Solvent Dependence on the Preference of Helical Screw Sense: Effect of L-Leu Residue Second from N-Terminal on Screw Sense in Achiral Peptide

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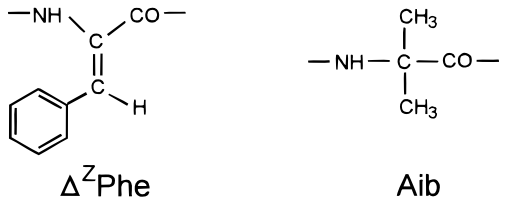
ABSTRACT: To clarify which helical screw sense is preferred when one L-residue is introduced into the second position from N-terminal of an achiral helical segment, we prepared Boc-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe (**1**: Boc = *t*-butoxycarbonyl; Aib = α-aminoisobutyric acid; Δ^ZPhe = *Z*-dehydrophenylalanine; OMe = methoxy). Here the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe was used as an achiral backbone composed of two "enantiomeric" (left-/right-handed) helices. Peptide **1** was found to form a 3_{10}-type helical conformation in chloroform, from FT IR (the position of amide I band) and ^{1}H NMR (difference nuclear Overhauser effect and solvent accessibility of NH resonances) measurements. Interestingly, CD patterns of peptide **1** changed with types of solvents: i.e., exciton couplets around 280 nm with a negative peak at longer wavelengths in chloroform and with a positive peak at longer wavelengths in methanol or in tetrahydrofuran. Consequently, the helical segment prefers a right-handed screw sense in chloroform and a left-handed one in methanol or in tetrahydrofuran. Also, the preference of a screw sense changed reversibly, depending on chloroform−methanol mixtures of varying concentrations. Conformational energy calculation was carried out on acetyl-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe, which was predicted to take both left- and right-handed helical conformations. The above experimental and theoretical results were compared with those of Boc-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe, in which the N-terminal L-Leu residue induces a left-handed helical screw sense preferentially.

Introduction

In general, a helical polymer takes a one-side (left- or right-handed) screw sense preferentially when chiral moieties are introduced into the main or side chains. As far as helical segments of proteins or peptides are concerned, most of the L-amino acid residues are well recognized to prefer a right-handed screw sense. On the other hand, the L-residue was rarely found to induce a left-handed screw sense for several peptides containing achiral helicogenic residues, *Z*-dehydrophenylalanine (Δ^ZPhe)[1−13] or α-aminoisobutyric acid (Aib)[14,15] (see Scheme 1).

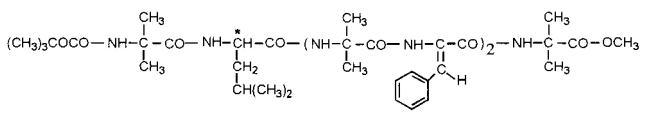
For example, a left-handed 3_{10}-helix in solution was seen in Ac-Δ^ZPhe-Gly-Δ^ZPhe-L-Ala-OMe (Ac, acetyl; OMe, methoxy)[13] or Boc-L-Pro-Aib-L-Ala-Aib-L-Ala-OMe (Boc, *t*-butoxycarbonyl).[16] Left-handed 3_{10}-helices have also been observed in the crystalline state for Aib peptides containing one L-residue in the C-terminal position.[16−20] Z-Aib-Aib-L-Ala-OMe (Z, benzyloxycarbonyl) took an incipient left-handed 3_{10}-helix (type III′ *β*-turn) in the solid state,[17] and Z-Aib-Aib-Aib-L-Val-OMe took a left-handed 3_{10}-helix in the solid state.[19] Also, N$^\alpha$-blocked pentapeptide esters containing four Aib residues and one chiral L-Val or C$^\alpha$-methyl-L-Val residue in the C-terminal position of the sequence took a left-handed screw in solution.[20] Furthermore, in proteins, Schellman noted that many right-handed helical segments ended with a residue in left-handed conformation.[21] Boc-L-Ala-Δ^ZPhe-Gly-Δ^ZPhe-L-Ala-OMe[5,13] and Boc-L-Val-Δ^ZPhe-Gly-Δ^ZPhe-L-Val-OMe[22] showed reversible screw sense inversion of the 3_{10}-helix, depending on solvent or temperature conditions. Also, Boc-L-Ala-Δ^ZPhe-Δ^ZPhe-NMA (NMA, *N*-methylamide) took an incipient 3_{10}-helix of both left- and right-handed screw senses in the solid state.[12] Thus, the L-residue seems to show the character of a left-handed screw sense when it will be introduced into relatively C-terminal positions of achiral helical peptides.

Scheme 1. Δ^ZPhe and Aib Residues



On the other hand, little is known about effects of the N-terminal L-residue on dominating a helical screw sense in achiral peptides. Recently, we demonstrated this effect using Boc-X-(Aib-Δ^ZPhe)$_2$-Aib-OMe,[23−25] in which one L-amino acid residue (X) is introduced into the N-terminal position in achiral helical segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe composed of Aib and Δ^ZPhe residues. As a result, an N-terminal L-residue was found to induce a left-handed screw sense preferentially, irrespective of types of L-residues and solvents.[23] On the other hand, an internal L-residue has been well recognized to take a right-handed screw sense from the viewpoint of conformational energy.[26] One might ask at this point which helical screw sense is preferred when one L-residue is introduced into the position second from N-terminal in an achiral peptide. To clarify this point, we prepared the following peptide **1**:

Boc−Aib−L- Leu−(Aib− Δ^ZPhe)$_2$−Aib−OMe **1**



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The conformation in solution was investigated by ^{1}H NMR, FT IR, and CD spectroscopy. Helical screw sense was determined by sign of exciton couplets around 280 nm (assignable to the Δ^ZPhe residue), according to the exciton chirality method.[27] These spectroscopic data were compared with Boc-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe **2**, in which one L-Leu residue is introduced into the N-terminal position of achiral helical segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe.

