12-Helix Formation in Aqueous Solution with Short β-Peptides Containing Pyrrolidine-Based Residues

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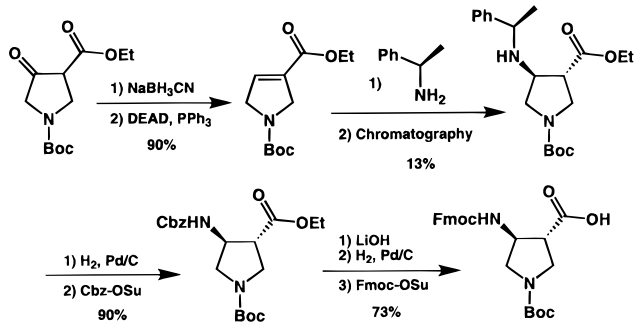
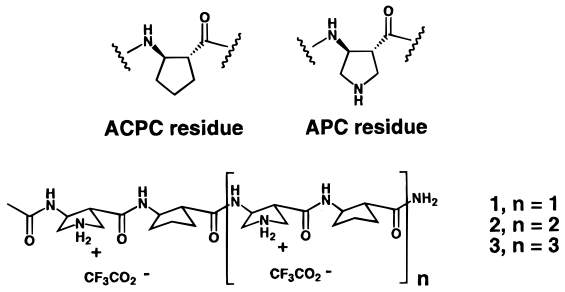
Oligomers that display well-defined and predictable conformations ("foldamers") have become subjects of widespread interest in recent years.[1,2] The foldamer strategy could be useful for creating specific molecular shapes or specific arrangements of functional groups, as required for many design goals. The traditional approach to controlling shape and surface functionality involves small, rigid skeletons, which are usually nonperiodic. In contrast, oligomers display high backbone periodicity, with functional group diversity arising from side chains. Use of oligomeric scaffolds could prove advantageous relative to traditional scaffolds because there is no intrinsic size limit for a periodic architecture, and because the combination of backbone periodicity and side chain variation is conducive to optimization of molecular properties via cycles of combinatorial variation and selection. Most known oligomers, however, are very conformationally mobile, at least when short (<10 residues), and this flexibility is disadvantageous in the context of many design goals.

We describe a set of β-amino acid oligomers ("β-peptides"[1,3]) that adopt a specific helical conformation in aqueous solution with as few as four residues. Conformational stability in aqueous solution is important with regard to biological applications. Among conventional peptides (α-amino acid residues) conformational stability is usually lower in water than in other common solvents.[4] Relatively few unnatural foldamers have been examined in aqueous solution,[5] and nearly all conformational analyses in water have involved low resolution methods. Only one nonassociated unnatural foldamer has been subjected to high-resolution structural analysis in water, to our knowledge, a hexa-β-peptide that adopts a 14-helical conformation in aqueous solution (*i* to *i* − 2 C═O- -H−N hydrogen bonds).[6−8] In this case, the foldamer's shape is enforced by cyclohexyl constraints at the residue level. We have previously shown that cyclopentyl constraints enforce a different β-peptide shape, the 12-helix (*i* to *i* − 3 C═O- -H−N hydrogen bonds), in organic solvents.[9] Here we show that use of pyrrolidine-based β-amino acid residue leads to formation of short 12-helices in aqueous solution.

Scheme 1



To probe for 12-helical folding in water, we required a hydrophilic β-amino acid residue with the proper conformational constraint, as a complement to the hydrophobic (*R*,*R*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) residue used previously.[9] Scheme 1 outlines the synthesis of enantiomerically pure (*R*,*S*)-*trans*-3-aminopyrrolidine-4-carboxylic acid (APC) in a protected form, from a known β-ketoester.[10] The key step is Michael addition of (*R*)-α-methylbenzylamine to the α,β-unsaturated ester. The desired (*R*,*S*,*R*) isomer was readily isolated via chromatography, and the absolute stereochemistry was confirmed by X-ray crystallographic analysis of the hydrochloride salt. The Fmoc/Boc-protected APC derivative was then used for solid-phase synthesis of oligomers **1**−**3** on Rink amide resin with PyBOP as



the coupling agent. After reverse-phase HPLC purification, the oligomers were isolated as trifluoroacetate salts (one TFA counterion per APC residue, as determined via ^{19}F NMR). Alternation of hydrophobic ACPC and hydrophilic APC residues should distribute the cationic groups around the circumference of the 12-helix, which has ∼2.5 residues per turn,[9] thereby minimizing the prospect of aggregation.

