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Total Helical-Sense Bias of an Achiral Peptide Main Chain Induced by a Chiral Side-Chain Bridge

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Naturally occurring helical structures such as protein and DNA play a crucial role in a biological system. The α- and 3_{10}-helices in protein almost always adopt a right-handed (*P*) helicity as dictated by the asymmetry of the α-carbon in L-α-amino acids. Out of 20 protein amino acids (except for Gly) two, Ile and Thr, possess an additional asymmetric carbon atom at the *β*-position of the side chain. In the artificial helical polymers and oligomers,[1] much attention has been paid to the effect of side-chain chirality on the helical sense of the achiral main chain. However, a few examples have been reported in the cases of the peptide backbones.[2] In principle, appropriate steric interactions between the side chains or between the side chain and the main chain in such helical molecules are required for efficient transfer of chiral information from the chiral side chains to the achiral backbone. Thus, the helical sense bias is much reduced on increasing conformational flexibility of the chiral side chains.

Herein, we report an unprecedented total helical-sense bias that a single intramolecular cross-linking[3] between the chiral side chains induces only one-handed helicity in a 3_{10}-helical peptide which has asymmetric carbon atoms not in the main chain but at the two side chains. We also compare the helical-sense bias of the peptides before and after the cross-linking.

We chose the same octapeptide sequence we studied previously, Cbz-[Aib_2-Api(Boc)]$_2$-Aib_2-OMe,[3c,4] which is shown to adopt the 3_{10}-helical structure in the crystalline and solution states,[3c] and the rigid backbone is able to incorporate chiral elements in specific positions of the side chains.[5] It is known that the C^{α}-tetrasubstituted amino acids such as Aib and Api residues strongly promote a helical structure.[6,7] The latter residue also possesses a functional group at the side chain.[6] Our modeling study indicates that a cystine residue is one of the most suitable chiral bridging moieties for the cross-linking between the side chains of Api residues at the *i* and *i* + 3 positions which are located on the same face of the 3_{10}-helix. Thus, chiral peptide Cbz-[Aib_2-Api(Boc-L-Cys(Acm)-)]$_2$-Aib_2-OMe (**1**)[4] was synthesized from the corresponding octapeptide after the removal of Boc-protecting groups. Then, the side-chain cross-linking peptide **2** was prepared from *S*-Acm-protected Cys **1** by direct oxidation with iodine (for details, see Supporting Information).[8]

The X-ray crystal structure of peptide **2**[9] shows there are three crystallographically independent molecules (A, B, and C). They adopt similar conformations, and the only difference is at one of the Cys residues in the side chains, i.e., the *cis*-amide isomer for B (*cis*-**2**) and the *trans* for A and C (molecule A is shown in Figure 1, and B and C in Figure S1).[10] Interestingly, the backbones of these molecules only take a regular (*P*) 3_{10}-helix except for the last residue, although the backbone is composed of an achiral chain.

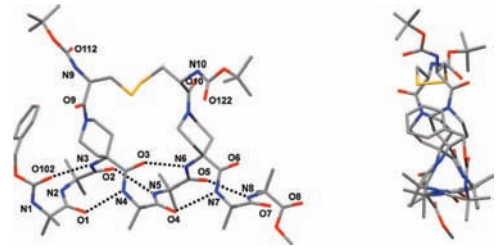
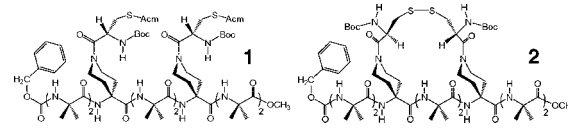


Figure 1. Side (left) and top (right) views of crystal structure of **2**. One of the three crystallographically independent molecules, molecule A, is shown. Hydrogen atoms and cocrystallized solvent molecules are omitted for clarity. Dotted lines represent intramolecular hydrogen bonds.

