ORGANIC LETTERS

2007
Vol. 9, No. 9
1801−1804

Origins of the High 14-Helix Propensity of Cyclohexyl-Rigidified Residues in β-Peptides

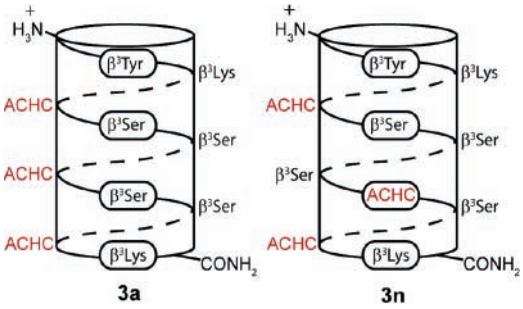
Myung-ryul Lee,[†,§] Tami L. Raguse,[†,§] Marina Schinnerl,[§] William C. Pomerantz,[§] Xiaodong Wang,[‡] Peter Wipf,*[,‡] and Samuel H. Gellman*[,‡]

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706, and Department of Chemistry and Center for Chemical Methodologies and Library Development, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

gellman@chem.wisc.edu; pwipf+@pitt.edu

Received February 28, 2007

ABSTRACT



β-Peptides containing residues derived from *trans*-2-aminocyclohexanecarboxylic acid (ACHC) display high population of 14-helical secondary structure in aqueous solution. We show that hydrophobic interactions between cyclohexyl rings are not responsible for this conformation-promoting effect, and that polar groups may be attached to the cyclohexyl ring without diminishing the effect.

Oligomers that adopt predictable conformations in aqueous solution, that can be prepared via straightforward modular chemistry, and that enable display of a wide range of functional groups are attractive as a basis for creating new types of biologically active agents.[1] β-Peptides have become increasingly popular in this regard because this class of foldamers allows access to a variety of backbone conformations.[2] The 14-helix is the most widely studied β-peptide folding pattern.[2,3] β-Peptides favoring the 14-helix have been reported to display antibacterial[4] and antifungal[5] activity, to enter cells,[6] to block protein−protein interactions,[7] to self-assemble into helix-bundle quaternary structures,[8] and to form lyotropic liquid crystalline phases.[9] In each case the ability of the β-peptide to display side chains in a particular three-dimensional arrangement, as a result of 14-helix

[†] These authors contributed equally.
[§] University of Wisconsin−Madison.
[‡] University of Pittsburgh.

(1) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (b) Barron, A. E.; Zuckermann, R. N. *Curr. Opin. Chem. Biol.* **1999**, *3*, 681. (c) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893. (d) Huc, I. *Eur. J. Org. Chem.* **2004**, 17.

(2) (a) Seebach, D.; Matthews, J. L. *J. Chem. Soc., Chem. Commun.* **1997**, 2015. (b) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219. (c) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, *1*, 1111.

(3) (a) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (b) Seebach, D.; Ciceri, P. E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 2043. (c) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071. (d) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 6206.

(4) (a) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1999**, *121*, 12200. (b) Liu, D.; DeGrado, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 7553. (c) Raguse, T. L.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12774.

(5) Karlsson, A. J.; Pomerantz, W. C.; Weisblum, B.; Gellman, S. H.; Palecek, S. P. *J. Am. Chem. Soc.* **2006**, *128*, 12630.

(6) Potocky, T. B.; Menon, A. K.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 3686.

