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Templating Peptide Folding on the Surface of a Micelle: Nucleating the Formation of a β-Hairpin

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Abstract—NMR spectroscopy is used to show that a 20-residue β-hairpin peptide sequence derived from ferredoxin I, with a Pro-Asp two-residue type I turn which is uncommon in β-hairpins, is unstructured in aqueous solution but shows NOE evidence for partial folding in the presence of sodium dodecylsulphate micelles. The peptide has a number of lysine residues in the N-terminal β-strand capable of interacting with the micelle surface and templating the partial folding of the hairpin by reducing the entropic cost of ordering the peptide backbone. © 2000 Published by Elsevier Science Ltd.

Peptide fragments have been widely used to model the interactions found in elements of secondary structure in native proteins,[1,2] to investigate the relationship between amino acid sequence and folded structure,[3,4] and as novel motifs in molecular recognition and catalysis.[5–7] Autonomous folding of protein fragments has provided insight into the possible nucleation events that promote protein folding.[1,2] We, and others, have investigated the folding of β-hairpin peptides to establish, in particular, the role of the β-turn sequence in dictating both hairpin stability and the alignment and register of β-strands in the folded structure.[8–13] The large database of protein structures now available enables statistical analysis to be used to determine most favoured turn sequences. Two-residue turns in β-hairpin peptides favour the type I′ variety (Asn-Xxx),[12,13] with Asn-Gly particularly prevalent. The frequency of occurrence seems to correlate well with their stability in model β-hairpin systems.[12] In contrast, type I ($^{\mathrm{L}}$Pro-Xxx) and type II ($^{\mathrm{L}}$Pro-Gly) turns are much less common in β-hairpins because these residues are not compatible with the right-handed twist found in most protein β-sheets. Again, studies of model β-hairpin systems containing these turns show that they are largely unfolded, but peptides with $^{\mathrm{D}}$Pro-Xxx or $^{\mathrm{D}}$Pro-Gly, which are able to adopt type I′ and II′ conformations promote the formation of stable folded hairpin structures.[11]

The N-terminal 20-residue β-hairpin peptide of ferredoxin I from the blue green alga *Aphanothece sacrum* (Fig. 1a,b) has been studied in solution by NMR and CD spectroscopy.[14] The β-hairpin carries the turn sequence Thr-Pro-Asp-Gly which folds in the native protein into the uncommon two-residue type I turn conformation. NMR studies of the isolated 20-residue peptide F(1–20) in aqueous solution show little evidence for contiguous positive deviations from random coil chemical shift values ($\Delta\delta^{\mathrm{H}\alpha} > 0.1$ ppm), that are considered a hallmark of β-sheet secondary structure formation (Fig. 2),[15] or any medium or long range NOEs that suggest a significant amount of folded structure in water. Given the molecular weight of the peptide, and its dynamic nature, ROESY experiments were also performed but revealed no evidence for folding under these conditions. These results are consistent with those presented previously, that the type I turn is not a good promoter of β-hairpin formation.[11] However, low levels of organic co-solvents such as methanol and trifluorethanol (30–40%) induce folding into a native-like conformation with large $\Delta\delta^{\mathrm{H}\alpha}$ values (Fig. 2) and many NOEs consistent with both formation of a type I turn and extensive cross-strand side chain interactions. Thus, the primary amino acid sequence appears to code for the formation of the native-like hairpin conformation, however, this propensity is only realised in a solvent that promotes structure formation. These conditions are, however, less than a realistic representation of the solvent medium in vivo.

An alternative approach to promoting folding of the peptide in aqueous solution is to reduce the number of degrees of conformational freedom of the polypeptide chain, and hence the entropic cost of folding, by partial