The averaged *ϕ*/*ψ* dihedral angles for Aib(1) to Aib(7) fell in the range −54° to −56°/−27° to −29° for all three structures. The selected torsion angles and hydrogen-bonding parameters are summarized in Tables S1−S6. All the piperidine rings have the amino group in an axial orientation, and the amide carbonyl orientations of the two side chains are in opposite directions to each other. This orientation was also observed in the case of previously reported systems.[3c]



A modeling study based on the crystal structure of **2** shows if the helical structure is maintained, the other orientations can not make a disulfide bond, because the S−S distance becomes too long. The averaged *ϕ*/*ψ* dihedral angles for the L-Cys residues at the side chains are −107°/131°, indicating that these adopt a *β*-sheet like conformation. It is known that the cyst(e)ine residue prefers a *β*-sheet conformation rather than helical ones.[11] These favorable *ψ* values for the side-chain L-Cys residues fit well with the (*P*) 3_{10}-helix, whereas they are unsuitable for the left-handed (*M*) helix (because the S−S distance becomes too long).

Solution conformations of peptides **1** and **2** were investigated by a ^{1}H NMR technique. As shown in Figure S2A, variable temperature ^{1}H NMR spectra of **1** in $CDCl_3$ show complicated spectral patterns, particularly splitting amide NHs (∼7−8 ppm), probably due to the presence of many possible orientations of side-chain amide carbonyl −CO-N$(CH_2)_2$− groups that slowly interconvert on the NMR time scale (Figure S2B).[12] The complicated spectral feature is retained even when the solution is heated up to 50 °C (Figure S2A).[12] In contrast, all the proton signals of **2** are very sharp (Figure 2). The urethane NH and other signals are at a relatively higher field (below 6.6 ppm) than other amide protons (above 7.2 ppm), and these two protons are assigned to N(1)H and N(2)H.[3c,13] Indeed, chemical shifts of the two

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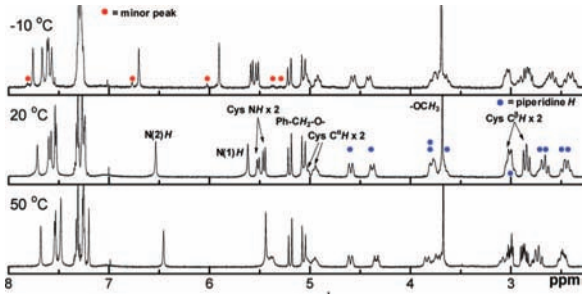

Figure 2. Part of variable temperature ^{1}H NMR (400 MHz) spectra of **2** in $CDCl_3$; [**2**] = 3.5 mM. Blue and red circles indicate protons of piperidine rings and minor peak, respectively. The full region spectra are provided in Figure S3.

NHs are sensitive to the DMSO-d_6 percentage in $CDCl_3$ solution (shown in red, Figure S4), indicating the lack of intramolecular H-bonding for the two protons.

The high $^3J_{HN\alpha}$ coupling constants of two L-Cys residues at 20 °C (around 5.5 ppm, Figure 2) are 8.8 and 9.0 Hz, implying the ϕ torsion angles of $\sim -100°$ or $-140°$,[14] that agree well with the averaged ϕ value of L-Cys residues in the crystalline state of **2**. All the proton signals of piperidine rings are very sharp and distributed over a wide chemical shift range, as assigned by a 2D TOCSY spectrum (Figure S5), because the motion of the two piperidine rings is constrained (Figure 2).[3c−e] Remarkably, N-terminal methylene proton resonances of the Cbz group (around 5.13 ppm) show a double doublet (Figure 2). Moreover, the *J* value of the methylene protons is almost independent of temperatures ranging from −10 to 50 °C. These data indicate that the rate of helix inversion of **2** is too slow on the NMR time scale or that peptide **2** only adopts either a (*P*) or an (*M*) helix. At lower temperatures, additional minor peaks appeared (Figure 2). These peaks correspond either to a minor conformation with the same helicity of the main peaks, namely, the *cis*-amide isomer observed in the crystal (molecule B, Figure S1), or to the opposite helicity. These minor peaks are not observed above 20 °C (Figure 2), contrary to expectations for the slow helix inversion of the cross-linked peptide.[15,16] The energy calculations revealed that the energy order (energy difference at the DFT level of theory) is (*P*)-**2** (0) < *cis*-(*P*)-**2** (+0.51) ≪ (*M*)-**2** (+13.8 kcal/mol) (for details, see Supporting Information). These data indicate that the minor peaks correspond to the *cis*-amide isomer with the same helicity of the major peaks. Therefore, peptide **2** adopts only a (*P*) helix in crystalline and solution states.

