Decelerated chirality interconversion of an optically inactive 3_{10}-helical peptide by metal chelation†

Naoki Ousaka,*[a] Norihiko Tani,[b] Ryo Sekiya[b] and Reiko Kuroda*[ab]

Received (in Cambridge, UK) 21st February 2008, Accepted 19th March 2008
First published as an Advance Article on the web 21st April 2008
DOI: 10.1039/b803080d

A dynamically optically inactive 3_{10}-helical peptide possessing metal chelating ability between the side-chains at the *i* and *i* + 3th residues was synthesized, and its metal complexes were shown to stabilize the peptide structure and the helical sense.

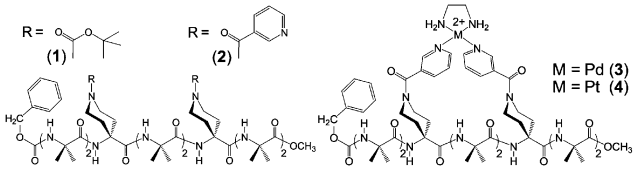
An intramolecular side-chain metal ligation is a useful method for stabilizing β-sheet, turn and helical structures in short peptides.[1] Metal ligation of an α-helix was achieved by coordination to two amino acid side-chains spanning *i* and *i* + 4 positions located on one face of the helix.[2,3] Similar attempts to stabilize α-helical peptides have been demonstrated by using side-chain constraints such as salt-,[4a] disulfide-,[4b,c] lactam-,[4d] hydrazone-bridges,[4e] hydrogen bond surrogate,[4f] and hydrocarbon crosslinks.[4g] The stabilization of the helical structure may enhance biological activities and protease resistance *in vitro* or *in vivo*.[4f,g] The side-chain constraint on residues *i* and *i* + 3 in the 3_{10}-helix[5] (a relatively common structural motif in protein helices[6]) has also been investigated by using covalent tether[7] and salt-bridge formation.[8] Fairlie and his co-workers have demonstrated that His–X–X–His motif binds to a transition metal ion and the resulting complex adopts some turn structures.[3d] Oku and his co-workers have also demonstrated that a helical structure is stabilized by complexation with Co(II) ion.[9] However, a well-defined 3_{10}-helical peptide with metal ligation to the side-chains has rarely been demonstrated.

Herein, we report the design, synthesis, and characterization of a dynamically optically inactive 3_{10}-helical peptide[7d,10] possessing a metal-chelating ability. The peptide-metal complexes with a 25-membered metallocycle to stabilize the entire peptide structure and decelerate helix-inversion of the peptide which is composed of only achiral C^{α}-tetrasubstituted α-amino acids.[5,11]

We chose the same octapeptide we studied previously, Cbz-[Aib_2-Api(Boc)]$_2$-Aib_2-OMe (Cbz = benzyloxycarbonyl; Api(Boc) = 1-Boc-piperidine-4-amino-4-carboxylic acid; Boc = *tert*-butoxycarbonyl; OMe = methoxy) (**1**),[7d] as a rigid backbone to design the metal-ligating 3_{10}-helical peptide. The high percentage of Aib residues in short peptides is known to show a strong preference for the 3_{10}-helix,[5,7–11] and the achiral Api residue possessing functional group at the side-chain is also known to promote the helical structure.[8,12] The peptide **1** was shown to adopt the 3_{10}-helical structure in the crystalline and solution states.[7d] A careful modeling study based on the crystal structure of **1** indicates that pyridine-3-carboxylic acid ($PyCO_2H$) is one of the most suitable and relatively small metal coordinating moieties for the metal chelation between the side-chains of Api residues at *i* and *i* + 3 positions which are located on the same face of the 3_{10}-helix. Thus, ligand peptide Cbz-[Aib_2-Api(PyCO)]$_2$-Aib_2-OMe (**2**) (Scheme 1) was synthesized from **1**[7d] by coupling with $PyCO_2H$ after removal of the protecting Boc groups.†

A solution structure of **2** was investigated by ^{1}H NMR technique, and additionally by computational analysis. ^{1}H NMR titration experiments in a CD_3CN solution were performed upon addition of DMSO-d_6 (strong hydrogen bond accepting solvent) to determine the helix type (3_{10}- or α-helix). The plots of NH chemical shifts against DMSO-d_6 percentage showed that two NH protons shifted substantially to lower field on increasing DMSO-d_6 ratio (shown in red in Fig. 1a), whereas the remaining six NH protons stayed almost constant (shown in black in Fig. 1a) due to intramolecular hydrogen bonding. One of the two solvent exposed NH protons located at higher field was assigned to the urethane N(1)H, and the other to N(2)H.[13] The structures of **2** optimized by semi-empirical MO calculation (PM6 method[14] in MOPAC2007) with the four possible starting orientations of side-chain amide carbonyl groups (types I–IV) afforded a typical 3_{10}-helical conformation; the averaged $|\phi|/|\psi|$ dihedral angles fell in the range of $|61| \sim |63|/|22| \sim |24|^{\circ}$ for all the four structures.‡ Only the lowest energy structure (type II) is shown in Fig. 2. The other optimized structures are shown in Fig. S2 (see ESI†).

