Dissecting the effects of cooperativity on the stabilisation of a *de novo* designed three stranded anti-parallel β-sheet

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Cooperative effects between two sets of weak interactions at different hydrogen bonded interfaces are shown to stabilise a *de novo* designed three-stranded anti-parallel β-sheet: NMR and CD (circular dichroism) spectroscopy have been used to compare and contrast the stability of the β-sheet (residues 1–24) and the isolated C-terminal β-hairpin (residues 9–24).

Molecular recognition phenomena, including host–guest interactions in chemical systems and the processes by which complex structures self-assemble in biology, are fundamentally dependent on large numbers of weak, non-covalent interactions and their cooperative interplay.[1,2] Cooperativity frequently complicates attempts at quantitative analysis of such interactions because ligand binding constants are seldom only a measure of the interactions occurring at one particular molecular interface but reflect some property of the whole set of linked weak interactions within the ligand complex.[1–7] Here we describe a peptide system that has enabled us to investigate the interplay between two sets of weak interactions that occur at two different hydrogen bonded interfaces within a *de novo* designed anti-parallel three stranded β-sheet (Fig. 1). To dissect-out possible cooperative interactions we have synthesised two peptides. The first peptide (9–24) of 16 residues was designed to form a β-hairpin. The second peptide (1–24) ends with the same 16 residues but has a preceding 8 amino acid sequence designed to form a second β-turn and the third strand (S3) of the anti-parallel β-sheet. The first strand (S1 in Fig. 1) is in the same environment in both folded structures and undergoes the same changes in backbone solvation and hydrophobic interactions when it interacts with S2. Thus, the indirect influence of the third strand on the stability of the interaction between S1 and S2 can be assessed in this system. We report here evidence from CD and NMR studies that 1–24 folds into a three stranded sheet in which the C-terminal hairpin is cooperatively stabilised with respect to the isolated hairpin (9–24). There is much current interest in the autonomous folding of β-hairpin peptides for probing the sequence-dependent factors important in their stabilisation.[8]

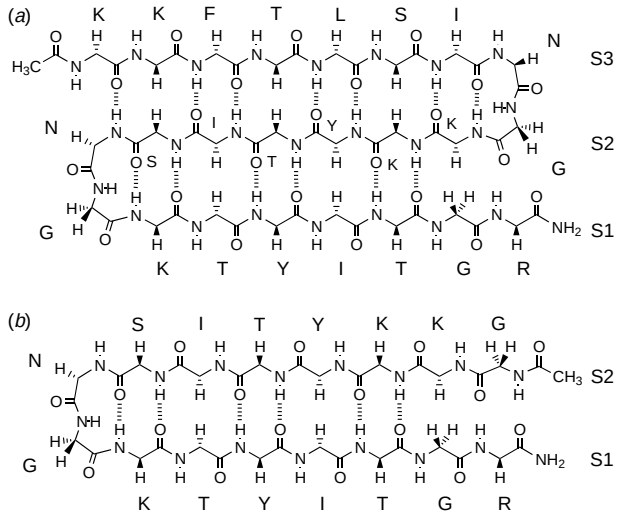

Fig. 1 Structures and sequences of (*a*) the designed three-stranded β-sheet (residues 1–24) and (*b*) β-hairpin (residues 9–24). The first strand (S1) is in an identical environment in both folded structures.

In aqueous solution, the two peptides showed limited evidence of folding. Qualitative analysis of CD (circular dichroism) spectra suggested that substantial populations of random coil conformations persist with both peptides showing very similar molar ellipticity values (per residue) at 198 nm. Addition of methanol (in the range 0–50% v/v) to the aqueous peptide solutions was found to promote secondary structure formation. In 50% aqueous methanol peptide 1–24 showed only a small negative ellipticity at 198 nm, but a pronounced minimum around 216 nm, characteristic of a substantial population of β-sheet and β-turn conformation.[9,10] In contrast, the changes in molar ellipticity (per residue) on addition of methanol are less marked for the shorter peptide (9–24), with residual random coil structure still evident.

Both peptides were subsequently investigated under identical conditions by ^{1}H NMR spectroscopy. H_α chemical shift deviations from random coil values have been shown to be an excellent probe of protein secondary structure.[11] In the mixed solvent system [50% CD_3OD–50% D_2O (or 50% H_2O) solution at pH 3.9 and 2 mM concentration], H_α shifts for peptide 1–24 (Fig. 2) were indicative of three β-strands (downfield shifts of up to 0.7 ppm) separated by two β-turns (upfield shifts of < 0.3 ppm).[12] These structural features were confirmed by the pattern and intensity of NOEs: strong inter-residue and weak intra-residue H_α–NH NOEs were observed within the strands, whereas these were of similar intensity within the turn regions. Also, the only significant sequential NH–NH NOEs were observed between residues 9–10 and 17–18, again characteristic of the two β-turns. The observation of long range NOEs provided the most conclusive evidence for the formation of folded structure in solution, consistent with the alignment of the peptide chain shown in Fig. 1(*a*). These included many of the

