Formation of Short, Stable Helices in Aqueous Solution by β-Amino Acid Hexamers

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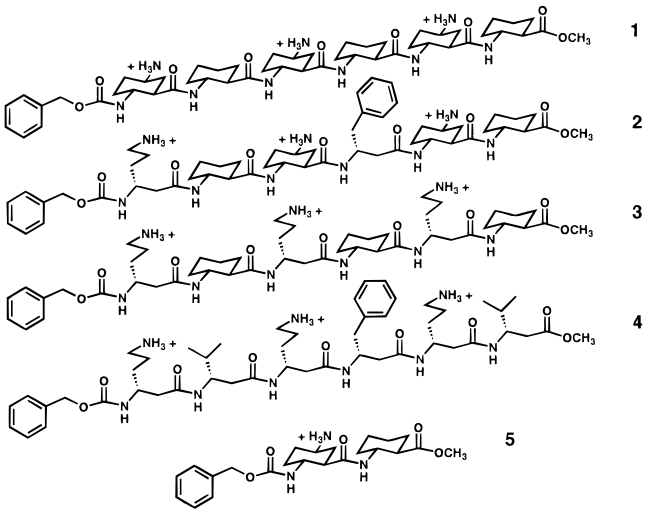
Received November 13, 1998

Biological systems rely on peptides and proteins for a wide variety of functions, but designing new activities into short, linear α-amino acid oligomers is difficult because of high intrinsic flexibility. Conformational instability arises because the forces that promote folding, like hydrogen bonds and hydrophobic interactions, are insufficient to overcome the entropic cost of ordering the oligo-α-amino acid backbone. β-Amino acid oligomers ("β-peptides") provide an interesting alternative to conventional peptides for design purposes, since the β-peptide backbone offers greater opportunity for conformational rigidification.[1] Short β-peptides have recently been shown to adopt each of the three types of regular secondary structure (helix, sheet, and turn) that are observed in proteins,[2−4] but these results have been obtained in organic solvents. High-resolution structural analysis of other unnatural oligomers with well-defined secondary structures has also been limited to organic solvents.[5,6] Organic solvents often exert very strong structure-stabilizing effects on conventional peptides, relative to water. Here we show that β-peptides with just six residues display a high population of a specific helical conformation in aqueous solution, if backbone flexibility is limited by careful residue choice.

Short oligomers of optically pure *trans*-2-aminocyclohexanecarboxylic acid (ACHC),[3] or of optically pure acyclic β-substituted β-amino acids,[2] adopt a helix defined by 14-membered ring hydrogen bonds ("14-helix") in organic solvents and in the solid state. We have previously proposed that the backbone constraint provided by the cyclohexane ring should confer very high conformational stability on ACHC oligomers.[3] To test this hypothesis in aqueous solution, we synthesized a *R*,*R*,*R*-2,5-diaminocyclohexanecarboxylic acid (DCHC) derivative with differential protection on the amino groups.[7] We then prepared a series of hexa-β-peptides, **1**−**4**, with varying proportions of cyclohexyl and acyclic β-amino acid residues. The acyclic residues were synthesized from the corresponding D-α-amino acids (ornithine, phenylalanine, or valine) by the elegant Seebach method.[2]



Hexa-β-peptides **1**−**4** were designed to bear a charge of +3 at pH ≤ 7, with the charges spread around the periphery of the 14-helix, to ensure aqueous solubility and discourage aggregation.

High-resolution structural analysis of all-cyclohexyl hexamer **1** by ^{1}H NMR was hampered because of poor spectral resolution; however, hexamer **2**, with a 2:1 cyclohexyl:acyclic ratio, could be analyzed in this way (100 mM CD_3CO_2D/CD_3CO_2Na, pH 3.9).[8] The six NH resonances were well resolved at 5 °C, but the $C_\beta H$ of residues 2 and 6 were completely overlapped, and there were several cases of partial overlap among the $C_\alpha H$ and the $C_\beta H$ resonances. In addition to the main set of resonances for **2**, a minor set of signals was observed in the amide region. The proportion of this minor component varied with temperature (maximum of 11%); the minor resonances broadened significantly above 40 °C and were undetectable at 55 °C. This behavior suggests that the minor species is a conformational isomer, presumably about the carbamate C−N bond.[9]

NOESY[10] data for **2** at 5 °C revealed numerous short- and long-range interactions consistent with 14-helix formation. ROESY[11] data obtained at 35 °C indicated that the 14-helical conformation of **2** is very stable, since many helix-defining correlations persisted

