J|A|C|S
COMMUNICATIONS

Published on Web 07/28/2006

Cyclic Peptide Helices: A Hybrid β-Hairpin/β-Helical Supersecondary Structure

Mallika Sastry,[†] Christopher Brown,[‡] Gerhard Wagner,[†] and Thomas D. Clark*,[‡]

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, and Division of Chemistry, Naval Research Laboratory, Washington, D.C. 20375-5342

Received April 19, 2006; E-mail: thomas.clark@nrl.navy.mil

In this communication, we describe the design of a new peptide motif that is a hybrid of β-hairpin and β-helical supersecondary structures, and we present the characterization of this motif using circular dichroic (CD), IR, and NMR spectroscopies. Helical segments in peptides and proteins fulfill important functions, including scaffolding, molecular recognition, and tailoring of electrostatic microenvironments.[1a] In addition to the familiar structures generated from α-amino acids, such as α, 3_{10}, and polyproline helices,[1a] much recent interest has focused on helices comprised of β-amino acids.[1b] By contrast, β helices—helices formed by peptides composed of alternating D- and L-α-amino acids (D,L-peptides) and stabilized by β-sheet hydrogen bonding[2a]—are currently receiving much less notice. This dearth of attention is likely due to the conformational promiscuity of β helices; for example, a given D,L-peptide often produces a mixture of single-stranded (ss), double-stranded (ds) parallel (↑↑), and ds antiparallel (↑↓) β-helical forms.[2a−c] Here, we present a strategy for limiting the number of conformations available to a D,L-peptide, resulting in a well-defined, antiparallel species having about 5.6 residues per turn (a ↑↓$\beta^{5.6}$ helix).

Figure 1 outlines our approach. We chose to examine oligo-D,L-valine peptides in our initial studies because prior work by others has shown that related sequences adopt ↑↓$\beta^{5.6}$-helical conformations in the solid state[2d] but exist in solution as a mixture of interconverting ss and ds helices, with ↑↓$\beta^{5.6}$-helical forms predominating (Figure 1a).[2c] Conceptually, we began with a ↑↓$\beta^{5.6}$ helix composed of two linear D,L-peptide strands and hypothesized that covalently tying the N and C termini together using two β turns[3a] would constrain the molecule into a single ↑↓$\beta^{5.6}$-helical species (Figure 1b). To test this hypothesis, we designed the 22-residue cyclic β-hairpin peptide *cyclo*{[(L-Val−D-Val)$_4$−(L-Val−D-Pro−Gly)]$_2$−} **2**. Model building indicated that right and left-handed ↑↓$\beta^{5.6}$-helical conformations of **2** would be stabilized by 16 and 14 hydrogen bonds, respectively; therefore, we expected the helical sense of **2** to be right-handed. As an initial probe of the sequence scope of the hybrid β-hairpin/β-helical motif, we also designed *cyclo*{[(D-Leu−L-Leu)$_4$−(D-Leu−L-Pro−Gly)]$_2$−} **3**. We note that the chiralities of the corresponding residues in **2** and **3** are opposite, and, thus, we anticipated that peptide **3** would form a left-handed ↑↓$\beta^{5.6}$ helix, also stabilized by 16 hydrogen bonds.

Peptides **2** and **3** were synthesized on 4-sulfamylbutyryl AM resin and cyclized with cleavage from the resin.[3b,4] Figure 2 shows the far-UV CD spectra of **2** and **3** in organic solvents. We note that the β-turn segments (D-Pro−Gly and L-Pro−Gly, respectively) comprise only 4 of the 22 residues of **2** and **3**; therefore, we expect CD and IR (vide infra) spectra to be dominated by features arising from the putative ↑↓$\beta^{5.6}$-helical regions. The CD spectrum of **3** in cyclohexane (Figure 2c, ca. 10 μM) has a negative Cotton effect

