

A four stranded β -sheet structure in a designed, synthetic polypeptide

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A designed four stranded β -sheet peptide has been constructed using three internal D-proline residues to nucleate β -hairpin formation.

De novo protein design provides a firm test of our understanding of the principles that govern the folding of polypeptide chains. The key element in this approach is the construction of synthetic peptide sequences which give rise to well characterized secondary and tertiary structures.¹ The choice of sequences is based on the propensities of specific sequences to adopt well defined local folds like helices, β -turns, and β -strands.² There has been considerable recent success in designing helical bundles³ and mixed α/β models,⁴ but attempts to design all β -sheet structures have been far fewer. β -Sheet formation has acquired a new dimension after the recognition that such structures are involved in the pathogenesis of Alzheimer's and prion diseases.⁵ Two major difficulties have retarded the development of design approaches to β -sheets. (i) The intrinsic tendency of sheet-like structures to aggregate, leading to solubility problems, was indeed recognized in early studies of the β -sheet model betabellin.¹ (ii) The absence of well determined cross-strand amino acid preferences in protein β -sheets⁶ limits rational design of sequences to facilitate long-range interactions. Recent success in development of soluble β -hairpin models has resulted from the recognition that nucleating β -turns of the appropriate stereochemistry (type I' and type II') can facilitate hairpin formation. The sequence choices at the turns have been limited to Asn-Gly and ^DPro-Gly; segments which have a strong tendency to adopt type I'/II' conformations. This is a consequence of the stereochemical imperative that the $i + 1$ residue in type I'/II' β -turns must adopt local left-handed helical (α_L) conformations. While in ^DPro, pyrrolidine ring formation restricts the N-C α torsion angle to the desired value of $+60 \pm 20^\circ$, Asn residues have the highest propensity to adopt α_L conformations, amongst the 20 protein amino acids.⁷ ^DPro-Gly segments have been shown to be superior to Asn-Gly segments in stabilizing β -hairpins.⁸ There have been several recent reports describing construction of three-stranded β -sheet structures soluble in both organic and aqueous media.⁹ We describe here the unambiguous characterization of a four-stranded β -sheet structure in a designed 26 residue synthetic peptide (Beta-4, Fig 1).[†] Three internal ^DPro-Xxx sequences were positioned to nucleate type II'/I' β -turn conformations. In the strands, a preponderance of β -branched residues (Ile, Val, Thr) was chosen to facilitate extended strand formation. Lys/Arg residues were introduced to promote solubility and reduce the chances of aggregation. The aromatic residues Phe/Tyr were positioned so as to permit observation of long-range side chain interactions as diagnostics for structure formation.

The CD spectrum of the peptide in water showed a negative band at *ca.* 213 nm. In MeOH and MeOH-water mixtures, there is considerable intensification of this negative CD band, a feature consistent with a β -sheet structure for the peptide Beta-4. The 500 MHz ¹H NMR spectrum for Beta-4 in MeOH was extremely well resolved, permitting sequence specific assignments using a combination of TOCSY and NOESY experiments. All proton resonances observed for the peptide Beta-4 were sharp and there was no appreciable concentration dependence of chemical shifts in the range of 3.2 to 0.1 mM,

suggesting the absence of aggregation effects. Several C α H resonances were observed at low field positions, ≥ 4.6 ppm [T3 (4.60), I4 (4.87), K5 (4.85), I8 (4.74), T9 (4.87), F10 (5.00), A11 (4.96), V15 (4.61), L16 (5.04), F17 (5.10), A18 (4.94), T23 (4.75), Y25 (5.15)]. Furthermore, large values of vicinal coupling constants $J_{\alpha\text{H}\text{NH}} \geq 8.5$ Hz were observed for several NH resonances which could be accurately measured from resolution enhanced 1D experiments [J (Hz): T3 (8.9), I4 (9.4), K5 (8.6), F10 (9.4), A11 (9.0), T14 (9.0), A18 (8.5), V19 (8.6), K22 (8.8), L24 (8.7), Y25 (8.5)]. These parameters are consistent with a large number of residues in Beta-4 adopting β -strand conformations. NOE data provide definitive confirmation of the β -sheet structure in the peptide. For all residues in the strand segments the observed C $_i$ α HN $_{i+1}$ H ($d_{\alpha\text{N}}$) NOE was appreciably more intense than the corresponding intraresidue N $_i$ HC $_i$ α H ($d_{\text{N}\alpha}$) NOE. This is a characteristic of residues adopting (ϕ , ψ) values in the β -strand region. Observation of several key long-range NH-NH NOEs (T3-F10, K5-I8, A11-T14, V19-K22, and V15-R26) in the NOESY spectra of Beta-4 provide evidence for the proper registry of the β -strands, as shown in Fig. 1. The location of the turn segments is confirmed