Variable temperature ^{1}H NMR spectra of **2** (Fig. 3a) showed that the relatively broad NH resonance at around 7.87 ppm at 20 °C becomes sharp at higher temperatures but splits at lower temperatures. In contrast, protons neighboring the N atom on the piperidine ring (around 4.4 ppm) are sharp at lower temperatures but disappear at higher temperatures, indicating that rotation of side-chain amide bonds is relatively slow on the NMR time scale. These data suggest that the split



Scheme 1

[a] *Japan Science and Technology Agency, ERATO-SORST Kuroda Chiromorphology Team, 4-7-6, Komaba, Meguro-ku, Tokyo, 153-0041, Japan. E-mail: ousaka@chiromor2.erato.rcast.u-tokyo.ac.jp; Fax: (+81) 3 5454 6601*
[b] *Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1, Komaba, Meguro-ku, Tokyo, 153-8902, Japan. E-mail: ckuroda@mail.ecc.u-tokyo.ac.jp; Fax: (+81) 3 5454 6600*

† Electronic supplementary information (ESI) available: Experimental details and additional spectroscopic data. See DOI: 10.1039/b803080d

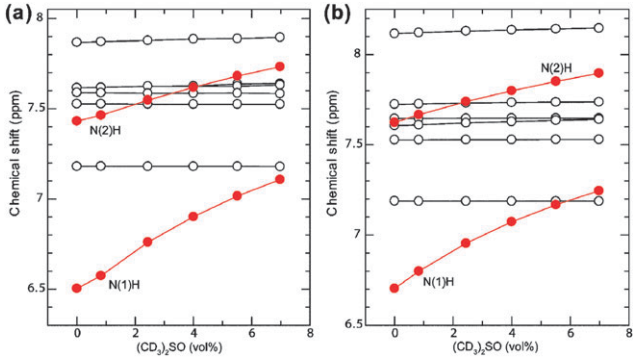


Fig. 1 Solvent dependence on NH chemical shifts of peptides (a) **2** and (b) **3** in CD_3CN/DMSO-d_6 mixture at 20 °C in 500 MHz ^{1}H NMR spectra.

NH resonance at lower temperatures corresponds to the side-chain amide orientations of Api residue.

We have studied the metal-chelation ability of **2**. Pd(en)(O-NO_2)$_2$ (en = ethylenediamine) (**5**) as the *cis*-blocked square-planar Pd(II) complex was added to a CD_3CN solution of **2**. In the ^{1}H NMR spectrum of 1 : 0.5 (**2** : **5**) mixture at 20 °C (Fig. S3†), two sets of resonances were clearly observed, one for **2** alone and the other for complex **2**[Pd(en)]$^{2+}$ (**3**) (Scheme 1). After further addition of 0.5 equiv of **5** (total of 1 : 1 (**2** : **5**) mixture), the resonances of **2** disappeared completely and only one set of resonances was observed (Fig. S3†). Similarly, the ^{1}H NMR spectrum of **2**[Pt(en)]$^{2+}$ (**4**) (Scheme 1) prepared from 1 : 1 **2** : Pd(en)$(ONO_2)_2$ stoichiometry showed a single set of resonances (Fig. S4†), indicating that these peptide–metal ions form a 1 : 1 complex. These 1 : 1 complexations are also supported by electron spray ionization mass spectra of **3** and **4**. As shown in Fig. 4, complex **3** exhibited an intense peak at m/z value of 652.05 and a moderate peak at m/z value of 1367.07. These signals are assigned to the positively charged species **3** and [**3**(ONO_2)]$^+$, respectively. Complex **4** gave a similar spectrum.