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(1) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173.
(2) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. 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. Seebach, D.; Matthews, J. L. *J. Chem. Soc., Chem. Commun.* **1997**, 2015. Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J. L.; Schreiber, J. V.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1998**, *81*, 932.
(3) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071.
(4) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, Huang, D. R. X.; Barchi, J. J. *Nature* **1997**, *387*, 381. Krauthäuser, S.; Christianson, L. A.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11719. Chung, Y. J.; Christianson, L. A.; Stanger, H. E.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 10555.
(5) Unnatural oligomers with discrete folding propensities in organic solvents: Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568. Smith, A. B.; Guzman, M. C.; Sprengler, P. A.; Keenan, T. P.; Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* **1994**, *116*, 9947. Gennari, C.; Salom, B.; Potenza, D.; Longari, C.; Fioravanzo, E.; Carugo, O.; Sardone, N. *Chem. Eur. J.* **1996**, *2*, 644−655. Nowick, J. S.; Mahrus, S.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 1066. Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1997**, *119*, 10587. Armand, P.; Kirshenbaum, K.; Goldsmith, R. A.; Farr-Jones, S.; Barron, A. E.; Truong, K. T.; Dill, K. A.; Mierke, D. F.; Cohen, F. E.; Zuckermann, R. N.; Bradley, E. K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4309. Hintermann, T.; Gademann, K.; Jaun, G.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 983. Hanessian, S.; Luo, X.; Schaum, R.; Michnick, S. *J. Am. Chem. Soc.* **1998**, *120*, 8569. Tanatani, A.; Yamaguchi, K.; Azumaya, I.; Fukutomi, R.; Shudo, K.; Kagechika, H. *J. Am. Chem. Soc.* **1998**, *120*, 6433. Smith, M. D.; Claridge, T. D. W.; Tranter, G. E.; Sansom, M. S. P.; Fleet, G. W. J. *J. Chem. Soc., Chem. Commun.* **1998**, 2041. Nelson, J. C.; Saven, J. G.; Moore, J. S.; Wolynes, P. G. *Science* **1997**, *277*, 1793−1796.
(6) Unnatural oligomers for which low-resolution structural data are available in aqueous solution: Bolli, M.; Micura, R.; Eschenmoser, A. *Chem. Biol.* **1997**, *4*, 309. Lokey, R. S.; Iverson, B. L. *Nature* **1995**, *375*, 303. Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074.
(7) Appella, D. H.; Gellman, S. H., manuscript in preparation.
(8) Data may be found in the Supporting Information.
(9) Benedetti, E.; Pedone, C.; Toniolo, C.; Nemethy, G.; Pottle, M. S.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1980**, *16*, 156

10.1021/ja983918n CCC: $18.00
Published on Web 02/24/1999

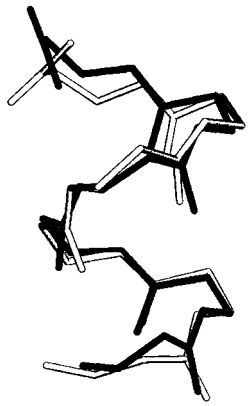

Figure 1. Backbone-only overlay of an NOE-derived structure (dark) and the crystal structure (light) of a 14-helical hexa-β-peptide comprised of cyclohexane-based residues (ref 3); the rms deviation between these two structures (heavy backbone atoms) is 0.27 Å. Details of the NMR and NOE-restrained dynamics analyses may be found in the Supporting Information.

at this elevated temperature. Only two cross-peaks inconsistent with the 14-helix were identified in any of the two-dimensional studies, very weak $NH_i \rightarrow C_\beta H_{i-1}$ NOEs involving the NHs of residues 5 and 6.

Restrained molecular dynamics calculations were performed with distance restraints derived from the NOE data.[8] These simulations identified a set of 16 structures with low restraint violation and minimum energy; the rms deviation among these structures was 0.1 Å. The conformations are very homogeneous except at the N terminus, where the benzyloxycarbonyl-protecting group displays considerable flexibility. Figure 1 is an overlay of the backbone of one of the NMR-derived structures and the backbone from the crystal structure of an ACHC hexamer.[3] This juxtaposition shows that despite the presence of two acyclic residues and aqueous solvation, hexa-β-peptide **2** displays a 14-helical conformation very similar to that of a β-peptide comprised exclusively of cyclohexane-based residues.

Circular dichroism (CD) allowed us to compare 14-helix stability among hexa-β-peptides **1**−**4**, i.e., to monitor the effect of incrementally relaxing backbone constraint by replacing cyclohexyl residues with acyclic residues (Figure 2). Recent experimental and theoretical work[2,12] has established the utility of CD for identification of helical structure in β-peptides. Hexa-β-peptide **1** displays a broad maximum at ca. 215 nm, which we

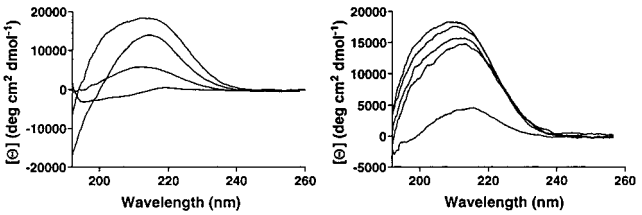

Figure 2. Circular dichroism data. Left: Hexa-β-peptides **1**−**4** (in order of decreasing ellipticity at 215 nm), 100 nM aqueous CH_3CO_2H/CH_3CO_2Na, pH 3.9, 25 °C. Right: Hexa-β-peptide **1** at 25, 45, 65, and 80 °C (upper set of curves, in order of decreasing ellipticity at 215 nm), and di-β-peptide **5** at 25 °C (lowest curve). Data were obtained on a Jasco J-715 instrument with 1-mm pathlength cells. The data have been normalized for β-peptide concentration and number of residues.

