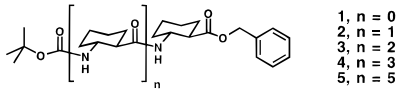
β-Peptide Foldamers: Robust Helix Formation in a New Family of *β*-Amino Acid Oligomers

Daniel H. Appella,† Laurie A. Christianson,†
Isabella L. Karle,*,‡ Douglas R. Powell,† and
Samuel H. Gellman*,†

Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706-1396
Laboratory for the Structure of Matter
Naval Research Laboratory, Washington, D.C. 20375-5341

Received September 19, 1996

Chemists have long sought to extrapolate the power of biological catalysis and recognition to synthetic systems. These efforts have focused largely on low molecular weight catalysts and receptors;[1−3] however, biological systems themselves rely almost exclusively on polymers, proteins and RNA, to perform complex chemical functions. Proteins and RNA are unique in their ability to adopt compact, well-ordered conformations, and specific folding provides precise spatial orientation of the functional groups that comprise the "active site". These features suggest that identification of new polymer backbones with discrete and predictable folding propensities ("foldamers") will provide a basis for design of molecular machines with unique capabilities. The foldamer approach complements current efforts to design unnatural properties into polypeptides and polynucleotides.[4−7] The first step in creating a foldamer is to identify polymeric backbones with well-defined secondary structural preferences.[8−18] Here we describe a new polyamide family (**1**−**5**) that strongly favors a specific helical secondary structure, which should ultimately serve as a building block for stable tertiary structures.



We have previously reported model studies that indicate *β*-amino acid oligomers ("*β*-peptides") to be well suited for adoption of compact secondary structures stabilized by intramolecular hydrogen bonds.[8] Figure 1 shows the hydrogen bonds that define the six narrowest helices available to poly-*β*-alanine, the simplest *β*-peptide polymer. The 12-, 16-, and 20-helices (nomenclature derived from hydrogen-bonded ring size) contain hydrogen bonds from carbonyls toward NH groups in the C-terminal direction, as observed for 3_{10}- and α-helices in proteins, while the 10-, 14-, and 18-helices contain hydrogen bonds from carbonyls to NH groups in the N-terminal direction. Molecular mechanics studies of a *β*-alanine decamer (AMBER*/MacroModel 3.5[19,20]) suggested that all six of these helices constitute local minima on the conformational energy surface. *β*-Alanine oligomers, however, have been shown experimentally to be unordered in solution and to adopt sheetlike packing patterns in the solid state.[21]

Incorporation of the two backbone carbons of a *β*-amino acid into a small carbocycle provides substantial rigidity, and we employed computational methods to evaluate whether any particular helix/small ring combination would lead to enhanced conformational stability. For each of the six minimized deca-*β*-alanine helices (Figure 1), each residue was modified by incorporation of the backbone carbons into a three-, four-, five-, or six-membered cycloalkyl ring. For each ring size, both *cis* and *trans* relationships between the amino and carboxyl substituents were examined, and for the *cis* rings, both of the possible ring orientations relative to the helix were examined. This process yielded 72 helical starting structures [six helices × four cycloalkyl ring sizes × (one *trans* + two *cis* forms)]. A combination of minimization and dynamics studies predicted that the 14-helical form of the decamer of *trans*-2-aminocyclohexanecarboxylic acid (*trans*-ACHC) would be the most stable among these hypothetical helices.

In order to test this computational prediction, we prepared optically active *trans*-ACHC by the reported route[22] and synthesized oligomers via standard methods. The crystal structures of tetramer **4** (not shown) and of hexamer **5** (Figure 2) reveal that these molecules adopt 14-helical conformations in the solid state. The hexamer crystal contains three independent but very similar molecules, each of which forms the four possible 14-membered ring hydrogen bonds. The regular helix revealed by the hexamer crystal structure matches the minimum energy conformation predicted for the decamer.

Amide proton exchange is one of the most powerful methods for assessing conformational stability of peptides and proteins;[23] adoption of a stable intramolecularly hydrogen-bonded conformation leads to diminution of the rate of exchange. NH/ND exchange behavior of *trans*-ACHC hexamer **5** relative to dimer **2**, which is too small to form a favorable internal hydrogen bond, suggests that the hexamer adopts a very stable intramolecularly hydrogen-bonded folding pattern in methanol solution. To ensure a direct comparison, these studies were conducted with solutions containing 2 mM **2** and 2 mM **5**. Upon dissolution of the 1:1 **2**/**5** mixture in CD_3OD, the amide proton and the urethane proton of dimer **2** are completely exchanged within 6 min, according to ^{1}H NMR (Figure 3). In contrast, three of

* Correspondence regarding the hexamer crystal structure should be sent to I.L.K. Correspondence on matters other than the hexamer crystal structure should be sent to S.H.G. at gellman@chem.wisc.edu.
† University of Wisconsin.
‡ Naval Research Laboratory.