Experimental Section

Measurement. ^{1}H NMR spectra were recorded using a JEOL JNM-GX400 spectrometer (400 MHz). The measurements were carried out with a peptide concentration of 10−30 mg mL^{-1} in $CDCl_3$ and a mixture of $CDCl_3$/$(CD_3)_2SO$. 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. A chloroform 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. A difference nuclear Overhauser effect (NOE) experiment was carried out on a Varian XL-200 spectrometer (200 MHz) using the standard Varian software library. The typical acquisition parameters were a 12.0 μs pulse width, a 5.0 s acquisition time, a 4.0 s delay time, and 300−600 accumulations. 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 Δ^ZPhe concentration was determined using maximum absorbance around 280 nm (assignable to a Δ^ZPhe residue) and its molar extinction coefficient ($\epsilon_{max} = 1.8 \times 10^4$).[28,29] 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) 1-butanol−acetic acid−water (7:2:1). Gel permeation chromatography (GPC) was recorded on a Tosoh HLC-803-D equipped with G1000-, G2500-, and G3000-HLX columns in series, using THF eluent. A single spot in the TLC and a single peak in the GPC were obtained for peptide **1**.

Peptide Synthesis. Boc amino acid and amino acid methyl ester were prepared by the standard procedure. Peptide **2** was prepared according to previous studies.[23,24]

Boc-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe 1. Peptide **2** (100 mg, 0.13 mmol) was dissolved in dichloromethane (2 mL)/trifluoroacetic acid (2 mL) at 0 °C; the solution stood for 24 h at room temperature and was concentrated in vacuo. After addition of 5% $NaHCO_3$ solution, the residue was extracted with ethyl acetate; the organic layer was washed with 5% $NaHCO_3$ and 10% NaCl solutions and dried over $MgSO_4$. Evaporation of solvent gave white crystals of H-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe (81 mg, 90%).

To a solution of Boc-Aib-OH (52 mg, 0.26 mmol) and 1-hydroxybenzotriazole hydrate (35 mg, 0.26 mmol) in DMF (0.1 mL) was added dicyclohexylcarbodiimde (54 mg, 0.26 mmol) at 0 °C, and the mixture was stirred for 2 h at 0 °C. After addition of H-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe (81 mg, 0.12 mmol), the mixture was stirred for 2 h at 0 °C and for 24 h at room temperature and concentrated in vacuo, and the residue was redissolved in ethyl acetate. After removing dicyclohexylurea by filtration, the solution was washed with 10% NaCl, 5% $KHSO_4$, 10% NaCl, 5% $NaHCO_3$, and 10% NaCl solutions successively and dried over $MgSO_4$. The product was purified by eluting through a silica gel column with ethyl acetate and further recrystallized from ethyl acetate/*n*-hexane. Yield 40 mg (39%); mp 163−165 °C; $R_f^A = 0.70$; $R_f^B = 0.91$; $R_f^C = 0.75$; $R_f^D = 0.96$; retention time = 22.0 min. ^{1}H NMR (400 MHz) (δ, in $CDCl_3$): 8.70 (1H, s, NH Δ^ZPhe), 8.63 (1H, s, NH Δ^ZPhe), 8.00 (1H, s, NH Aib), 7.87 (2H, s, NH Aib), 7.55−7.17 (12H, m, 2 × (phenyl H + C$^\beta$H) Δ^ZPhe), 6.72 (1H, bs, NH Leu), 5.25 (1H, bs, NH Boc-NH), 3.91 (1H, m, C$^\alpha$H Leu), 3.70 (1H, s, $COOCH_3$), 1.85−1.6 (3H, m, C$^\beta$H$_2$−C$^\gamma$H Leu), 1.45 (9H, s, 3 × CH_3 Boc), 1.60 + 1.58 + 1.54 + 1.47 + 1.41 + 1.20 + 1.07 (24H, s + s + s + s + s + s + s, 8 × CH_3 Aib), and 0.98 + 0.92 (6H, d + d, 2 × CH_3 Leu). FT IR (cm^{-1}, in chloroform): 3304, 1736, 1701, 1664, 1629, and 1534.

