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Induction of γ-Turn-Like Structure in Ferrocene Bearing Dipeptide Chains via Conformational Control

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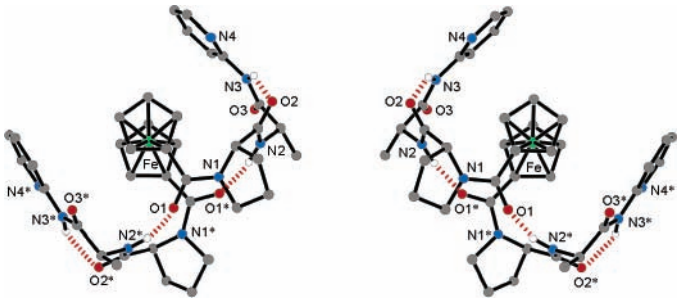
ABSTRACT



A combination of the ferrocene scaffold as a central reverse-turn unit with the dipeptide chains (-L-Pro-L-Ala-NHPy) was demonstrated to induce both inverse γ-turn-like and antiparallel β-sheet-like structures. Only the antiparallel β-sheet-like structure was formed in the ferrocene bearing the heterochiral dipeptide chains (-L-Pro-D-Ala-NHPy), in which highly organized self-assembly was achieved through a network of intermolecular hydrogen bonds.

Highly ordered molecular assemblies are present in proteins that fulfill various functions as observed in enzymes, receptors, etc., which stimulate the construction of bio-inspired systems by utilizing self-assembling properties of short peptides. Turns are a key structural element in three-dimensional peptide structures.[1] Although considerable efforts have been devoted to design β-turn mimics,[2,3] γ-turn mimicry has attracted less attention.[2b,3b,h−i,4] Recently, the research field of bioorganometallic chemistry, which is a hybrid area between biochemistry and organometallic chemistry, has received extensive interest. Conjugation of organometallic compounds with biomolecules such as amino acids and peptides has been used to design bioconjugates.[5] Ferrocenes are recognized as organometallic scaffolds with potential to mimic the central reverse-turn unit because the inter-ring spacing of ferrocene is about 3.3 Å, which is appropriate for hydrogen bonding of the attached peptide strands as observed in β-sheets. A variety of ferrocene-peptide bioconjugates have been designed to induce ordered structures and develop new biomaterials.[6−8] We have already demonstrated that the introduction of dipeptide chains

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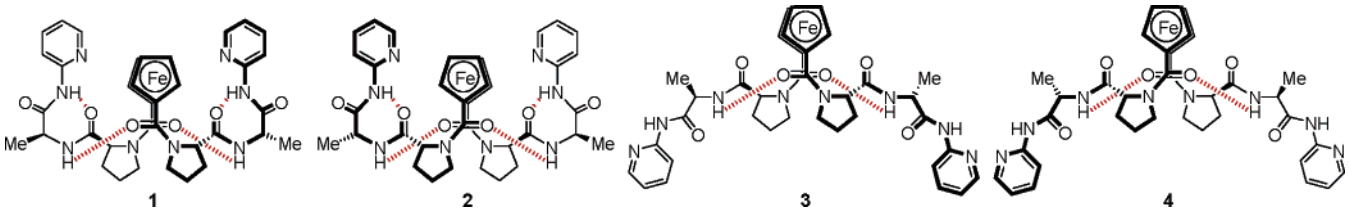

Figure 1. Ferrocenes **1**−**4** bearing the dipeptide chains (-Pro-Ala-NHPy).

(-L-Ala-L-Pro-) onto the ferrocene scaffold permits the formation of intramolecular antiparallel β-sheet-like hydrogen bonds between the NH of the Ala and the CO of the Ala of the opposite peptide chain to induce secondary structures.[7a−c,e,g,h] The sequence and configuration of the amino acids are considered to contribute to the organization of ordered hydrogen bonding. We herein report the effect of the configuration of the dipeptide chains on the organization of the templated structure, which adopted both inverse γ-turn-like and antiparallel β-sheet-like structures when L-Pro-L-Ala-NHPy was introduced onto the ferrocene scaffold as a central reverse-turn unit (Figure 1).