(1) Ball, P. *Designing the Molecular World*; Princeton University Press: Princeton, NJ, 1994.
(2) Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: Weinheim, 1995.
(3) Kirby, A. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 707.
(4) Krejchi, M. T.; Atkins, E. D. T.; Waddon, A. J.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Science* **1994**, *265*, 1427.
(5) Thorn, S. N.; Daniels, R. G.; Auditor, M. T.; Hilvert, D. *Nature* **1994**, *373*, 228.
(6) Wilson, C.; Szostak, J. W. *Nature* **1995**, *374*, 777.
(7) Robertson, D. E.; Farid, R. S.; Moser, C. C.; Urbauer, J. L.; Mulholland, S. E.; Pidikiti, R.; Lear, J. D.; Wand, A. J.; DeGrado, W. F.; Dutton, P. L. *Nature* **1994**, *368*, 425.
(8) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054.
(9) Chan, H. S.; Dill, K. A. *Proteins: Struct., Funct., Genet.* **1996**, *24*, 335.
(10) Eschenmoser, A. *Origins Life* **1994**, *24*, 389.
(11) Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568.
(12) Nowick, J. S.; Mahrus, S.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 1066.
(13) Hamuro, Y.; Geib, S. J.; Hamilton, A. H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 446.
(14) Gennari, C.; Salom, B.; Potenza, D.; Williams, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2067.
(15) 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.
(16) Lokey, R. S.; Iverson, B. L. *Nature* **1995**, *375*, 303.
(17) Cho, C. Y.; Moran, E. J.; Cherry, S. R.; Stephans, J. C.; Fodor, S. P. A.; Adams, C. L.; Sundaram, A.; Jacobs, J. W.; Schultz, P. G. *Science* **1993**, *261*, 1303.
(18) Krämer, R.; Lehn, J.-M.; Marquis-Rigault, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5394.
(19) Weiner, S.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230. McDonald, D. Q.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 7743.
(20) Mohamdi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
(21) Narita, M.; Doi, M.; Kudo, K.; Terauchi, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3553.
(22) Nohira, H.; Ehara, K.; Miyashita, A. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 2230.
(23) Englander, S. W.; Kallenbach, N. R. *Q. Rev. Biophys.* **1984**, *16*, 521.

S0002-7863(96)03290-8 CCC: $12.00

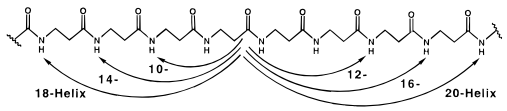


Figure 1. Hydrogen bonds defining the six narrowest helices available to a poly-β-alanine backbone. The helix designations are based on the number of atoms in the hydrogen-bonded ring.

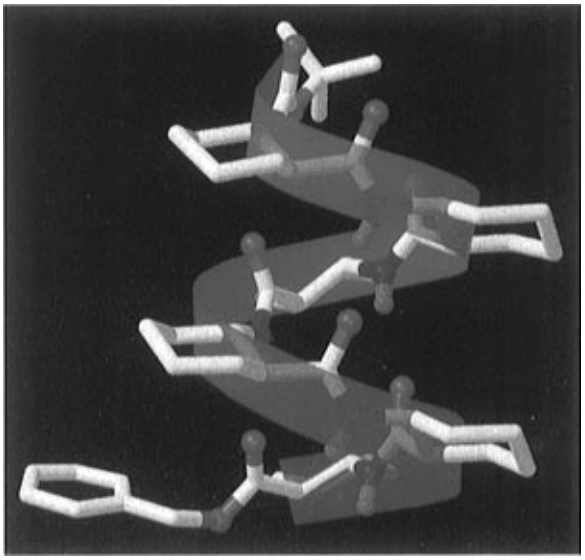


Figure 2. Solid state conformation of hexamer **5**; only one of the three independent molecules is shown. Crystallographic data: $3(C_{54}H_{82}H_6O_9)\cdot 2(CH_3OH)\cdot 3(C_2H_4Cl_2)$ per asymmetric unit, space group $P2_1$ with $a =$ 15.3932(3) Å, $b = 21.7642(4)$ Å, $c = 27.8692(3)$ Å, $\beta = 101.457(1)°$, $R = 11.8\%$ for 13335 data observed $F > 4\sigma(F)$ (resolution 1 Å). The structure of the three independent hexamer molecules was solved by vector search and translation[30] of a model consisting of a 34 atom fragment from the tetramer **4** structure and consequent development of the correctly oriented and placed fragment to the full structure of >200 atoms by the use of the tangent formula.[31] The atoms in one of the disordered phenyl groups and the five solvent molecules were located in difference maps. The image was produced with Raster3D v.2.2.[32]