ascribe to 14-helix formation. Hexa-β-peptide **2** also displays a maximum at 215 nm. The intensity at 215 nm is modestly diminished for **2** relative to **1**, suggesting that replacement of two cyclohexyl residues by acyclic residues has modestly decreased 14-helix stability. Further diminution of 14-helical stability is observed for **3**, in which half of the residues are acyclic. Hexa-β-peptide **4**, with a completely unconstrained backbone, no longer displays the maximum at 215 nm. Previous data have suggested that β-peptides containing exclusively acyclic residues and β-peptides containing exclusively rigidified cyclic residues adopt helices with comparable stability in organic solvents.[2,3] In contrast, the present results suggest that rigidified residues are essential for short well-defined helices in water.

Variable-temperature CD data for **1** suggest that heating from 25 to 80 °C causes only modest diminution of 14-helical folding in aqueous solution (Figure 2). Even at 80 °C, the maximum at 215 nm is considerably more intense for **1** than for di-β-peptide **5** at room temperature. The CD spectrum of hexa-β-peptide **2** also manifested relatively little change between 25 to 80 °C (not shown). These findings indicate that use of conformationally constrained β-amino acid residues allows creation of a small and specific three-dimensional structure, in this case just two turns of 14-helix, that is extremely stable in water.

The 14-helices adopted by hexa-β-peptides **1** and **2** are remarkable because, at most, only four intramolecular hydrogen bonds can form, and small hydrogen-bonded structures are virtually never stable in water. α-Helical α-amino acid oligomers display much less conformational stability on a per-residue basis. α-Peptides 6−8 residues in length are typically used as standards for the unfolded ("random coil") state, because even when these conventional peptides are comprised of residues with high α-helical propensity, they display no detectable folding in water.[13] Short oligoprolines have been proposed to adopt polyproline II helical conformations in aqueous solution, but these conclusions have been based exclusively on low-resolution structural data.[14,15] Despite widespread recent interest in unnatural oligomers with well-defined folding propensities, only a few conformational studies have been reported in aqueous solution.[6] No high-resolution structural data were available for an unnatural oligomer in water prior to this report.

The high conformational stability of short oligomers of properly chosen β-amino acids in aqueous solution suggests that β-peptides will provide useful scaffolds for creation of biologically active molecules with predetermined shapes. In particular, the β-peptide 14-helix (1.6 Å rise per residue; 4.0 Å internal diameter) could mimic α-helices (1.5 Å rise per residue; 3.2 Å internal diameter), providing a new strategy for targeted disruption of protein−protein complexes.[16] Biological applications of β-peptides should be facilitated by their resistance to protease degradation.[17,18]

Supporting Information Available: Description of NMR and modeling studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA983918N

(10) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95.
(11) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.
(12) Bode, K. A.; Applequist, J. *Macromolecules* **1997**, *30*, 2144. Applequist, J.; Bode, K. A.; Appella, D. H.; Christianson, L. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4891.
(13) Rohl, C. A.; Baldwin, R. L. *Biochemistry* **1994**, *31*, 7760.
(14) Rothe, M.; Rott, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 770. Dukor, R. A.; Kiederling, T. A.; Gut, V. *Int. J. Pept. Protein Res.* **1991**, *38*, 198. Williamson, M. P. *Biochem. J.* **1994**, *297*, 249. McMafferty, D. G.; Slate, C. A.; Nakhle, B. M.; Graham, H. D.; Austell, T. L.; Vachet, R. W.; Mullis, B. H.; Erickson, B. W. *Tetrahedron* **1995**, *51*, 9859. Computational evidence for conformational flexibility in short oligoprolines: Sneddon, S. F.; Brooks, C. L. *J. Am. Chem. Soc.* **1992**, *114*, 8220.
(15) High-resolution NMR studies suggest that short proline-rich oligomers, which also contain other residues, are disordered in water: Pons, M.; Feliz, M.; Celma, C.; Giralt, E. *Magn. Reson. Chem.* **1987**, *25*, 402. Murray, N. J.; Williamson, M. P. *Eur. J. Biochem.* **1994**, *219*, 915. Chu, S. S.; Vander Velde, D.; Shobe, D.; Balse, P.; Doughty, M. B. *Biopolymers* **1995**, *35*, 583.
(16) Clackson, T.; Wells, J. A. *Science* **1995**, *267*, 383. Huang, J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13396. Judice, J. K.; Tom, J. Y. K.; Huang, W.; Wrin, T.; Vennari, J.; Petropoulos, C. J.; McDowell, R. S. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13426. Zutshi, R.; Brickner, M.; Chmielewski, J. *Curr. Med. Chem.* **1998**, *2*, 62.
(17) Hintermann, T.; Seebach, D. *Chimia* **1997**, *51*, 244.
(18) This work was supported by the National Institutes of Health (GM56414, to S.H.G.). D.H.A. was supported in part by a Chemistry-Biology Interface Training Grant from NIGMS and by a fellowship from Procter & Gamble.