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Environment-Independent 14-Helix Formation in Short β-Peptides: Striking a Balance between Shape Control and Functional Diversity

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β-Peptides are attracting increasing interest because they are able to adopt a variety of secondary structures, and choice of residue substitution pattern controls folding preference in a predictable way.[1] In contrast to conventional peptides (i.e., peptides that consist of α-amino acid residues), β-peptides are not subject to proteolytic degradation,[2] and they can display substantial conformational stability at relatively short lengths in water.[3] These characteristics suggest that β-peptides are good scaffolds for the creation of biologically active molecules (see ref 4 for examples). Further development for biomedical applications requires efficient strategies for placing diverse sets of functional groups at specific sites along the β-peptide sequence; folding translates sequential relationships among side chains into spatial relationships.

Here we show that short β-peptide 14-helices, defined by 14-membered ring $i \rightarrow i + 2$ N−H··O═C backbone hydrogen bonds, can be easily decorated with diverse side chains without sacrificing conformational stability. Initial studies suggested that it was difficult to achieve high 14-helix stability and extensive side chain diversity simultaneously.[1b,3a] The largest 14-helix propensity is displayed by residues preorganized by a six-membered ring constraint (e.g., *trans*-2-aminocyclohexanecarboxylic acid (ACHC)),[3a] but these are difficult to functionalize.[5] β-Substituted β-amino acids ("β^3-residues"), which can be prepared rapidly and enantiospecifically from α-amino acids,[6] support the 14-helix under some conditions, but oligomers containing only β^3-residues generally show little or no tendency to fold in water.[3a,7] Cheng and DeGrado[8] and Seebach et al.[9] have recently demonstrated that cyclic residues are not absolutely required for substantial 14-helicity in water: ion pairing between side chains of appropriately spaced residues can induce high 14-helix population in β-peptides containing only β^3-residues. Both groups recognized that i, $i + 3$ relationships between residues with complementary basic and acidic side chains (e.g., β^3-homoornithine (β^3-hOrn) and β^3-hGlu) would allow intramolecular ion pair formation to promote 14-helical folding at neutral pH, because the 14-helix has ca. three residues per turn and a pitch of ca. 5.0 Å. The crucial structural role of the ion pairs was demonstrated by the precipitous decline or disappearance of 14-helicity at pH extremes.[8,9] Dramatic pH effects led Cheng and DeGrado to conclude that the 14-helical propensity of β^3-residues in water is lower than the α-helical propensity of α-amino acid residues.[8]

We began by comparing the zwitterionic hepta-β-peptide of Seebach et al. (**1**; Figure 1) with an analogue in which the three β^3-hVal residues have been replaced with ACHC residues (**2**).[10] Figure 2 juxtaposes the far-UV circular dichroism of **1** and **2** (0.2 mM) under four sets of conditions. Methanol is a strongly structure-promoting solvent for β-peptides,[7] and the similarity of the CD signatures of **1** and **2** in this solvent (Figure 2a) suggests similar extents of 14-helicity. In pH neutral aqueous buffer (Figure 2b),

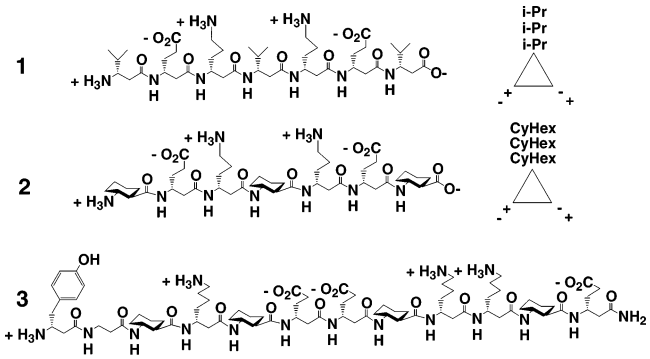

Figure 1. β-Peptides **1**−**3**. For **1** and **2**, the image on the right is an idealized view along the axis of the 14-helical conformation available to the β-peptide. This end-on view highlights the prospect of internal ion pairing. The 14-helical wheel projection for **3** may be found in the Supporting Information.

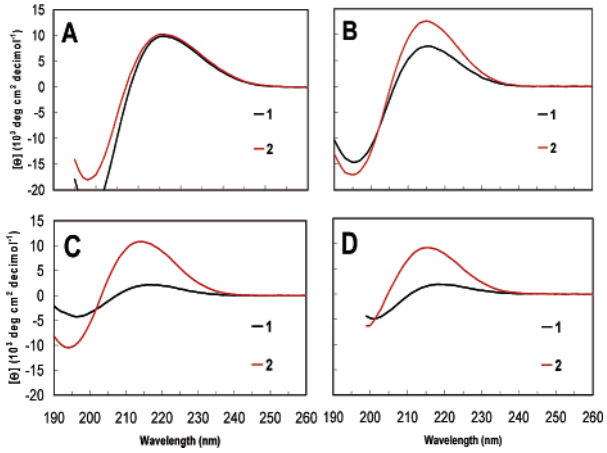

Figure 2. Far-UV circular dichroism data for 0.2 mM **1** (black) and **2** (red) in various solvents at room temperature: (a) methanol; (b) aqueous solution, 10 mM Tris, pH 7.2; (c) aqueous solution, 10 mM HCl, pH 2; (d) aqueous solution, 10 mM NaOH, pH 12.