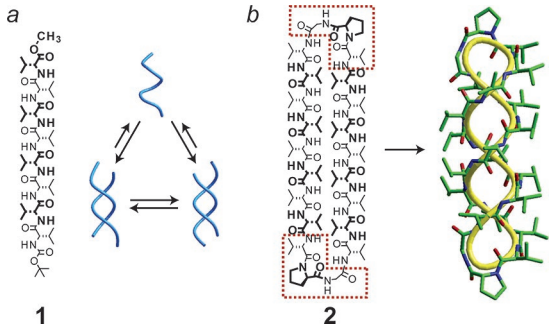

Figure 1. (a) Boc−(L-Val−D-Val)$_4$−OMe **1** exists in $CDCl_3$ solution as a mixture of interconverting ss and ds β-helices.[2c] (b) *cyclo*{[(L-Val−D-Val)$_4$−(L-Val−D-Pro−Gly)]$_2$−} **2** comprises two copies of peptide **1** and two copies of the sequence L-Val−D-Pro−Gly (boxed in red) and was designed to form a single, well-defined β-hairpin/↑↓$\beta^{5.6}$-helical species (right). For **1** and **2**, D- and L-residues are shown in bold and normal line width, respectively.

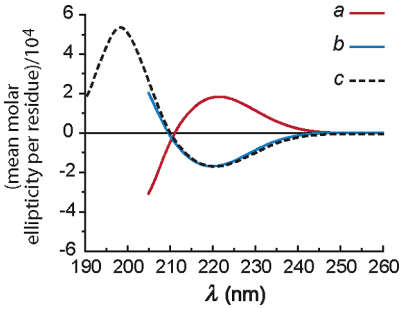

Figure 2. CD spectra of **2** and **3** in organic solvents (295 K). (a) Peptide **2** in $CDCl_3$ (c = 10 mM, l = 10 μm). (b) Peptide **3** in $CDCl_3$ (c = 10 mM, l = 10 μm). (c) Peptide **3** in cyclohexane (c = ca. 10 μM, l = 5 mm).

near 220 nm and a large positive Cotton effect below 200 nm; these features agree with the CD spectra of D,L-peptides that form predominantly left-handed ↑↓$\beta^{5.6}$-helical species.[5] The CD spectrum of **3** in $CDCl_3$ (Figure 2b, 10 mM) is almost overlapping with the spectrum in cyclohexane, indicating that **3** has the expected left-handed ↑↓$\beta^{5.6}$-helical structure in both solvents and that this structure is independent of concentration in a range spanning 3 orders of magnitude (10 μM to 10 mM; the CD spectra of **2** and **3** in $CDCl_3$ are truncated at 205 nm due to strong absorbance by the solvent at shorter wavelengths). Peptide **2** proved insoluble in cyclohexane, but its CD spectrum in $CDCl_3$ (Figure 2a, 10 mM) is nearly a mirror image of the spectrum of **3** in the same solvent. This observation indicates that **2** has the expected right-handed ↑↓$\beta^{5.6}$-helical conformation.

The IR spectra[4] of **2** and **3** in $CDCl_3$ solution (ca. 2 mM) show amide A bands at 3278 and 3267 cm^{-1}, respectively, corresponding to strongly hydrogen-bonded NH groups.[6,7a,b] The position of the

[†] Harvard Medical School.
[‡] Naval Research Laboratory.

10.1021/ja062737f CCC: $33.50

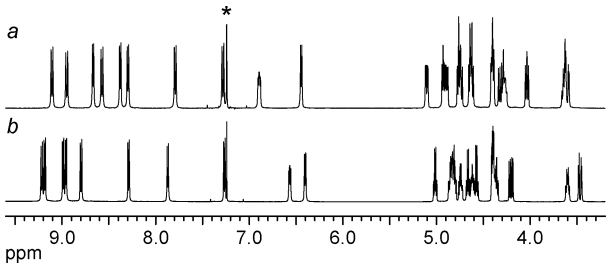

Figure 3. NH and H^{α} region of the 1D ^1H NMR spectra of **2** and **3** (295 K, $CDCl_3$, residual $CHCl_3$ at 7.24 ppm is indicated with an asterisk). (a) Peptide **2**, 500 MHz, 10 mM. (b) Peptide **3**, 600 MHz, 12 mM.