Conformational Energy Calculation. Empirical conformational energy calculations were carried out using structural and energy parameters based on the ECEPP system.[30] The parameters of Δ^ZPhe residue were determined in the previous study.[28,31−33] The program PEPCON,[30,34] written by Sisido[35] for conformational energy calculation and graphics of a given peptide, was modified to be applicable to Δ^ZPhe-containing peptides. On the basis of many crystallographic data of Δ^Z-Phe- or Aib-containing peptides,[1,4,6,7,10,14,15] all amide groups were fixed to the trans conformation ($\omega = 180°$) and each Δ^Z-Phe side chain (χ^1) was fixed to 0°. Energy minimization was carried out for Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe to predict stable conformations of the N-terminal segment Ac-Aib-L-Leu- attached to a left- or right-handed 3_{10}-helical segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe. Namely, the minimization was carried out with 10 variables of the Leu residue (ϕ_{Aib}, ψ_{Aib}, $\chi^{1,1}_{Aib}$, $\chi^{1,2}_{Aib}$, ϕ_{Leu}, ψ_{Leu}, χ^1_{Leu}, χ^2_{Leu}, $\chi^{3,1}_{Leu}$, $\chi^{3,2}_{Leu}$), using the Simplex algorithm, while the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe was fixed to a standard left- or right-handed 3_{10}-helix: (ϕ,ψ) = (60°, 30°) or (−60°, −30°).[36,37] Here all energy minima of the N-terminal segment -Aib-L-Leu- ($28 \times 81 = 2268$)[38,39] were used as starting conformations. For comparison, the energy minimization of Ac-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe was carried out with six variables (ϕ_{Leu}, ψ_{Leu}, χ^1_{Leu}, χ^2_{Leu}, $\chi^{3,1}_{Leu}$, $\chi^{3,2}_{Leu}$), while the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe was fixed to a standard left- or right-handed 3_{10}-helix: (ϕ,ψ) = (60°, 30°) or (−60°, −30°).[36,37] Here all energy minima of the L-Leu residue (81)[39] were used as starting conformations. The conformations of the -Aib-L-Leu- segment and L-Leu residue were expressed by the conformational letter code that divides 16 regions in conformational space.[39]

Results and Discussion

Confirmation of Helical Conformation in Peptide 1. The segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe can be expected to generate two "enantiomeric" (left- and right-handed) helices, since Aib and Δ^ZPhe residues are achiral ones and well-known to be strong inducers for taking a 3_{10}-helix (sometimes an α-helix, depending on peptide sequence, chain length, or environment). Already, the segment -(Aib-Δ^ZPhe)$_2$-OMe was found to take a 3_{10}-helix, from ^{1}H NMR spectroscopy on Boc-(Aib-Δ^ZPhe)$_2$-Aib-OMe.[23] The previous study[23,24] indicated that peptide **2**, in which one L-Leu residue is introduced into the N-terminal position of segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe, takes a 3_{10}-type helix in chloroform.

Peptide **1** showed the amide I absorption band characteristic of a 3_{10}-type helix in its FT IR spectrum: 1664 and 1629 cm^{-1}, which can be assigned to saturated amino acid residues and Δ^ZPhe residues in helical segments, respectively.[40,41] Such a helical conformation was also supported by ^{1}H NMR spectroscopy on peptide **1**. The difference NOE experiment in $CDCl_3$ showed marked positive signals between neighboring NH resonances as shown in Table 1, indicating that peptide **1** takes a 3_{10}- or α-helix.[42−44] Figure 1 shows the variation in NH chemical shifts for peptide **1** with concentration of $(CD_3)_2SO$.[45] Two NH resonances, Aib(1) NH and Leu-(2) NH, were markedly shifted to a lower field with increasing concentration of $(CD_3)_2SO$. This means no marked conformational change, but the absence of hydrogen bonding in Aib(1) and Leu(2) NHs, since the analogous peptide **2** did not cause conformational change in 0−100 vol % $(CD_3)_2SO$.[23] These NMR results indicate that the NHs except for Aib(1) NH and Leu(2) NH were relatively unaffected by the addition of strong hydrogen-accepting $(CD_3)_2SO$ and thus should be shielded from solvent due to intramolecular hydrogen bonding.[45]

Table 1. Difference NOEs of NH and C$^{\alpha}$H Resonance Observed for Peptide 1 in $CDCl_3$ at 40 °C[a]

	% NOE for resonance							
irradiated NH resonance	Aib(1) NH	L-Leu(2) NH	L-Leu(2) C$^{\alpha}$H	Aib(3) NH	Δ^ZPhe(4) NH	Aib(5) NH	Δ^ZPhe(6) NH	Aib(7) NH
Aib(1)	×	1.2						
L-Leu(2)	1.2	×	6.4	1.7				
Aib(3)		1.2	1.6	×	2.4			
Δ^ZPhe(4)				2.1	×	2.5		
Aib(5)					2.6	×	2.7	
Δ^ZPhe(6)						2.5	×	3.0
Aib(7)							2.7	×

[a] × shows irradiated NH resonance.

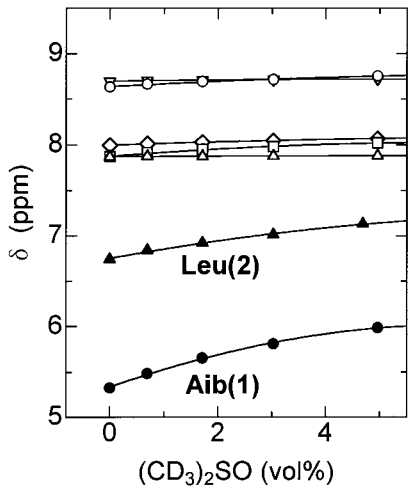

Figure 1. Solvent dependence on NH chemical shifts in peptide **1** in $CDCl_3$−$(CD_3)_2SO$ mixtures of varying concentrations: (●) for Aib(1), (▲) for Leu(2), (□) for Aib(3), (○) for Δ^Z-Phe(4), (◇) for Aib(5), (▽) for Δ^ZPhe(6), and (△) for Aib(7).