The advantage in the use of L-prolyl-L-alanine as a dipeptide chain depends on a sterically constrained proline as a well-known turn inducer in proteins and a hydrogen bonding alanyl moiety. The ferrocenes **1** and **2** bearing the dipeptide chains L-Pro-L-Ala-NHPy and D-Pro-D-Ala-NHPy were synthesized from 1,1′-bis(chlorocarbonyl)ferrocene and the corresponding dipeptide derivative. X-ray crystallographic analyses were performed in order to determine the conformation of the ferrocene-peptide conjugates **1** and **2**.[9] The single-crystal X-ray structure determination of the ferrocene **1** bearing L-Pro-L-Ala-NHPy revealed that the NH adjacent to the pyridyl moiety participated in an intramolecular hydrogen bond with the CO of the Pro of the same peptide chain (N(3)···O(2), 3.032 Å; N(3*)···O(2*), 2.996 Å) to nucleate a γ-turn-like structure in each dipeptide chain (Figure 2). The torsion angles ϕ_2 ($\phi_2 = -90.5°$ and $\phi_2* = -89.5°$) and ψ_2 ($\psi_2 = 60.9°$ and $\psi_2* = 58.4°$) of **1** indicated an inverse γ-turn-like structure similar to an ideal inverse γ-turn ($\phi_2 = -70°$ to $-85°$ and $\psi_2 = 60°$ to 70°). Another remarkable feature of the structure was interchain intramolecular antiparallel β-sheet-like hydrogen bonding between the NH of the Ala and the CO of the ferrocene unit attached to the opposite peptide chain (N(2)···O(1*), 2.978 Å; N(2*)···O(1), 2.836 Å). The ferrocene scaffold acts as a central reverse turn. The combination of the ferrocene scaffold as a central reverse-turn unit with the L-Pro-L-Ala-NHPy dipeptide chains permits the formation of an artificial inverse γ-turn-like and antiparallel β-sheet-like structures.

The molecular structure of **2** composed of the D-Pro-D-Ala-NHPy dipeptide chains was in a mirror image relationship with **1**, indicating that **1** and **2** were conformational isomers (Figure 2). The opposite values of the torsion angles of **2** ($\phi_2 = 90.6°$, $\phi_2* = 89.4°$, $\psi_2 = -60.7°$, and $\psi_2* = -57.9°$) as compared with those of **1** was in agreement with conformational isomers. The ferrocenyl moiety was restricted about the Cp(centroid)-Fe-Cp(centroid) axis and the C(ipso)-CO bond because of intramolecular hydrogen bonds between the peptide chains.

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(9) Crystal data for **1**: $C_{38}H_{42}N_8O_6Fe$, $M_r = 762.65$, monoclinic, space group $P2_1$ (No. 4), $a = 10.3173(9)$ Å, $b = 16.659(1)$ Å, $c = 11.241(1)$ Å, $\beta = 110.415(2)°$, $V = 1810.7(3)$ Å^3, $Z = 2$, $T = 23.0$ °C, $D_{calc} = 1.399$ g cm^{-3}, μ(Mo Kα) = 4.75 cm^{-1}, Mo Kα radiation ($\lambda = 0.71069$ Å), $R1 = 0.039$, $wR2 = 0.108$. Crystal data for **2**: $C_{38}H_{42}N_8O_6Fe$, $M_r = 762.65$, monoclinic, space group $P2_1$ (No. 4), $a = 10.2587(6)$ Å, $b = 16.5151(9)$ Å, $c = 11.203(1)$ Å, $\beta = 110.663(3)°$, $V = 1776.0(2)$ Å^3, $Z = 2$, $T = 4.0$ °C, $D_{calc} = 1.426$ g cm^{-3}, μ(Mo Kα) = 4.84 cm^{-1}, Mo Kα radiation ($\lambda = 0.71069$ Å), $R1 = 0.040$, $wR2 = 0.117$. Crystal data for **3**: $C_{38}H_{42}N_8O_6Fe$, $M_r = 762.65$, orthorhombic, space group $P2_12_12$ (No. 18), $a = 12.5539(6)$ Å, $b = 15.1712(9)$ Å, $c = 9.5405(4)$ Å, $V = 1817.1(3)$ Å^3, $Z = 4$, $T = 4.0$ °C, $D_{calc} = 2.788$ g cm^{-3}, μ(Mo Kα) = 9.47 cm^{-1}, Mo Kα radiation ($\lambda = 0.71069$ Å), $R1 = 0.061$, $wR2 = 0.162$. Crystal data for **4**: $C_{38}H_{42}N_8O_6Fe$, $M_r = 762.65$, orthorhombic, space group $P2_12_12$ (No. 18), $a = 12.5476(4)$ Å, $b = 15.1704(7)$ Å, $c = 9.5407(3)$ Å, $V = 1816.1(2)$ Å^3, $Z = 4$, $T = 4.0$ °C, $D_{calc} = 2.789$ g cm^{-3}, μ(Mo Kα) = 9.47 cm^{-1}, Mo Kα radiation ($\lambda = 0.71069$ Å), $R1 = 0.062$, $wR2 = 0.160$.