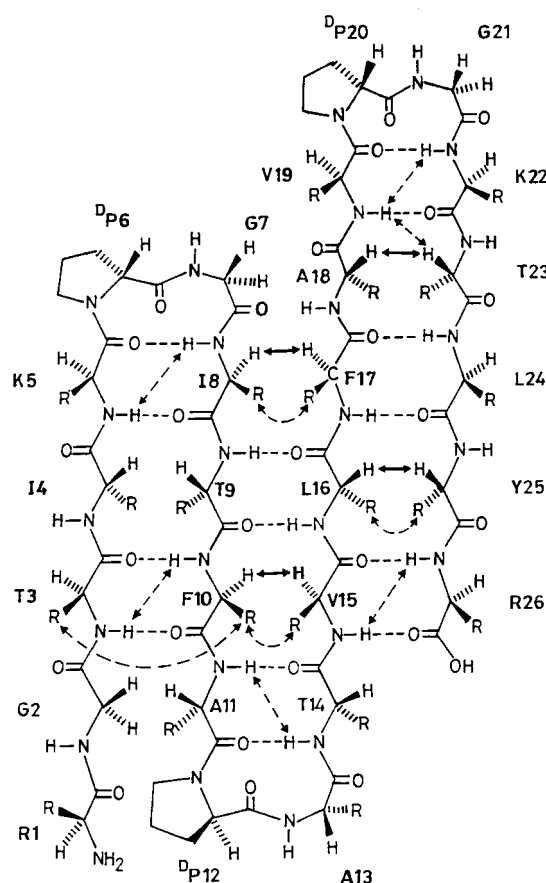


Fig. 1 Schematic representation of the target structure for the peptide Beta-4. A summary of some important long range NOEs observed for the peptide are indicated by arrows. Solid arrows indicate strong NOEs and broken arrows indicate weak NOEs.

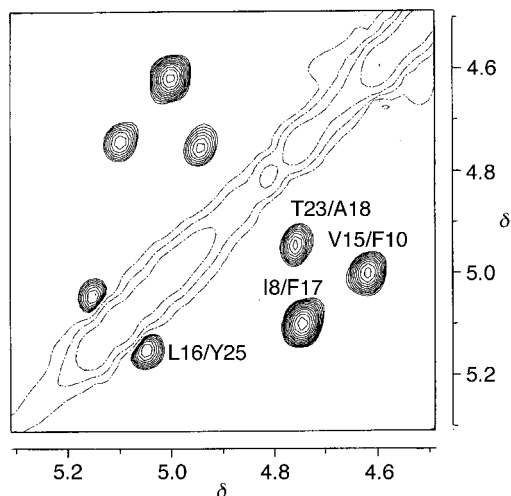


Fig. 2 Portion of the ROESY spectrum of Beta-4 in CD₃OD at 300 K, highlighting long-range C^αH–C^αH NOEs.