10.1021/ol070511r CCC: $37.00
Published on Web 03/30/2007

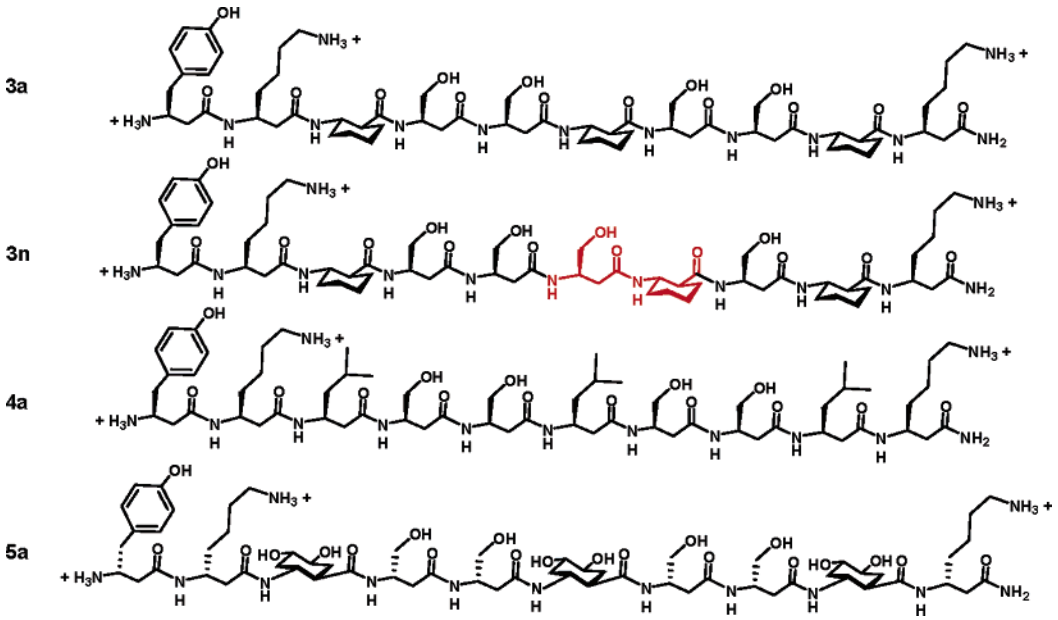


Figure 1. Structures of β-peptides **3**−**5**. The difference between the "a" (aligned) and "n" (non-aligned) series is illustrated for **3a/n**; the sequential swap between residues 6 and 7 is highlighted in red. Structures **4n** and **5n** (not shown) have a comparable sequential swap between residues 6 and 7 relative to **4a** and **5a**, respectively.

formation, appears to be crucial for activity. The expanding range of applications among 14-helical molecules highlights a need for understanding the factors that stabilize this β-peptide secondary structure.

Here we describe experiments that probe the contribution of ring-constrained β-amino acid residues to 14-helix propensity in aqueous solution. *trans*-2-Aminocyclohexanecarboxylic acid (ACHC) residues favor 14-helical folding in water more strongly than do analogous β^3-residues (which bear a single side chain adjacent to the backbone nitrogen atom).[10] Specialized strategies to promote 14-helicity in water with exclusively β^3-residues have recently been identified.[11] Most prominent among these strategies is *i*, *i* + *3* positioning of complementary ionic side chains, which allows intrahelical salt bridge formation (the 14-helix has ca. 3 residues per turn), and incorporating β^3-residues with side chains branched adjacent to the backbone, such as β^3-homovaline (β^3-hVal). Employing side chain ion pairing and/or branching generally requires that two-thirds of the residues be devoted to 14-helix stabilization. To assess the helix-promoting effects of ACHC relative to ion pairing and side chain branching, we have previously compared β-peptide **1** (β^3hVal-β^3hGlu-β^3-hOrn-β^3hVal-β^3hOrn-β^3hGlu-β^3hVal; β^3-residues derived from D-α-amino acids), the enantiomer of which was originally described by Seebach et al.,[11a] and ACHC-containing analogue **2** (ACHC-β^3hGlu-β^3hOrn-ACHC-β^3-hOrn-β^3hGlu-ACHC).[10b] We found that **2** displays a greater extent of 14-helicity than does **1**, especially under conditions that preclude salt bridge formation. The behavior of **2** suggests that rigidification of fewer than half of the residues can strongly promote 14-helicity in short β-peptides, and that use of ACHC for this purpose is advantageous relative to other strategies, because a majority of the residues can bear side chains selected for function rather than structure stabilization.

Our observations with **2** raise a question: does the alignment of ACHC residues along one side of the 14-helix contribute to conformational stability in water? The cyclohexyl unit is hydrophobic, and therefore the stacking of ACHC rings upon one another in the folded state might bury considerable hydrophobic surface. A requirement for ACHC alignment would be a significant constraint on the design of

(7) (a) Werder, M.; Hauser, H.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774. (b) Kritzer, J. A.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. *J. Am. Chem. Soc.* **2004**, *126*, 9468. (c) Stephens, O. M.; Kim, S.; Welch, B. D.; Hodsdon, M. E.; Kay, M. S.; Schepartz, A. *J. Am. Chem. Soc.* **2005**, *127*, 13126. (d) Murray, J. K.; Farooqi, B.; Sadowsky, J. D.; Scalf, M.; Freund, W. A.; Smith, L. M.; Chen, J.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 13271.

