De Novo Design of a Monomeric Helical β-Peptide Stabilized by Electrostatic Interactions

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The de novo design of peptides and proteins has provided an approach to critically assess the features that are responsible for the folding and function of proteins.[1] Recent successes in this endeavor suggest that it should now be possible to extend this approach to the design of nonbiological polymers with well-defined tertiary structures and activities. Indeed, early work with a variety of sequence-specific polymers has shown the feasibility of designing sequence-specific polymers with well-defined secondary structures and properties.[2−5] In particular, peptides composed of β-amino acids (β-peptides) hold particular promise for molecular design;[2,4,5] β-amino acids can be synthesized by homologation of α-amino acids as well as other routes providing a convenient and highly diverse source of monomers.[6] Further, like peptides composed of α-amino acids, they are intrinsically flexible, but nevertheless adopt well-defined secondary structures through the cooperative accrual of weak interactions throughout the sequence.[2,4,5] Thus, β-peptides provide an excellent framework for extending our understanding of protein structure and stabilization into the realm of folded, nonbiological polymers.

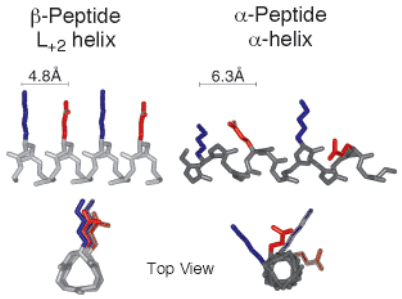

Figure 1. Electrostatic interactions in idealized models of the L_{+2} helix[2c] and α-helix. Charged residues are shown in blue and red (hLys and hGlu, three residues apart for the L_{+2} helix; Lys and Glu, four residues apart for the α-helix).

The L_{+2} helix is a particularly stable and frequently observed conformation in synthetic β-peptides (Figure 1),[2,4,5] which is reasonably similar to the α-helix in its overall dimensions. The stereochemical requirements for the formation of L_{+2} helices in organic solvents have emerged from pioneering studies from the groups of Seebach and Gellman.[2,4,5] However, the design of β-peptides that adopt stable L_{+2}-helical conformations in water has been observed only for a class of peptides with conformationally restricted cyclic amino acids.[4a,d] Here, we demonstrate that electrostatic interactions between the side chains of acyclic β-amino acids can be used to drive the formation of L_{+2} helices in water.

Electrostatic interactions between oppositely charged side chains placed one turn apart in an α-helix are able to stabilize monomeric helices,[7] allowing for the design of approximately 15-residue peptides that are partially helical in water. A comparison of the structure of the L_{+2} helix with the α-helix suggests that similar interactions may be even more favorable in β-peptides (Figure 1). In the L_{+2} helix, side chains separated by a single helical turn (positions i and i+3) are separated by 4.8 Å, and the side chains project in the same direction. In contrast, the distance between residues at positions i and i+4 of an α-helix is 6.3 Å, and the side chains project at a 40° angle from one another. Interestingly, however, the geometric arrangement of neighboring side chains in β-peptides is similar to the spacing in antiparallel and parallel β-sheet conformations of α-peptides, in which side chains project in similar directions at a distance of approximately 5 Å (not shown). Thus, one would expect that the rules of L_{+2} helix formation in β-peptides may be similar to those for β-sheet

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(9) The β^3-amino acids are referred to as homologues of the natural α-amino acids bearing the same side chain by adding the letter "h" preceding the three-letter code of the natural amino acid; for example, the β^3-amino acid with a methyl group side chain would be hAla while the natural amino acid is alanine (Ala).

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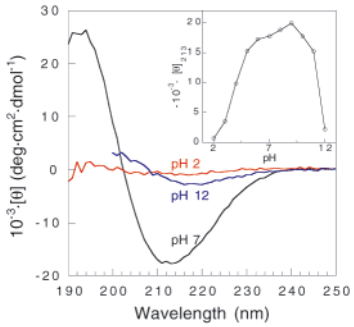
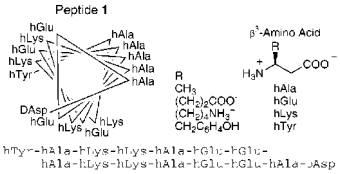


Figure 2. The CD spectra of peptide **1** at pH 2, 7, and 12 in mean residue ellipticity. The inset plots the mean residue ellipticity at 213 nm vs pH.

formation in α-peptides;[8] one should observe very extensive side chain−side chain interactions leading to a greater sequence dependence of L_{+2} helix formation than was observed in the α-helical peptides.



