Induction of One-Handed Helical Screw Sense in Achiral Peptide through the Domino Effect Based on Interacting Its N-Terminal Amino Group with Chiral Carboxylic Acid

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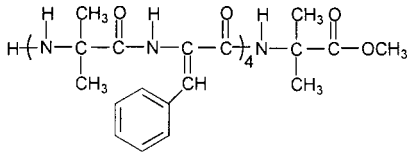
A helical chain being the most common secondary structure in biopolymers prefers one-handed (left- or right-handed) screw sense, when chiral moieties are incorporated into the main or side chain through the covalent bond. We here report that an achiral helical peptide prefers the one-handed helical screw sense by noncovalent interaction of its N-terminal amino group with a chiral carboxylic acid. Little is known about such phenomena in peptide systems typical of biopolymers, although it has been reported that synthetic helical polymers bearing carboxyl or amino groups in the repeating units induce the one-handed screw sense by addition of chiral small amines or acids to interact on their polymer side or main chains.[1] In our system, the acid−base interaction occurring in the N-terminal position of the peptide chain will lead to the predominance of one-handed screw sense of the entire peptide chain, namely through *domino effect*. For our purpose, the following N-deprotected nonapeptide **1** consisting of nonprotein amino acids [α-aminoisobutyric acid (Aib) and α,*β*-dehydrophenylalanine (Δ^ZPhe)] was synthesized.[2]



H-(Aib-Δ^ZPhe)$_4$-Aib-OMe (**1**) (OMe = methoxy)

Peptide **1** can be expected to generate two "enantiomeric" (left- and right-handed) helices, since Aib and Δ^ZPhe residues are achiral ones and strong inducers for forming a 3_{10}-helix.[3] Actually, a helical conformation was evidenced by ^{1}H NMR spectroscopy on peptide **1** in $CDCl_3$. In the NOESY experiment, marked cross-peaks were observed for the $N_iH-N_{i+1}H$ resonances in the segment of Aib(3) to Aib(9),[2] indicating that peptide **1** forms a 3_{10}- or α-helix.[4] The solvent dependence on amide NH chemical shifts in $CDCl_3/(CD_3)_2SO$ mixtures revealed that six NH resonances of Δ^ZPhe(4) to Aib(9) residues are shielded from solvent due to intramolecular hydrogen bonding,[2] of which the pattern corresponds to a 3_{10}-helix. The helical conformation was also supported by the amide I absorption bands of its FT-IR spectrum in chloroform: 1660 and 1627 cm^{-1}, which can be assigned to saturated amino acid and Δ^ZPhe residues in a helical segment, respectively.[5] Furthermore, energy minimization of peptide **1** by the semiempirical molecular orbital method[6] gave a 3_{10}-helical conformation (Figure 1) characterized by $\langle\phi\rangle = \pm 41.1°$, $\langle\psi\rangle = \pm 37.6°$, and $\langle\omega\rangle = 180.0°$ for average values of Δ^ZPhe(2) to Δ^Z-Phe(8) residues, and the main-chain energy contour map was severely restricted into right- and left-handed helical regions ($\phi = \pm 60$ to $\pm 40°$, $\psi = \pm 60$ to $\pm 30°$).[2] Therefore, peptide **1** having a strong helix-forming tendency forms a 3_{10}-helical conformation in chloroform.[7]

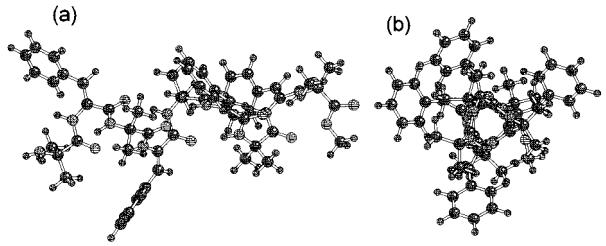


Figure 1. Energy-minimized peptide **1** by the AM1 method: (a) side and (b) top views of the right-handed one.

Peptide **1** could not show any CD signals due to the absence of chiral residues, thus taking both left- and right-handed helices with the same content in an equilibrium state (Figure 2, dotted line). However, intense split CD signals were induced around 282 nm assignable to Δ^ZPhe residues by the addition of enantiomerically pure Boc-L-Pro-OH (Boc = *t*-butoxycarbonyl), as shown in Figure 2. The mirror image was obtained by the addition of Boc-D-Pro-OH, thus indicating that the induced CD signals are responsible for the chiral interaction of peptide **1** with Boc-Pro-OH, but not for some accidental impurities.