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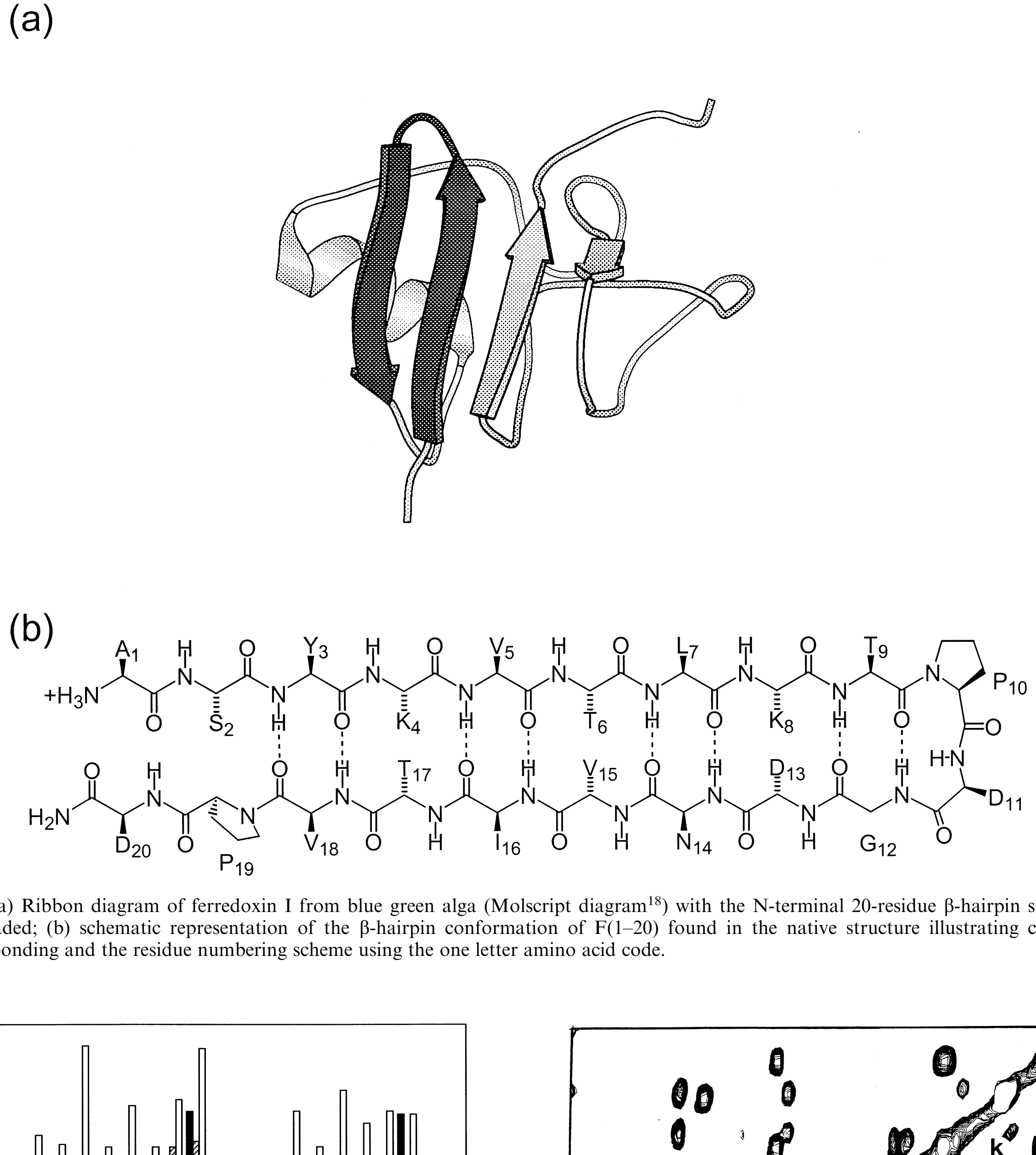

Figure 1. (a) Ribbon diagram of ferredoxin I from blue green alga (Molscript diagram[18]) with the N-terminal 20-residue β-hairpin shown more heavily shaded; (b) schematic representation of the β-hairpin conformation of F(1–20) found in the native structure illustrating cross strand hydrogen bonding and the residue numbering scheme using the one letter amino acid code.