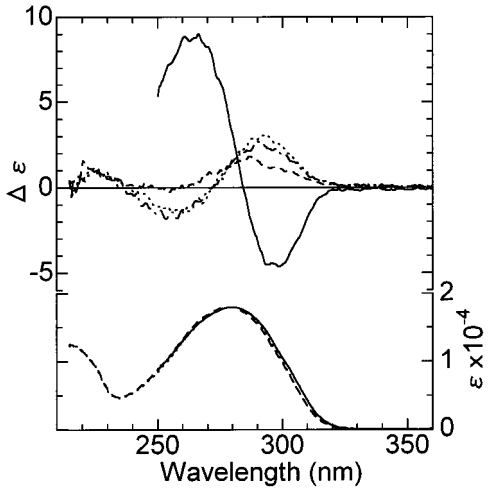

Figure 2. CD (top) and UV absorption (bottom) spectra of peptide **1** in chloroform (—), acetonitrile (- - -), methanol (···), and THF (- · -).

This hydrogen-bonding pattern corresponds to a 3_{10}-type helical (4 → 1 hydrogen-bonding) mode, in which all the NHs except for Aib(1) NH and Leu(2) NH participate in intramolecular hydrogen bonding. Consequently, helical conformations were retained by the introduction of the segment -Aib-L-Leu- into the N-terminal position of segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe.

Determination of Helical Screw Sense. The peptide Boc-(Aib-Δ^ZPhe)$_2$-Aib-OMe cannot show any CD signal due to the absence of chiral residues. It is nonsense to measure its CD spectrum. Thus, it takes left- and right-handed helices with the same contents. On the other hand, marked CD signals were observed for peptide **1**, in which the segment -Aib-L-Leu- is attached to the N-terminal position of the achiral helical segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe. Figure 2 shows CD and UV absorption spectra of peptide **1** in chloroform, acetonitrile, methanol, and THF. The $\Delta\epsilon$ ($=\epsilon_L - \epsilon_R$) was expressed with respect to the molar concentration of Δ^Z-Phe residue.

All UV absorption spectra showed intense maxima (λ_{max}) around 280 nm (band I) assignable to the Δ^ZPhe residue. The UV pattern did not change in peptide **1** but resembled that of Boc-L-Leu-Δ^ZPhe-L-Leu-OMe[29] having a single Δ^ZPhe residue. Thus, no strong ground-state interaction between the Δ^ZPhe−Δ^ZPhe pair exists in peptide **1**. Such interaction was also not observed in 3_{10}-helical peptides containing Δ^ZPhe residues.[23−25,28,29]

The corresponding CD spectra of peptide **1** showed marked exciton couplets around 280 nm in chloroform, methanol, and THF, as shown in Figure 2. Interestingly, the sign of couplets changed with the solvents: a negative peak at longer wavelengths for chloroform and a positive peak at longer wavelengths for methanol and THF. Peptides containing Δ^ZPhe residues show intense absorption maxima around 220 and 280 nm (band I).[13,28] The former absorption band precludes a far-UV CD analysis usually used to 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 the styryl moiety to the vacant orbital of carbonyl group in the Δ^ZPhe residue.[46,47] The transition moment was estimated from molecular orbital calculations to lie on the styryl−carbonyl line.[28] By applying the exciton chirality method[27] to the present system, the sign of a split CD pattern with a positive peak at longer wavelengths corresponds to a left-handed helical arrangement of the transition moment, namely to a left-handed helical segment, and the sign with a negative peak at longer wavelengths corresponds to a right-handed helical segment. This assignment has also been used for 3_{10}-helical peptides containing Δ^ZPhe-X-Δ^ZPhe unit(s).[1,2,5,13,23−25,28,33] Moreover, according to theoretical CD calculations on Δ^ZPhe-containing peptides,[28,31,40] the sign of exciton couplets at band I does not change with strict (ϕ,ψ) values but depends on the sign of (ϕ,ψ) values. Namely, theoretical CD spectra of exciton couplets with a negative peak at longer wavelengths were obtained at band I for Boc-(Aib-Δ^ZPhe)$_2$-Aib-OMe in five right-handed 3_{10}-type (4 → 1 hydrogen-bonded) helices (1)−(5) and in three right-handed α-type (5 → 1 hydrogen-bonded) helices (6)−(8): (1) $\phi = -44°$, $\psi = -33°$;[48] (2) $\phi = -54°$, $\psi = -28°$;[49] (3) $\phi = -71°$, $\psi = -18°$;[50] (4) $\phi = -53°$, $\psi = -36°$;[51] (5) $\phi = -60°$, $\psi = -30°$;[36,37] (6) $\phi = -53°$, $\psi =$

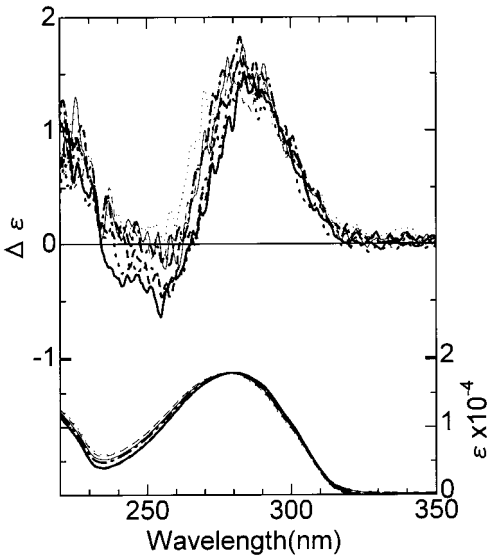

Figure 3. CD (top) and UV absorption (bottom) spectra of peptide **1** in acetonitrile at −30 (bold —), −20 (bold - - -), −10 (bold ···), 0 (- · -), 10 (—), 20 (- - -), and 40 °C (···).

