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A DESIGNED β - HAIRPIN PEPTIDE

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A synthetic octapeptide, Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe (**1**) has been designed as a model for a β-hairpin conformation. Circular dichroism spectra in various organic solvents reveal a single negative band at 214-217 nm consistent with β-sheet structures. NMR studies in $CDCl_3$ and C_6D_6 establish the solvent shielded nature of the Leu(1), Val(3), Leu(6) and Val (8) NH groups. Nuclear Overhauser effects are observed between Val(7) $C^{\alpha}H$ and Val(2) $C^{\alpha}H$ protons providing strong support for a β-hairpin conformation. Several important diagnostic interresidue NOEs establish a Type II' β-turn conformation for the D-Pro-Gly segment and extended conformations for the amino and carboxyl terminal tripeptide arms. The high solubility of the β-hairpin peptide in organic solvents holds promise for the development of models for three and four stranded β-sheets.

The design of short peptide sequences adopting well defined conformations is a critical element in the development of synthetic protein mimics (1-4). The stabilization of helical conformations in short peptides in aqueous solution, (5-7) organic solvents(8-10) and crystals (11-13) have been achieved by diverse strategies. The rational construction of β - hairpins has attracted lesser attention, although they form one of the simplest elements of supersecondary structure in globular protein (14-16). Recent studies have focussed on the stabilization of β - hairpins in short peptides (17-18) and in development of novel non-peptide scaffolding for the generation of β-sheet mimics (19-21). Early work elaborated the nucleating role of tight turns (22-25). An analysis of β-hairpins in protein crystal structures reveals that two residue hairpin loops consist almost exclusively of type I' / type II' β - turns, which provide the right orientation of the extended arms (14-16).

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In this communication, we describe the characterization of a β - hairpin structure in a hydrophobic octapeptide Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe **(1)** in organic solvents. The central D-Pro-Gly segment facilitates nucleation of a type II' or type I' β -turns, (26-28) since the torsion angle ϕ is constrained to a value of $+60^{o} \pm 20^{o}$ in D-proline. Pro-Gly sequences have earlier been shown to have a high β–turn propensity (29-31).

Materials and Methods

The octapeptide 1 was synthesized by conventional solution phase procedures, using a racemization free strategy and a final 3+5 coupling. The peptide was purified by medium pressure liquid chromatography on a C_{18}(40-60 μ) column, followed by HPLC purification on a C_{18} (10 μ) column, using methanol-water gradient elution. Peptide homogenity was demonstrated by analytical HPLC (C_{18}, 5 μ) and complete assignment of 400 ^{1}H NMR spectra. All NMR studies were carried out on a Bruker AMX-400 spectrometer. Resonance assigments were done using DQF COSY and ROESY spectra. All 2D. experiments were recorded in phase sensitive mode by using the time proportional phase incrementation. 1024 and 512 data points were used in t_2 and t_1 dimensions, respectively. The resultant data set was zero filled to finally yield 1K x 1K data points. A shifted square sine bell window was used in both dimensions. Spectral widths were in the range of 4500 Hz. Peptide concentration was ~8 mM and the probe temperature was maintained at 296K. CD spectra were recorded on a JASCO-J-500 spectropolarimeter using 1 mm pathlength cells.

Results and Discussion

Figure 1 shows the CD spectrum of peptide **1** in several solvents. The presence of a single negative band at 214 - 217nm is consistent with spectra reported for polypeptide β-sheets (32,33). The absence of pronounced solvent dependence favours population of a stable conformational species. ^{1}H NMR studies at 400 MHz were carried out in three solvents, $CDCl_3$, C_6D_6 and $(CD_3)_2SO$. In the polar solvent $(CD_3)_2SO$, evidence for two species, corresponding to the trans (80 %) and cis (20 %) conformations about the Val-D-Pro bond were observed (34). Conformational studies discussed below are consequently, restricted to $CDCl_3$ and C_6D_6. Assignments of resonances was accomplished by a combination of double quantum filtered (DQF)COSY and ROESY spectra.

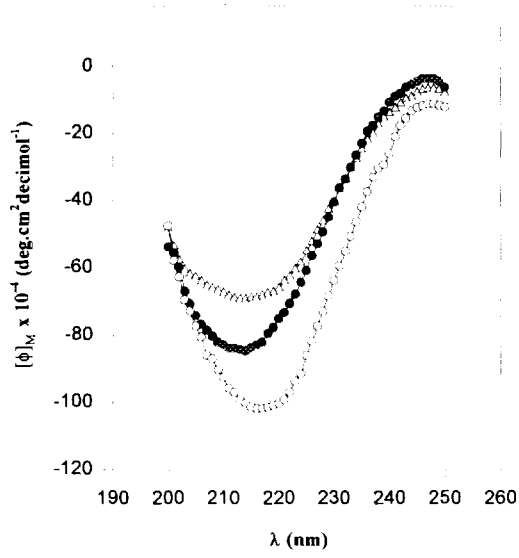


Figure 1. CD spectra of **1** in methanol (Δ), 2,2,2-trifluoroethanol (•) and trimethylphosphate (o).Peptide concentration, 3.44×10^{-4}M, 4.4×10^{-4}M, 5.5×10^{-4} M.

