A Study of Chain-Length Effect on Helical Screw Sense in Peptides Having an *N*-Terminal L-Leu Residue

Yoshihito INAI,[†] Satoru ASHITAKA, and Tadamichi HIRABAYASHI

Department of Environmental Technology and Urban Planning,
Graduate School of Engineering, Nagoya Institute of Technology,
Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan

(Received July 29, 1998)

ABSTRACT: In order to clarify which helical screw sense is dominated depending on chain-length of peptides when an L-residue is introduced into the *N*-terminal position of achiral helical segments, we prepared three kinds of peptides **I–III**: Boc-L-Leu-(Aib-ΔPhe)_{*n*}-Aib-OMe (*n* = 2–4) **I–III** (Boc, *t*-butoxycarbonyl; Aib, α-aminoisobutyric acid; ΔPhe, Z-dehydrophenylalanine; OMe, methoxy). Here the segment -(Aib-ΔPhe)_{*n*}-Aib-OMe was used for an achiral backbone composed of two “enantiomeric” (left-/right-handed) helices. Peptides **I–III** were found to form a 3₁₀-type helical conformation in chloroform, from the position of amide I band in FT-IR spectra and solvent accessibility of NH resonances in ¹H NMR measurement. CD spectra of peptides **I–III** showed exciton couplets around 280 nm with a positive peak at longer wavelengths in chloroform, acetonitrile, methanol, and tetrahydrofuran. The sign of exciton couplets indicates that the main-chains prefer a left-handed screw sense. Consequently, an *N*-terminal L-Leu residue induces a left-handed screw sense preferentially, irrespective of chain lengths for achiral helical segments (5–9 residues). This result could be supported by conformational energy calculation on acetyl-L-Leu-(Aib-ΔPhe)₄-Aib-OMe, in which a left-handed helical conformation was predominant. Also, in the lowest-energy left-handed helix, an *N*-terminal L-Leu residue takes an irregular conformation that deviates from a left-handed 3₁₀-α-helical region in conformational space of (φ, ψ).

KEY WORDS Helical Screw Sense / Chain-Length Effect / Achiral Helical Segment / Effect of *N*-Terminal L-Residue / CD Spectroscopy / Conformational Energy Calculation /

Most of L-amino acid residues are well recognized to prefer a right-handed screw sense in helical segments of proteins or peptides. On the other hand, L-residue was rarely found to induce a left-handed screw sense for several peptides containing achiral helicogenic residues, Z-dehydrophenylalanine (ΔPhe)^{1–13} or α-aminoisobutyric acid (Aib)^{14,15}:

Scheme 1. ΔPhe and Aib residues.

For example, a left-handed 3₁₀-helix in solution was seen in Ac-ΔPhe-Gly-ΔPhe-L-Ala-OMe (Ac, acetyl; OMe, methoxy)¹³ or Boc-L-Pro-Aib-L-Ala-Aib-L-Ala-OMe (Boc, *t*-butoxycarbonyl).¹⁶ Left-handed 3₁₀-helices have also been observed in the crystalline state for Aib peptides containing an L-residue in the C-terminal position.^{16–18} Boc-L-Ala-ΔPhe-Gly-ΔPhe-L-Ala-OMe showed reversible screw sense inversion of 3₁₀-helix, depending on solvent and temperature conditions.^{5,13} Also, Boc-L-Ala-ΔPhe-ΔPhe-NMA (NMA, *N*-methyl amide) took an incipient 3₁₀-helix of both left- and right-handed screw senses in the solid.¹² Thus, L-residue seems to show the character of left-handed screw sense when it is relatively C-terminally introduced to achiral helical peptides.

On the other hand, little is known about effect of *N*-terminal L-residue on dominating helical screw sense in achiral peptides. Recently,¹⁹ we investigated this effect

using Boc-X-(Aib-ΔPhe)₂-Aib-OMe, in which an L-amino acid residue (X) is introduced into the *N*-terminal position of achiral helical segment -(Aib-ΔPhe)₂-Aib-OMe composed of Aib and ΔPhe residues. Here X residue is Ala, Leu, Val, Phe, 1-naphthylalanine, and Pro. As a result, an *N*-terminal L-residue, irrespective of types of L-residues, induces a left-handed screw sense preferentially. However, systematic data on the left-handed screw sense induced by L-residue have not been fully accumulated in terms of varying amino acid residues, chain-lengths, peptide sequences, environments (solvent and temperature), and so on.

We here focused on chain-length effect on dominating helical screw sense in peptides having only an L-Leu residue in the *N*-terminal position. For the purpose, three kinds of peptides **I–III** were prepared:

Here the repeating unit (*n*) in achiral helical segment -(Aib-ΔPhe)_{*n*}-Aib-OMe increases from 2 to 4 in peptides **I–III**, and an L-Leu residue is introduced into the *N*-terminal positions. The conformations in solution were investigated by FT-IR, ¹H NMR, and CD spectroscopy. Helical screw sense was determined by sign of exciton couplets around 280 nm (assignable to ΔPhe residue), according to exciton chirality method.²⁰

[†] To whom correspondence should be addressed.