Initial studies involved conformational analysis of hexamer **2** in CD_3OH (10 mM). β-Peptide conformational propensity is very sensitive to residue substitution pattern,[3] and it was therefore

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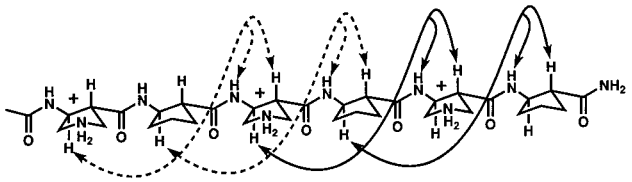

Figure 1. Selected NOEs ($C_\beta H_i \rightarrow NH_{i+2}$ and $C_\beta H_i \rightarrow C_\alpha H_{i+2}$) for hexa-$\beta$-peptide **2**. These NOEs are consistent with the 12-helical conformation. The dotted lines indicate NOEs observed in CD_3OH but obscured by resonance overlap in 9:1 H_2O:D_2O; the solid lines indicate NOEs observed in both solvents.

necessary to determine whether the APC residue is conducive to 12-helix formation in a solvent that is permissive[11] with regard to β-peptide folding. ^{1}H NMR data obtained at both 500 and 750 MHz (3 °C) were used for structural analysis. All resonances could be assigned based on COSY, TOCSY, ROESY and NOESY data.[12] The 32 interresidue NOEs included $C_\alpha H_i \rightarrow NH_{i+1}$, $C_\beta H_i \rightarrow NH_{i+1}$, $C_\beta H_i \rightarrow NH_{i+2}$, $C_\beta H_i \rightarrow C_\alpha H_{i+2}$, $C_\beta H_i \rightarrow C_\alpha H_{i+1}$, $C_\epsilon H_i \rightarrow NH_{i+3}$ and $C_\epsilon H_i \rightarrow C_\gamma H_{i+3}$, all of which are consistent with the 12-helical conformation (no interresidue NOEs inconsistent with the 12-helix conformation were observed). The NOEs were classified as strong, medium, weak-medium, or weak and used as distance restraints for structure determination with the program DYANA.[13] The best 40 structures were then used as starting structures for NOE-restrained simulated annealing/molecular dynamics with the Tripos force field in the SYBYL 6.4 program (Tripos Software, St. Louis, MO). The rmsd for the heavy atoms among the 10 best structures was 0.61 ± 0.33 Å. The NMR structure closest to the average of the 10 was compared with the crystal structure of an ACPC hexamer;[9] the rmsd between these two structures was 0.95 Å (backbone atoms only).