by the observation of the sequential d_{NN} connectivities, G7-I8, A13-T14 and G21-K22. In all three turns the $i + 2$ residue must adopt local helical conformations in which these NOEs are expected. Fig. 2 shows an expanded view of the NOEs observed between C^αH protons. The observed cross-stranded interactions between I8-F17, F10-V15, A18-T23 and L16-Y25 provide clear support for the structure in Fig. 1. The $d_{\alpha\alpha}$ NOE between I4 and T9 could not be detected due to overlap of these resonances with the residual OH resonance of the solvent. However, the proper registry of the two N-terminal strands is supported by the observation of both the expected cross-strand NH–NH NOEs (K5-I8 and T3-F10). Identification of several sidechain–sidechain NOEs between methyl resonances at high field and the aromatic resonances at low field provides strong evidence of compact structure of the peptide in solution. Most importantly, the NOEs observed between the aromatic protons of F10 and the methyl protons of V15 and T3 clearly support the alignment of the first three N-terminal strands. Inter-residue aromatic proton–methyl proton NOEs between I8-F17 and L16-Y25 are also clearly indicative of proper strand registry. Using a total of 100 observed NOEs, 11 dihedral angle constraints from J values, and 10 hydrogen bonding constraints obtained from H/D exchange data, structure calculations were performed using DYANA.¹⁰ Fig. 3 shows a superposition of 12 calculated structures with a mean RMSD of 0.59 Å for all backbone atoms in the well-ordered region of residues 3–25 and 1.32 Å for all heavy atoms in the same region of residues. In 50% MeOH–water most of the long range NOEs characteristic of the β -sheet structure are retained. Methyl resonances of I8 and V15 appear at extremely high field due to the shielding of aromatic rings of F10 and F17. This feature is characteristic of sidechain clustering in the β -sheet. In 50% MeOH–water both these resonances move to slightly lower field. In water these ring current effects are significantly reduced [I8 C^γH₃ (0.40 ppm) in MeOH, (0.50 ppm) in 50% MeOH–water, (0.67 ppm) in water]. In water, NOEs characteristic of the three β -turn segments are retained (NH–NH NOEs between G7-I8, A13-T14 and G21-K22). However, long range interstrand NOEs diagnostic of the β -sheet structure were abolished suggesting that solvent invasion disrupts the proper strand registry for β -sheet formation. Since cooperative cross-strand hydrogen bonding may be the dominant cementing force in β -sheets, competition from a strongly hydrogen bonding solvent like water may disrupt sheet formation unless the segment is sequestered in a relatively apolar environment. It is not surprising that β -sheets in proteins are often largely solvent inaccessible. The recent observation of completely solvent exposed β -sheet¹¹ holds promise for further refining of Beta-4 to provide additional stability for a sheet in a completely aqueous environment. Rational design of β -sheet structures will prove useful in understanding the forces of

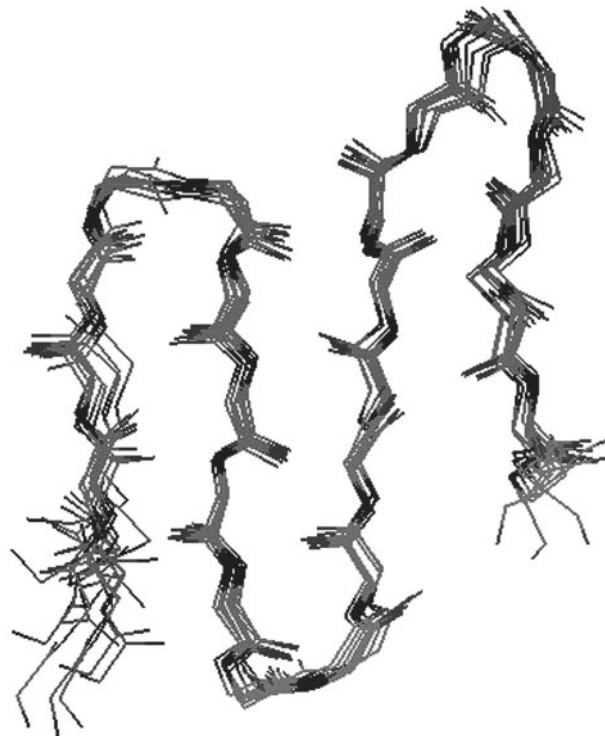


Fig. 3 Superposition of the best 12 structures of Beta-4 obtained from NMR restraints. Backbone traces illustrating the four-stranded β -sheet fold of the molecule are shown.

nucleation and structure propagation in addition to throwing light on the factors that determine the tendency of these structures to aggregate, a factor of particular importance in diseases caused by polypeptide precipitation.

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Notes and references

† The 26-residue peptide (Beta-4) was synthesized by standard solid-phase procedures based on Fmoc chemistry and purified by reverse phase HPLC (C18, 10 μ) using MeCN–water (containing 0.1% TFA) gradients for elution. The peptide was characterized by its electrospray ionization mass spectrum (M observed 2775.10, M calculated 2775.30) and by complete assignment of the 500 MHz ¹H NMR spectrum. All NMR studies were carried out on a Bruker DRX 500 spectrometer.

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