EXPERIMENTAL

Measurement

^1H NMR spectra were recorded using a JEOL JNM-GX400 (400 MHz), and a Hitachi R-90 spectrometer (90 MHz). The measurements were carried out with a peptide concentration of 10–20 mg mL⁻¹ in CDCl₃, and a mixture of CDCl₃/(CD₃)₂SO. All of the chemical shifts were expressed as δ downfield from tetramethylsilane (TMS). FT-IR spectra were recorded in chloroform using a JASCO FT/IR-430 spectrometer. In chloroform, peptide solution of 1–10 mM peptide concentration was prepared and transferred to NaCl cell with 0.1 mm cell length, and 100% chloroform was used as blank. CD and UV absorption spectra were simultaneously recorded using a JASCO J-600 spectrometer in chloroform, acetonitrile, methanol, and tetrahydrofuran (THF). These solvents were purified by distillation before use. The ΔPhe concentration was determined using maximum absorbance around 280 nm (assigned to a ΔPhe residue) and its molar extinction coefficient ($\epsilon_{\text{max}} = 1.8 \times 10^4$).^{21,22} Thin-layer chromatography (TLC) was carried out on precoated silica plates in the following solvent systems: (A) ethyl acetate, (B) methanol, (C) chloroform–methanol (9:1), and (D) *n*-butanol–acetic acid–water (7:2:1). Gel permeation chromatography (GPC) was recorded on a Tosoh HLC-803-D equipped with G5000-, G4000-, G3000-, G2000-HLX columns in series, by using THF eluent. Single spot in the TLC and single peak in the GPC were obtained for all peptides I–III.

Peptide Synthesis

Boc-L-Leu-(Aib- ΔPhe)₂-Aib-OMe I. Boc amino acid and amino acid methyl ester were prepared by the standard procedure. Peptide I was prepared according to the previous study¹⁹: Boc-Aib- ΔPhe azlactone was prepared *via* Boc-Aib-DL- β -phenylserine-OH, according to ref 23. Boc-Aib- ΔPhe -Aib-OMe was prepared by ring-opening Boc-Aib- ΔPhe azlactone with amino group of H-Aib-OMe. Likewise, Boc-(Aib- ΔPhe)₂-Aib-OMe was prepared by ring-opening the azlactone with *N*-deprotected H-Aib- ΔPhe -Aib-OMe. To obtain peptide I, Boc-L-Leu-OH was coupled with H-(Aib- ΔPhe)₂-Aib-OMe using dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole hydrate (HOBt). The preparation and characterization of peptide I were described in detail previously.¹⁹

Boc-(Aib- ΔPhe)₃-Aib-OMe. Boc-(Aib- ΔPhe)₂-Aib-OMe (0.80 g, 1.1 mmol) was dissolved in dichloromethane (5 mL)/trifluoroacetic acid (TFA; 5 mL) at 0°C, the solution stood for 24 h at room temperature, and concentrated *in vacuo*. After addition of 5% NaHCO₃ solution, the residue was extracted with ethyl acetate, the organic layer was washed with 5% NaHCO₃ and 10% NaCl solutions, and dried over MgSO₄. Evaporation of solvent gave white crystals of H-(Aib- ΔPhe)₂-Aib-OMe [Yield: 0.56 g (82%)].

Boc-(Aib- ΔPhe)₃-Aib-OMe was prepared by ring-opening Boc-Aib- ΔPhe azlactone with H-(Aib- ΔPhe)₂-Aib-OMe: Boc-Aib- ΔPhe azlactone (0.57 g, 1.7 mmol) and H-(Aib- ΔPhe)₂-Aib-OMe (0.97 g, 1.7 mmol) were dissolved in *N,N*-dimethylformamide (DMF; 5 mL), and

stirred for 24 h at room temperature. To increase the basicity of reaction mixture, *N*-methylmorpholine (NMM; 55 μL , 0.51 mmol) was added, and the reaction mixture was stirred for 36 h at room temperature. And then NMM (55 μL , 0.51 mmol) was added, the mixture was stirred for 42 h at room temperature, and evaporated *in vacuo*. The residue was extracted with ethyl acetate, the organic layer was washed with 10% NaCl, 5% KHSO₄, 10% NaCl, 5% NaHCO₃, and 10% NaCl solutions, and dried over MgSO₄. The product was purified by recrystallization from dichloromethane/*n*-hexane. Yield 0.74 g (49%); mp 164–166°C; $R_f^A = 0.70$; $R_f^B = 0.84$; $R_f^C = 0.53$; $R_f^D = 0.93$; retention time in GPC (t_R) = 36.8 min. 400 MHz ^1H NMR (δ , in CDCl₃): 8.99 (1H, s, NH ΔPhe), 8.77 (1H, s, NH ΔPhe), 8.15 (1H, s, NH Aib), 8.06 (1H, s, NH Aib), 7.88 (1H, s, NH Aib), 7.79 (1H, s, NH ΔPhe), 7.57–7.18 (18H, m, 3 $\times$ (phenyl H + C ^{β} H) ΔPhe), 5.18 (1H, s, NH Boc-NH), 3.71 (3H, s, COOCH₃), 1.68 + 1.63 + 1.59 + 1.49 + 1.29 (24H, s + s + s + s + s, 8 $\times$ CH₃ Aib), and 1.44 (9H, s, 3 $\times$ CH₃ Boc). FT-IR (cm⁻¹, in chloroform): 3202, 1735, 1699 (shoulder), 1664, 1628, and 1534.

Boc-L-Leu-(Aib- ΔPhe)₃-Aib-OMe II. Boc-(Aib- ΔPhe)₃-Aib-OMe (0.50 g, 0.55 mmol) was dissolved in dichloromethane (4 mL)/TFA (4 mL) at 0°C, the solution stood for 24 h at room temperature, and concentrated *in vacuo*. After addition of 5% NaHCO₃ solution, the residue was extracted with ethyl acetate, the organic layer was washed with 5% NaHCO₃ and 10% NaCl solutions, and dried over MgSO₄. Evaporation of solvent gave white crystals of H-(Aib- ΔPhe)₃-Aib-OMe [Yield: 0.40 g (91%)].

