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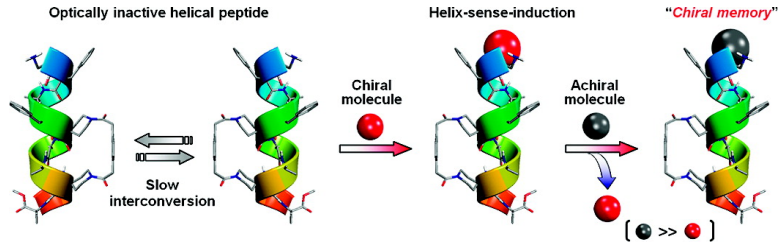

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Chain-Terminus Triggered Chiral Memory in an Optically Inactive 3_{10}-Helical Peptide

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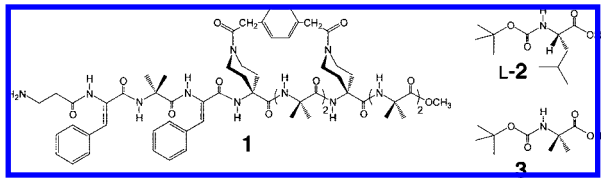
Control of screw sense of helical polymers and oligomers that are composed of an achiral backbone by covalent or noncovalent chiral interactions has been developed mostly in the past two decades.[1] In 1999, Yashima and his co-workers demonstrated that external chiral stimuli induced helical sense in a dynamically optically inactive helical polymer and the induced macromolecular helicity was maintained in a solution for a long period of time after the complete replacement of the chiral molecule with an achiral one ("chiral memory").[2a] Such a memory system in helical molecules has so far been limited to artificial polymeric backbones (polyacetylene[2a−c] and polyisocyanide[2d]), although there have been many reports on supramolecular systems.[3]

Herein, we report "chiral memory" of an optically inactive 3_{10}-helical peptide[4] which is composed of only achiral amino acids and possesses a chiral recognition site at the N-terminal region with a single intramolecular side-chain cross-linking.

A N-terminal free decapeptide, H-β-Ala-Δ^ZPhe-Aib-Δ^ZPhe-Api-Aib_2-Api-Aib_2-OMe with the side-chain cross-linking between Api[5] residues (**1**) [Δ^ZPhe = (*Z*)-α,β-dehydrophenylalanine, Aib = α-aminoisobutyric acid, Api = 4-aminopiperidine-4-carboxylic acid, OMe = methoxy], was designed based on the following facts. (i) C^α-Tetrasubstituted α-amino acids such as Aib and Api residues promote a helical structure strongly.[4,5] (ii) Noncovalent multipoint interaction between the N-terminal segment H-β-Ala-Δ^ZPhe-Aib-[6] in 3_{10}-helix and an N-protected amino acid preferentially induces a one-handed helix in the achiral peptide chain (termed as a "noncovalent chiral domino effect"[6a,b,7]).[8] (iii) Absorption band of Δ^ZPhe residues ($\lambda_{max} \sim 280$ nm) is located away from those of the usual solvents and peptide bond, and the residues in every other place enable us to identify induced helicity.[6,7,9] (iv) The side-chain cross-linking[9] of Api residues at *i* and *i* + 3 in a 3_{10}-helix stabilizes the entire structure and decelerates the helix inversion of the optically inactive 3_{10}-helical peptide.[10c,d]



The solution structure of **1** was investigated by a ^{1}H NMR technique and additionally by computational analysis. Variable temperature ^{1}H NMR spectra of **1** in $CDCl_3$ are shown in Figure S2. The two amide NH resonances (8.09 and ~9.76 ppm at 20 °C) depend heavily on temperature.[11] One [Aib(3)] of the two resonances shows a significant downfield shift with decreasing temperature, and the other [Δ^ZPhe(2)] becomes sharp at lower temperatures but disappears at higher temperatures.[11] Chemical shift of the former NH is sensitive to DMSO-d_6 percentage in $CDCl_3$ solution (Figure S3). These data suggest the lack of intramolecular H-bonding for the two protons.[13] The structure of **1** optimized by semiempirical MO calculation (AM1 method[14] in MOPAC2007) afforded a typical 3_{10}-helical conformation; the averaged $|\phi|/|\Psi|$ torsion angles for Δ^ZPhe(2) to Aib(9) were |50|°/|33|° (Figure 1). Additionally, the side-chain constraint in peptide **1** promotes the 3_{10}-helical structure and destabilizes an α-helix due to severe strain.[10c]