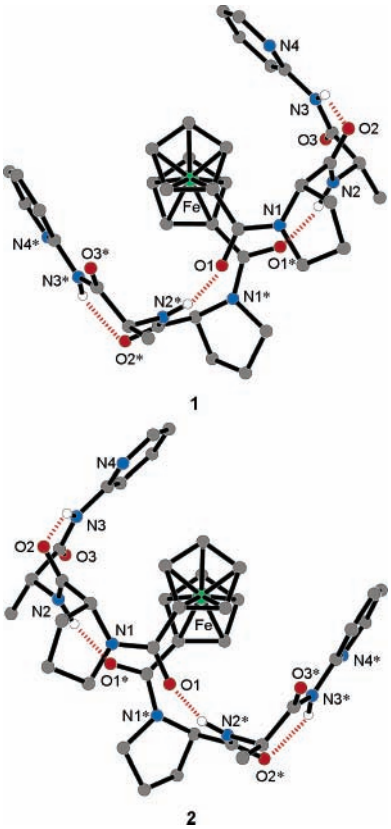

Figure 2. X-ray structures of **1** and **2**.

To evaluate the effect of the diastereomeric dipeptide configurations, ferrocenes **3** and **4** bearing the dipeptide chains L-Pro-D-Ala-NHPy and D-Pro-L-Ala-NHPy were made using a similar strategy as mentioned above. In the X-ray structure of ferrocene **3**[9] bearing L-Pro-D-Ala-NHPy, interchain intramolecular antiparallel β-sheet-like hydrogen bonds were observed between the NH of the Ala and the CO of the ferrocene unit attached to the opposite peptide chain (N(2)···O(1*), 2.928 Å; N(2*)···O(1), 2.928 Å), similar to as observed in the structure of **1** (Figure 3). In contrast to **1**, the NH adjacent to the pyridyl moiety participated in an intermolecular hydrogen bond with the CO of the Pro of the neighboring molecule (N(3)···O(2a*), 3.012 Å; N(3a*)···O(2), 3.012 Å; N(3*)···O(2b), 3.012 Å; N(3b)···O(2*), 3.012 Å) to form a 14-membered intermolecularly hydrogen-bonded ring. In the crystal packing, each molecule was connected to two neighboring molecules (Figure 4). The molecular structure of **4**[9] composed of the dipeptide chains (-D-Pro-L-Ala-NHPy) was a mirror image of **3** (Figure 3), indicating that **3** and **4** are conformational isomers. These results indicate that symmetrical introduction of the homochiral dipeptide chains (-L-Pro-L-Ala-NHPy or -D-Pro-D-Ala-NHPy) onto the ferrocene scaffold as a central reverse-turn unit favored an inverse γ-turn-like and antiparallel β-sheet-like structures.

The structures were also studied in solution by ^{1}H NMR, FT-IR, and CD analyses. In the ^{1}H NMR spectra of **1** in

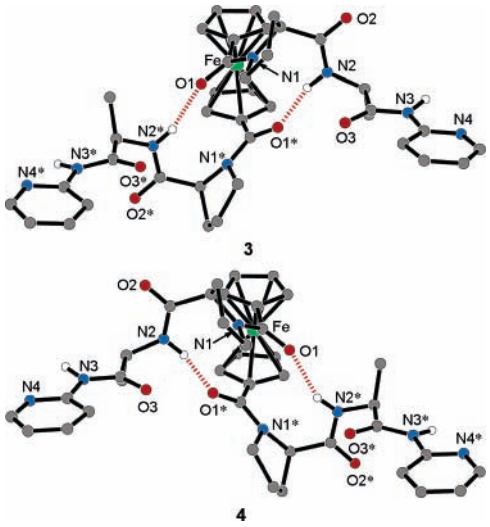

Figure 3. X-ray structures of **3** and **4**.