To a solution of Boc-L-Leu-OH (43 mg, 0.19 mmol) and HOBt (28 mg, 0.19 mmol) in DMF (0.1 mL) was added DCC (38 mg, 0.19 mmol) at 0°C, and the mixture was stirred for 0.5 h at 0°C. After addition of H-(Aib- ΔPhe)₃-Aib-OMe (100 mg, 0.12 mmol), the mixture was stirred for 2 h at 0°C, and for 48 h at room temperature, concentrated *in vacuo*, and the residue was redissolved in ethyl acetate. After removing dicyclohexylurea by filtration, the solution was washed with 10% NaCl, 5% KHSO₄, 10% NaCl, 5% NaHCO₃, and 10% NaCl solutions, and dried over MgSO₄. The product was purified by eluting through a silica-gel column with ethyl acetate. Yield 72 mg (57%); mp 243–246°C; $R_f^A = 0.67$; $R_f^B = 0.85$; $R_f^C = 0.63$; $R_f^D = 0.91$; $t_R = 36.4$ min. 400 MHz ^1H NMR (δ , in CDCl₃): 8.92 (1H, s, NH ΔPhe), 8.78 (1H, s, NH ΔPhe), 8.67 (1H, s, NH ΔPhe), 8.14 (1H, s, NH Aib), 8.05 (1H, s, NH Aib), 7.90 (1H, s, NH Aib), 7.6–7.19 (18H, m, 3 $\times$ (phenyl H + C ^{β} H) ΔPhe), 6.76 (1H, s, NH Aib), 5.18 (1H, d, Boc-NH), 3.76–3.73 (1H, m, C ^{α} H Leu), 3.75 (3H, s, COOCH₃), 1.90–1.82 (2H, m, C ^{β} H₂ Leu), 1.72–1.65 (1H, C ^{γ} H Leu), 1.63 (9H, s, 3 $\times$ CH₃ Boc), 1.59 + 1.58 + 1.55 + 1.53 + 1.32 + 1.28 + 1.15 (24H, s + s + s + s + s + s + s, 8 $\times$ CH₃ Aib), 0.97 + 0.93 (6H, d + d, 2 $\times$ CH₃ Leu). FT-IR (cm⁻¹, in chloroform): 3295, 1734, 1661, 1627, and 1535.

Boc-(Aib- ΔPhe)₄-Aib-OMe. Boc-(Aib- ΔPhe)₄-Aib-OMe was prepared in a similar manner to Boc-(Aib- ΔPhe)₃-Aib-OMe. Yield 77%; mp 244–246°C; $R_f^A = 0.62$; $R_f^B = 0.89$; $R_f^C = 0.62$; $R_f^D = 0.92$; $t_R = 36.2$ min. 400 MHz ^1H NMR (δ , in CDCl₃): 9.13 (1H, s, NH ΔPhe), 9.10 (1H, s, NH ΔPhe), 8.81 (1H, s, NH ΔPhe),

8.23 (1H, s, NH Δ Phe), 8.22 (1H, s, NH Aib), 8.15 (1H, s, NH Aib), 7.94 (1H, s, NH Aib), 7.92 (1H, s, NH Aib), 7.57–7.18 (24H, m, $4 \times$ (phenyl H + C $^\beta$ H) Δ Phe), 5.41 (1H, s, Boc–NH), 3.69 (3H, s, COOCH $_3$), 1.73 + 1.63 + 1.58 + 1.5–1.2 (30H, s + s + s + m, $10 \times$ CH $_3$ Aib), and 1.42 (9H, s, $3 \times$ CH $_3$ Boc). FT-IR (cm $^{-1}$, in chloroform): 3289, 1734, 1698 (shoulder), 1660, 1626, and 1535.

Boc-L-Leu-(Aib- Δ Phe) $_4$ -Aib-OMe **III.** Boc-L-Leu-(Aib- Δ Phe) $_4$ -Aib-OMe was prepared in a similar manner to Boc-L-Leu-(Aib- Δ Phe) $_3$ -Aib-OMe. Yield 45%; mp 244–246°C; R_f^A = 0.59; R_f^B = 0.84; R_f^C = 0.57; R_f^D = 0.92; t_R = 36.0 min. 400 MHz 1 H NMR (δ , in CDCl $_3$): 9.09 (1H, s, NH Δ Phe), 9.02 (1H, s, NH Δ Phe), 8.77 (1H, s, NH Δ Phe), 8.66 (1H, bs, NH Δ Phe), 8.21 (1H, s, NH Aib), 8.19 (1H, s, NH Aib), 7.99 (1H, bs, NH Aib), 7.92 (1H, s, NH Aib), 7.6–7.15 (24H, m, $4 \times$ (phenyl H + C $^\beta$ H) Δ Phe), 6.59 (1H, s, NH Aib), 4.95 (1H, s, Boc–NH), 3.8–3.7 (1H, m, C $^\alpha$ H Leu), 3.72 (3H, s, COOCH $_3$), 1.60 (9H, s, $3 \times$ CH $_3$ Boc), 1.75–1.05 + 1.26 + 1.17 (33H, m + s + s, C $^\beta$ H $_2$ –C $^\gamma$ H Leu and $10 \times$ CH $_3$ Aib), and 1.0–0.8 (6H, m, $2 \times$ CH $_3$ Leu). FT-IR (cm $^{-1}$, in chloroform): 3283, 1739, 1661, 1625, and 1538.