NMR data for hexa-β-peptide **2** in aqueous solution (10 mM)[14] showed that the 12-helix forms in this competitive solvent, although the folded population is diminished relative to methanol. Analysis in 9:1 H_2O:D_2O (100 mM acetate, pH 3.8) was more challenging than in CD_3OH because the resonance dispersion was poorer in aqueous solution. The conformationally characteristic network of $C_\beta H_i \rightarrow NH_{i+2}$ and $C_\beta H_i \rightarrow C_\alpha H_{i+2}$ NOEs appeared to be retained in water (Figure 1). For $C_\beta H_1 \rightarrow NH_3$, $C_\beta H_1 \rightarrow C_\alpha H_3$, $C_\beta H_2 \rightarrow NH_4$ and $C_\beta H_2 \rightarrow C_\alpha H_4$ definitive assignment of the cross-peaks was hindered by resonance overlap, but the $C_\beta H_3 \rightarrow NH_5$, $C_\beta H_3 \rightarrow C_\alpha H_5$, $C_\beta H_4 \rightarrow NH_6$ and $C_\beta H_4 \rightarrow C_\alpha H_6$ cross-peaks could be unambiguously discerned in aqueous solution. Internal NOE comparisons indicated that the 12-helical population was lower in water than in methanol, because in methanol the interresidue $C_\beta H_i \rightarrow C_\alpha H_{i+2}$ NOEs were comparable in intensity to the intraresidue $C_\beta H_i \rightarrow C_\alpha H_i$ NOEs, while in water the intraresidue NOEs were stronger than the interresidue NOEs. In addition, none of the weak $C_\epsilon H_i \rightarrow NH_{i+3}$ or $C_\epsilon H_i \rightarrow C_\gamma H_{i+3}$ NOEs observed in methanol could be detected in water. The deleterious effect of water relative to an alcohol solvent on secondary structural stability is well-precedented among β-peptides[11] and conventional peptides;[4] however, it is remarkable that hexamer **2** retains sufficient helicity in water to give rise to NOEs between non-neighboring residues.

Circular dichroism was used to compare the extent of 12-helix formation among oligomers **1**−**3**. In the far-UV region (<250 nm), the CD signal arises largely from backbone amide groups

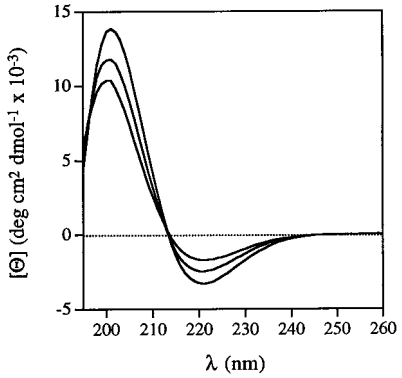

Figure 2. Circular dichroism data for β-peptides **1**−**3** (1 mg/mL each) at 25 °C in H_2O. Data were obtained on an Aviv instrument with 1 mm path length cells. The data are normalized for β-peptide concentration and number of residues (i.e., the vertical axis is mean residue ellipticity).

of conventional peptides[15] and β-peptides.[16] We have previously shown that an ACPC hexamer in methanol displays a maximum at ∼207 nm and a minimum at ∼222 nm,[9] and that these features agree well with theoretical predictions for the 12-helical conformation.[16] Figure 2 provides CD data for **1**−**3** in aqueous solution; the data have been normalized for concentration and number of residues to facilitate direct comparisons. Oligomers **1**−**3** all display a CD signature very similar to that previously observed for an ACPC hexamer in methanol,[9] except that the maximum is somewhat red-shifted (∼201 nm for **1**−**3**). As oligomer length increases, the intensities of the 201 nm maximum and the 221 minimum also increase, with an isodichroic point at 213 nm. This trend suggests that 12-helical stability increases as the helical segment becomes longer. Analogous length-dependent cooperativity is observed among α-helix-forming conventional peptides.[17] Among conventional peptides a minimum of 10−15 residues is required for detectable α-helicity in aqueous solution, and peptides containing only 4−8 residues are typically used as "benchmarks" for the fully unfolded state.[18] The observation of significant 12-helicity even in tetra-β-peptide **1** indicates high intrinsic stability for this β-peptide secondary structure.

We have shown that the heterocyclic APC residue has a high propensity for 12-helix formation, like carbacyclic analogue ACPC,[9] and that this new residue confers water-solubility on β-peptides. The availability of APC has allowed us to establish that the 12-helical conformation is populated with as few as four residues in aqueous solution. The ring nitrogen of the APC residue should provide an attachment point for diverse side chains. Therefore, β-peptides composed of cyclopentane- and pyrrolidine-based residues could be useful scaffolds for creating specific functional group arrays for biomedical applications.[19]

Supporting Information Available: Synthetic protocol for APC and NMR data for hexamer **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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