amide I band for peptides and proteins is known to be sensitive to structure;[7] the positions of this band for **2** and **3**—1635 and 1639 cm^{-1}, respectively—are similar to those observed for D,L-peptides that form predominantly $\uparrow\downarrow\beta^{5.6}$-helical structures[7a,b] and are close to the theoretical value of 1636 cm^{-1} calculated for an idealized $\uparrow\downarrow\beta^{5.6}$ helix.[7c] For both **2** and **3**, we also observed a shoulder near 1682 cm^{-1} that is consistent with the amide I parallel component, the presence of which is a hallmark of well-defined antiparallel β-sheet structure.[7a]

The 1D ^1H NMR spectra of **2** and **3** in $CDCl_3$ both comprise a single set of sharp, well-dispersed resonances, with no evidence of interconverting conformers or oligomers (Figure 3). The NH region of each spectrum (δ 6.44−9.23) shows a total of 10 peaks, in agreement with the expected 2-fold-symmetrical structure of the 22-mers. In both cases, eight of these NH peaks appear downfield from 7.2 ppm and are independent of concentration in the range examined (2−20 mM); these chemical shifts are consistent with those of hydrogen-bonded NH groups in $CDCl_3$, as observed in closely related peptides[2c,6a,8a] and model systems.[6b] The δ NH values thus support the existence of 16 hydrogen bonds, as expected for the right and left-handed $\uparrow\downarrow\beta^{5.6}$-helical structures of **2** and **3**, respectively. Furthermore, the majority of the $^3J_{NH-H\alpha}$ values for the D- and L-residues are greater than 8 Hz, which is typical for residues having β-sheetlike ϕ dihedral angles.[9a]

We obtained the ^1H NMR assignments of **2** and **3** using 2D NMR spectroscopy (TOCSY, DQF−COSY, ROESY).[4] The ROESY spectra revealed cross-peaks characteristic of an antiparallel β sheet, including cross-strand $d_{\alpha\alpha}$, $d_{\alpha}N$, and d_{NN} ROEs.[9a] We also observed ROEs between protons on residues that are distant in the covalent structures and that support the expected $\uparrow\downarrow\beta^{5.6}$-helical conformations of **2** and **3**. Furthermore, the patterns of ROEs between main-chain protons for **2** and **3** were nearly identical to one another and closely resembled those of known $\uparrow\downarrow\beta^{5.6}$-helical structures having 2-fold symmetry.[8] The spectra of **2** were better dispersed than those of **3**; therefore, we selected **2** for more detailed characterization via structure calculations.

We performed structure calculations for **2** using NMR-derived restraints and employing a simulated annealing protocol within Xplor-NIH.[4,9b] Figure 4a shows a family of 12 low-energy structures with an average backbone RMSD to mean of 0.36 Å. The structures all exhibit a well-defined right-handed $\uparrow\downarrow\beta^{5.6}$ helix flanked by more flexible type I′ β turns.[4] These results validate our design of a hybrid β-hairpin/β-helical motif.

The success of our design confirms our hypothesis that two β turns are *sufficient* to constrain a D,L-peptide into a single $\uparrow\downarrow\beta^{5.6}$-helical species. As an initial test of whether both turns are *necessary*, we prepared a peptide having only one β turn: Boc−D-Val−(L-Val−D-Val)$_3$−L-Pro−Gly−(D-Val−L-Val)$_4$−OMe **4**.[4] The 1D ^1H NMR spectrum of **4** in $CDCl_3$ is poorly dispersed and extremely broad,[4] implying multiple conformations and aggregations states.