Conformational Energy Calculation

Empirical conformational energy calculations were carried out using structural and energy parameters based on ECEPP system.²⁴ The parameters of Δ Phe residue were determined in the previous study.^{21,25–28} The program PEPCON,^{24,29} written by M. Sisido³⁰ for conformational energy calculation and graphics of a given peptide, was modified to be applicable to Δ Phe-containing peptides. Based on many crystallographic data of Δ Phe-containing peptides,^{4,6,7,10} all amide groups were fixed to the *trans* conformation (ω = 180°) and each Δ Phe side chain (χ^1) was fixed to 0°. First, main-chain energy contour maps were calculated for Ac-(Aib- Δ Phe) $_n$ -Aib-OMe (n = 2–4). Here in each (ϕ, ψ), the χ^2 angle in Δ Phe side chain was taken as the value that gives the minimal conformational energy.

Second, the energy minimization was carried out for Ac-L-Leu-(Aib- Δ Phe) $_4$ -Aib-OMe to predict conformations of L-Leu residue at *N*-terminal position of left- or right-handed helix. The two ways were chosen. One is to energy-minimize Ac-L-Leu-(Aib- Δ Phe) $_4$ -Aib-OMe with six variables of Leu residue (ϕ_{Leu} , ψ_{Leu} , χ_{Leu}^1 , χ_{Leu}^2 , $\chi_{\text{Leu}}^{3,1}$, $\chi_{\text{Leu}}^{3,2}$) using the Simplex algorithm, while segment -(Aib- Δ Phe) $_4$ -Aib-OMe was fixed to a standard left- or right-handed 3_{10} -helix: *i.e.*, (ϕ, ψ) = (60°, 30°) or (–60°, –30°).^{31,32} Here all energy minima of L-Leu residue (81)³³ were used as starting conformations. The other is to energy-minimize Ac-L-Leu-(Aib- Δ Phe) $_4$ -Aib-OMe with 36 variables of all main- and side-chains (ϕ_{Leu} , ψ_{Leu} , χ_{Leu}^1 , χ_{Leu}^2 , $\chi_{\text{Leu}}^{3,1}$, $\chi_{\text{Leu}}^{3,2}$, $5 \times \phi_{\text{Aib}}$, $5 \times \psi_{\text{Aib}}$, $5 \times \chi_{\text{Aib}}^1$, $5 \times \chi_{\text{Aib}}^{1,2}$, $4 \times \phi_{\Delta\text{Phe}}$, $4 \times \psi_{\Delta\text{Phe}}$, $4 \times \chi_{\Delta\text{Phe}}^2$). Also, all energy minima of L-Leu residue (81) in a standard left- or right-handed 3_{10} -helix, (ϕ, ψ) = (60°, 30°) or (–60°, –30°),^{31,32} were used as starting conformations. The conformations of L-Leu residue were expressed by the conformational letter code that divides 16 regions in conformational space.³³

RESULTS AND DISCUSSION

Confirmation of Helical Conformation

Segments -(Aib- Δ Phe) $_n$ -Aib-OMe (n = 2–4) can be expected to generate two “enantiomeric” (left- and right-handed) helices, since Aib and Δ Phe residues are achiral ones and well known to be strong inducers that form a 3_{10} -helix (sometimes an α -helix depending on peptide sequence, chain-length, or environment). Already, segment -(Aib- Δ Phe) $_2$ -OMe was found to take a 3_{10} -helix, from 1 H NMR spectroscopy on Boc-(Aib- Δ Phe) $_2$ -Aib-OMe.¹⁹ Such a helical conformation will also be expected for segments -(Aib- Δ Phe) $_n$ -OMe (n = 3 and 4) due to longer chain lengths. Actually, Boc-(Aib- Δ Phe) $_n$ -Aib-OMe (n = 3 and 4) showed the position of amide I band characteristic of such a 3_{10} -type helix: 1664 and 1628 cm $^{-1}$ for n = 3, and 1660 and 1626 cm $^{-1}$ for n = 4.³⁴ Helix-forming tendency in segments -(Aib- Δ Phe) $_n$ -Aib-OMe (n = 2–4) was also confirmed by conformational energy calculation. Figure 1 shows main-chain energy contour maps of Ac-(Aib- Δ Phe) $_n$ -Aib-OMe (n = 2–4), indicating left- and right-handed (3_{10} - or α -) helices as the most stable region.

The previous study¹⁹ indicated that peptide **I**, in which an L-residue is introduced into the *N*-terminal position of segment -(Aib- Δ Phe) $_2$ -Aib-OMe, takes a 3_{10} -type helix in chloroform. Peptides **II** and **III** should also take such a helix, based on their FT-IR spectra in chloroform³⁴: 1661 and 1627 cm $^{-1}$ for peptide **II**, and 1661 and 1625 cm $^{-1}$ for peptide **III**. Such a helical conformation was also supported by measuring the degree of solvent exposure of each NH group in 1 H NMR spectroscopy.³⁵ The assignment of NH chemical shifts in peptide **I** in CDCl $_3$ were already done by difference Nuclear Overhauser effect spectra obtained by irradiation of each NH resonance.^{19,36} The assignment of NH chemical shifts in peptides **II** and **III** in CDCl $_3$ was done with reference to that in peptide **I**, since peptides **II** and **III** have analogous peptides of peptide **I** and were found to take a helical conformation similar to peptide **I**, from their FT-IR spectra in chloroform. First, the NH resonances at a higher field (*ca.* 5.0 ppm) in peptides **I–III** can be assigned to urethane NH,^{37–40} *i.e.*, Leu(1)’s. For peptide **I**, the Aib(2) NH, which does not participate in intramolecular hydrogen-bonding, showed a singlet at *ca.* 6.6 ppm. A similar singlet for peptides **II** and **III** was seen at *ca.* 6 ppm, and thus can be assigned to Aib(2) NH, which does not participate in intramolecular hydrogen-bonding. For peptide **I**, two Aib NHs participating in hydrogen-bonding appeared at *ca.* 8 ppm as a singlet. In this region (*ca.* 8 ppm), peptide **II** showed three singlets, and peptide **III** did four singlets, indicating that these singlets can be assigned to the remaining Aib NHs participating in hydrogen-bonding. Also peptide **I** showed two singlets at a lower field (*ca.* 8.5 ppm) characteristic of hydrogen-bonding Δ Phe NHs.^{19,22} In this region (*ca.* 8.5–9.0 ppm), peptide **II** showed three singlets, and peptide **III** did four singlets, indicating that these singlets can be assigned to the hydrogen-bonding Δ Phe NHs. The above assignment of hydrogen-bonding NHs in peptides **II** and **III** can be supported by the solvent dependence on NH chemical

