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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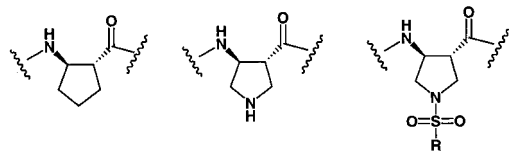
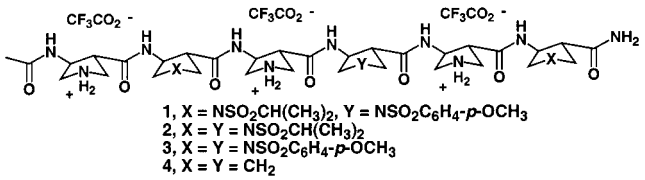
Oligomers that adopt well-defined conformations in solution ("foldamers") have been a subject of increasing interest.[1] Results from many laboratories demonstrate that shape control can be achieved with a wide variety of backbones, and recent efforts have shown that foldamers can be endowed with useful 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-aminocyclopentanecarboxylic acid (ACPC)[3a] and *trans*-3-aminopyrrolidine-4-carboxylic acid (APC),[3d] each of which can be prepared efficiently in large quantities in either enantiomeric form.[4] We have now explored sulfonylated APC (S-APC) residues for functionalization of water-soluble 12-helices. Attachment of side chains via N-sulfonylation should be advantageous relative to N-alkylation, which would introduce cationic charge, or N-acylation, which would interfere with structural characterization because of cis−trans rotamer equilibria of the resulting tertiary amide groups. However, sulfonylation introduces a non-sp^3 atom[5] into the five-membered ring of the β-amino acid residue and might therefore adversely affect 12-helix stability by altering the $C_\alpha-C_\beta$ torsional preference of S-APC residues relative to APC and ACPC residues.



Fmoc-protected S-APC residues were prepared via straightforward methods[6] 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 H_2O:D_2O to determine whether a backbone comprised solely of APC and S-APC residues adopts the 12-helical conformation. ^{1}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 ^{1}H NMR resonances of **1** in water are more dispersed than those of previously examined[3d] 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



NOESY[7a] and ROESY[7b] spectra) between protons on nonadjacent residues were observed along the backbone of **1** (Figure 1a). All three types of nonadjacent NOEs, $C_\beta H_i \rightarrow NH_{i+2}$, $C_\beta H_i \rightarrow C_\alpha H_{i+2}$, and $C_\beta H_i \rightarrow NH_{i+3}$, are characteristic of the 12-helical folding pattern.[3a,d] 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[3d] showed that **4** in water displayed a set of four weak $C_\beta H_i \rightarrow C_\alpha H_{i+1}$ NOEs (only $C_\beta H_5 \rightarrow C_\alpha H_6$ was missing). In contrast, no $C_\beta H_i \rightarrow C_\alpha H_{i+1}$ 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 **1** in 9:1 H_2O:D_2O 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

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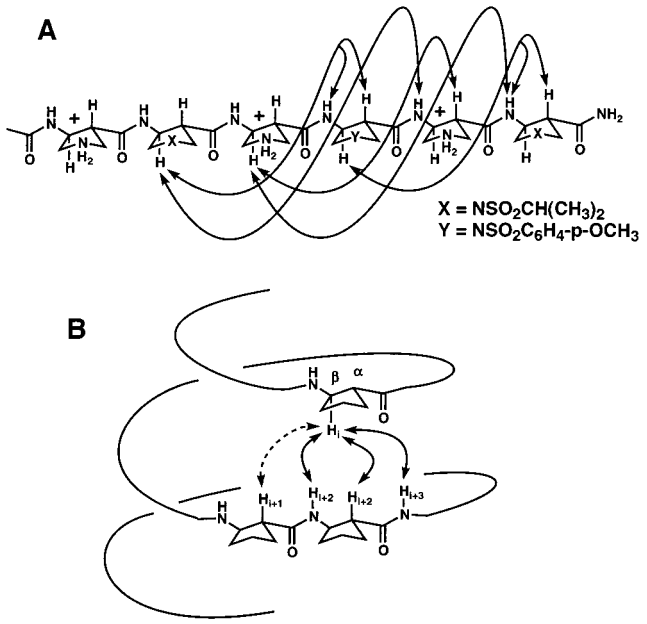

