Diversity in Short β-Peptide 12-Helices: High-Resolution Structural Analysis in Aqueous Solution of a Hexamer Containing Sulfonylated Pyrrolidine Residues

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Oligomers that adopt well-defined conformations in solution ("foldamers") have been a subject of increasing interest.1 Results from many laboratories demonstrate that shape control can be achieved with a wide variety of backbones, and recent efforts have shown that foldamers can be endowed with useful activities.2 Many applications require placement of specific functional groups at defined positions along the foldamer backbone, so that folding brings these groups into the arrangement necessary for activity.

Described here is a strategy for functionalizing the 12-helix, a secondary structure defined by 12-membered-ring hydrogen bonds ([C=O(i) → N–H(i+3)]) that is formed by β- amino acid oligomers in which the residues are constrained by five-membered rings.3 Two appropriately constrained residues have been identified, trans-2-amino cyclopentanecarboxylic acid (ACPC)4 and trans-3-aminopyrrolidine-4-carboxylic acid (APC),5 each of which can be prepared efficiently in large quantities in either enantiomeric form.6 We have now explored sulfonylated APC (S-APC) residues for functionalization of water-soluble 12-helices. Attachment of side chains via N-sulfonation should be advantageous relative to N-alkylation, which would introduce cationic charge, or N-acetylation, which would interfere with structural characterization because of cis–trans rotamer equilibria of the resulting tertiary amide groups. However, sulfonylation introduces a non-s¹ atom into the five-membered ring of the β-amino acid residue and might therefore adversely affect 12-helix stability by altering the Cα–ε² torsional preference of S-APC residues relative to APC and ACPC residues.

Fmoc-protected S-APC residues were prepared via straightforward methods8 and used for solid-phase synthesis of hexamers 1–3. The two S-APC residues in 1–3 illustrate that alkyl and aromatic side chains can be introduced in this way: a variety of polar S-APC residues should also be readily available (for example, we have prepared a taurine-derived S-APC residue, with which it should be possible to replace the cationic APC residues of 1–3). Hexamer 1 was analyzed by 2D NMR in 9:1 H2O:D2O to determine whether a backbone comprised solely of APC and S-APC residues adopts the 12-helical conformation.9 H chemical shifts of 1 did not change significantly upon dilution from 2.5 mM to 0.3 mM, which suggests that the hexamer does not self-associate in this concentration range. The 1H NMR resonances of 1 in water are more dispersed than those of previously examined SΛ APC/ACPC hexamer 4, presumably because of the greater residue diversity of 1 relative to 4 and ring current effects from the aromatic side chain. Seven NOEs (present in both NOE SY5¹ and ROE SY5² spectra) between protons on nonadjacent residues were observed along the backbone of 1 (Figure 1a). All three types of nonadjacent NOEs, CαH → NH13, CαH → CαH12, and CαH → NH13, are characteristic of the 12-helical folding pattern.5,6 The lack of NOEs in 1 between residues 1 and 3 (numbered from N-terminus) suggests that the N-terminus of the 12-helix is frayed; terminal fraying is observed among α-helices formed by conventional peptides in aqueous solution.8

Comparison of NOE data for hexamers 1 and 4 suggests that incorporating S-APC residues (in 1) in place of ACPC residues (in 4) leads to subtle differences in 12-helix geometry. Previous data showed that 4 in water displayed a set of four weak CαH → CαH11 NOEs (only CαH → CαH6 was missing). In contrast, no CαH → CαH11 NOEs were observed for 1, which suggests that the 12-helix formed by 1 is slightly more tightly wound than the 12-helix formed by 4 (Figure 1b). The subtle differences between the 12-helical conformations of 1 and 4 are comparable to differences among α-helices formed by conventional peptides.9 NOE data obtained for 4 in 9:1 H2O:D2O were used for NOE-restrained dynamics simulations with the program DYANA.10 This approach was used to generate 400 structures, the best 10 of which (all 12-helical) were used as starting points for NOE-restrained

(6) Please see the Supporting Information.


(10) Gunter, P.; Mumenthaler, C.; Wüthrich, K. J. Mol. Biol. 1997, 273, 283. The structure library in DYANA was modified to include cyclopentane and pyrrolidine rings for these calculations.
It was not possible to determine a structure of 4 in water because of overlap among crucial proton resonances and a general decrease in the number of characteristic NOEs, relative to methanol.\textsuperscript{3d} In contrast, the proton resonance dispersion of 1 was comparable in water and methanol, and the number and intensities of the helix-defining NOEs were comparable in the two solvents.

Circumstantial dichroism is widely used to assess the secondary structures of conventional peptides,\textsuperscript{14} and CD has also been useful for \(\beta\)-peptides\textsuperscript{12,13} and other unnatural oligomers.\textsuperscript{14} CD spectra of 1–3 in \(\text{H}_2\text{O}\) and in \(\text{CH}_3\text{OH}\) show a characteristic maximum near 200 nm and a minimum near 220 nm,\textsuperscript{9} which is consistent with theoretical predictions\textsuperscript{53} and previously reported CD data\textsuperscript{9} for 12-helical \(\beta\)-peptides. In \(\text{CH}_3\text{OH}\) the maximum and minimum are somewhat more intense, and the maximum is slightly shifted to the red, relative to \(\text{H}_2\text{O}\). The intensity trends suggest that addition of methanol enhances 12-helix population, which mirrors well-established trends for alcohol cosolvents among \(\alpha\)-helix-forming conventional peptides;\textsuperscript{55} however, strong similarities between NOE data sets for 1 in water and methanol\textsuperscript{56} suggest that the solvent effect on 12-helix population is not large.\textsuperscript{56} The similarities among CD data for 1–4 indicate that neither the sulfonamide groups nor the aromatic side chains exert a large effect on the 12-helical CD signature.\textsuperscript{6}

We have shown that S-APC residues represent a general strategy for introducing specific side chains at defined positions along the surface of 12-helical \(\beta\)-peptides. The \(\beta\)-peptide 12-helix and the \(\alpha\)-helix of conventional peptides have similar geometric features (inner diameter: 3.1 vs 3.2 Å; rise per turn: 5.5 vs 5.4 Å; helix dipole: positive to negative from N-terminus to C-terminus), which suggests that specifically functionalized 12-helices may be able to mimic the structure of \(\alpha\)-helical segments of natural proteins. 12-Helical \(\beta\)-peptides may therefore offer a rational approach to development of specific inhibitors of protein–protein interactions that depend on \(\alpha\)-helix recognition.\textsuperscript{17} \(\beta\)-Peptides containing S-APC and other five-membered-ring-constrained residues are especially interesting from this perspective because of their high conformational stability relative to conventional peptides, which do not form \(\alpha\)-helices with fewer than 10–15 residues.\textsuperscript{8,18}

**Supporting Information Available:** CD data for 1–4 in water and in MeOH; NOE lists for 1 in water and MeOH; summary of Fmoc-S-APC residue synthesis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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\textsuperscript{(16)} Switching from water to methanol exerts a profound effect on \(\beta\)-peptides lacking cyclic backbone constraints, which usually display little or no secondary structure in water: ref 13a and references therein.
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