Figure 2 shows an assigned partial ROESY spectrum of **1** in C_6D_6 containing ~1.7% $(CD_3)_2SO$ (35). A noteworthy feature is the extremely large dispersion of $C^{\alpha}H$ and NH chemical shifts, suggesting an ordered, stable conformation in solution. The low field positions of several NH and $C^{\alpha}H$ protons is also characteristic of a β-sheet conformation (36,37). The partial ROESY spectrum in Figure 2 shows a strong Val (7)$C^{\alpha}H \leftrightarrow$ Val(2)$C^{\alpha}H$ interstrand NOE. Other important diagnostic interresidue NOEs observed between backbone protons are: Leu(1)$C^{\alpha}H \leftrightarrow$Val(2)NH, Val(2)$C^{\alpha}H \leftrightarrow$Val(3)NH, Gly(5)$C^{\alpha}H \leftrightarrow$Leu(6)NH, Leu(6)$C^{\alpha}H \leftrightarrow$Val(7)NH and Val(7)$C^{\alpha}H \leftrightarrow$Val(8)NH. A strong NOE between Val(3)$C^{\alpha}H$ and Pro(4) $C^{\delta}H$ confirms the trans geometry of the Val(3)-D-Pro(4) peptide bond. The delineation of solvent shielded NH groups in $CDCl_3$ was achieved using solvent perturbation of NH chemical shifts. Figure 3 clearly shows that Val(3), Leu(6) and Val(8) NH groups are strongly solvent shielded showing almost no downfield movement on addition of $(CD_3)_2SO$. Leu(1)NH is moderately shielded while Val(2), Gly(5) and Val(7) NH are appreciably perturbed, indicating solvent exposure. These results are consistent with the β-hairpin conformation shown in Figure 4.

The weak Val(3)NH ↔Leu(6)NH and the strong Pro(4)$C^{\alpha}H \leftrightarrow$Gly(5)NH and the Gly(5)NH↔Leu(6)NH NOEs, together with the solvent shielded, nature of

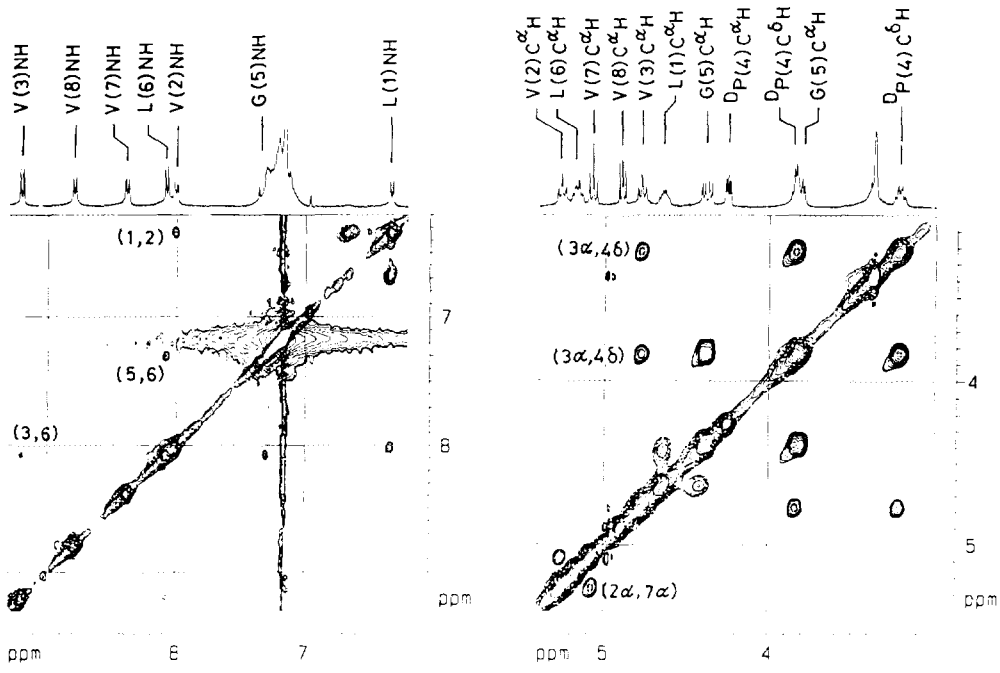
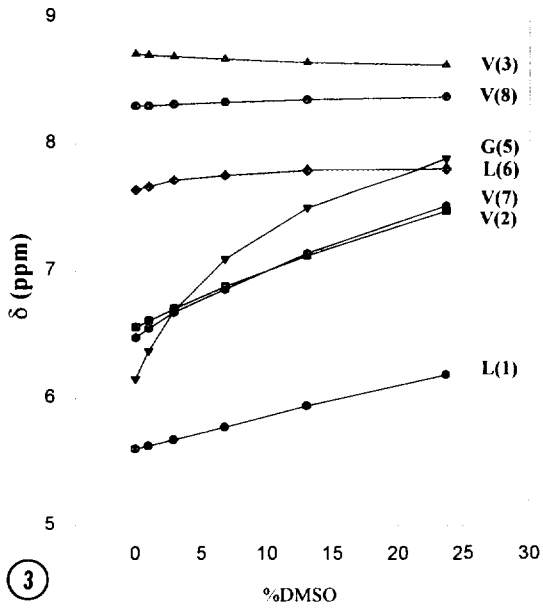