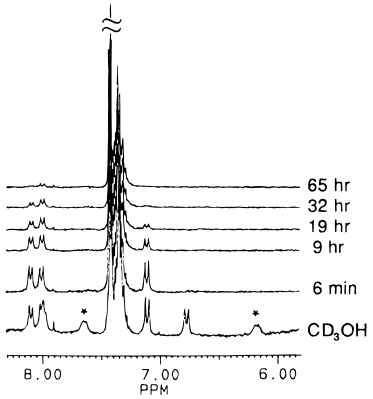


Figure 3. ^{1}H NMR data for a solution containing 2 mM dimer **2** and 2 mM hexamer **5**. The bottom spectrum was obtained in CD_3OH with solvent suppression. The two NH resonances from the dimer are indicated with an asterisk (*). All other spectra were obtained in CD_3-OD at the times indicated after dissolution of the sample. Data obtained on a Bruker 300 MHz spectrometer at 20 °C.

the six amide protons of hexamer **5** show strong resonances at this point. One of these protected protons exchanges within a ca. 20 h, but the other two require >2 days for complete exchange. Thus, two of the amide protons of **5** display >100-fold protection from NH/ND exchange with CD_3OD, which suggests remarkable conformational stability for this six-residue foldamer in a hydrogen-bonding solvent. We tentatively assign the two most protected protons of hexamer **5** to the amide groups of residues 2 and 3 (numbering from the N-terminus). The amide protons of residues 5 and 6 should exchange rapidly because they cannot be involved in 14-helical hydrogen bonds. The protons of residues 1 and 4 occur at the ends of the 14-helix in crystalline **5**. The ends of α-helices in short α-peptides are "frayed" in solution,[24] and similar fraying in **5** would presumably enhance the NH/ND exchange rate. This conclusion is supported by the observation that only one of the NH resonances of tetramer **4** can be detected by ^{1}H NMR within a few minutes of dissolution in CD_3OD, and this proton exchanges completely in less than an hour. Adoption of a stable folding pattern in solution requires the intrinsic rigidity of the *trans*-ACHC residue; dissolution of a β-alanine hexamer analogous to **5** in CD_3OD causes all NH groups to exchange within 6 min.

We have shown that β-peptide oligomers constructed from an appropriately rigidified residue are highly predisposed to form a specific helix. The conformations of more flexible β-amino acid polymers have previously been examined, but only low-resolution structural data are available for these materials, and conflicting deductions have been reported.[25−27] After the present work was completed, a report appeared from Seebach et al. describing oligomers constructed from optically active β-substituted β-amino acids.[28] These workers deduced 14-helix formation for a hexamer in pyridine, although a trimer displayed sheet packing in the crystalline form. The high folded stability of the *trans*-ACHC hexamer in methanol raises the possibility that analogues constructed from more highly functionalized monomers (e.g., carbohydrate derivatives[29]) will adopt stable folding patterns in aqueous solution. It will be particularly interesting to see whether heteropolymers containing both hydrophilic and lipophilic residues display hydrophobically driven tertiary structure formation. The defined conformation conferred by a small number of unadorned *trans*-ACHC residues suggests that combinatorial exploration of functionalized oligomers could lead to medicinally useful compounds.

Acknowledgment. We thank H. Nohira and J. Wuest for helpful comments. This work was supported by the U.S. National Science Foundation. D.A. was supported by a Chemistry Biology Interface Training Grant from NIGMS. L.C. was supported in part by a Graduate Fellowship from the Office of Naval Research.

Supporting Information Available: Crystallographic data for hexamer **5** (33 pages). See any current masthead page for ordering and Internet access instructions.

JA963290L

(24) Rohl, C. A.; Scholtz, J. M.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Biochemistry* **1992**, *31*, 1263.
(25) Yuki, H.; Okamoto, Y.; Taketani, Y.; Tsubota, T.; Marubayashi, Y. *J. Polym. Sci., Polym. Chem. Ed.* **1978**, *16*, 2237.
(26) Fernández-Santin, J. M.; Muñoz-Guerra, S.; Rodríguez-Galán, A.; Aymamí, J.; Lloveras, J.; Subirana, J. A.; Giralt, E.; Ptak, M. *Macromolecules* **1987**, *20*, 62.
(27) López-Carrasquero, F.; Alemán, C.; Muñoz-Guerra, S. *Biopolymers* **1995**, *36*, 263.
(28) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913.
(29) Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575.
(30) Egert, E.; Sheldrick, G. M. *Acta Crystallogr.* **1985**, *A41*, 262.
(31) Karle, J. *Acta Crystallogr.* **1986**, *B24*, 182.
(32) (a) Bacon, D. J.; Anderson, W. F. *J. Mol. Graphics* **1988**, *6*, 219. (b) Merrit, E. A.; Murphy, M. E. P. *Acta Crystallogr.* **1994**, *D50*, 869.