(8) (a) Raguse, T. L.; Lai, J. R.; LePlae, P. R.; Gellman, S. H. *Org. Lett.* **2001**, *3*, 3963. (b) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 11564. (c) Chakraborty, P.; Diederichsen, U. *Chem. Eur. J.* **2005**, *11*, 3207. (d) Qiu, J. X.; Petersson, E. J.; Matthews, E. E.; Schepartz, A. *J. Am. Chem. Soc.* **2006**, *128*, 11338. (e) Daniels, D. S.; Pettersson, E. J.; Qiu, J. X.; Schepartz, A. *J. Am. Chem. Soc.* **2007**, *129*, 1532.

(9) Pomerantz, W. C.; Abbott, N. L.; Gellman, S. H. *J. Am. Chem. Soc.* **2006**, *128*, 8730. For self-association of ACHC oligomers, see: Martinek, T. A.; Hetényi, A.; Fülöp, L.; Mándity, I. M.; Tóth, G. K.; Dekany, I.; Fülöp, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 2396.

(10) (a) Appella, D. H.; Barchi, J. J.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309. (b) Raguse, T. L.; Lai, J. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2003**, *125*, 5592.

(11) (a) Arvidsson, P. I.; Rueping, M.; Seebach, D. *J. Chem. Soc., Chem. Commun.* **2001**, 649. (b) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 5162. (c) Raguse, T. R.; Lai, J. R.; Gellman, S. H. *Helv. Chim. Acta* **2002**, *85*, 4154. (d) Hart, S. A.; Bahadoor, A. B. F.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. *J. Am. Chem. Soc.* **2003**, *125*, 4022. (e) Guarracino, D. A.; Chiang, H. R.; Banks, T. N.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. *Org. Lett.* **2006**, *8*, 807. (f) Vaz, E.; Brunsveld, L. *Org. Lett.* **2006**, *8*, 4199.

β-peptides that are intended to adopt the 14-helix. To address this question we have prepared and examined three pairs of deca-β-peptide sequence isomers, **3a/n**, **4a/n**, and **5a/n** (Figure 1).

The sequence of **3a** (**a** for "aligned") was designed so that in the 14-helical conformation the three ACHC residues would be aligned along the helix axis. Sequence isomer **3n** (**n** for "non-aligned") does not allow direct contact of ACHC rings along the side of the 14-helix. Two β^3-hLys residues were incorporated because we believed that a net charge, arising from protonation of the side chain amino groups, would be important for conferring aqueous solubility.[12] Most residues in **3a/n** are β^3-hSer, which was chosen because the side chain is small, polar, and uncharged. ACHC is expected to be the dominant 14-helix-promoting component of β-peptides **3a/n**. If stacking of cyclohexyl rings upon one another is important for 14-helical stability in water, then **3a** should display a larger extent of folding than does sequence isomer **3n**.

We used circular dichroism (CD) for qualitative comparison of secondary structure among β-peptides in solution. Figure 2A shows far-UV CD signatures for 0.1 mM **3a** or **3n** in aqueous buffer (10 mM Tris, pH 7.2) or in methanol. In water, **3a** and **3n** display a minimum around 214 nm and a maximum around 195 nm, a signature that is characteristic of the 14-helix.[2,3,10,11,13] In methanol, the corresponding minima and maxima are at similar wavelengths. Two conclusions can be drawn from this comparison. First, since CD intensities observed in aqueous buffer for **3n** are nearly identical with those observed for **3a**, it appears that cyclohexyl stacking does *not* play a major role in stabilizing the 14-helical conformation. Second, since the CD intensities in water are comparable to those in methanol, it is possible that these β-peptides are fully folded in water. This conclusion arises from extensive literature on β-peptides constructed exclusively from β^3-residues, for which CD intensities in methanol are always greater than CD intensities in water, often dramatically so.[14] This effect is illustrated by the CD data for β-peptide **4a** (Figure 2B), the analogue of **3a** in which ACHC has been replaced by β^3-hLeu. No sign of 14-helicity can be detected for **4a** in water, but a strong 14-helical signature is observed in methanol. Identical behavior was observed for **4n**.[15] Monitoring the CD minimum at 214 nm for **4a** or **4n** as a function of water:methanol ratio reveals a sigmoidal relationship (Figure 3). Thus, water appears to be a potent denaturant for the 14-helix formed by the all-β^3 sequences. Comparisons between series **3** and **4** illustrate the profoundly stabilizing effect of a small proportion (30%) of ACHC residues on folding in aqueous solution.