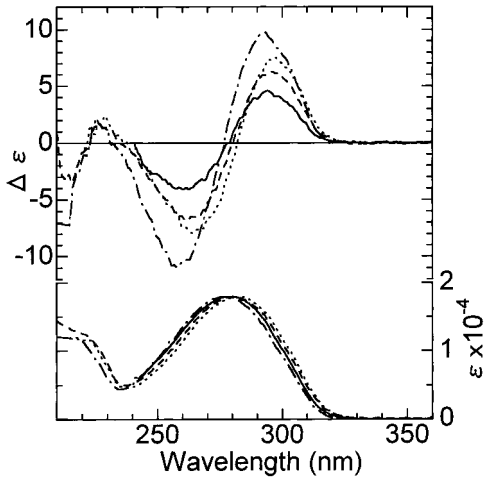

Figure 4. CD (top) and UV absorption (bottom) spectra of peptide **2** in chloroform (—), acetonitrile (- - -), methanol (···), and THF (- · -).

−52°;[52] (7) $\phi = -57°$, $\psi = -47°$;[53] (8) $\phi = -63°$, $\psi = -42°$.[54] Conversely, for the corresponding eight left-handed helices (ϕ, ψ), theoretical CD spectra of exciton couplets with a positive peak at longer wavelengths were obtained.

Therefore, the main chain of peptide **1** should prefer a right-handed helix in chloroform and a left-handed one in methanol and THF. In acetonitrile, peptide **1** showed no marked exciton couplets, but a positive signal, suggesting the small difference between contents of left- and right-handed helices. Figure 3 shows the temperature dependence on CD spectra of peptide **1** in acetonitrile. The spectra did not change with temperatures (20 to −30 °C) essentially, although the splitting sign characteristic of a left-handed screw sense seemed to appear at −30 °C.

On the other hand, peptide **2** taking a 3_{10}-type helix showed exciton couplets with a positive peak at longer wavelengths in all the solvents used above as shown in Figure 4. This indicates that the helical segment of peptide **2** prefers a left-handed screw sense. On the basis of a comparison between peptides **1** and **2** in helical

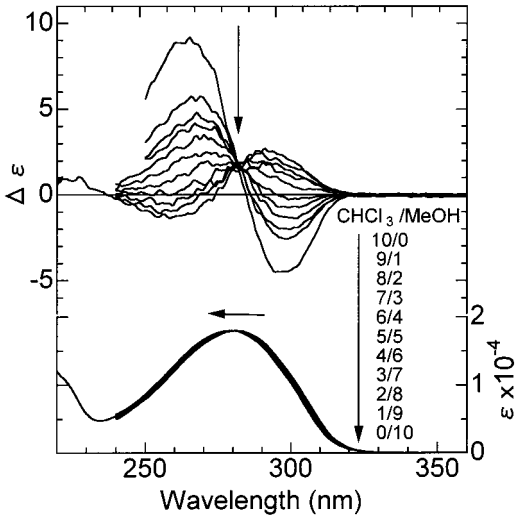

Figure 5. CD (top) and UV absorption (bottom) spectra of peptide **1** in chloroform−methanol (MeOH) mixtures of varying compositions (v/v %).

screw sense, the L-Leu residue second from the N-terminal position was found to induce both left- and right-handed screw senses, depending on types of solvents. On the other hand, an N-terminal L-Leu residue induces a left-handed screw sense preferentially, irrespective of types of solvents.[23−25] Consequently, an L-Leu residue second from the N-terminal position should induce both helical screw senses due to small energy difference between left- and right-handed helical conformations.

Figure 5 shows the solvent dependence on CD spectra for peptide **1** in chloroform−methanol mixtures of varying compositions (v/v %). With increasing methanol compositions, the preference of a helical screw sense changed from right- to left-handed screw senses. Conversely, with increasing chloroform compositions, the preference changed from left- to right-handed screw senses. In these CD spectra, an isodichronic point was seen around 282 nm, indicating that peptide **1** should compose of two conformations in chloroform−methanol mixtures, i.e., left- and right-handed helices. Such reversible inversion was rarely found in oligopeptides: e.g., Boc-L-Ala-Δ^ZPhe-Gly-Δ^ZPhe-L-Ala-OMe[5,13] and Boc-L-Val-Δ^ZPhe-Gly-Δ^ZPhe-L-Val-OMe[22] showed reversible screw sense inversion of the 3_{10}-helix, depending on solvent or temperature conditions. Also, Figure 6 shows the solvent dependence on CD spectra for peptide **1** in chloroform−acetonitrile mixtures of varying compositions (v/v %). With increasing acetonitrile compositions, the preference of a screw sense changed from right-handed to both screw senses. The solvent dependence on the preference of a screw sense and the appearance of an isodichronic point around 282 nm were also seen in chloroform−acetonitrile mixtures. Thus, peptide **1** showed reversible screw sense inversion, depending on solvent conditions.