To test this hypothesis, we prepared peptide **1**, analogous to α-helical peptides in which Lys and Glu side chains interact between adjacent turrns of the helix.[7e,g] Thus, alternating layers of β^3-hGlu and β^3-hLys residues were positioned along a 12-residue helix.[9] Analogous to previous studies, the sequence was filled in at the remaining positions with β^3-hAla; β^3-hTyr was attached to the *N*-terminus to facilitate concentration determination, a D-Asp was added to the *C*-terminus as a potentially stabilizing *C*-capping interaction,[10] both termini were left uncapped, and the charged residues (β^3-hGlu and β^3-hLys) were arranged to optimize interactions with the helix dipole.

The circular dichroism (CD) spectrum of the resulting peptide at pH 7 is typical of the L_{+2} helix (Figure 2). Furthermore, the peptide is monomeric as assessed by the concentration independence of its CD spectrum between 10 μM and 0.3 mM and analytical ultracentrifugation (data not shown). Depending on the assumed mean residue ellipticity for 100% helicity,[11] we calculate that between 10 and 13 residues are helical. This range is in reasonable agreement with the 12 residue-helical segment intended in our original design. Unfortunately, NMR spectra for β-peptides larger than 6−7 residues are severely congested because of the α-methylenes, which has impeded progress in determining the solution structure of peptide **1**.

The pH dependence of the CD spectrum of the peptide further supports the hypothesis that electrostatic interactions strongly stabilize the L_{+2} conformation of peptide **1**. The magnitude of the ellipticity decreases at pH values above and below the pK_a values expected for the basic (hLys side chain and *N*-terminal amine) and acidic groups (hGlu and D-Asp side chains and

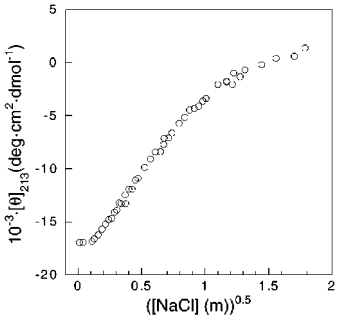


Figure 3. The mean residue ellipticity peptide **1** at 213 nm plotted against the square root of NaCl concentration in molality.

C-terminal carboxylate), respectively. A small increase near pH 8 may be associated with partial deprotonation of the hTyr side chain, as aromatic side chains have been shown to contribute to the CD spectra of helical peptides.[12] Similarly, the CD spectrum of the peptide at pH 7 depended markedly on the concentration of added electrolyte. Significantly, a plot of $[\theta]_{213}$ versus the square root of the molality of NaCl (by Debye−Hückel theory, the energy of electrostatic interaction between ions scales negatively with the square root of [NaCl] in molality) is approximately sigmoidal (Figure 3), with a midpoint near 0.4 M NaCl. At low salt concentration, the magnitude of the ellipticity appears to level off, suggesting that a fully stable conformation is formed under these conditions.

These results with β-peptides are different from earlier studies of α-helical peptides of similar length[7e,g,h] in two important regards: First, at very low salt concentrations, the stability of the L_{+2} helix formation appears to be greater for the β-peptides than analogous α-helical peptides at room temperature. Second, and more importantly, disruption of the electrostatic interactions results in a much greater decrease in helical content for the β-peptide as compared to earlier α-helical peptides. Neutralization of the acidic or basic functionalities at low and high pH, respectively, gives rise to a virtually complete loss of ellipticity, as does the addition of high concentrations of NaCl. By contrast, considerable residual structure is observed under equivalent conditions for α-helical peptides.[7e,g,h] This finding suggests that L_{+2} helix formation may be intrinsically less favorable for this class of β-amino acids, as might be expected from the increased conformational freedom associated with the insertion of an unsubstituted methylene in their structures. By contrast, the extent of side chain−side chain interactions is accentuated in β-peptides, as has previously been observed in β-sheets of natural proteins.[8] The availability of peptide **1**, whose conformational stability can be varied by varying the salt concentration, provides an excellent model for testing these and other features stabilizing the L_{+2} helix in β-peptides.

Note Added in Proof: A similar design has also been published recently by Seebach and co-workers: Arvidsson, P. I.; Rueping, M.; Seebach, D. *Chem. Commun.* **2001**, 649−650.

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Supporting Information Available: Experimental procedures are provided (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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