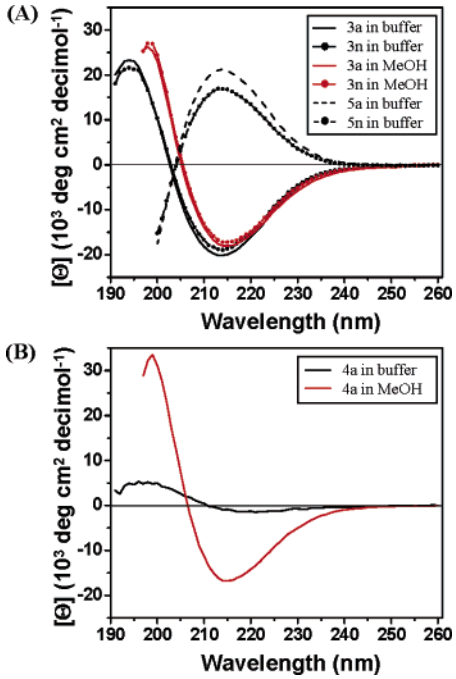


Figure 2. (A) CD data for **3a** and **3n** in aqueous buffer and in methanol; CD data for **5a** and **5n** in aqueous buffer. (B) CD data for **4a** in aqueous buffer and in methanol.

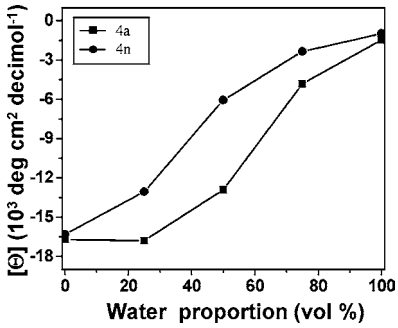


Figure 3. CD intensity at 214 nm of **4a** and **4n** as a function of solvent composition, varying between pure methanol and pure aqueous buffer.

The interpretation offered above regarding CD data for **3a** and **3n** is based on the assumption that neither molecule self-associates in aqueous solution. This assumption is supported by our finding that 8-fold dilution of each β-peptide caused no change in mean residue ellipticity at 214 nm.[15] We probed further for self-association in **3a** by examining an analogue in which the cyclohexane-based residues do not present a hydrophobic outer edge to the solvent. This effort involved replacing ACHC with a derivative bearing hydroxyl groups on carbons 4 and 5. The necessary β-amino acid building block was available only

(12) We prepared the analogues of **3a** and **3n** in which the two β^3h-Lys residues were replaced with β^3h-Ser. These compounds were quite water-soluble, up to at least 1 mM, and their CD signatures matched those of **3a** and **3n**.

(13) (a) Abele, S.; Guichard, G.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 187. (b) Gung, B. W.; Zou, D.; Stalcup, A. M.; Cottrell, C. E. *J. Org. Chem.* **1999**, *64*, 2176.

(14) Glaettli, A.; Daura, X.; Seebach, D.; van Gunsteren, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 12972.

(15) Relevant data may be found in the Supporting Information.

with *R* configuration at the ring positions bearing the amino and carboxyl groups;[16] to generate **5a/n**, this novel β-amino acid was combined with β^3-amino acids derived from D-α-amino acids to achieve the homochirality required for 14-helical folding. The hydroxyl groups are equatorial on the cyclohexyl ring; therefore, when the dihydroxylated ACHC residues are aligned along one side of the 14-helix, as in **5a**, they will not form an outward-facing hydrophobic surface of the type that might support self-association. The CD signatures displayed by **5a** and **5n** in aqueous buffer (Figure 2A) are similar to one another and, in mirror image fashion, to the CD signatures of **3a** and **3n** under the same conditions. These similarities show that the dihydroxylated ACHC residue supports 14-helix formation, and they strongly suggest that self-association does not play a role in determining the extent of 14-helix formation in **3a**.

