Design, Synthesis, and Partial Characterization of Water-Soluble β-Sheets Stabilized by a Dibenzofuran-Based Amino Acid

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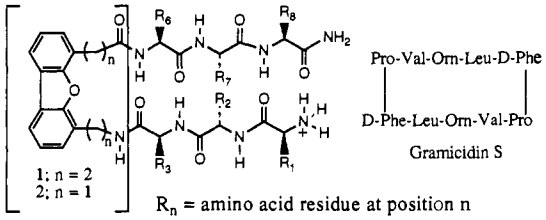
β-Sheets are found in almost all proteins, yet this secondary structure is poorly understood when compared with our comprehension of the α-helical structure.[1] Attempts to make small antiparallel β-sheet structures have been hindered by their propensity to self-associate into high molecular weight aggregates.[2,3] We have reported evidence that simple amide derivatives composed of 4-(2-aminoethyl)-6-dibenzofuranpropionic acid (**1**) adopt a hydrogen-bonded conformation which could support β-sheet formation.[4] We now report evidence that peptides composed of **1** adopt a β-sheet structure in aqueous buffers at room temperature.[5]



R_n = amino acid residue at position n

				Turn Residue(s)					
PEPTIDE	R1	R2	R3	R4		R5	R6	R7	R8
Peptide A	Val	Lys	Leu	-	1	-	Val	Lys	Leu-NH_2
Peptide B	Val	Lys	Leu	D-Phe		Pro	Val	Lys	Leu-NH_2
Peptide C	Val	Lys	Leu	-	2	-	Val	Lys	Leu-NH_2

Since little is known about the propensity of amino acid sequences to adopt an antiparallel β-sheet structure, we based our model β-sheet polypeptides on the amphiphilic β-sheet portion of the monomeric cyclic peptide gramicidin S.[6] Conceptually, the linear gramicidin S analogs were prepared by excising the two D-Phe-Pro reverse turns and replacing one with either residue **1** or **2**. The Orn residues in gramicidin S were replaced with structurally similar Lys residues. The positive charges in peptides **A–C** may prevent unwanted self-association and enhance solubility. Peptides **A–C** were synthesized on benzhydrylamine resin using *t*-Boc chemistry, deprotected and cleaved from the resin employing HF, and purified by reverse-phase HPLC.[7] Residues **1** and **2** were incorporated into peptides using pentafluorophenyl and BOP activation, respectively.[8] All peptides were characterized by matrix-assisted laser desorption mass spectrometry.[9]

The circular dichroism (CD) spectra of peptides **A–C** were recorded in aqueous buffers, Figure 1. Residue **1** is expected to exist in either of two low-energy intramolecularly hydrogen-bonded conformations.[4a] One of these conformations facilitates a hydrophobic interaction between the dibenzofuran skeleton and the side chains of the flanking amino acid residues, affording a hydrophobic cluster which may be important for β-sheet nucleation.[4a,e] The CD spectrum of peptide **A**, which incorporates **1** in the turn region, has minima at 197 and 214 nm, suggesting both random coil and β-sheet contributions, respectively. Peptide **A** exhibits pH-independent CD spectra in the range of pH 2.9–7.0. These spectra are nearly identical with those that have been previously recorded for an equilibrium mixture of unordered and β-sheet conformers.[3c,h] It is significant that such a short sequence exhibits a CD spectrum which is consistent with a partial β-sheet structure because β-sheet–random coil transition theory predicts that the N-terminal and C-terminal residues should be disordered.[10,11]

Replacement of **1** with D-Phe-Pro affords peptide **B**, which exhibits a random coil conformation by CD. Peptide **B** demonstrates that the sequence of the linear gramicidin S analog is not in itself sufficient to adopt a β-sheet structure, supporting the interpretation that **1** induces sheet structure in peptide **A**. Peptide **C** was prepared because modeling suggested[4a] that the putative hydrophobic cluster could not form in a peptide incorporating residue **2**.[8b] The spectrum of peptide **C** is that expected for an unordered chain,[12] indicating that **2** is not capable of sheet nucleation. It is possible that the 214-nm CD signal from peptide **A** could, in part, originate from electronic transitions within the dibenzofuran chromophore; however, independent experiments demonstrate that the major contributor is the amide chromophore.[14b]

