Antiparallel Sheet Formation in β-Peptide Foldamers: Effects of β-Amino Acid Substitution on Conformational Preference[1]

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Among proteins, only two types of secondary structure with long range order are observed, helices and sheets.[2] Seebach et al.[3a−c] and we[3d,e] have recently shown that short oligomers of β-amino acids ("β-peptides") can adopt stable helical conformations. Here we describe model studies that allow us to identify optimal residues for sheet secondary structure in β-peptide foldamers.[4]

There are two types of antiparallel β-peptide sheet structure. In **I**, the $NC_\beta-C_\alpha C(=O)$ torsion angle of each residue is anti, while in **II**, these torsion angles are gauche. (For an extended
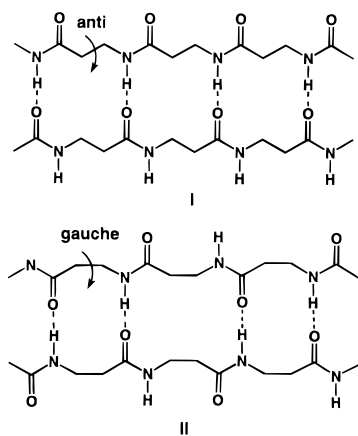
strand of type **II**, the $NC_\beta-C_\alpha C(=O)$ torsion angles must alternate between g^+ and g^-; repeated g^+ or g^- leads to helix formation.) The type **I** sheet has a net dipole while type **II** sheet does not, because the carbonyls are parallel in the former but antiparallel in the latter. This dimension of variation does not exist among sheets formed from α-amino acids, for which only an antiparallel arrangement of carbonyls is possible.

Type **I** sheets are particularly interesting, because the conformation-dependent dipole should allow appended ionic

groups to influence sheet stability.[5] Previous work on helix-forming β-peptide oligomers has focused on residues predisposed toward gauche $NC_\beta-C_\alpha C(=O)$ torsion angles, i.e., residues bearing a single substituent, at either the α- or β-carbon[3a−c] or residues in which $C_\alpha-C_\beta$ is incorporated into a small ring.[3d,e] We reasoned that an anti $NC_\beta-C_\alpha C(=O)$ torsion angle would be promoted by placing an alkyl substituent at C_α and another at C_β, with relative configurations such that anti disposition of the alkyl substituents (favored sterically) would correspond to anti disposition of N and C=O.

Testing this hypothesis requires a small increment of β-peptide sheet that forms in solution. Creation of discrete β-sheet model systems, containing conventional α-amino acid residues, has been a long-standing challenge in protein science. Only recently have peptide segments been identified that bring two attached strands together in a defined orientation (to form a "β-hairpin") without inducing uncontrolled strand aggregation.[6] Our laboratory's solution to this problem,[6c] use of a D-Pro-Xxx linker to connect L-residue strands, was initially demonstrated and optimized in a minimal model system in which each strand comprised only one residue.[7] We have now applied this minimal approach to the study of β-peptide sheet formation, with the goal of identifying optimal structures for the strand residues.

Our first target structure, **1**, contains two α,β-disubstituted β-amino acid residues with configurations predicted to promote anti $NC_\beta-C_\alpha C(=O)$ torsion angles. Synthesis of the C-terminal
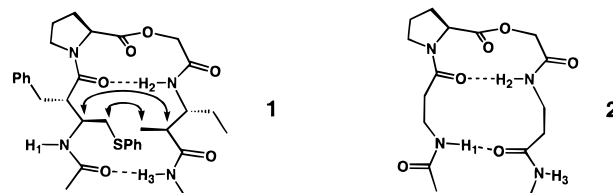
residue from L-aspartic acid has been reported,[8] and we modified the published route to prepare the N-terminal residue.[9] The L-proline-glycolic acid segment was previously used in our minimal α-amino acid hairpin studies[7] because this depsipeptide unit is known to form a stable β-turn-like conformation containing a 10-membered-ring hydrogen bond.[10] The crystal structure of **1** (Figure 1) displays the expected conformation, with the two β-amino acid residues intramolecularly arranged in type **I** antiparallel sheet fashion.

Spectroscopic data suggest that the conformation observed for **1** in the solid state is also highly populated in methylene chloride and methanol. Experiments in methylene chloride, which is nonpolar, must be conducted at sufficient dilution to preclude intermolecular hydrogen bonding of the solute (solute−solute hydrogen bonding is of less concern in methanol). The onset of intermolecular hydrogen bonding can be detected by

(1) Dedicated to Professor Dr. Dieter Seebach on the occasion of his 60th birthday.

(2) (a) Creighton, T. E. *Proteins: Structures and Molecular Principles*, 2nd ed.; Freeman: New York, 1993 and references therein. (b) It has been predicted that helix and sheet secondary structures will characterize all compactly folded polymers: Chan, H. S.; Dill, K. A. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 447.