The split CD pattern with a negative peak at longer wavelengths was obtained for the Boc-L-amino acids shown in Table 1. Based on the exciton chirality method,[8] the sign of split CD means a left-handed helical arrangement of the transition moment at 282 nm, thus corresponding to a right-handed screw sense for a 3_{10}-

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(1) Elegant examples for the phenomena have been reported. See: (a) Yashima, E.; Maeda, K.; Okamoto, Y. *Nature* **1999**, *399*, 449. (b) Yashima, E.; Matsushima, T.; Okamoto, Y. *J. Am. Chem. Soc.* **1997**, *119*, 6345. (c) Yashima, E.; Matsushima, T.; Okamoto, Y. *J. Am. Chem. Soc.* **1995**, *117*, 11596. (d) Yashima, E.; Maeda, Y.; Matsushima, T.; Okamoto, Y. *Chirality* **1997**, *9*, 593. (e) Maeda, K.; Yamamoto, N.; Okamoto, Y. *Macromolecules* **1998**, *31*, 5924. (f) Schlitzer, D. S.; Novak, B. M. *J. Am. Chem. Soc.* **1998**, *120*, 2196. (g) Majidi, M. R.; Kane-Maguire, L. A. P.; Wallace, G. G. *Polymer* **1994**, *35*, 3113.

(2) See the Supporting Information.

(3) (a) Prasad, B. V. V.; Balaram, P. *CRC Crit. Rev. Biochem.* **1984**, *16*, 307. (b) Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Crisma, M.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1982**, *104*, 2437. (c) Jain, R.; Chauhan, V. S. *Biopolymers* **1996**, *40*, 105. (d) Pieroni, O.; Fissi, A.; Pratesi, C.; Temussi, P. A.; Ciardelli, F. *J. Am. Chem. Soc.* **1991**, *113*, 6338.

(4) Wüthrich, K.; Billeter, M.; Braun, W. *J. Mol. Biol.* **1984**, *180*, 715.

(5) (a) Inai, Y.; Sakakura, Y.; Hirabayashi, T. *Polym. J.* **1998**, *29*, 828. (b) Inai, Y.; Kurokawa, Y.; Hirabayashi, T. *Macromolecules* **1999**, *32*, 4575. (c) Kennedy, D. F.; Crisma, M.; Toniolo, C.; Chapman, D. *Biochemistry* **1991**, *30*, 6541.

(6) The AM1 method (Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.) in MOPAC 97 (Stewart, J. J. P. MOPAC97, Fujitsu Ltd, Tokyo, Japan, 1998.) was used with a key word of MMOK. An initial conformation for the AM1 calculation was obtained using the modified PEPCON for conformational energy calculation on Δ^ZPhe-containing peptides. A stereoview of the energy-minimized peptide **1** was shown in the Supporting Information. For PEPCON, see: (a) Momany, F.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1975**, *79*, 2361. (b) Beppu, Y. *Comput. Chem.* **1989**, *13*, 101. (c) Sisido, M. *Peptide Chem. 1991* **1992**, *29*, 105. For the modified one, see: (d) Inai, Y.; Kurashima, S.; Hirabayashi, T.; Yokota, K. *Biopolymers* **2000**, *53*, 484.

(7) The crystal structure of peptide **1** cannot be determined due to its low single crystallinity, for the present. However, the crystal state of Boc-(Aib-Δ^ZPhe)$_2$-Aib-OMe having the same sequence as **1** was found in a 3_{10}-helical structure characterized by $\langle\phi\rangle = 52.1°$, $\langle\psi\rangle = 29.9°$, and $\langle\omega\rangle = -174.0°$ for average values of Aib(1) to Δ^ZPhe(4) residues: Inai, Y.; Hirabayashi, T.; Oshikawa, T.; Yamashita, M. *Polym. Prepr., Jpn.* **2000**, *49*, 959. For 3_{10}-helical structures, see: Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350.

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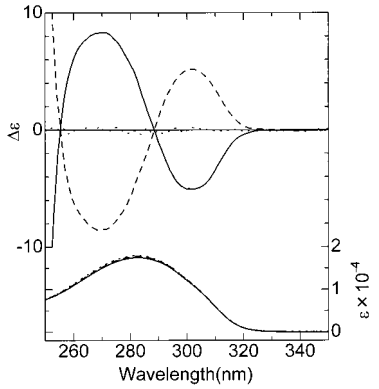


Figure 2. CD (top) and UV absorption (bottom) spectra of peptide **1** (dotted line) in chloroform and of that in the presence of Boc-L-Pro-OH (solid line) and Boc-D-Pro-OH (broken line): [**1**] = 0.16 mM and [Boc-Pro-OH] = 65 mM. $\Delta\epsilon$ and ϵ are expressed with respect to the molar concentration of Δ^ZPhe residues.