Peptide **A** is monomeric in aqueous solution as determined by equilibrium ultracentrifugation studies at pH 4.1. The weight-averaged MW is ≈1000, consistent with a nonassociated solution structure.[13] Further evidence for a monomeric solution structure comes from the lack of a concentration-dependent change in the far-UV CD spectrum over a concentration range from 5.0 μM to 1.25 mM.[14] In addition, the 500-MHz ^1H NMR spectrum of peptide **A** (10 mM in 9:1 H_2O–D_2O, 50 mM deuterated acetate buffer, pH 4.6, 25 °C) exhibits narrow line widths and spectra that do not change over a time period of 4 weeks.

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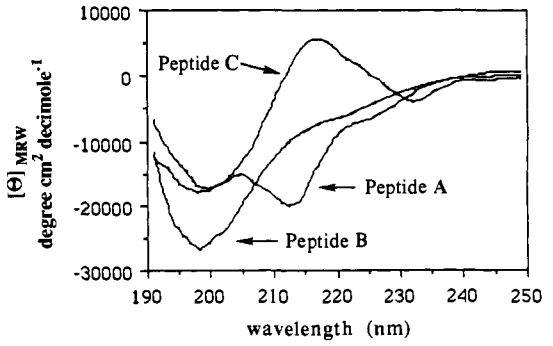
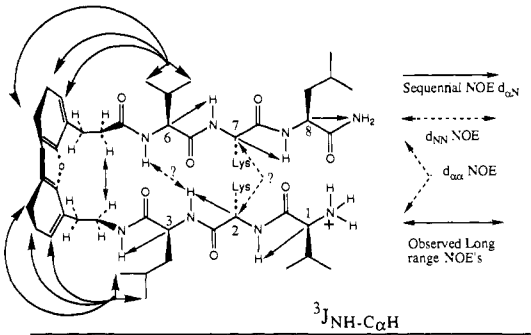


Figure 1. Circular dichroism spectra of a 0.2 mM solution of peptides **A**, **B**, and **C** at pH 2.9 in 10 mM phosphate buffer employing a 1-mm path length quartz cell at 25 °C. Spectra are reported as residue ellipticity.[14]



Lys-2, 7.33Hz; Leu-3, 7.32Hz; Val-6, 7.81Hz; Lys 7, 7.33Hz*; Leu 8, 6.84Hz* * assigments may be interchangeable

Figure 2. Intrastrand NOEs observed in the NOESY spectrum with τ_m = 300 ms (solid arrows). Dotted arrows indicate that corresponding cross peaks are present but have not been uniquely assigned due to resonance overlap.

Sequence-specific resonance assignments have been made in peptide **A** using classical 2D-COSY, NOESY, RELAYED-COSY, and TOCSY experiments.[15] The intra- and interstrand 2D-NOE assignments that are certain are shown in Figure 2. Strong NOEs between the dibenzofuran protons and protons from the diastereotopic methyl groups on the flanking Val and Leu residues confirms the presence of a hydrophobic cluster.[4a,e] The upfield shifts of the methyl groups in Val-6 (0.36 ppm) and Leu-3 (0.45 ppm) relative to those in Val-1 and Leu-8 suggest that they are shielded by the dibenzofuran ring. The ability to form this hydrophobic cluster may explain the efficacy of **1** in nucleating β-sheet structure and the near-UV CD spectrum of peptide **A**.[4e] A strong NOE between the β-carbons of **1** and the strong sequential NOEs shown in Figure 2 support an extended conformation for each strand, consistent with an antiparallel β-sheet structure.[15] An NOE cross peak is observed where the interstrand d_{NN} signal is expected; however, it cannot be proven that this peak results from the d_{NN} NOE because the amide N–H resonances from Lys-7 and Leu-3 overlap. Nitrogen-15-labeled Leu-3 has been incorporated into peptide **A**, which should facilitate the acquisition of ^{15}N-edited spectra to resolve the NOE assignment.