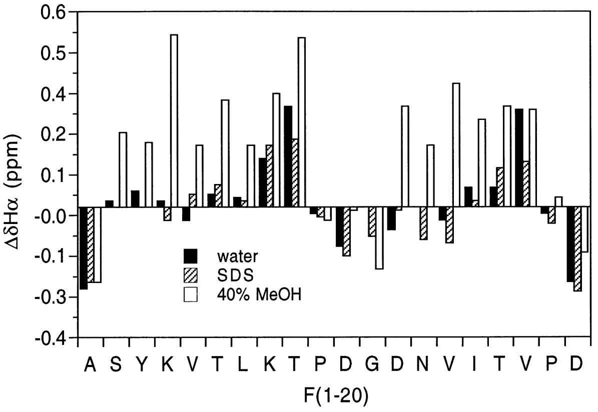

Figure 2. Deviations of Hα chemical shifts ($\Delta\delta^{H\alpha}$) from random coil values for F(1–20) in water, 40% aqueous methanol solution and in the presence of 200 mM sodium [$^2H_{25}$]-dodecylsulphate micelles. F(1–20) was synthesised using standard solid-phase *F*moc chemistry, as previously described.[14] NMR data were collected at 500 MHz at 298K.

immobilisation on a molecular surface through weak non-covalent interactions. To this end, we have examined the conformation of F(1–20) in aqueous solution at 5 mM concentration in the presence of 200 mM sodium [$^2H_{25}$]-dodecylsulphate at pH 5.8 under micelle forming

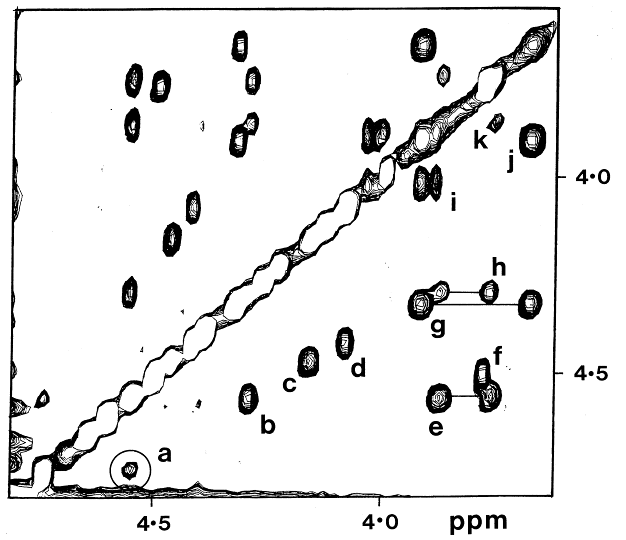

Figure 3. Portion of the 200 ms NOESY spectrum (3.6 to 4.8 ppm) of F(1–20) in the presence of 200 mM sodium [$^2H_{25}$]-dodecylsulphate micelles at 288 K in D_2O solution: cross-peaks in the spectrum are labelled as follows. (a) Hα→Hα NOE for K8→D13 (circled); (b) T9 Hα →Hβ; (c) T17 Hα →Hβ; (d) T6 Hα →Hβ; (e) T9 Hα →P10 Hδs; (f) S2 Hα →Hβ; (g) V18 Hα →P19 Hδs; (h) T9 Hβ →P10 Hδs; (i) G12 Hα →Hα; (j) P19 Hδ →Hδ; (k) P10 Hδ →Hδ.

conditions. Line widths for F(1–20) in the ^1H NMR spectrum were found to be significantly increased compared to D_2O solution, indicative of association with the micelle affecting the overall rate of tumbling of the peptide. A full assignment of the spectrum was possible using homonuclear 2D (NOESY and TOCSY) experiments. While $\Delta\delta^{H\alpha}$ values are still relatively small, the observation of a number of medium and long range NOEs between non-sequential residues can only be rationalised if a β-turn-like conformation is populated in solution. The $H\alpha \rightarrow H\alpha$ NOE for Lys8→Asp13 (Fig. 3) suggests native-like alignment of the peptide backbone, as do a number of other medium range main chain–side chain and side chain–side chain NOEs (Asp13 $H\alpha \rightarrow$ Leu7 $C\delta H_3$ and Asn14 $C\beta H_2 \rightarrow$ Thr9 $C\gamma H_3$). These interactions are localised to residues around the turn region and to residues immediately adjacent to the turn (see Fig. 4a), and are consistent with interactions observed in the native folded β-hairpin conformation.[14] The absence of extensive cross-strand interactions suggests that the two anti-parallel β-strands are at best weakly associated and substantially disordered in solution. This is consistent with small deviations of chemical shifts from random coil values. An ensemble of structures calculated from the limited NOE data enables the turn-like conformation to be visualised (Fig. 4b).