Figure 1. (a) Graphical summary of NOEs between nonadjacent residues observed for hexa-β-peptide **1** (2.5 mM, trifluoroacetate salt) in 9:1 H_2O:D_2O, 4 °C. Three types of NOE were observed: $C_\beta H_i \rightarrow NH_{i+2}$, $C_\beta H_i \rightarrow C_\alpha H_{i+2}$, and $C_\beta H_i \rightarrow NH_{i+3}$ (solid arrows in part b). All of these NOEs are consistent with the 12-helical conformation; no NOEs inconsistent with the 12-helical conformation were observed. (b) Cartoon showing the spatial relationship between protons on residue *i* and protons on residues *i*+2 and *i*+3 in the 12-helix; as mentioned in the text, $C_\beta H_i \rightarrow C_\alpha H_{i+1}$ NOEs (dashed arrow) were observed for **4** (ref 3d) but not for **1**, which suggests that the helix formed by **1** is slightly more tightly wound.

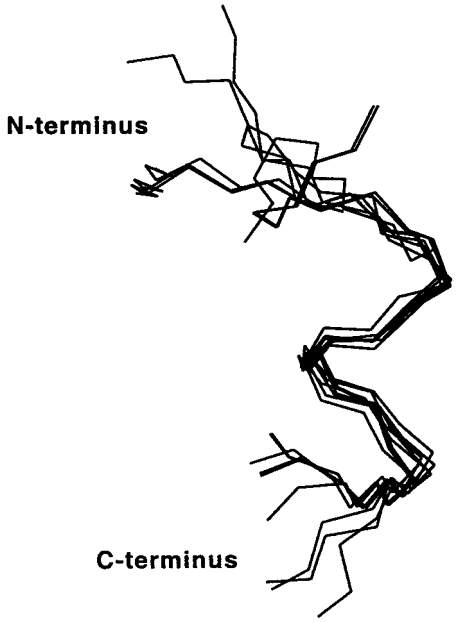

Figure 2. NOE-restrained dynamics results for hexa-β-peptide **1**, based on NMR data obtained in 9:1 H_2O:D_2O, 4 °C (details in text). Overlay of 10 structures, backbone atoms only, with low energy and NOE restraint violation. The N-termini are at the top.

simulated annealing with the SYBYL 6.4 program (Tripos Software, St. Louis, MO). Figure 2 shows an overlay of the resulting 10 structures, backbone atoms only. Fraying, apparent at both ends, is pronounced at the N-terminus, but the 12-helix is well-formed in the central portion. Among the 10 structures shown root-mean-squared deviation is 0.69 ± 0.51 Å for residues 3−6 (backbone atoms). The 12-helical conformation deduced for **1** in water agrees well with the conformation previously deduced for **4** in methanol[3d] and with crystal structures of ACPC oligomers.[3a,b] 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.[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.

Circular dichroism is widely used to assess the secondary structures of conventional peptides,[11] and CD has also been useful for β-peptides[12,13] and other unnatural oligomers.[14] CD spectra of **1**−**3** in H_2O and in CH_3OH show a characteristic maximum near 200 nm and a minimum near 220 nm,[6] which is consistent with theoretical predictions[12] and previously reported CD data[3] for 12-helical β-peptides. In CH_3OH the maximum and minimum are somewhat more intense, and the maximum is slightly shifted to the red, relative to H_2O. The intensity trends suggest that addition of methanol enhances 12-helix population, which mirrors well-established trends for alcohol cosolvents among α-helix-forming conventional peptides;[15] however, strong similarities between NOE data sets for **1** in water and methanol[6] suggest that the solvent effect on 12-helix population is not large.[16] 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.[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 β-peptides. The β-peptide 12-helix and the α-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 α-helical segments of natural proteins. 12-Helical β-peptides may therefore offer a rational approach to development of specific inhibitors of protein−protein interactions that depend on α-helix recognition.[17] β-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 α-helices with fewer than 10−15 residues.[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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