$CDCl_3$ (1.0×10^{-2} M), only one kind of resonance of the Ala N−H and the N−H adjacent to the pyridyl moiety were detected at a lower field (9.48 and 9.57 ppm). The N−H resonances of **1** were not perturbed by the addition of aliquots of DMSO-d_6 to $CDCl_3$ ($CDCl_3$/DMSO-d_6 (9:1); 9.46 and 9.66 ppm). These results indicate that the amides were present in intramolecular hydrogen bonds in solution. Temperature dependencies ($\Delta\delta/\Delta T$) obtained from variable temperature ^{1}H NMR studies of **1** in DMSO-d_6 (−3.1 ppb/K for Ala N−H and −6.3 ppb/K for the N−H adjacent to the pyridyl moiety) indicated, however, that only antiparallel β-sheet-like hydrogen bonds were formed in DMSO. The FT-IR spectrum of **1** in CH_2Cl_2 (1.0×10^{-2} M) showed only one N−H stretch at 3250 cm^{-1}, which also supported the hydrogen bonding in **1**. Ferrocene **1** exhibited an induced circular dichroism (ICD) at the absorbance region of the ferrocenyl moiety (Figure 5). Furthermore, the mirror-imaged CD signals were obtained in the case of **2**. The intramolecular hydrogen bonds were likely present in solution. The protons of the ferrocenyl moiety of **1** (4.87−4.86, 4.17−4.16, 3.54−3.53, and 3.40−3.38 ppm) were observed at a higher field as compared with **3** (5.04−5.03, 4.61−4.59, 4.35−4.33, and 4.31−4.30 ppm) in the ^{1}H NMR, probably due to the ring-current effect of the pyridine π-ring. This finding supported an inverse γ-turn-like structure as observed in the crystal structure. Proton magnetic resonance nuclear Overhauser effect (NOE) of **1** in $CDCl_3$ at 25 °C also provided diagnostic evidence for this structure. Irradiation of the Cp proton at the β position enhanced the pyridyl protons (Figure S1, Supporting Information). Irradiation of the Cp proton at the α position also enhanced the Ala NH, NH adjacent to the pyridyl moiety, Pro α-CH, and the pyridyl proton at the 3-position (Figure S2, Supporting Information). An inverse γ-turn-like structure was found to be achieved in solution.

On the other hand, ferrocene **3** showed resonances of the Ala N−H and the N−H adjacent to the pyridyl moiety at 9.44 and 8.99 ppm, respectively. A downfield shift of the

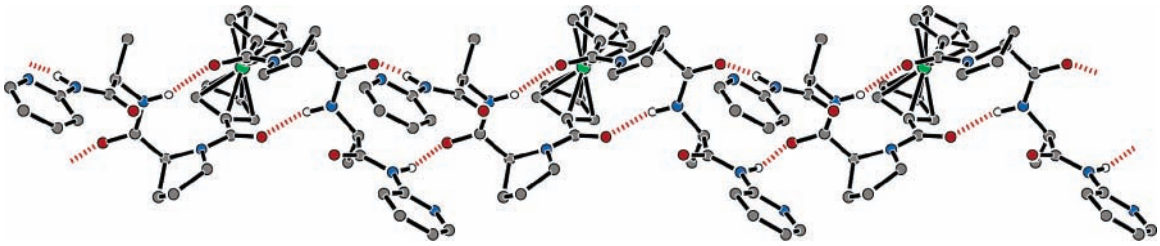

Figure 4. Self-assembly of **3** via the formation of a 14-membered intermolecularly hydrogen-bonded ring in the crystal packing.