The screw sense inversion seemed to somewhat depend on the peptide length. As a preliminary experiment, CD spectra were measured for Boc-Aib-L-Leu-(Aib-Δ^ZPhe)$_3$-Aib-OMe **3** having a longer achiral segment than peptide **1**. Peptide **3** showed a right-handed screw sense in chloroform and in tetrahydrofuran and a left-/right-handed one in acetonitrile and in methanol. However, the preference of a left-handed screw sense was not markedly seen in peptide **3**, unlike in peptide

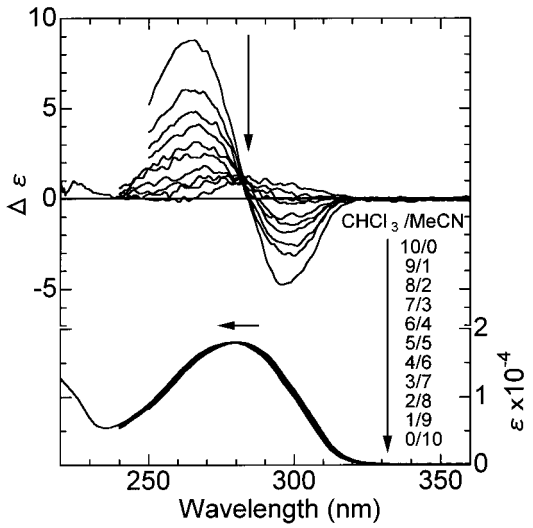

Figure 6. CD (top) and UV absorption (bottom) spectra of peptide **1** in chloroform−acetonitrile (MeCN) mixtures of varying compositions (v/v %).

Table 2. Energy-Minimized Conformations for Segment -Aib-L-Leu- in Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe in a Standard 3_{10}-Helix[a]

conformational letter code[b]	Aib		Leu		helical screw sense[c]	ΔE_{res}[d], kcal mol^{-1}
	ϕ	ψ	ϕ	ψ		
AA	−57	−35	−67	−50	RH	
A*A*	56	34	57	63	LH	0.17
A*A*	56	35	58	60	LH	0.22
AA	−58	−33	−69	−50	RH	0.22
A*C	53	47	−64	109	LH	0.28
A*A*	56	34	59	62	LH	0.36
AA	−57	−35	−68	−50	RH	0.36
A*A*	56	34	60	60	LH	0.37
AA	−57	−35	−68	−49	RH	0.37
A*A*	55	33	57	64	LH	0.38

[a] Energy minimization was carried out with 10 variables in segment -Aib-L-Leu- (ϕ_{Aib}, ψ_{Aib}, $\chi^{1,1}{}_{Aib}$, $\chi^{1,2}{}_{Aib}$, ϕ_{Leu}, ψ_{Leu}, $\chi^1{}_{Leu}$, $\chi^2{}_{Leu}$, $\chi^{3,1}{}_{Leu}$, $\chi^{3,2}{}_{Leu}$), while the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe is fixed to a standard left- or right-handed 3_{10}-helix: (60°, 30°) or (−60°, −30°),[32,33] respectively. [b] For segment -Aib-L-Leu-. [c] LH and RH represent left- and right-handed helical screw senses for segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe, respectively. [d] $\Delta E_{res} = (E - E_0)/7$. E_0 is the lowest energy.

1. The peptide length effect is under detailed investigation.

Conformational Energy Calculation. In the preceding section, it should be concluded that an L-Leu residue second from the N-terminal position induces left-handed as well as right-handed helical screw senses. Table 2 shows energy-minimized conformations of the segment -Aib-L-Leu- in Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe, in which the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe is fixed to a standard left- or right-handed 3_{10}-helix, and lists from the lowest-energy to tenth low-energy conformations. ΔE_{res} is the energy difference per residues from the lowest energy. In Table 2, Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe showed the right-handed helix for the lowest-energy conformation, while Ac-L-Leu-(Aib-Δ^Z-Phe)$_2$-Aib-OMe did the left-handed helix for the lowest-energy conformation in Table 3. Also, four right-handed helices were present among 10 conformers ($\Delta E_{res} \leq 0.38$ kcal mol^{-1}) in Table 2, while three right-handed ones were present among 10 conformers ($\Delta E_{res} \leq 0.42$ kcal mol^{-1}) in Table 3. Obviously, the L-Leu residue second from the N-terminal position has more marked preference of a right-handed screw sense than the N-terminal L-Leu residue. However, Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe should take both left- and right-handed screw helices due to four right- and six left-handed helices in $\Delta E_{res} \leq 0.38$ kcal mol^{-1} as shown in Table 2. This small energy difference between left- and right-handed helices will be ascribed to reversible screw sense inversion for peptide **1**, as described in the preceding section.