Figure 1. Main-chain energy contour map of Ac-(Aib- Δ Phe) $_n$ -Aib-OMe: (a) $n=2$; (b) $n=3$; (c) $n=4$. The contours (10 lines) are drawn in 1.0 kcal [mol(per residues)] $^{-1}$ increments from energy minimum points (-55° , -50°) and (55° , 50°). In each (ϕ , ψ), $\chi_{\Delta\text{Phe}}^2$ angle(s) was taken as the value to give the minimal conformational energy, while its $\chi_{\Delta\text{Phe}}^1$ was fixed to 0° .

Figure 2. Solvent dependence of NH chemical shifts in peptides (a) **I**, (b) **II**, and (c) **III** in CDCl_3 -(CD_3) $_2\text{SO}$ mixtures of varying concentrations: (●) for L-Leu(1), (▲) for Aib(2), (△) for the other Aib's, and (○) for Δ Phe's.

shifts as follows.

Figure 2 shows variation in NH chemical shifts for peptides **I**–**III** with concentration of (CD_3) $_2\text{SO}$.⁴¹ Two NH resonances, Leu(1) NH and Aib(2) NH, were markedly shifted to a lower field with increasing concentration of (CD_3) $_2\text{SO}$. This means no marked conformational change, but the absence of hydrogen-bonding in Leu(1) and Aib(2) NHs, since similar sequential peptide, Boc-(L-Leu- Δ Phe) $_6$ -L-Leu-OMe, did not cause conformational change in 0–9 vol% (CD_3) $_2\text{SO}$.²² Also, similar results were obtained in dependence of NH resonance line widths in peptide **I**–**III** on the addition of 2,2,6,6-tetramethylpiperidine (TEMPO)⁴² in CDCl_3 . There was remarkably pronounced line broadening of Leu(1) NH and Aib(2) NH resonances, while the other NHs remain almost unaffected. These NMR results indicate that the NHs except for Leu(1) NH and Aib(2) NH were relatively unaffected by the addition of strong hydrogen-accepting (CD_3) $_2\text{SO}$ or paramagnetic TEMPO, and thus should be shielded from solvent due to intramolecular hydrogen-bonding.^{41,42} This hydrogen-bonding pattern corresponds to 3_{10} -type helical [4 $\rightarrow$ 1 hydrogen-bonding] mode,^{21,22} in which all the NHs except for Leu(1) NH and Aib(2) NH participate in intramolecular hydrogen bonding. Consequently, helical conformations were retained by the introduction of an L-Leu residue into the *N*-terminal position of helical segment -(Aib- Δ Phe) $_n$ -Aib-OMe ($n=2$ –4).

Determination of Helical Screw Sense

As expected, peptides Boc-(Aib- Δ Phe) $_n$ -Aib-OMe ($n=2$ –4) showed no CD signals due to no chiral residues

Figure 3. CD (top) and UV absorption (bottom) spectra of peptides **I** (-----), **II** (---), **III** (—), **IV** (— · —), and **V** (—) in chloroform.

being included, indicating they take left- and right-handed helices with the same contents. On the other hand, marked CD signals were observed for peptides **I**–**III**, in which an L-Leu residue is attached to the *N*-terminal position of enantiomeric helical segment -(Aib- Δ Phe) $_n$ -Aib-OMe. Figure 3 shows CD and UV absorption spectra of peptides **I**–**III** in chloroform. The $\Delta\epsilon$ ($=\epsilon_L - \epsilon_R$) is expressed with respect to the molar concentration of Δ Phe residue.

All UV absorption spectra showed intense maxima (λ_{max}) around 280 nm (band I) assignable to Δ Phe residue.