The solution conformations of these complexes were determined by the same method used for **2**. Only two NHs are clearly exposed to solvent, indicating that **3** and **4** maintain the 3_{10}-helical structure (Fig. 1b, S5†). Some of the NHs exhibit slight downfield shift compared to **2**, however, similar tendency was found in the case of the covalent system,[7d] probably

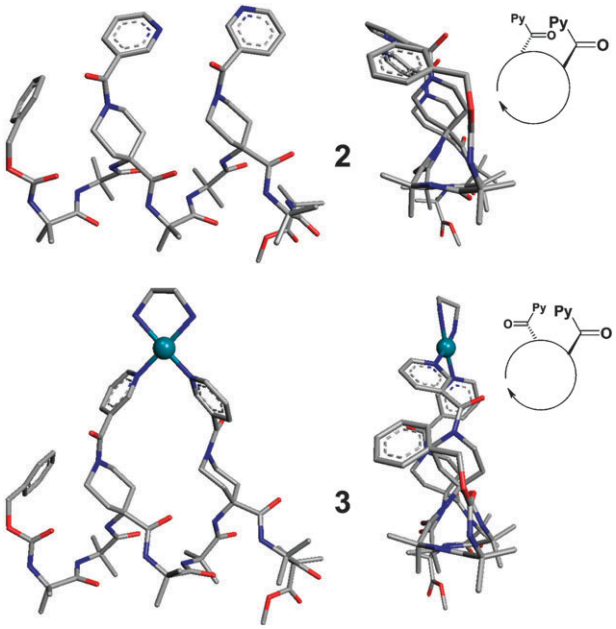


Fig. 2 Side (left) and top (right) views of the right-handed structures of **2** and **3** as energy-minimized by the semi-empirical MO calculation (PM 6 method[14]).[17] Hydrogen atoms are omitted for clarity. The schematic representations indicate the side-chain orientations of amide carbonyl.

due to slightly stronger hydrogen bonding. The theoretical study of **3** also supports the 3_{10}-helical structure. The optimized structure of **3** is shown in Fig. 2 (PM6 method[14]).‡ The starting model was based on type I of **2**, which has two amide carbonyls in opposite orientations. The other orientations (types II–IV) cannot make metal coordination without severe strain. In fact, only single set of resonances is observed in the NMR spectrum, although the side-chain amide bonds exhibit slow rotation on the NMR time scale. This orientation was also observed in the case of previously-reported covalent system.[7d] The averaged $|\phi|/|\psi|$ dihedral angles of optimized structure, (|63|/|23|), are similar to the values of **2**. These results suggest that the backbone structures are almost unchanged upon the metal ligation.§

In the ^{1}H NMR spectra of complexes **3** and **4**, all the protons of piperidine rings are very sharp and distributed over

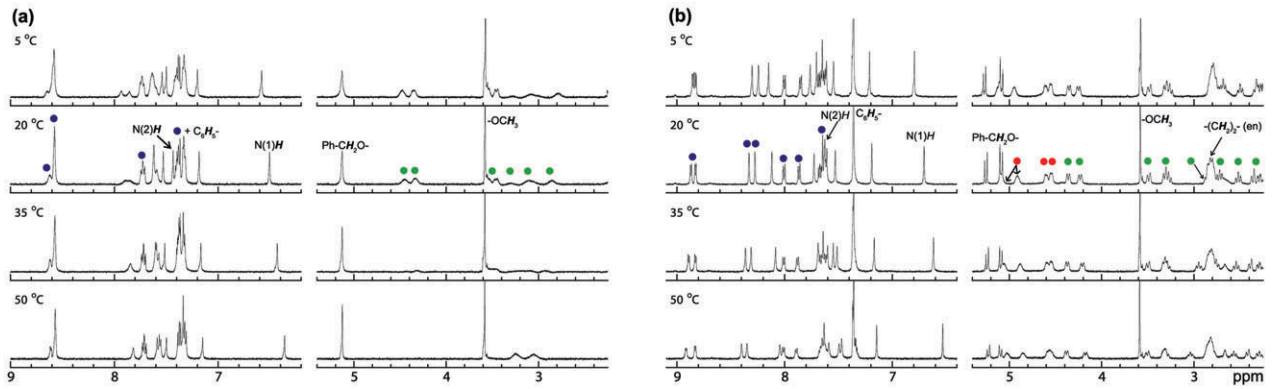


Fig. 3 Variable temperature ^{1}H NMR (500 MHz) spectra of **2** (a) and **3** (b) in CD_3CN; [**2**] = 3.5 mM, [**3**] = 4.0 mM. Blue, green, and red circles indicate protons of pyridine rings, piperidine rings, and amino groups (en), respectively. The full region spectra are provided in Fig. S6.†