Table 3. Energy-Minimized Conformations for L-Leu Residue in Ac-L-Leu-(Aib-Δ^ZPhe)$_2$-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	φ		
C	−64	114	LH	
A	−65	−53	RH	0.15
A*	56	69	LH	0.25
C	−78	101	RH	0.27
C	−66	119	LH	0.28
A*	56	67	LH	0.36
C	−81	83	LH	0.37
A	−71	−52	LH	0.39
C	−76	95	LH	0.40
D	−148	102	RH	0.42

[a] Energy minimization was carried out with six variables in L-Leu residue (ϕ_{Leu}, ψ_{Leu}, $\chi^1{}_{Leu}$, $\chi^2{}_{Leu}$, $\chi^{3,1}{}_{Leu}$, $\chi^{3,2}{}_{Leu}$), while the segment -(Aib-Δ^ZPhe)$_2$-Aib-OMe is fixed to a standard left- or right-handed 3_{10}-helix: (60°, 30°) or (−60°, −30°).[32,33] [b] For L-Leu residue. [c] LH and RH represent left- and right-handed helical screw senses, respectively. [d] $\Delta E_{res} = (E - E_0)/6$. E_0 is the lowest energy.

Figures 7 and 8 show the main-chain energy contour map (ϕ, ψ) of the L-Leu residue in Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe and Ac-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe, respectively. In both peptides, the moiety except for the L-Leu residue was fixed to the lowest-energy left- or right-handed helix shown in Table 2 or 3. For each (ϕ, ψ), the side chain of the L-Leu residue ($\chi^1{}_{Leu}$, $\chi^2{}_{Leu}$) was taken as the value that gives the minimal conformational energy on changing $\chi^1{}_{Leu}$ and $\chi^2{}_{Leu}$ at 5° in −180° to +180°. The conformational space (ϕ, ψ) of the L-Leu residue was more restricted to the left- or right-handed helical region in Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe, compared with Ac-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe. Namely, the conformational freedom of the L-Leu residue second from the N-terminal position was found to decrease much more than that of the N-terminal L-Leu residue.

As shown in Table 3, the N-terminal L-Leu residue in the lowest-energy left-handed helix took a conformational letter code of "C", but not of "A*",[39] which corresponds to a region for the left-handed 3_{10}- or α-helical conformation. Here the -Leu(1)-Aib(2)- sequence forms a type II-like β-turn conformation supported by hydrogen bonding between C═O (Boc) and NH [Δ^ZPhe(3)]. The β-turn ($\phi_{Leu} = -64°$, $\psi_{Leu} = 114°$, $\phi_{Aib} = 60°$, and $\psi_{Aib} = 30°$) is somewhat deviant from the standard one ($\phi_{Leu} = -60°$, $\psi_{Leu} = 120°$, $\phi_{Aib} = 80°$, and $\psi_{Aib} = 0°$).[55] Consequently, the N-terminal L-Leu residue is not incorporated into a left-handed helical conformation (A*) but takes an irregular conformation (C) that deviates from a left-handed helix.[24] On the other hand, the L-Leu residue second from the N-terminal position in the lowest-energy left- or right-handed helix took a conformational letter code of "A*" or "A", which corresponds to a region for left- or right-handed 3_{10}-/α-helical conformations, respectively. It should be noted that the L-Leu residue second from the N-terminal position is incorporated into a left (A*)- or right (A)-handed helix, unlike the N-terminal L-Leu residue. These results of

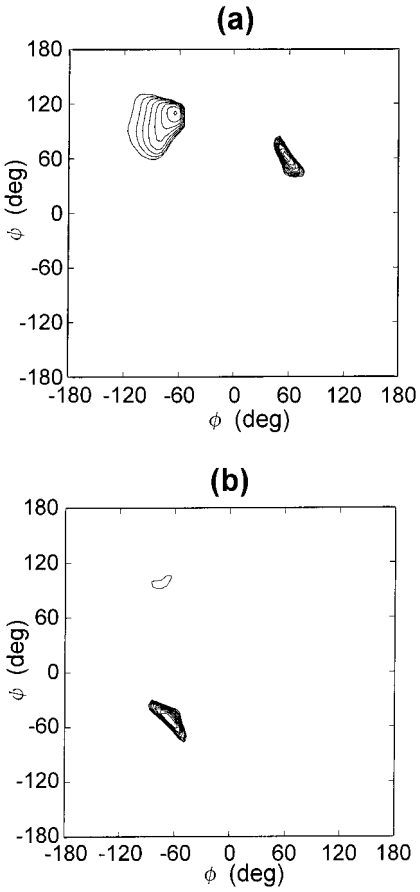

Figure 7. Main-chain energy contour map (ϕ, ψ) of L-Leu residue for Ac-Aib-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe in the lowest-energy **(**a**)** left- or **(**b**)** right-handed helix in Table 2. The contours are drawn in 0.5 kcal mol^{-1} increments from the minimum points **(**a**)** (55°, 65°) and **(**b**)** (−65°, −50°) to 5 kcal mol^{-1}.