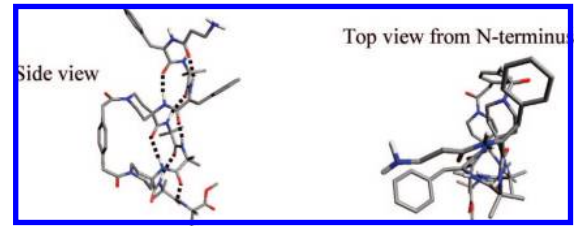


Figure 1. Side and top views of the right-handed structure of **1** as energy-minimized by the semiempirical MO calculation (AM1 method[14]). Hydrogen atoms except for the amino and amide protons were omitted for clarity. Dotted lines represent intramolecular hydrogen bonds.[15]

All the proton signals of the piperidine rings of **1** are very sharp and distributed over a wide chemical-shift range, because the motion of the two piperidine rings is constrained (Figure S2).[10c] Both methylene protons of $-CH_2-C_6H_4-CH_2-$ (~3.6−3.8 ppm) in the bridging moiety show diastereotopic splitting. Moreover, N-terminal α, β-methylene protons of the β-Ala residue (~2.4 and 3.0 ppm) are observed individually (Figure S2), as assigned by a 2D TOCSY spectrum (see Figure S4), although they are placed distant from the bridging region. Observation of these diastereotopic protons indicates peptide **1** as a whole exhibits slow helix inversion.

The optically inactive peptide **1** shows no CD signal in achiral media; however, the copresence of Boc-L-Leu-OH (L-**2**, Boc = *tert*-butoxycarbonyl) in the chloroform solution induced a split CD pattern around 286 nm as shown in Figure 2A.[16,17] This is assignable to the Δ^ZPhe residues adopting a right-handed helix.[6,7,9] The mirror image CD corresponding to a left-handed helix was observed in the presence of D-**2** (Figure 2A). A small amount (30 μL) of the chloroform solution containing **1** and L-**2** as described above was added to a solution (770 μL) of excess Boc-Aib-OH (**3**), an achiral guest, at 20 °C, and the time dependence of the CD intensity of **1** at 271 nm was measured (Figure 2B). Interestingly, under a large excess of **3** ([**3**]/[L-**2**] = ca. 445/1), the induced CD signal slowly reduced its intensity and reached an undetectable level only after ca. 20 min (Figure 2B). The apparent binding constant

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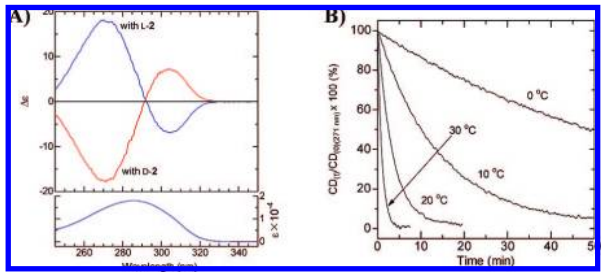

Figure 2. (A) CD (top) and absorption (bottom) spectra of **1** in the presence of L-**2** (blue line) or D-**2** (red line) in chloroform at room temperature; [**1**] = 0.94 mM and [**2**] = 6.0 mM. $\Delta\epsilon$ and ϵ were expressed with respect to the molar concentration of Δ^ZPhe residues. Solutions were left to stand for over 30 min at room temperature prior to measurements to reach an equilibrium state. (B) Time dependence of the CD intensity at 271 nm of **1** in the presence of L-**2** and large excess of **3** at 0, 10, 20, and 30 °C; [**1**] = 3.5 × 10^{-2} mM, [L-**2**] = 0.23 mM, [**3**] = 100 mM, [**3**]/[L-**2**] = ca. 445/1.

