** were 1.0, while those of the Aib and Ac₅c peptides **1** and **2** were 0.5 (red-shift of the maximum at 222 nm was observed). These *R* values mean that the Ac₅c^{dOM} hexapeptides form the (*P*) α-helices and the Aib and Ac₅c hexapeptides form the (*P*) ₃₁₀-helices. The CD spectra of prototype Ac₅c (non-MeO-substituent) peptide are more similar to those of Aib peptides than those of Ac₅c^{dOM} peptides.

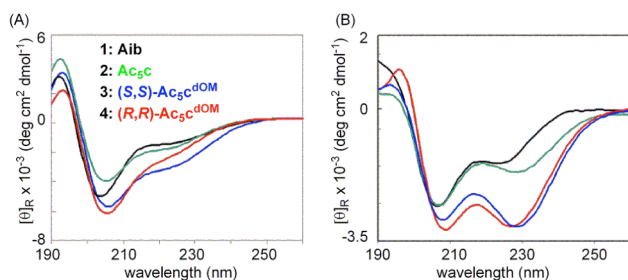


Fig. 3. CD spectra of Leu-hexapeptides
(A) Hexapeptides **1-4** in TFE solution; (B) **1-4** in KCl

The crystal structures of Aib hexapeptide **1** and (*S,S*)-Ac₅c^{dOM} hexapeptide **3** were determined by the X-ray crystallographic analysis, as shown in Figure 4. In the crystal structure, the Aib hexapeptide **1** formed right-handed (*P*) ₃₁₀-helix. Contrary to the ₃₁₀-helix of Aib peptide **1**, in the crystal structure of (*S,S*)-Ac₅c^{dOM} peptide **3**, right-handed (*P*) α-helix (3.6₁₃-helix) existed. Interestingly, the (*S,S*)-Ac₅c^{dOM} peptide **3** crystallized to give two shapes of crystals, plates and needles. The latter seems to have different lattice parameters.

In conclusion, we have disclosed that the propensity of Ac₅c^{dOM} is an α-helix formation, whereas that of Aib is a ₃₁₀-helix formation. Although it has been believed that the α-helix formation needs a peptide composed of more than seven amino acid residues [4,6,7], the L-Leu-hexapeptides containing Ac₅c^{dOM} assumed the right-handed (*P*) α-helices in the crystal state. Study of the detailed effect of substituents at the cyclopentane rings on the secondary structures is currently underway.

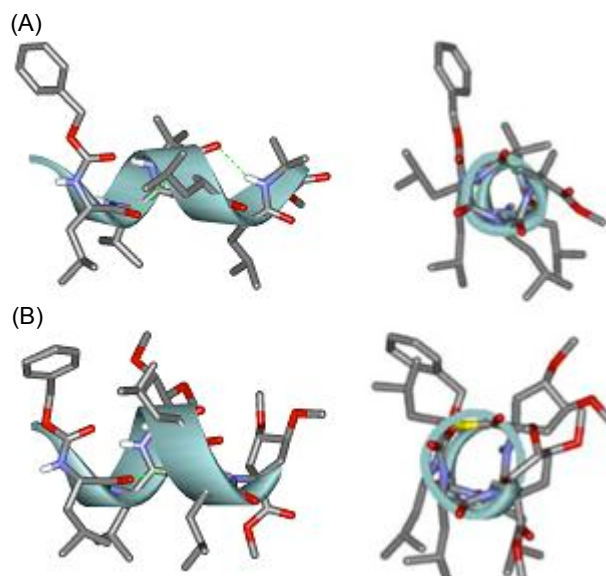


Fig. 4. X-ray structures of Leu heteropeptides containing dAAs.

- (A) (*P*) ₃₁₀-helix of Cbz-[L-Leu-L-Leu-Aib]₂-OMe;
(B) (*P*) α-helix of Cbz-[L-Leu-L-Leu-(*S,S*)-Ac₅c^{dOM}]₂-OMe.

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References

- Kale I. L., Balaram P., (1990) *Biochemistry*, **29**, 6747-6756.
- Heimgartner H., (2004) *Angew. Chem. Int. Ed.*, **30**, 238-264.
- Wysong C. L., Yokum T. S., McLaughlin M. L., Hammer R. P., (1997) *Chemtech*, **27**, 26-33.
- Toniolo C., Crisma M., Formaggio F., Peggion C., Broxterman Q., Kaptein B., (2005) *J. Incl. Phenom. Macro. Chem.*, **51**, 121-136.
- Tanaka, M., Demizu, Y., Doi, M., Kurihara, M., Suemune, H., (2004) *Angew. Chem. Int. Ed.*, **43**, 5360-5363.
- Crisma M., Formaggio F., Moretto A., Toniolo C., (2006) *Biopolymers (Peptide Science)*, **84**, 3-12.
- Demizu, Y., Tanaka, M., Nagano, M., Kurihara, M., Doi, M., Suemune, H., (2007) *Chem. Pharm. Bull.*, **55**, 840-842.