Table I. Spectroscopic properties of peptides **I**–**V** in solution

Peptide	Solvent	UV Data		CD Data	
		$\lambda_{\max}/\text{nm}$	$\Delta\epsilon_{\max}/\lambda_{\max}/\text{nm}$	$\Delta\epsilon_{\min}/\lambda_{\min}/\text{nm}$	A
I ^a	Chloroform	280	4.6/294	– 4.1/260	8.7
II	Chloroform	281	3.2/300	– 2.5/268	5.7
III	Chloroform	282	5.0/300	– 5.6/267	10.6
IV ^a	Chloroform	280	4.2/260	– 4.2/292	8.4
V	Chloroform	284	31.5/270	– 19.5/303	51.0
I ^a	Acetonitrile	280	6.3/296	– 6.8/262	13.1
II	Acetonitrile	280	7.9/298	– 10.0/266	17.9
III	Acetonitrile	281	9.2/298	– 11.1/265	20.3
IV ^a	Acetonitrile	280	7.0/260	– 6.2/295	13.2
V	Acetonitrile	281	20.6/270	– 10.8/295	31.4
I ^a	Methanol	282	7.5/297	– 7.9/264	15.4
II	Methanol	280	6.8/298	– 9.2/267	16.0
III	Methanol	282	7.7/299	– 9.1/267	16.8
IV ^a	Methanol	281	8.1/263	– 7.8/295	15.9
V	Methanol	281	16.2/270	– 7.0/303	23.2
I ^a	THF	277	9.8/292	– 10.9/258	20.7
II	THF	280	11.2/298	– 12.3/266	23.5
III	THF	280	14.6/299	– 16.5/265	31.1
IV ^a	THF	276	11.1/258	– 9.4/291	20.5
V	THF	281	24.2/268	– 15.6/303	39.8

^a From ref 36.

The UV pattern did not change in all peptides **I**–**III**, but resembled that of Boc–L–Leu– Δ Phe–L–Leu–OMe²² having a single Δ Phe residue. Thus, no strong ground-state interaction between Δ Phe– Δ Phe pair(s) exists in peptides **I**–**III**. Such interaction was also not observed in 3_{10} -helical peptides containing Δ Phe residues.^{21,22,27}

The corresponding CD spectra of peptides **I**–**III** showed marked exciton couplets around 280 nm with a positive peak at longer wavelengths, as shown in Figure 3. Peptides containing Δ Phe residues show intense absorption maxima around 220 nm and around 280 nm (band I).^{13,21} The former absorption band precludes a far-UV CD analysis to usually investigate conformations of peptides or proteins. On the other hand, the latter absorption band has been assigned to charge transfer from the highest occupied orbital localized on styryl moiety to the vacant orbital of carbonyl group in Δ Phe residue.^{43,44} The transition moment was estimated from molecular orbital calculation to lie on the styryl-carbonyl line.²¹ By applying the exciton chirality method²⁰ to the present system, the sign of split CD pattern corresponds to a left-handed helical arrangement of the transition moment, meaning left-handed helical main chain. This assignment has also been used for 3_{10} -helical peptides containing Δ Phe–X– Δ Phe unit(s).^{1,2,5,13,22,27}; *i.e.*, the sign of exciton couplets at band I is a positive peak at longer wavelengths for a left-handed helix and a negative peak at longer wavelengths for a right-handed helix. Moreover, the sign of exciton couplets observed here (a positive peak at longer wavelengths) should be ascribed to left-handed 3_{10} - or α -helices, based on theoretical CD calculation.^{21,25} Namely, exciton couplets with a positive peak at longer wavelengths were obtained at band I for Boc–(Aib– Δ Phe)_{*n*}–Aib–OMe (*n* = 2–4) in five left-handed 3_{10} -type (4 $\rightarrow$ 1 hydrogen bonded) helices (1)–(5) and in three left-handed α -type (5 $\rightarrow$ 1 hydrogen bonded)

helices (6)–(8): (1) $\phi = 44^\circ$, $\psi = 33^\circ$; (2) $\phi = 54^\circ$, $\psi = 28^\circ$; (3) $\phi = 71^\circ$, $\psi = 18^\circ$; (4) $\phi = 53^\circ$, $\psi = 36^\circ$; (5) $\phi = 60^\circ$, $\psi = 30^\circ$; (6) $\phi = 53^\circ$, $\psi = 52^\circ$; (7) $\phi = 57^\circ$, $\psi = 47^\circ$; (8) $\phi = 63^\circ$, $\psi = 42^\circ$. These eight left-handed helices (ϕ, ψ) correspond to the right-handed ones ($-\phi, -\psi$) reported.^{45–48,31,32,49–51} Therefore, the main chains of peptides **I**–**III** prefer the left-handed screw sense to the right-handed one. Obviously, the preference of left-handed screw sense by an *N*-terminal L-residue is retained, irrespective of chain-lengths of achiral helical segments (5–9 residues). On the other hand, peptide Boc–D–Leu–(Aib– Δ Phe)₂–Aib–OMe (**IV**) containing a D-Leu residue in the *N*-terminal position gave almost completely asymmetric CD spectrum, compared with peptide **I** containing an L-Leu residue in the *N*-terminal position: *i.e.*, peptide **IV** showed marked exciton couplets with a negative peak at longer wavelengths and gave the split amplitude value similar to peptide **I**, as shown in Figure 3 and Table I.

Figures 4–6 show CD and UV absorption spectra of peptides **I**–**III** in acetonitrile, methanol, and THF, respectively. In these solvents, CD patterns at band I were similar to those in chloroform. It should also be concluded that an *N*-terminal L-Leu residue induces a left-handed screw sense preferentially. Here the split amplitude (A)^{21,25} is defined as the difference between the maximum and minimum $\Delta\epsilon$ ($\Delta\epsilon_{\max}$ and $\Delta\epsilon_{\min}$) values in the couplets of band I. In all solvents, peptide **III** showed the largest A value, since it might have the strongest dipole–dipole interaction due to the longest chain. On the other hand, peptide **III** showed smaller A values in all solvents than Boc–(L–Leu– Δ Phe)₄–L–Leu–OMe (**V**) that takes a right-handed 3_{10} -type helical conformation in chloroform,^{22,27} as shown in Figures 3–6 and Table I. Peptide **V** is regarded as analogous peptide of Boc–(Aib– Δ Phe)₄–Aib–OMe in which all Aib

Figure 4. CD (top) and UV absorption (bottom) spectra of peptides I (-----), II (---), III (—), IV (— · —), and V (—) in acetonitrile.