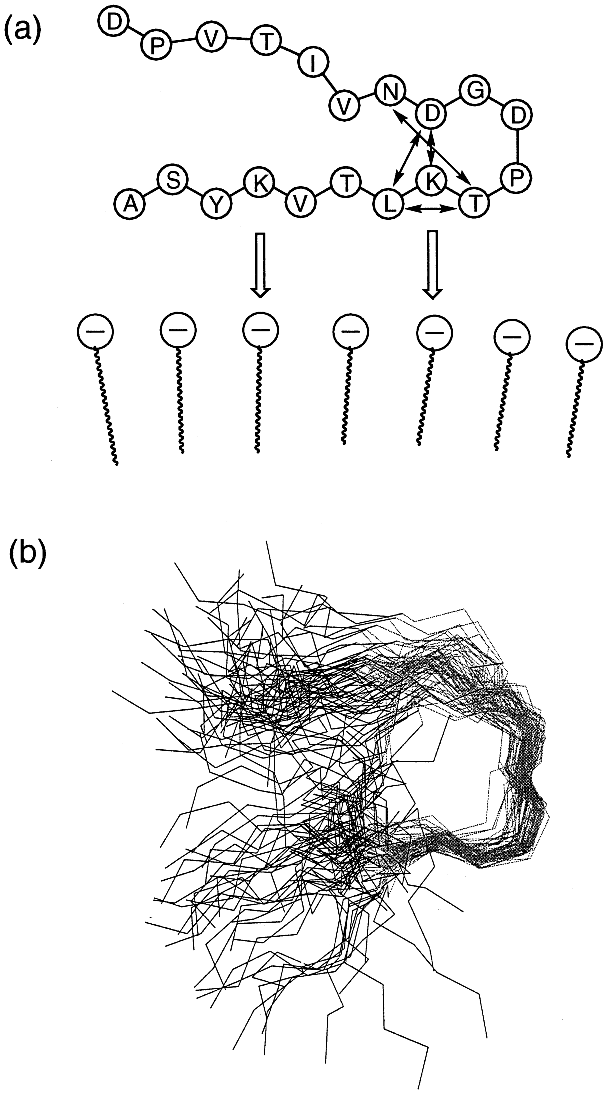

Figure 4. (a) Schematic representation of the medium/long range NOEs identified in NOESY spectra of the peptide in the presence of 200 mM sodium [$^2H_{25}$]-dodecylsulphate at pH 5.8: NOEs are consistent with the proximity of residue side chains in the native structure; (b) ensemble of 60 structures of residues 7 to 14 of F(1–20) calculated using previously described protocols,[7,13] and NOE restraints with an upper distance bound of <5 Å. The turn conformation (residues 9–12) is reasonably well-defined by the restraints, with the native type I geometry highly represented; the β-strands are highly disordered (not shown).

A detailed model of the interaction of the peptide with the micelle remains unclear. The turn region of the peptide appears to be 'ordered' to the greatest extent, and this region contains a high proportion of polar side chains, suggesting that the peptide may be associated with the surface of the micelle rather than buried within its interior. It is particularly notable that the N-terminal β-strand carries two positively charged Lys residues (see Fig. 1b) suggesting that interaction of this strand with the anionic surface of the micelle may provide sufficient restriction of conformational freedom to template the folding of the turn region by reducing the entropic cost of ordering the peptide backbone. In relation to current models of protein folding, nucleation condensation mechanisms suggest that collapse of the polypeptide chain through long range interactions produces a compact state which is subsequently consolidated in the native folded protein with concomitant formation of regular secondary structure.[16,17] These initial events bring different parts of the peptide chain into contact to reduce the amount of conformational freedom. The micelle surface, in these studies, may mimic non-specific interactions that promote folding and secondary structure nucleation by reducing the entropic cost of ordering the peptide backbone making the weak interactions, even in unfavourable turn sequences, sufficient to stabilise some degree of folded structure.

Acknowledgements

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