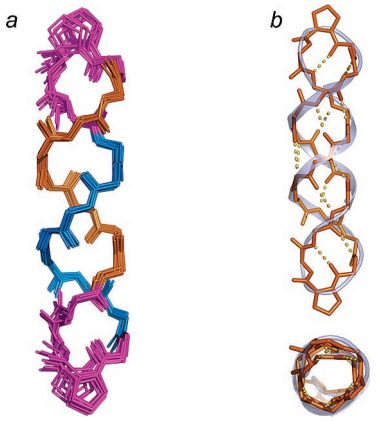

Figure 4. (a) A family of 12 low-energy structures calculated for **2** using NMR-derived restraints; the average RMSD to mean for backbone atoms is 0.36 Å. (b) Side and top view of the lowest energy structure of the family, including hydrogen-bonding interactions.[4] Side chains of D- and L-Val residues point away from the helical axis and are omitted for clarity.

This result suggests that in some cases two β turns may be necessary to obtain a unique $\uparrow\downarrow\beta^{5.6}$-helical species.

We emphasize that the hybrid β-hairpin/β-helical constructs described here were characterized only in nonpolar organic solvents. Given the highly constrained nature of **2** and **3** and the compactness of the resulting structures, we hypothesize that appropriately designed polar derivatives will adopt similar folds in water. We are currently working to test this hypothesis by preparing water-soluble variants of **2** and **3** for possible use as ligands for macromolecular targets and building blocks for new protein architectures. In addition, we are working to more fully define the sequence scope of this new peptide motif.

Acknowledgment. We thank Greg Heffron for assistance with instrumentation and Federico Del Rio for helpful discussions. This work was supported by the Office of Naval Research and the NIH (Grant GM47467 to G.W.).

Supporting Information Available: Details concerning the structural characterization of **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Creighton, T. E. *Proteins: Structures and Molecular Properties*, 2nd ed.; W. H. Freeman: New York, 1992. (b) Seebach, D.; Hook, D. F.; Glattli, A. *Biopolymers* **2006**, *84*, 23−37.

(2) (a) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988−1011. (b) Navarro, E.; Fenude, E.; Celda, B. *Biopolymers* **2004**, *73*, 229−241. (c) Lorenzi, G. P.; Jackle, H.; Tomasic, L.; Rizzo, V.; Pedone, C. *J. Am. Chem. Soc.* **1982**, *104*, 1728−1733. (d) Di Blasio, B.; Benedetti, E.; Pavone, V.; Pedone, C.; Spiniello, O.; Lorenzi, G. P. *Biopolymers* **1989**, *28*, 193−201.

(3) (a) Stanger, H. E.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4236−4237. (b) Yang, L.; Morriello, G. *Tetrahedron Lett.* **1999**, *40*, 8197−8200.

(4) See Supporting Information for details.

(5) (a) Lorenzi, G. P.; Tomasic, L. *J. Am. Chem. Soc.* **1977**, *99*, 8322−8323. (b) O'Boyle, F.; Wallace, B. A. *Protein Pept. Lett.* **2003**, *10*, 9−17.

(6) (a) Clark, T. D.; Buriak, J. M.; Kobayashi, K.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 8949−8962. (b) Akiyama, M.; Ohtani, T. *Spectrochim. Acta* **1994**, *50A*, 317−324.

(7) (a) Naik, V. M. *Vib. Spectrosc.* **1992**, *3*, 105−113. (b) Naik, V. M. *Int. J. Pept. Protein Res.* **1993**, *42*, 125−131. (c) Naik, V. M.; Krimm, S. *Biophys. J.* **1986**, *49*, 1131−1145.

(8) (a) Navarro, E.; Fenude, E.; Celda, B. *Biopolymers* **2002**, *64*, 198−209. (b) Zhang, Z. L.; Pascal, S. M.; Cross, T. A. *Biochemistry* **1992**, *31*, 8822−8828.

(9) (a) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986. (b) Schwieters, C. D.; Kuszewski, J. J.; Tjandra, N.; Clore, G. M. *J. Magn. Reson.* **2003**, *160*, 65−73.

JA062737F