(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) Hintermann, T.; Seebach, D. *Synlett* **1997**, 437. (d) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071. (e) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. *Nature* **1997**, *387*, 381.

(4) Early work on β-amino acid homopolymers led to the conclusion that these materials adopt sheet structures: (a) Schmidt, E. *Angew. Makromol. Chem.* **1970**, *14*, 185. (b) Chen, F.; Lepore, G.; Goodman, M. *Macromolecules* **1974**, *7*, 779. (c) Yuki, H.; Okamoto, Y.; Taketani, Y.; Tsubota, T.; Marubayashi, Y. *J. Poly. Sci., Poly. Chem. Ed.* **1978**, *16*, 2237. More recent studies, however, suggest that some or all of these polymers adopt helical rather than sheet structures: (d) Bella, J.; Alemán, C.; Fernández-Santin, J. M.; Alegre, C.; Subirana, J. A. *Macromolecules* **1992**, *25*, 5225. (e) Bode, K. A.; Applequist, J. *Macromolecules* **1997**, *30*, 2144.

(5) Influence of terminal charges on α-helix stability: (a) Blagdon, D. E.; Goodman, M. *Biopolymers* **1975**, *14*, 241. (b) Fairman, R.; Shoemaker, K. R.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Proteins: Struct. Funct. Genet.* **1989**, *5*, 1. (c) Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 11560.

(6) For leading references, see: (a) Ramírez-Alvarado, M.; Blanco, F. J.; Serrano, L. *Nat. Struct. Biol.* **1996**, *3*, 604. (b) de Alba, E.; Jiménez, M. A.; Rico, M. *J. Am. Chem. Soc.* **1997**, *119*, 175. (c) Haque, T. S.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 2303. (d) Maynard, A. J.; Searle, M. S. *J. Chem. Soc., Chem. Commun.* **1997**, 1297. (e) β-Hairpin promotion in aqueous solution by unnatural turn segments: Nesloney, C. L.; Kelly, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 5836 and references therein.

(7) (a) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4105. (b) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975.

(8) Jefford, C. W.; McNulty, J. *Helv. Chim. Acta* **1994**, *77*, 2142.

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

(10) Boussard, G.; Marraud, M.; Neel, J.; Maigret, M.; Aubry, A. *Biopolymers* **1977**, *16*, 1033.
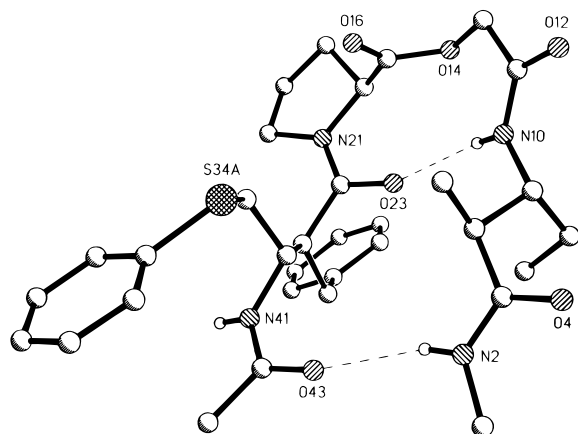
Figure 1. Ball-and-stick representation of the solid state conformation of **1**. Two distinct but similar conformations are observed in crystal; only one is shown. For clarity, all hydrogen atoms, except those attached to nitrogen, have been omitted. Hydrogen bonds are indicated with dotted lines.

examining the amide proton NMR chemical shift as a function of peptide concentration. For **1**, aggregation occurs only above ca. 5 mM in CD_2Cl_2.[9] The concentration-independent $\delta(NH)$ values for all three amide protons of **1** (measured at 2 mM) are consistent with the intramolecular hydrogen bonding pattern observed in the solid state as a major contributor to the time-averaged structure detected by NMR. NH(2) and NH(3) occur at δ 7.40 and 7.27, respectively, both values suggesting extensive internal hydrogen bonding, while NH(1) occurs at δ 5.81, suggesting little or no hydrogen bonding. The $^3J(\alpha,\beta)$ values for each β-amino acid residue of **1** are 10−11 Hz, in CD_2Cl_2 and in CD_3OD, which is consistent with the anti torsion angles observed in the crystal structure.[11] N−H stretch region IR data in CH_2Cl_2 (1 mM) reveal extensive intramolecular hydrogen bonding.[9] NOESY[12] measurements in both CD_2Cl_2 and CD_3-OD reveal two long-range NOEs between the β-amino acid residues, both of which are consistent with the solid state conformation (curved arrows in the drawing of **1**). The presence of these NOEs in CD_3OD suggests that this small β-peptide sheet is particularly stable.