Finally, we confirmed the helical sense of peptides **1** and **2** in solution. CD spectra of the both peptides in TFE[4] at room temperature (Figure S7A) exhibit no characteristic CD patterns for the α- and 3_{10}-helices, due to a large contribution of the side-chain amide chromophores. Hence, we prepared *p*-bromo-benzoylated (pBrBz) peptides which were incorporated into the N-terminus of both peptides instead of the Cbz group (pBrBz-**1** and pBrBz-**2**).[17] CD spectra of the pBrBz-peptides show a splitting pattern with a positive cotton effect at the longer wavelength region, indicating that these peptides prefer the (*P*) 3_{10}-helix (Figure S7A).[17] To compare the helical-sense bias of **1** with that of **2**, CD spectra of the peptides were subtracted from those of the corresponding pBrBz-peptides (Figure S7B). These resultant CD spectral patterns are very similar to each other, but the intensity at 247 nm for **2** is three times larger than that for **1**. This indicates that the cross-linking between the chiral side chains can greatly increase the helical-sense bias of the achiral peptide main chain.

In summary, we have demonstrated that a single intramolecular cross-linking between the side-chain L-Cys residues at the *i* and *i* + 3 positions induces only a (*P*) helix in the 3_{10}-helical peptide and transforms the "dynamic" helix to a "static" one. Our results may provide insights into the role of the side-chain chirality in the handedness of the helical molecules and, hence, a guide for the construction of one-handed helical architectures.

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

References

(1) For reviews: (a) *Foldamers: Structure, Properties, and Applications*; Hecht, S., Huc, I., Eds.; Wiley-VCH: Weinheim, 2007. (b) Nolte, R. J. M. *Chem. Soc. Rev.* **1994**, *23*, 11. (c) Green, M. M.; Peterson, N. C.; Sato, T.; Teramoto, A.; Cook, R.; Lifson, S. *Science* **1995**, *268*, 1860. (d) Nakano, T.; Okamoto, Y. *Chem. Rev.* **2001**, *101*, 4013. (e) Fujiki, M. *Macromol. Rapid Commun.* **2001**, *22*, 539. (f) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893. (g) Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M.; Sommerdijk, N. A. J. M. *Chem. Rev.* **2001**, *101*, 4039. (h) Palmans, A. R. A.; Meijer, E. W. *Angew. Chem., Int. Ed.* **2007**, *46*, 8948. (i) Yashima, E.; Maeda, K. *Macromolecules* **2008**, *41*, 3.

(2) (a) Tanaka, M.; Demizu, Y.; Doi, M.; Kurihara, M.; Suemune, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 5360. (b) Tanaka, M.; Anan, K.; Demizu, Y.; Kurihara, M.; Doi, M.; Suemune, H. *J. Am. Chem. Soc.* **2005**, *127*, 11570. (c) Royo, S.; Borggraeve, W. M. D.; Peggion, C.; Formaggio, F.; Crisma, M.; Jiménez, A. I.; Cativiela, C.; Toniolo, C. *J. Am. Chem. Soc.* **2005**, *127*, 2036.

(3) The side-chain cross-linking in the 3_{10}-helical peptides : (a) Boal, A. K.; Guryanov, I.; Moretto, A.; Crisma, M.; Lanni, E. L.; Toniolo, C.; Grubbs, R. H.; O'Leary, D. J. *J. Am. Chem. Soc.* **2007**, *129*, 6986. (b) Schievano, E.; Bisello, A.; Chorev, M.; Bisol, A.; Mammi, S.; Peggion, E. *J. Am. Chem. Soc.* **2001**, *123*, 2743. (c) Ousaka, N.; Sato, T.; Kuroda, R. *J. Am. Chem. Soc.* **2008**, *130*, 463. (d) Ousaka, N.; Tani, N.; Sekiya, R.; Kuroda, R. *Chem. Commun.* **2008**, 2894. (e) Ousaka, N.; Inai, Y.; Kuroda, R. *J. Am. Chem. Soc.* **2008**, *130*, 12266.