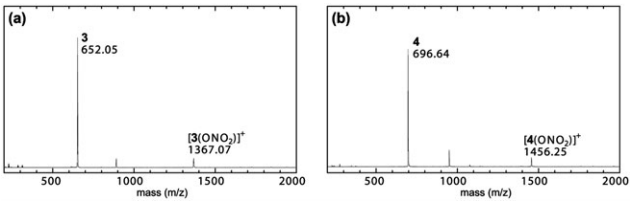


Fig. 4 Electron spray ionization mass spectra of **3** and **4**.

a wide chemical-shift range (Fig. 3b and S4†).¶ Positions and half bandwidths of these resonances are less sensitive to temperature changes relative to those of **2**. Thus, the motion of the piperidine rings and the rotation of side-chain amides are constrained in **3** and **4**. The protons of pyridine rings also show similar trends and lower magnetic field shift due to the coordination with the metal ion.¶ The resonances of the four amino protons of ethylenediamine appear at different chemical shifts due to the broken symmetry caused by complexation with **2** (Fig. 3b, red circles). Remarkably, N-terminal methylene protons of Cbz group (around 5.17 ppm) show diastereotopic splitting (Fig. 3b), although they are placed distant from the metallocyclic region. The coalescence temperature of the splitting resonance is above 70 °C (Fig. S6†). This observation indicates that **3** and **4** exhibit slow helix-inversion.‖ In other words, deformation and re-formation of the hydrogen bonds and reversal of ϕ/ψ dihedral angles are necessary for the helix-inversion, and the helix sense and the structure are stabilized by the metal coordination.

In summary, we have designed and characterized the first case of a well-defined 3_{10}-helical peptide possessing the metal chelating site. The side-chain metal ligation of i and $i + 3$ residues not only stabilizes the structure but also decelerates the helix-inversion in the optically inactive 3_{10}-helical peptide. To the best of our knowledge, this is the stiffest helical metallopeptide which is composed of achiral C^{α}-tetrasubstituted amino acids with side-chain metal ligation. Control of the helical structure and its sense by metal-ligation is preferable to covalent-bond formation in terms of by-product formation in preparation. The Api(PyCO) residue possessing high propensity for helix formation and metal coordination may serve as a key residue for the *de novo* design of metal-binding peptides.

Notes and references

‡ The starting conformations of **2** and **3** are modeled based on the crystal structure of **1**[7d] without changing the backbone dihedral angles.
§ Single crystals of these peptides suitable for X-ray diffraction analysis could not be obtained.
¶ The protons of piperidine rings, pyridine rings, and en are assigned by 2D COSY spectra after the DMSO-d_6 titration experiments (Fig. S7).† TOCSY measurements for peptides **2** and **3** were performed (Fig. S8).†
‖ The artificial helical molecules composed of achiral non-biological backbone sometimes exhibit slow helix-inversion,[15] and even retain the helix sense for a long period of time.[16] However, in the case of biological backbone, only one example has been reported.[7d]

