**De novo** design of a five-stranded β-sheet anchoring a metal-ion binding site

Janani Venkatraman, a G. A. Naganagowda, a R. Sudha a and Padmanabhan Balaram a∗

a Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560012, India.
E-mail: pb@mbu.iisc.ernet.in; Fax: 91 80 3600683/91 80 3600535; Tel: 91 80 3602741
b Sophisticated Instrumentation Facility, Indian Institute of Science, Bangalore-560012, India

Received (in Cambridge, UK) 5th September 2001, Accepted 1st November 2001
First published as an Advance Article on the web 23rd November 2001

A five-stranded β-sheet bearing two histidine residues as part of a metal-binding site has been designed, synthesised and characterised using NMR and electrospray ionization mass spectrometry techniques.

Construction of well-defined structural elements such as isolated helices,1,2 helical bundles,3,4 β-turns and hairpins,5,6 mixed α/β structures7 and β-sheets6,8 have employed common design principles such as (a) incorporation of residues with appropriate secondary structural propensities,1 (b) utilisation of sequence motifs observed in protein three-dimensional structures as structural templates4,9 or (c) nucleation of specific conformations by sterically constrained non-protein amino acids.5,10 Indeed, the use of both normal as well as unusual amino acids that nucleate turns with requisite stereochemistry (type I and type II ) have been responsible for the successful design of β-hairpins and small sheets that are stable in aqueous6,8,11 and organic solvents.5,12 Attempts at the construction of large multistranded sheets as precursors for the rational design of β-sandwiches and β-barrels have been plagued by problems of solubility and difficulty in characterising the designed structures.13

We demonstrate in this communication, the construction of a five stranded β-sheet, whose stable, extended backbone can be further utilised as a template onto which a metal-binding site can be grafted.† The designed 34-residue peptide, B5 [RGKVPQGETNTPVQFHTPQGYKTLHPARIVLKL], has four ’Pro-Xxx segments designed to nucleate type II/β-turns (Fig. 1), β-branched residues such as Val/Ile/Thr, which are known to promote β-sheet conformation have been appropriately positioned in the strands. A large number of positively charged residues (Arg/Lys) have been included in the designed sequence to enhance solubility and discourage aggregation.

The design of metal binding sites has often stressed the His,Cys, motif as appropriate ligands for the chelation of transition metals.14 We have chosen to position two histidine residues (His 17 and His 26) on adjacent (third and fourth) strands of the five-stranded β-sheet towards the construction of a minimal metal-binding motif. Folding of the B5 peptide sequence into the target structure would bring the two His residues into the required structural juxtaposition for metal-ion chelation. In addition, the Glu8-His17 pair may also serve as a metal binding site. Tridentate coordination involving Glu8, His17 and His26 is impossible if the constraints of the β-sheet are maintained.

The structure of the B5 peptide was characterized in detail using high-resolution NMR techniques in methanol, as CD studies indicated the peptide to be poorly structured in water. TOCSY and NOESY experiments permitted complete sequence-specific assignments for B5. Spectra derived from NOESY experiments revealed various features consistent with β-sheet structure, such as significantly more intense CαHN,H,C,H NOEs as compared to the corresponding intraresidue N,H,C,H NOEs. The folding of B5 into β-sheet conformation with the desired strand registry was corroborated by the presence of several key, long-range NH/NH NOEs and medium intensity, cross-strand CαH,CαH NOEs (Fig. 1, 2) between residues E8/H17, N10/Q15, F16/L25, T18/K23 and H26/I31. The observation of sequential dHα NOEs between residues G37/E9, S13/V14, G21/Y22 and A29/R30 served to confirm the position of the turns. H/D exchange experiments

![Fig. 1 Schematic representation of the target structure for B5. Important long range NOEs observed are indicated by arrows (darker arrows denote medium NOEs and lighter arrows represent weak NOEs).](Image 376x114 to 523x262)

![Fig. 2 Partial expansion of the NOESY spectrum of B5 in CD3OD at 300 K, indicating long range CαH,CαH NOEs.](Image 337x299 to 571x578)
performed on the B5 peptide in CD$_3$OD revealed the presence of some slow exchanging amide protons corresponding to residues involved in hydrogen bonding in the β-strands (I3, V5, T9, T11, V14, F16, I19, Y22, T24, R30 and V32). Structure calculations were performed using DYANA$^{15}$ and a total of 228 distance constraints (114 upper and 114 lower), derived from 90 NOEs and H/D experiments. A superposition of ten best structures is shown in Fig. 3a, with a mean RMSD of 1.04 Å for all back bone atoms. Fig. 3b is a ribbon diagram of the mean structure for B5, illustrating probable orientations of the mean structure for B5, illustrating probable orientations of His17 and His26 (side chain positions modeled).

The ability of the peptide to bind a single nickel ion between the two imidazole groups of His17 and His26 was demonstrated by electrospray ionization mass spectrometry (ESIMS) in methanol. Fig. 4 is the ESI mass spectrum of the free peptide and B5 bound to Ni$^{2+}$. The mass of the free peptide as measured by ESIMS is 3785 (Calc. mass = 3785.4). The mass observed for B5 treated with nickel acetate is 3842, which corresponds to the mass of the peptide + the average mass of Ni$^{2+}$ for + metal ion. Also, attempts to detect sandwich complexes (data not shown). It is worthy of remark that the +5 charge state as the most abundant suggests that two negative charges have been acquired by the complex. This is consistent with the loss of two protons by the imidazole groups of His17 and His26 (resulting in two negative charges), rendering the charge of the [His$_2$M$^{2+}$] moiety zero, and maintaining the overall charge of the complex at +5. Involvement of one His and one Glu residue in metal binding would have resulted in the creation of only one new negative charge; in this case the charge state distribution might be expected to be different, with the +6 state being most abundant. CD spectra of peptide–metal complexes closely resembled the parent spectrum, suggesting no major conformational change on metal ion binding, supporting the existence of a preformed template and further indicating the absence of tridentate coordination.

The present design strategy has successfully resulted in the characterisation of a five-stranded β-sandwich capable of metal ion coordination. In principle, a metal ion template can be used to assemble individual β-sheet structures to form β-sandwiches and closed β-bars.

Notes and references

† The B5 peptide was synthesised by standard solid-phase methods using Fmoc chemistry and purified by reverse phase HPLC (C18, 10 µ) on acetamidite–water–0.1% TFA gradients. The peptide was characterised by electrospray mass spectrometry and complete NMR analysis on a Bruker 500 MHz spectrometer. Mass spectrometric experiments were carried out on a Hewlett Packard series 1100 MSD mass spectrometer.