We chose **5a** for qualitative correlation of CD and 2D NMR (ROESY) data because of the novel β-amino acid residues in this β-peptide. Extensive precedent has shown that $C_\alpha H(i) \rightarrow C_\beta H(i + 3)$ NOEs are a hallmark of 14-helical secondary structure.[3,10] There are seven possible $C_\alpha H(i) \rightarrow C_\beta H(i + 3)$ pairings in deca-β-peptide **5a** (Figure 4). We

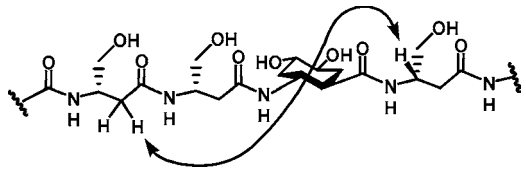


Figure 4. Graphical representation of a $C_\alpha H(i) \rightarrow C_\beta H(i + 3)$ NOE observed for **5a**.

examined **5a** in 9:1 H_2O:D_2O and in CD_3OH (the CD signature for **5a** in methanol matched that of **3a** and **3n** (not shown)). At 14 °C in 9:1 H_2O:D_2O, four of the seven possible NOEs could be identified, those involving residue pairs 1/4, 5/8, 6/9, and 7/10. The other three NOEs might have been present, but resonance overlap prevented unambiguous assignment. At 14 °C in CD_3OH, five of the seven NOEs could be identified, for pairs 1/4, 2/5, 4/7, 5/8, and 7/10; the other two NOEs might have been present.[15] Despite problems arising from partial resonance overlap, these ROESY data provide strong evidence for a high population of the 14-helical conformation because both terminal $C_\alpha H(i) \rightarrow C_\beta H(i + 3)$ NOEs could be clearly observed in each solvent. For partially folded helices, from both α- and β-peptides, it is common *not* to detect characteristic NOEs at termini, even if NOEs from central regions are strong, because helix termini are often "frayed".[17]

We applied several standard techniques to try to disrupt 14-helicity of **3a** in aqueous solution, with results monitored by CD. Heating from 25 to 80 °C caused only a modest diminution in the intensity of the 14-helical signature. Introduction of 8 M urea, a common protein denaturant, had virtually no effect on the CD signature of **3a**, and introduction of guanidinium chloride, a more potent denaturant, led to only modest changes. Nearly identical behavior was observed for **3n** under these various conditions.[15] Overall, these results indicate that the ACHC-stabilized 14-helix is a very robust molecular scaffold.

We have shown that the very high 14-helix stability manifested by short β-peptides containing ACHC residues does not require that the cyclohexyl residues be aligned to allow cyclohexyl−cyclohexyl stacking in the helical conformation. In addition, we have shown that introduction of polar functional groups as equatorial substituents on the cyclohexyl ring does not diminish the strong 14-helical propensity provided by the cyclic constraint. These results suggest that promotion of 14-helicity by ACHC and derivatives arises from conformational preorganization of the backbone. A number of strategies based purely on acyclic residues have been reported for stabilizing the 14-helix in water,[11] but these approaches typically require that a large proportion (e.g., two-thirds) of the residues be tailored for this purpose or that additional synthetic steps be introduced to connect side chains covalently. Our results suggest that incorporation of a small number of cyclohexyl-rigidified residues at any positions within a short β-peptide will lead to high 14-helicity in water, and that a wide variety of residues may be placed at the remaining positions. The ability to distinguish between a small number of structure-driven residue choices and a large number of function-driven residues should enable ACHC-containing β-peptides to display diverse functions.

Acknowledgment. This research was supported by NIGMS (GM56414 (to S.H.G.) and GM067082 (to P.W.)). L.M.R. was supported in part by a Korea Research Foundation Grant funded by the Korean Goverment (MOEHRD) (KRF-2006-214-C00053). T.L.R. was supported in part by an NSF pre-doctoral fellowship. M.S. was supported in part by a fellowship from the DAAD (German Academic Exchange Service; D/01/05604). The authors kindly thank PepTech for discounted prices for β-amino acids.

Supporting Information Available: Structure of **1** and **2**, CD spectra for **4n**, **3a**, and **3n**, and the 2D-ROESY spectrum of **5a**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL070511R

(16) Wipf, P.; Wang, X. *Tetrahedron Lett.* **2000**, *41*, 8747.

(17) (a) Rohl, C. A.; Baldwin, R. L. *Biochemistry* **1994**, *33*, 7760. (b) Scholtz, J. M.; Baldwin, R. L. *Annu. Rev. Biophys. Biomol. Struct.* **1992**, *21*, 95.