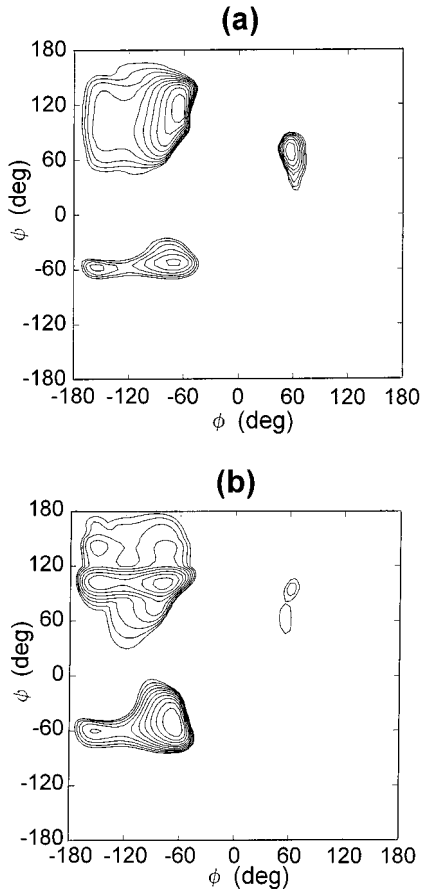

Figure 8. Main-chain energy contour map (ϕ, ψ) of L-Leu residue for Ac-L-Leu-(Aib-Δ^ZPhe)$_2$-Aib-OMe in the lowest-energy **(**a**)** left- or **(**b**)** right-handed helix in Table 3. The contours are drawn in 0.5 kcal mol^{-1} increments from the minimum points **(**a**)** (−65°, 115°) and **(**b**)** (−65°, −55°) to 5 kcal mol^{-1}.

calculation might be supported by NMR data. In the lowest-energy left-handed helical conformation shown in Table 3, the distance of Leu(1) C$^\alpha$H-Aib(2) NH is very small (2.2 Å). Actually, a remarkable NOE signal of 7.9% was observed for the proton pair of peptide **2** in $CDCl_3$ at 40 °C.[24] On the other hand, the distance of Leu(2) C$^\alpha$H-Aib(3) NH in the lowest-energy right-handed helical conformation in Table 2 is larger (3.5 Å). Correspondingly, the NOE signal for the proton pair in peptide **1** was much smaller (1.6%) as shown in Table 1.

The preferred screw sense of 3_{10}-helical peptides has been investigated by other groups[20,56,57] systematically using four helicogenic achiral Aib residues. Peptide *p*BrBz-L-Pro-D-Ala-(Aib)$_4$-O*t*Bu (*p*BrBz, *p*-bromobenzoyl; O*t*Bu, *t*-butoxy) in the solid state took a type II β-bend conformation in the -L-Pro-D-Ala-, followed by a left-handed 3_{10}-helix spanning the sequence.[56] Conversely, *p*BrBz-D-Pro-D-Ala-(Aib)$_4$-O*t*Bu adopted a distorted type II′ β-bend conformation in the -D-Pro-D-Ala-, which induces a right-handed 3_{10}-helix in the following -D-Ala-(Aib)$_3$- sequence.[56] Also, N$^\alpha$-blocked pentapeptide esters containing four Aib residues and one chiral L-Val or C$^\alpha$-methyl-L-Val residue in the C-terminal position of the sequence took a left-handed screw sense in solution.[20] In the solid state, the pentapeptides adopted a right-handed screw sense for the C-terminal L-Val residue and both right- and left-handed screw senses for the C$^\alpha$-methyl-L-Val residue.[57] On the other hand, a right-handed 3_{10}-helix in solution was taken for a variety of N$^\alpha$-blocked pentapeptide esters containing four Aib residues and one chiral L-Val or C$^\alpha$-methyl-L-Val residue in the N-terminal or internal (third form the N-terminal) position of the sequence, i.e., Bz-Y-(Aib)$_4$-O*t*Bu or Bz-(Aib)$_2$-Y-(Aib)$_2$-O*t*Bu (Bz, benzoyl or *p*-substituted benzoyl; Y, L-Val or C$^\alpha$-methyl-L-Val residue).[20]

These results differ from our present results essentially in two points. First, the N-terminal Leu residue in peptide **2** induces a left-handed screw sense, while the N-terminal L-Val or C$^\alpha$-methyl-L-Val residue (Y) in Bz-Y-(Aib)$_4$-O*t*Bu induces a right-handed screw sense. Second, the effect of the shift of the L-Leu residue from the N-terminal to second position in our present sequence on screw sense was dramatic, while a right-handed screw sense was observed in both the N-terminal and internal positions in the pentapeptides containing four Aib residues. Our conformational energy calculations (in Tables 2 and 3 and Figures 7 and 8) seem to support the dramatic difference between the N-terminal and second positions in a screw sense. Here the stable conformation and conformational freedom of the L-Leu residue in the second position differed from those in the N-terminal position dramatically, as described above.

However, for the present, we cannot explain the discrepancy unambiguously, while the discrepancy might be ascribed to the difference in chemical structures of achiral helical segments: i.e., -(Aib-Δ^ZPhe)$_2$-Aib- for our

present study and -(Aib)$_4$- for the other groups' studies.[20,56,57] To clarify the reason, a number of screw sense data about helical peptides with varying types of residues, sequences, and chain lengths should be accumulated. However, the screw sense inversion was found in peptide **1** containing the achiral helical segment -(Aib-Δ^ZPhe)$_2$-Aib- and one L-Leu residue second from the N-terminal position.

Conclusions

In the present paper, we focused on which helical screw sense is preferred in peptide **1** having one L-Leu residue introduced into the position second from the N-terminal of the achiral helical segment. The L-Leu residue induces both left- and right-handed screw senses preferentially, depending on types of solvents. The preference of a helical screw sense changed reversibly, with varying solvent compositions. This screw sense inversion was not observed for peptide **2** having one L-Leu residue in the N-terminal position of the achiral helical peptide. From conformational energy calculations, the L-Leu residue second from the N-terminal position was found to induce both screw senses due to the small energy difference between left- and right-handed helices.

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