Figure 2. Partial ROESY spectra (400 MHz) of **1** in C_6D_6 containing 1.7 % $(CD_3)_2SO$. Peptide concentration 2.64 x 10^{-3} M. Left panel shows NH-NH NOEs. Right panel shows $C^{\alpha}H \leftrightarrow C^{\alpha}H$ and $C^{\alpha}H \leftrightarrow C^{\delta}H$ (Pro) NOEs. Assignments are marked on the 1D spectrum using the one letter code.

Leu(6)NH confirms the type II' β-turn conformation for the D-Pro-Gly segment. Further, the strong $C^{\alpha}{}_iH \leftrightarrow N_{i+1}H$ NOES observed in the segment Leu(1)-Val(2)-Val(3) and Leu(6) -Val(7)-Val(8) confirms the extended conformation at these residues. The $J_{NH\text{-}C^{\alpha}H}$ values (Hz) in C_6D_6 ($CDCl_3$) are: Leu(1) 9.0 (8.5), Val(2) 9.5 (9.1), Val(3) 9.4 (9.0), Leu(6) 8.9 (8.0), Val(7) 8.6 (8.6), Val(8) 8.6 (8.0). The large values of $J_{NH\text{-}C^{\alpha}H}$ ranging from 8.0 to 9.5 Hz are consistent with ϕ values close to -120° and are a characteristic of β-sheet conformations (38). The temperature coefficients (dδ/dT) of chemical shifts in aromatic solvents can be a sensitive probe of NH group accessibility (39,40). The values dδ/dT (ppb/K) measured in C_6D_6 are: Leu(1) 10.8, Val(2) 12.5, Val(3) 7.4, Gly(5) 13.9, Leu(6) 5.5,Val(7) 18.0, Val(8) 10.2. The internal NH groups Leu(1), Val(3), Leu(6) and Val(8) have appreciably lower temperature dependences. The NOE data together with the observed coupling constants and the solvent shielded nature of Leu(1), Val(3), Leu(6) and Val(8) NH groups (39,40) are in full agreement with a well defined β-hairpin conformation (Figure 4) in apolar solvents like C_6D_6 and $CDCl_3$.



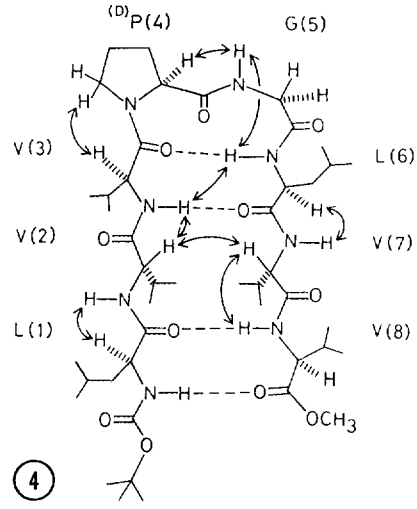


Figure 3. Solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures. NH assignments are indicated using the one letter code.

Figure 4. β-hairpin conformation of **1**. Double-edged arrows indicate observed interproton NOEs. Hydrogen bonds are indicated as broken lines.

The observed NH chemical shifts were found to be largely concentration independent (7.3 x 10^{-3} to 3.6 x 10^{-4} M) in C_6D_6 containing ~1.7 % $(CD_3)_2SO$, suggesting that aggregation effects are not prominent. Oligopeptides having extended conformations generally aggregate to form insoluble sheets, whereas peptide **1** is highly soluble in a variety of organic solvents. The success of the design strategy using a nucleating D-Pro-Gly segment augurs well for further development of peptide mimics for three and four stranded β - sheet segments, containing two or three chain reversals. Attempts in this direction are in progress.

Acknowledgments

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