Table 1. Signs of Splitting Cotton Effects and $\Delta\epsilon$ Values for Induced CD of Peptide **1** with Chiral Carboxylic Acid[a]

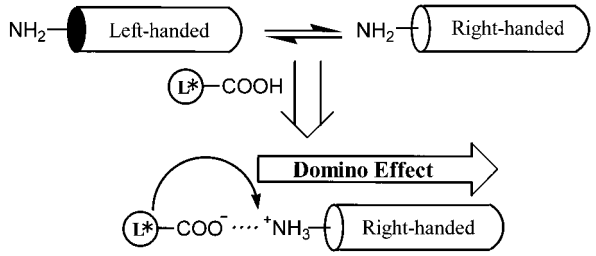
	first Cotton		second Cotton	
acid	sign	$\Delta\epsilon(\lambda$/nm)	sign	$\Delta\epsilon(\lambda$/nm)
Boc-D-Pro-OH	+	4.4/302	−	8.5/269
Boc-L-Pro-OH	−	4.3/302	+	8.4/270
Boc-L-Ala-OH	−	1.5/303	+	3.8/270
Boc-L-Val-OH	−	1.7/303	+	4.7/270
Boc-L-Leu-OH	−	2.4/303	+	5.5/270
Boc-L-Phe-OH	−	1.2/303	+	*b*

[a] All spectra were recorded using JASCO-J500 and -J600 in chloroform with [**1**] = 0.16 and [carboxylic acid] = 65 mM at 296−298 K. [b] Overlapped with the CD signal of Boc-L-Phe-OH.

or α-helix. The right-handed screw sense was also evidenced by theoretical CD calculation on peptide **1**.[9]

The induced CD amplitude increased markedly with an increase in Boc-L-Pro-OH concentration of 0 to 65 (400-fold) mM, and reached a saturation value over 100 mM essentially.[2] The CD amplitude could be essentially attributed to the fraction of peptide **1** complexed.[2,10] No CD signals were observed when N-Boc-protected peptide **1** was used with a large excess (400-fold) of Boc-L-Pro-OH in chloroform. Obviously, the N-terminal amino group interacting with a chiral carboxylic acid is required for the predominance of one-handed screw sense. In our previous works,[11] a left-handed 3_{10}-helical conformation was found for Boc-X-(Aib-Δ^ZPhe)$_2$-Aib-OMe (X = L-amino acid residues) and Boc-L-Leu-(Aib-Δ^ZPhe)$_4$-Aib-OMe in solution. Interestingly, the screw sense was inverted depending on how Boc-L-amino acid works in the N-terminal position of the achiral helical peptide, i.e., covalently or noncovalently.

Chart 1. Helical Screw Sense Induced by the Domino Effect



In conclusion, we realize that the chiral environment created by chiral acid−base interaction in the N-terminal position induces the excess of one-handed screw sense of the achiral peptide chain,[12] probably through the domino effect proposed by Chart 1. A detailed mechanism for the induced CD signals is under investigation. The present work, however, should provide not only a significant example for controlling helical screw sense of an achiral host peptide through the noncovalent domino effect of a chiral small guest, but also novel type chiral interactions between a helical segment and a chiral molecule in peptide and protein science.

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Supporting Information Available: Synthesis and characterization of peptide **1**, experimental details of ^{1}H NMR measurement, and additional figures for the results of ^{1}H NMR and CD measurements and energy calculation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Theoretical CD spectra of exciton couplets with a negative peak at longer wavelengths were obtained around 280 nm for peptide **1** in five right-handed 3_{10}-type (4 → 1 hydrogen-bonded) helices and three right-handed α-type (5 → 1 hydrogen-bonded) helices adopted in ref 5b. The sign of exciton couplets does not change with strict (ϕ, ψ) values, but depends on the sign of (ϕ, ψ) values. For theoretical CD calculation on Δ^ZPhe-containing peptides, see: Inai, Y.; Ito, T.; Hirabayashi, T.; Yokota, K. *Biopolymers* **1993**, *33*, 1173.

(10) The binding constant (*K*) between Boc-L-Pro-OH and H-Aib-OMe for the model compound of peptide **1** was determined to be 28 (M^{-1}) in $CDCl_3$ by ^{1}H NMR titration experiment. A 1:1 complexation was also found for the Boc-Pro-OH and H-Aib-OMe system, from a good linearity of the Benesi−Hildebrand plot (correlation coefficient = 0.99) and the slope of the Hill plot (slope = 1.01). The *K* value was used to calculate the fraction of peptide **1** complexed in the CD experiment.

(11) The left-handed screw sense is due to the presence of an N-terminal L-protein residue. This type of residue tends to prefer a semiextended conformation, which in turn favors the nonhelical type II β-bend structure. This β-bend structure is responsible for the left-handed helix of the following achiral residues. See: (a) Inai, Y.; Kurokawa, Y.; Hirabayashi, T. *Biopolymers* **1999**, *49*, 551. (b) Inai, Y.; Kurokawa, Y.; Ida, A.; Hirabayashi, T. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 55. (c) Inai, Y.; Ashitaka, S.; Hirabayashi, T. *Polym. J.* **1999**, *31*, 246.

(12) In a preliminary experiment, a split CD pattern similar to that of peptide **1** was induced for heptapeptide H-(Aib-Δ^ZPhe)$_3$-Aib-OMe by the addition of Boc-L-Pro-OH, but the CD amplitude was larger for peptide **1** than the heptapeptide ($\Delta\epsilon_{303}$= −1.9 and $\Delta\epsilon_{270}$ = +5.8). This means the persistence length for the helix-forming tendency in sequential peptide H-(Aib-Δ^ZPhe)$_n$-Aib-OMe should be equal to or above that for the nonapeptide (n = 4).