(4) Abbreviations: Cbz, benzyloxycarbonyl; Aib, α-aminoisobutyric acid: Api(Boc), 1-Boc-piperidine-4-amino-4-carboxylic acid; Boc, *tert*-butoxycarbonyl; OMe, methoxy; Acm, acetamidomethyl; TFE, 2,2,2-trifluoroethanol.

(5) For examples of site-specific incorporation of chiral side chains in foldamers: (a) Prince, R. B.; Moore, J. S.; Brunsveld, L.; Meijer, E. W. *Chem.—Eur. J.* **2001**, *7*, 4150. (b) Wu, C. W.; Sanborn, T. J.; Huang, K.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 6778.

(6) (a) Wysong, C. L.; Yokum, T. S.; Morales, G. A.; Gundry, R. L.; McLaughlin, M. L.; Hammer, R. P. *J. Org. Chem.* **1996**, *61*, 7650. (b) Yokum, T. S.; Gauthier, T. J.; Hammer, R. P.; McLaughlin, M. L. *J. Am. Chem. Soc.* **1997**, *119*, 1167.

(7) For comprehensive review of C^{α}-tetrasubstituted α-amino acids, see: Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers* **2001**, *60*, 396.

(8) Sieber, P.; Kamber, B.; Riniker, B.; Rittel, W. *Helv. Chim. Acta* **1980**, *63*, 2358.

(9) Structure determination is described in the Supporting Information. A single crystal of peptide **1** suitable for X-ray diffraction analysis could not be obtained probably due to conformational flexibility of the side-chain Cys residues.

(10) For observation of a *cis*-amide isomer within linear peptide composed of only nonproline amino acid residues, see: Bairaktari, E.; Mierke, D. F.; Mammi, S.; Peggion, E. *J. Am. Chem. Soc.* **1990**, *112*, 5383.

(11) For examples: (a) Nagano, N.; Ota, M.; Nishikawa, K. *FEBS Lett.* **1999**, *458*, 67. (b) Minor, D. L., Jr.; Kim, P. S. *Nature* **1994**, *367*, 660.

(12) The similar behavior was also observed in the case of 3-pyridine-carbonyl-functionalized peptide.[3d] It is to be noted that such a modification did not change the 3_{10}-helical conformation of the peptide.[3d]

(13) Polese, A.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. *Chem.—Eur. J.* **1996**, *2*, 1104.

(14) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1987; p 167.

(15) Recently, we have reported that a single side-chain cross-linking significantly decelerates the rate of helix inversion of the optically inactive 3_{10}-helical peptides; see refs 3c−e. In the case of an optically active helical oligomer that slowly interconverts between (*P*) and (*M*) helices, diastereomers are readily distinguishable in a ^{1}H NMR spectrum at above ambient temperature; see: Jiang, H.; Dolain, C.; Léger, J.-M.; Gornitzka, H.; Huc, I. *J. Am. Chem. Soc.* **2004**, *126*, 1034.

(16) In the case of ^{1}H NMR spectra of Boc-deprotected **2** (peptide **3**) which was prepared to exclude the possibility of the *cis*-amide isomer, the minor peaks were not observed at temperatures ranging from−20 to 50 °C (Figure S6). Additionally, the methylene proton signals of Cbz group in peptides **2** and **3** show a similar trend.

(17) Toniolo and his co-workers have demonstrated that the pBrBz chromophore at the N-terminus of the peptide backbone is an excellent CD probe for the assignment of the screw sense of 3_{10}-helical peptides: Toniolo, C.; Formaggio, F.; Crisma, M.; Schoemaker, H. S.; Kamphuis, J. *Tetrahedron: Asymmetry* **1994**, *5*, 507, and ref 13.

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