1. For selected recent reviews, see: (*a*) M. Albrecht and P. Stortz, *Chem. Soc. Rev.*, 2005, **34**, 496; (*b*) J. Garner and M. M. Harding, *Org. Biomol. Chem.*, 2007, **5**, 3577.
2. For examples, see (*a*) M. R. Ghadiri and C. Choi, *J. Am. Chem. Soc.*, 1990, **112**, 1630; (*b*) M. R. Ghadiri and A. K. Fernholz, *J. Am. Chem. Soc.*, 1990, **112**, 9633; (*c*) F. Ruan, Y. Chen and P. B. Hopkins, *J. Am. Chem. Soc.*, 1990, **112**, 9403; (*d*) W. D. Kohn, C. M. Kay, B. D. Sykes and R. S. Hodges, *J. Am. Chem. Soc.*, 1998, **120**, 1124; (*e*) M. Kohtani, B. S. Kinnear and M. F. Jarrold, *J. Am. Chem. Soc.*, 2000, **122**, 12377.
3. (*a*) M. J. Kelso, H. Hoang, T. G. Appleton and D. P. Fairlie, *J. Am. Chem. Soc.*, 2000, **122**, 10488; (*b*) M. J. Kelso, H. N. Hoang, W. N. Oliver, N. Sokolenko, D. R. March, T. G. Appleton and D. P. Fairlie, *Angew. Chem., Int. Ed.*, 2003, **42**, 421; (*c*) M. J. Kelso, R. L. Beyer, H. N. Hoang, A. S. Lakdawala, J. P. Snyder, W. P. Oliver, T. A. Robertson, T. G. Appleton and D. P. Fairlie, *J. Am. Chem. Soc.*, 2004, **126**, 4828; (*d*) R. L. Beyer, H. N. Hoang, T. G. Appleton and D. P. Fairlie, *J. Am. Chem. Soc.*, 2004, **126**, 15096.
4. For examples, see (*a*) S. Marqusee and R. L. Baldwin, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 8898; (*b*) M. Pellegrini, M. Royo, M. Chorev and D. F. Mierke, *J. Pept. Res.*, 1997, **49**, 404; (*c*) D. Y. Jackson, D. S. King, J. Chmielewski, S. Singh and P. G. Schultz, *J. Am. Chem. Soc.*, 1991, **113**, 9391; (*d*) J. W. Taylor, *Biopolymers*, 2002, **66**, 49; (*e*) E. Cabezas and A. C. Satterthwait, *J. Am. Chem. Soc.*, 1999, **121**, 3862; (*f*) D. Wang, M. Lu and P. S. Arora, *Angew. Chem., Int. Ed.*, 2008, **47**, 1879; (*g*) L. D. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine and S. J. Korsmeyer, *Science*, 2004, **305**, 1466.
5. C. Toniolo and E. Benedetti, *Trends Biochem. Sci.*, 1991, **16**, 350.
6. D. J. Barlow and J. M. Thornton, *J. Mol. Biol.*, 1988, **201**, 601.
7. (*a*) A. K. Boal, I. Guryanov, A. Moretto, M. Crisma, E. L. Lanni, C. Toniolo, R. H. Grubbs and D. J. O'Leary, *J. Am. Chem. Soc.*, 2007, **129**, 6986; (*b*) E. Schievano, K. Pagano, S. Mammi and E. Peggion, *Biopolymers*, 2005, **80**, 294; (*c*) E. Schievano, A. Bisello, M. Chorev, A. Bisol, S. Mammi and E. Peggion, *J. Am. Chem. Soc.*, 2001, **123**, 2743; (*d*) N. Ousaka, T. Sato and R. Kuroda, *J. Am. Chem. Soc.*, 2008, **130**, 463.
8. T. S. Yokum, M. G. Bursavich, T. Gauthier, R. P. Hammer and M. L. McLaughlin, *Chem. Commun.*, 1998, 1801.
9. H. Oku, Y. Kimura, M. Ohama, N. Ueyama, K. Yamada and R. Katakai, *J. Organomet. Chem.*, 2007, **692**, 79.
10. For examples, see: (*a*) R.-P. Hummel, C. Toniolo and G. Jung, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 1150; (*b*) M. Kubasik, J. Kotz, C. Szabo, T. Furlong and J. Stace, *Biopolymers*, 2005, **78**, 87; (*c*) Y. Inai, H. Komori and N. Ousaka, *Chem. Rec.*, 2007, **7**, 191.
11. I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747.
12. (*a*) C. L. Wysong, T. S. Yokum, G. A. Morales, R. L. Gundry, M. L. McLaughlin and R. P. Hammer, *J. Org. Chem.*, 1996, **61**, 7650; (*b*) L. G. J. Hammarström, T. J. Gauthier, R. P. Hammer and M. L. McLaughlin, *J. Pept. Res.*, 2001, **58**, 108; (*c*) T. S. Yokum, T. J. Gauthier, R. P. Hammer and M. L. McLaughlin, *J. Am. Chem. Soc.*, 1997, **119**, 1167.
13. A. Polese, F. Formaggio, M. Crisma, G. Valle, C. Toniolo, G. M. Bonora, Q. B. Broxterman and J. Kamphuis, *Chem.–Eur. J.*, 1996, **2**, 1104.
14. J. J. P. Stewart, *J. Mol. Model.*, 2007, **13**, 1173.
15. For examples, see: (*a*) H. Jiang, J.-M. Léger and I. Huc, *J. Am. Chem. Soc.*, 2003, **125**, 3448; (*b*) C. Dolain, J.-M. Léger, N. Delsuc, H. Gornitzka and I. Huc, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 16146.
16. For examples, see: (*a*) E. Yashima, K. Maeda and Y. Okamoto, *Nature*, 1999, **399**, 449; (*b*) H.-Z. Tang, P. D. Boyle and B. M. Novak, *J. Am. Chem. Soc.*, 2005, **127**, 2136; (*c*) T. Hasegawa, Y. Furusho, H. Katagiri and E. Yashima, *Angew. Chem., Int. Ed.*, 2007, **46**, 5885.
17. The molecular graphics were drawn with: M. A. Thompson, ArgusLab 4.0.1; Planaria Software LLC: Seattle, WA, 2004 (http://www.arguslab.com).