resonance of the N−H adjacent to the pyridyl moiety of **3** was observed by the addition of aliquots of DMSO-d_6 to $CDCl_3$ ($CDCl_3$/DMSO-d_6 (9:1); 9.44 ppm). The Ala N−H resonance was not perturbed by addition of DMSO-d_6 ($CDCl_3$/DMSO-d_6 (9:1); 9.34 ppm). Temperature dependencies for the Ala N−H and the N−H of the adjacent amino pyridyl moiety of **3** in DMSO-d_6 were −3.6 ppb/K and −4.7 ppb/K, respectively. The ferrocene **3** exhibited hydrogen-bonded (3257 cm^{-1}) and non-hydrogen-bonded N−H stretching bands (3409 cm^{-1}) in FT-IR. These results indicated that the NH adjacent to the pyridyl moiety did not participate in intramolecular hydrogen bonding in solution as observed in the crystal structure. Ferrocene **3** exhibited an ICD at the absorbance region of the ferrocene moiety, which was in a mirror image relationship with **4** (Figure 5). From these findings, ferrocenes **3** and **4** were likely to form intramolecular hydrogen bonds between the NH of the Ala and the CO of the ferrocene unit attached to the opposite peptide chain.

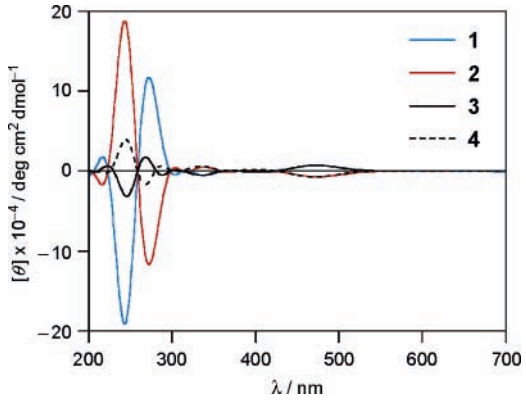

Figure 5. CD spectra of **1**−**4** in dichloromethane (1.0×10^{-4} M).

Compared with the ferrocenes bearing the Ala-Pro-NHPy chains ($E_{1/2}$: Fc(-L-Ala-L-Pro-NHPy)$_2$, 0.28 V; Fc(-L-Ala-D-Pro-NHPy)$_2$, 0.28 V; Fc(-D-Ala-L-Pro-NHPy)$_2$, 0.28 V vs Fc/Fc^+),[7e,i] the ferrocene-peptide conjugates **1**−**4** exhibited a reversible Fc/Fc^+ redox wave at a more positive value ($E_{1/2}$: **1**, 0.35 V; **2**, 0.35 V; **3**, 0.32 V, **4**, 0.32 V vs Fc/Fc^+), probably due to the absence of NH in Pro moieties. The difference of $E_{1/2}$ between **1** and **3** is assumed to be based on the ring-current effect of the pyridine π-ring.

In conclusion, ferrocene-peptide conjugates have been constructed and studied in both the solid and solution state. The combination of the ferrocene scaffold as a central reverse-turn unit with the L-prolyl-L-alanine homochiral sequence as a dipeptide unit was found to induce formation of both inverse γ-turn-like and antiparallel β-sheet-like structures. The configuration of the dipeptide chains played an important role on the creation of an inverse γ-turn-like structure. Self-assembly was observed through a network of intermolecular hydrogen bonds in the ferrocene bearing the heterochiral dipeptide chains (-L-Pro-D-Ala-NHPy), in which an antiparallel β-sheet-like structure was formed. Control of intra- and/or intermolecular hydrogen bond was exhibited in the design of these foldamers.[10] The present architectural control of dimensional structures utilizing minimum-sized peptide chains possessing chiral centers and hydrogen bonding sites is considered to be a useful approach to artificial ordered systems. The introduced C-terminal amido pyridyl moiety is envisioned to serve as a binding site for metal complexation. Studies on the application of ferrocenes **1**−**4** for molecular recognition and molecular dynamics are now in progress.

Acknowledgment. Thanks are due to the Analytical Center, Graduate School of Engineering, Osaka University, for the use of the NMR and MS instruments.

Supporting Information Available: Experimental details for the syntheses and characterization of **1**−**4**, difference NOE of **1**, and X-ray crystallographic data for **1**−**4** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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