CD data suggest that **1** retains considerable 14-helicity, as previously reported.[9] The decrease in the intensity of the characteristic CD maximum of **1** in pH 7.2 aqueous buffer relative to methanol, however, indicates diminished 14-helical folding in water. In contrast, the CD spectrum of **2** is slightly *more* intense in pH 7.2 aqueous buffer relative to methanol. Thus, the 14-helical population of **2** may be maximal in both solvents. β-Peptide **1** displays little or no 14-helicity at pH extremes, but **2** retains a strong 14-helical CD signature (Figure 2c,d). Thus, the CD data suggest that alterations in pH exert relatively little effect on 14-helix population when three of the seven β-amino acid residues are preorganized.

We turned to two-dimensional NMR analysis for further insight on **2** because CD is an intrinsically low-resolution method.[11] ROESY[12] data for **2** in CD_3OH (1.8 mM) and in 9:1 H_2O:D_2O

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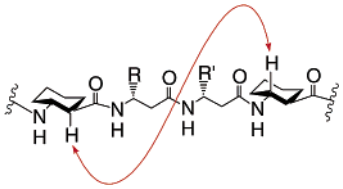

Figure 3. Graphical representation of a $C_\alpha H(i) \rightarrow C_\beta H(i + 3)$ NOE.

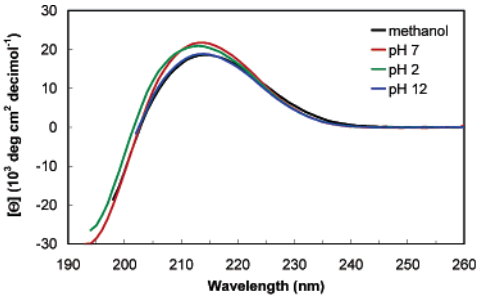

Figure 4. Far-UV circular dichroism data for 0.1 mM **3** in various solvents at room temperature: black, methanol; red, aqueous solution, 10 mM Tris, pH 7.2; green, aqueous solution, 10 mM HCl, pH 2; blue, aqueous solution, 10 mM NaOH, pH 12.

(3.6 mM; 40 mM perdeuterated Tris, pH 7.2) revealed $C_\alpha H(i) \rightarrow C_\beta H(i + 3)$ NOEs (Figure 3), which are characteristic of the 14-helical conformation. In methanol, three of the four possible NOEs were observed (residue pairs 1/4, 2/5, and 3/6); the fourth might have been present but was ambiguous because of resonance overlap. In aqueous solution, two of the $C_\alpha H(i) \rightarrow C_\beta H(i + 3)$ NOEs were observed (1/4 and 4/7), and the other two were ambiguous because of overlap. No NOEs inconsistent with the 14-helix were detected. Overall, these ROESY results support the conclusions derived from CD data for **2**.

We examined 12-mer **3** as a further test of the role of ion pairing in 14-helix formation by β-peptides containing a few preorganized residues. This β-peptide resembles the design of Cheng and DeGrado[8] in that it contains β^3-hLys rather than β^3-hOrn residues, and in the arrangement of the basic and acidic residues. A β^3-hTyr residue was placed at the N-terminus to facilitate analytical ultracentrifugation (UV detection). The CD spectra of 0.1 mM **3** in methanol and in aqueous buffer at neutral or extreme pH overlay almost perfectly (Figure 4). Thus, the extent of 14-helix formation by **3** in aqueous solution appears to be independent of internal ion pairing. Analytical ultracentrifugation of **3** at 0.3 mM showed no evidence of self-association,[13] indicating that the 14-helicity detected by CD arises from purely intramolecular factors. The environmental independence of 14-helix formation by **3** in water contrasts with the behavior of the β-peptide studied by Cheng and DeGrado, which folds significantly in water only at neutral pH.[8]

Our data show that substantial population of the β-peptide 14-helix in water can be induced by preorganizing fewer than one-half of the residues. It was not obvious from previous work[1,3,7−9] that such a small proportion of preorganized residues would be so effective. Because this shape stability does not require attractive interactions between the side chains of the β^3-residues, these nonpreorganized residues are free to display functionality that serves other purposes such as catalysis or recognition. ACHC itself is readily prepared in either enantiomeric form,[14] but access to functionalized derivatives is limited.[5] Our results show that a few ACHC residues can compensate for the high intrinsic flexibility of β^3-residues, which allows one to take full advantage of the broad synthetic accessibility of β^3-residues in the design of β-peptides with defined shape and function in aqueous solution.

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Note Added in Proof. Schepartz et al. have recently reported (Hart, S. A.; Bahadoor, A. B. F.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. *J. Am. Chem. Soc.* **2003**, *125*, 4022) that 14-helicity in β-peptides containing only β^3-residues can be promoted in water by combining favorable design features including side chain ion pairing and side chain branching adjacent to the β-peptide backbone. (For another recent example of side chain branching effects, albeit not in water, see: Raguse, T. L.; Lai, J. R.; Gellman, S. H. *Helv. Chim. Acta* **2002**, *85*, 4154.)

Supporting Information Available: A complete description of the NMR analysis of **2** and the equilibrium ultracentrifugation studies on **3** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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