Removal of all side chains from the β-amino acid residues, to generate **2**, leads to a substantial change in folding propensity. The concentration-independent $\delta(NH)$ values in CD_2Cl_2 for the three amide protons suggest type **II** β-peptide sheet formation, in which the β-alanine residues adopt gauche $NC_\beta-C_\alpha C(=O)$ torsion angles. NH(2) occurs at δ 7.68, consistent with extensive formation of the β-turn-like 10-membered-ring hydrogen bond across the L-prolylglycolyl segment, as also seen for **1**. In contrast to **1**, however, **2** displays downfield-shifted NH(1), δ 7.05, and upfield-shifted NH(3), δ 5.92. N−H stretch region IR data in CH_2Cl_2 (1 mM) indicated a smaller extent of intramolecular hydrogen bonding in **2** than in **1** (based on the relative heights of the non-hydrogen-bonded N−H bands),[9] which suggests that the type **II** sheet of **2** is less conformationally stable than the type **I** sheet of **1**.

Compound **3** contains β-amino acid strand residues bearing only one substituent each, at the β-positions. The N-terminal residue was generated from L-phenylalanine and the C-terminal
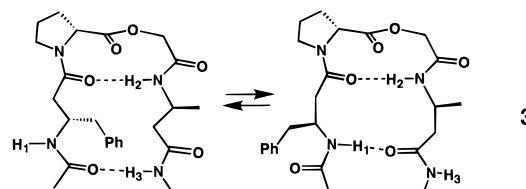
residue from L-alanine, using the elegant methodology of Seebach et al.[3a] To maintain the relative configurations among the analogous stereogenic centers in **3** and **1**, **3** was prepared from D-proline. The concentration-independent $\delta(NH)$ values in CD_2Cl_2 for the three amide protons of **3** suggest that neither the type **I** nor the type **II** sheet is strongly favored in this case. As observed for **1** and **2**, NH(2) is downfield-shifted, δ 7.37, which implies that the β-turn across the L-prolylglycolyl segment is intact. NH(1) and NH(3), however, occur at intermediate positions, δ 6.33 and 6.43, which suggest only partial hydrogen bonding in each case. One explanation for these NMR data is that both the type **I** and the type **II** sheet conformations are partially populated, as illustrated above. The four $^3J(\alpha,\beta)$ values for **3** are all 5−7 Hz in CD_2Cl_2, which is consistent with conformational averaging about the $C_\alpha-C_\beta$ bonds. NOESY analysis of **3** in CD_2Cl_2 revealed numerous short-range NOEs involving protons on each β-amino acid residue, but no long-range NOEs from one β-amino acid residue to the other. N−H stretch region IR data in CH_2Cl_2 (1 mM)[9] indicated that the extent of intramolecular hydrogen bonding in **3** is similar to that in **2** and somewhat less than that in **1**.

Our data suggest that α,β-disubstituted β-amino acid residues with appropriate relative stereochemistry at C_α and C_β (as in **1**) are particularly well suited for formation of polar antiparallel β-peptide sheets (type **I**). Interestingly, β-alanine residues (as in **2**) seem to prefer the type **II** sheet in a nonpolar solvent. This behavior may reflect optimization of internal dipole−dipole interactions in a nonpolar environment,[13] which is possible with highly flexible β-amino acid residues. Type **II** sheet preference of **2** could also arise from a proclivity of β-alanine residues for gauche $NC_\beta-C_\alpha C(=O)$ torsion angles.[14,15] β-Substituted β-amino acid residues (as in **3**), previously shown to be well-suited for helical secondary structure,[3a,b] appear to have a smaller intrinsic propensity for polar type **I** sheets than do appropriate α,β-disubstituted residues.[16]

(11) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.
(12) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95.

(13) For previous discussion of the influence of conformation-dependent dipoles on small molecule folding preferences, see ref 5c and the following: Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9774.
(14) Christianson, L. A. Unpublished ab initio computational results for acetyl-β-alanine-*N*-methyl amide. For analogous computational studies of β-alanine itself, see: Heal, G. A.; Walker, P. D.; Ramek, M.; Mezey, P. G. *Can. J. Chem.* **1996**, *74*, 1660.
(15) Despite the possible preference of β-alanine residues for gauche $NC_\beta-C_\alpha C(=O)$ torsion angles, these residues resist short-range intramolecular hydrogen bonding: (a) Marraud, M.; Neel, J. *J. Polym. Sci.* **1975**, *271*. (b) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054. (c) Gung, B. W.; Zhu, Z. *J. Org. Chem.* **1997**, *62*, 6100.
(16) This research was supported by the NIH (GM56414). S.K. thanks the Fonds der Chemischen Industrie for a fellowship. NMR equipment was purchased in part with funds from the NSF (CHE-8813550) and NIH (SIO RRO 4981), and crystallographic equipment with funds from the NSF (CHE-9310428).