Figure 5. CD (top) and UV absorption (bottom) spectra of peptides I (-----), II (---), III (—), IV (— · —), and V (—) in acetonitrile.

Figure 6. CD (top) and UV absorption (bottom) spectra of peptides I (-----), II (---), III (—), IV (— · —), and V (—) in THF.

Table II. Energy-minimized conformations for L-Leu residue in Ac-L-Leu-(Aib-ΔPhe)₄-Aib-OMe in a standard 3_{10} -helix^a

Conformational letter code ^b	Leu		Helical screw sense ^c	ΔE_{res}^d kcal mol ⁻¹
	ϕ	ψ		
C	-64	115	LH	—
A	-65	-53	RH	0.09
A*	56	70	LH	0.15
C	-78	101	RH	0.16
C	-66	120	LH	0.17
A*	56	67	LH	0.22
C	-81	83	LH	0.23
A	-70	-52	LH	0.24
D	-148	103	RH	0.25
F	-63	134	LH	0.25

^a Energy-minimization was carried out for six variables of L-Leu residue (ϕ_{Leu} , ψ_{Leu} , χ_{Leu}^1 , χ_{Leu}^2 , $\chi_{\text{Leu}}^{3,1}$, $\chi_{\text{Leu}}^{3,2}$), while the segment -(Aib-ΔPhe)₄-Aib-OMe is fixed to a standard left- or right-handed 3_{10} -helix: (60°, 30°) or (-60°, -30°),^{31,32} respectively. ^b For Leu residue. ^c LH and RH represent left- and right-handed helical screw senses for segment -(Aib-ΔPhe)₄-Aib-OMe, respectively. ^d $\Delta E_{\text{res}} = (E - E_0)/10$. E_0 is the lowest energy.

residues are replaced by L-Leu residue. Assuming that the *A* values of peptide V correspond to those for 100% right-handed helical conformation, smaller *A* value of peptide III than peptide V should mean that peptide III contains not only a left-handed helix, but a right-handed one in an equilibrium state. Particularly in chloroform in which both peptides III and V take a 3_{10} -type helical conformation, peptide III was estimated to contain 60% left-handed helix and 40% right-handed one, based on an *A* value of 10.6 for peptide III and 51.0 for peptide V in Table I. From the similar calculation, peptide III was also estimated to contain 82% left-handed helix in chloroform, 86% left-handed helix in methanol, and 89% left-handed helix in THF. In the three solvents, a left-handed helix in peptide III was taken more preferentially. For peptides I and II, such estimation could not be done due to lack of appropriate model peptides showing almost 100% right- or left-handed helical conformation: e.g., Boc-(L-Leu-ΔPhe)₂-L-Leu-OMe for peptide I showed solvent dependence on CD patterns, and Boc-(L-Leu-ΔPhe)₃-L-Leu-OMe for peptide II showed exciton couplets at a negative peak at longer wavelengths, but somewhat distorted splitting patterns.²²

Conformation of N-Terminal L-Leu Residue in Left-Handed Helix

In the preceding section, it should be concluded that an N-terminal L-Leu residue induces a left-handed screw sense preferentially. Table II shows energy-minimized conformations of L-Leu residue in Ac-L-Leu-(Aib-ΔPhe)₄-Aib-OMe, in which segment -(Aib-ΔPhe)₄-Aib-OMe is fixed to a standard left- or right-handed 3_{10} -helix,^{31,32} and lists from the lowest-energy to tenth low-energy conformations. ΔE_{res} is the energy difference per residue from the lowest energy. In Table II, the left-handed helix was found as the lowest-energy conformation, and seven left-handed helices were present among ten conformers ($\Delta E_{\text{res}} \leq 0.25$ kcal mol⁻¹), thus indicating that the left-handed helix is predominant. This prediction agreed well with the preceding experimental results. In the lowest-energy right-handed helix (second

low-energy conformation in Table II), *N*-terminal L-Leu residue showed a conformational letter code of "A",³³ which corresponds to a region for a right-handed 3_{10} - or α -helical conformation. Namely, an *N*-terminal L-Leu residue is incorporated into a right-handed helical conformation of $-(\text{Aib}-\Delta\text{Phe})_4\text{-Aib-OMe}$. On the other hand, interestingly, an *N*-terminal L-Leu residue in the lowest-energy left-handed helix took a conformational letter code of "C", but not of "A*",³³ which corresponds to a region for left-handed 3_{10} - or α -helical conformation. It should be noted that an *N*-terminal L-Leu residue is not incorporated into a left-handed helical conformation (A*), but takes an irregular conformation that deviates from a left-handed helix.