C_αH resonance overlap also makes the definitive assignment of the $d_{\alpha\alpha}$ NOE difficult at this time. The absence of interstrand NOEs between residues Val-1 and Leu-8 suggest that they are not in a well-defined conformation, which is verified by the low $^3J_{NH-C_\alpha H}$ coupling constant of Leu-8 (6.84 Hz). The $^3J_{NH-C_\alpha H}$ coupling constants are ≥7.2 Hz (Figure 2) for the remaining residues in peptide **A**, which are on the low side of the acceptable range for a β-sheet (7.0–10.0 Hz).[16] The relatively low coupling constants appear to result from conformational averaging. A small β-sheet structure like peptide **A** would be expected to be more dynamic than a β-sheet in a native protein. The presence of one resonance line for each nonequivalent ^{1}H shows that all conformations present are in rapid exchange on the NMR time scale. The NMR data suggest that the hydrophobic cluster/β-sheet conformation is significantly populated in solution.[4e]

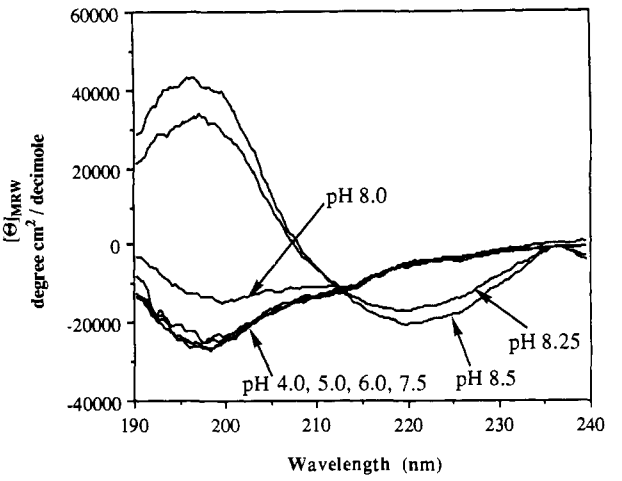


Figure 3. Circular dichroism spectra of a 0.10 mM solution of peptide **D** in either 10 mM borate buffer or acetate buffer at 25 °C as a function of pH employing a 1-mm path length quartz cell.

Peptide **D**, K-V-K-V-K-V-**1**-V-K-V-K-V-K-V-K-NH_2, was synthesized in an effort to make a homogeneous β-sheet in aqueous solution. The CD spectra of peptide **D** as a function of pH suggest that this 13-residue peptide containing **1** adopts a well-defined β-sheet structure at pH 8.5, Figure 3. It is likely that removal of charge from Lys-3 and Lys-12 facilitates the transition from a random coil to a β-sheet structure at pH 8.5 (note the isodichroic point), since replacement of these residues with Thr affords a spectroscopically analogous coil-to-β-sheet transition at pH 5.0.[17] The pH 8.5 spectrum of peptide **D** is very similar to the poly-Lys spectrum which has long been the standard for antiparallel β-sheet structure.[17] Equilibrium ultracentrifugation and concentration-dependent CD studies at pH 8.5 suggest a small quaternary structure <5000 MW, which is significant since poly-K and poly-VK are highly associated β-sheets.[3,18b]

The data suggest that residue **1** may be generally useful for the nucleation of β-sheet secondary structure via hydrophobic cluster formation. It appears that **1** stabilizes a partial antiparallel β-sheet structure in heptapeptides and a well-defined β-sheet structure in larger peptides. To the best of our knowledge, well-defined intramolecularly folded antiparallel β-sheets have not previously been observed in small peptides in H_2O.

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