Table 1. Pseudo Rate Constant (k_{rac}, s^{-1}) and Half-Life Time ($t_{1/2}$, min) for the Racemization of Complex **1·3**[a]

temp (°C)	k_{rac} (s^{-1})	$t_{1/2}$ (min)[b]
0	1.17×10^{-4}	49.3
10	5.46×10^{-4}	10.6
20	2.32×10^{-3}	2.5
30	8.29×10^{-3}	0.7

[a] Conditions: in chloroform, [**1**] = 3.5 × 10^{-2} mM, [L-**2**] = 0.23 mM, [**3**] = 100 mM, [**3**]/[L-**2**] = ca. 445/1. [b] $t_{1/2}$ (min) = ln 2/($2k_{rac}$ · 60).

(K_{app}, M^{-1}) of **1** to **3** was estimated to be similar to that of **1** to L-**2** by competition experiments.[18] In this condition, contribution of L-**2** to the decay of the CD intensity is almost negligible, and the complexation between **1** and **3** (**1·3**) is judged to be complete based on the K_{app} value. Additionally, the rate of guest−guest exchange in the complex is faster than the NMR time scale even at low temperature (−10 °C).[7c] Thus, there is no attribute of the slow exchange between **1·**L-**2** and **1·3** complexes to the decay of the CD intensity. Consequently, the racemization of the **1·3** complex obeys pseudofirst-order kinetics in this condition.

The pseudofirst-order rate constants (k_{rac}, s^{-1}) and half-life time for the racemization of the **1·3** complex at several temperatures (0, 10, 20, and 30 °C) were estimated by linear regression analysis for the initial slope (r^2 = 0.999) (Table 1). It is surprising that k_{rac} of the **1·3** complex is 10^5 times smaller than the related 3_{10}-helical decapeptide Z-Aib$_{10}$-O*t*Bu (Z = benzyloxycarbonyl, O*t*Bu = *tert*-butoxy).[19] Arrhenius and Eyring plots of the kinetic data from Table 1 provide the following thermodynamic parameters (Figure S7), E_a = 97.7 kJ mol^{-1}, $\Delta G^{\ddagger}_{20}$ = 86.6 kJ mol^{-1}, $\Delta H^{\ddagger}$ = 95.4 kJ mol^{-1}, and $\Delta S^{\ddagger}$ = 30.0 J mol^{-1} K^{-1}. A positive value for the entropy of activation indicates the transition state of the helix reversal process is more disordered than the folded state. The slow process and high barrier of helix inversion originate from the severe strain in the bridging region.[20] The obtained parameters suggest that the induced helicity is preserved for ∼1 week at −30 °C (half-life time).

In summary, we have demonstrated the first example of a "chiral memory" system in a helical oligomer, which is composed of a biological backbone with a single side-chain cross-linking. In this system, noncovalent chiral interaction at the N-terminal region leads to modulation of the original chain chirality, and the induced helix sense preference is stored in the peptide backbone assisted by the side-chain cross-linking. We are currently studying the effects of peptide main-chain length, solvents, and an additional cross-linking on the rate of helix inversion to achieve a complete memory of induced helicity (asymmetric synthesis) in peptide helices.

Supporting Information Available: Synthetic procedure, characterization, and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Similar broadening was also observed in N-terminal free peptide possessing Δ^ZPhe residue at the second position from the N-termimus.[6,7]

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(16) The solution stood for over 30 min at room temperature before CD measurement to establish an equilibrium state.

(17) The apparent binding constant (K_{app}, M^{-1}) of **1** for L-**2** was estimated to be 2.3 × 10^3 M^{-1}, which is obtained from nonlinear fitting of the corresponding CD data (1:1 binding model[6a]) (Figure S5).

(18) Induced CD intensity of **1** with equimolar mixture of L-**2** and **3** was about one-half the value as compared with that of **1** with L-**2** (Figure S6).

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(20) In methanol/chloroform (77/3; v/v) mixed solution which prevents interaction between **1** and L-**2**, kinetic and thermodynamic parameters of **1** were also obtained; E_a = 93.4 kJ mol^{-1}, $\Delta G^{\ddagger}_{20}$ = 85.1 kJ mol^{-1}, $\Delta H^{\ddagger}$ = 91.1 kJ mol^{-1}, and $\Delta S^{\ddagger}$ = 20.5 J mol^{-1} K^{-1} (Figures S8−S9 and Table S1). These values are comparable to those of the complex **1·3**. Thus, there is little effect of guest binding on the rate of helix inversion of **1**.

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