Table III shows energy-minimized conformations of $\text{Ac-L-Leu-(Aib-}\Delta\text{Phe)}_4\text{-Aib-OMe}$. Unlike in Table II, a right-handed helix was found as the lowest-energy conformation. However, the energy difference between the lowest-energy left- and right-handed helices was very small ($\Delta E_{\text{res}} = 0.06 \text{ kcal mol}^{-1}$), and seven left-handed helices were present among ten conformers ($\Delta E_{\text{res}} \leq 0.23 \text{ kcal mol}^{-1}$), thus indicating that a left-handed helix is also predominant. The energy-minimized conformations ($\langle\phi\rangle$ and $\langle\psi\rangle$) for segment $-(\text{Aib-}\Delta\text{Phe})_4\text{-Aib-OMe}$ appear to have a character of 3_{10} - and α -type helices in the hydrogen-bonding mode, irrespective of conformations of Leu residue and of helical screw sense. This energy-minimized helix differed somewhat from a 3_{10} -helix obtained in the preceding ^1H NMR study. On the other hand, 3_{10} - and α -helices take similar conformations: *e.g.*, (ϕ, ψ) is $(-60^\circ, -30^\circ)^{31,32}$ for standard 3_{10} -helix, and $(-57^\circ, -47^\circ)^{50}$ for standard α -helix. Also, the energy difference between both helices should be small, since 3_{10} - to α -helical transitions occur

with solvents and chain-lengths in Aib-containing peptides.^{14,52-59} Thus, it seems difficult to predict both helical structures. However, the conformations of *N*-terminal L-Leu residue showed the tendency similar to those in Table II: *i.e.*, a conformational letter code of "A" for the lowest-energy right-handed helix, and of "C" for the lowest-energy left-handed helix (second low-energy conformation in Table III).

To obtain further information, main-chain contour map of L-Leu residues was calculated on $\text{Ac-L-Leu-(Aib-}\Delta\text{Phe)}_4\text{-Aib-OMe}$. Here the segment $-(\text{Aib-}\Delta\text{Phe})_4\text{-Aib-OMe}$ was fixed to a standard 3_{10} -helix $(-60^\circ, -30^\circ)^{31,32}$ based on ^1H NMR results, and side

Table III. Energy-minimized conformations of $\text{Ac-L-Leu-(Aib-}\Delta\text{Phe)}_4\text{-Aib-OMe}^a$

Conformational letter code ^b	Leu		Helical screw sense ^c	Aib- ΔPhe^d		ΔE_{res}^e kcal mol ⁻¹
	ϕ	ψ		$\langle\phi\rangle$	$\langle\psi\rangle$	
A	-63	-57	RH	-54	-48	—
C	-65	109	LH	54	47	0.06
A	-65	-57	RH	-56	-47	0.06
A*	56	64	LH	54	49	0.07
C	-73	98	LH	54	49	0.09
C	-66	109	LH	54	49	0.13
A*	55	54	LH	53	49	0.14
A*	57	67	LH	54	46	0.15
C	-75	97	LH	54	49	0.21
E	-147	127	RH	-52	-52	0.23

^a Energy-minimization was carried out for 36 variables of all main- and side-chains. ^b For Leu residue. ^c LH and RH represent left- and right-handed helical screw senses for segment $-(\text{Aib-}\Delta\text{Phe})_4\text{-Aib-OMe}$, respectively. ^d $\langle\phi\rangle$ and $\langle\psi\rangle$ values were averaged for five Aib and four ΔPhe residues in segment $-(\text{Aib-}\Delta\text{Phe})_4\text{-Aib-OMe}$. ^e $\Delta E_{\text{res}} = (E - E_0)/10$. E_0 is the lowest energy.

Figure 7. Main-chain energy contour map of L-Leu residue on $\text{Ac-L-Leu-(Aib-}\Delta\text{Phe)}_4\text{-Aib-OMe}$ in terms of (a) total energy, (b) electrostatic energy, (c) nonbonded energy, and (d) hydrogen-bond energy. The contours (10 lines) are drawn in $0.5 \text{ kcal mol}^{-1}$ increments from energy minimum points of (a) $(-65^\circ, 115^\circ)$, (b) $(55^\circ, -45^\circ)$, (c) $(-70^\circ, -55^\circ)$, and (d) $(55^\circ, 0^\circ)$. The segment $-(\text{Aib-}\Delta\text{Phe})_4\text{-Aib-OMe}$ was fixed to a standard 3_{10} -helix $(-60^\circ, -30^\circ)^{31,32}$ based on ^1H NMR results, and side chain of L-Leu (χ^1, χ^2) in each (ϕ, ψ) was taken as the value giving the minimal conformational energy when χ^1 and χ^2 angles were changed from 0° to 350° at 10° .

chain of L-Leu (χ^1 , χ^2) in each (ϕ , ψ) was taken as the value that gives the minimal conformational energy when χ^1 and χ^2 angles were changed from 0° to 350° at 10° . As shown in Figure 7 (a), (-65° , 115°) was found as the minimum energy point, corresponding to that in Table II. Figures 7 (b)–(d) show the corresponding contour maps in terms of electrostatic energy, nonbonded energy, and hydrogen-bond energy, respectively. The map of total energy (Figure 7(a)) was similar to that of nonbonded energy (Figure 7(c)) in the pattern. Thus, nonbonded energy term is mainly ascribed to that the *N*-terminal L-Leu residue in the lowest-energy 3_{10} -helix takes a conformation deviating from the left-handed helix (A^*).

CONCLUSIONS

In the present paper, we focused on which helical screw sense is dominated depending on chain lengths when an L-Leu residue is introduced into the *N*-terminal position of achiral helical segments. First, an *N*-terminal L-Leu residue induces a left-handed screw sense preferentially, irrespective of chain lengths of achiral helical segments (5–9 residues). Second, in the lowest-energy left-handed helical conformation, an *N*-terminal L-Leu residue takes an irregular conformation that deviates from a left-handed 3_{10} - α -helical region in conformational space of (ϕ , ψ) (a conformational letter code of “ A^* ”).

Acknowledgments. We express our sincere gratitude to Professor M. Kawai in Department of Applied Chemistry, Nagoya Institute of Technology for the use of CD apparatus.

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