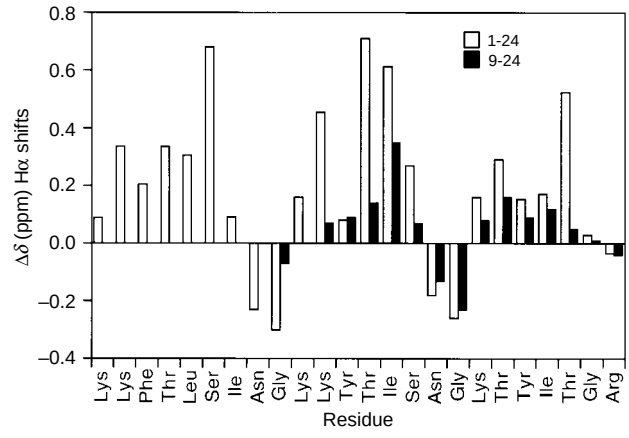

Fig. 2 H_α chemical shift deviations from random coil values for sheet (1–24) and hairpin (9–24) in 50% v/v aq. methanol, 2 mM peptide, pH 3.9, 283 K

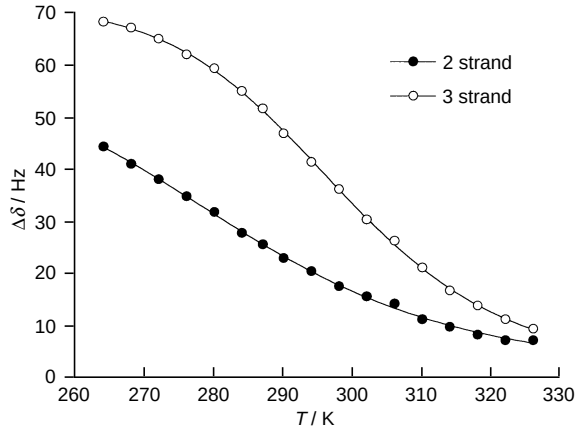

Fig. 3 Melting curves for 1–24 and 9–24 determined from the temperature-dependence of the chemical shift difference ($\Delta\delta$/Hz) between the H_ε resonances of Y12 and Y20. Data were fitted to a two-state model and the limiting values for $\Delta\delta$ determined iteratively. In both cases correlation coefficients (R) were > 0.99. At 298 K, peptide 1–24 was estimated to be 50% folded, while peptide 9–24 was only 25% folded.

expected cross-strand H_α–H_α NOEs (K2→S15, T4→T13, S6→K11, K10→G23, I14→T19), NH–NH NOEs (F3→I14, I7→K10, T13→Y20, S15→K18) and interactions between side chains in close proximity (F3→I14 and L5→Y12→I21).

The shorter peptide (9–24) (Fig. 1) showed evidence of a rather less folded structure. Much less pronounced downfield shifts are observed for H_α resonances of residues within the strands (Fig. 2). NOE intensity measurements indicate a less ordered more dynamic structure than observed for the same residues in the three stranded sheet. In contrast to the large number of long range interstrand NOEs identified for peptide 1–24, no long range H_α–H_α or NH–NH NOEs were detected, although the side chain interaction between Y12 and I21 was still apparent, suggesting a small population of a less well-ordered β-hairpin. Despite the apparently weak interaction between the two proposed β-strands, the observation of a strong NH–NH NOE between residues G17–K18, and similar shift perturbations for G17 H_α and K18 NH in both peptides, suggests that the β-turns may be populated to similar extents in the two structures.

To illustrate further the cooperative nature of the interactions, we have measured the temperature-dependence of the stability of both peptides (Fig. 3). The folded population in each case was conveniently monitored by measuring the chemical shifts of the aromatic protons of the two tyrosine residues (Y12 and Y20) which are common to both the hairpin and sheet, and whose chemical shifts diverge as the temperature is lowered. Sigmoidal melting curves are observed for both peptides indicative of a cooperative unfolding process. However, a sharper transition is evident for the three-stranded sheet, together with a higher mid-point transition temperature (298 *versus* 278 K). Thus, the larger number of hydrogen bonding interactions within the sheet gives rise to a greater degree of cooperativity.[2] The data are reminiscent of DNA melting curves where the transition also becomes sharper as the number of base pairs (interacting units) increases.[2]

The above data lead us to conclude that the stability of the C-terminal hairpin is cooperatively enhanced by interaction with the N-terminal portion of the sequence. This may in part be due to a chelate-like effect, in that the first strand can be envisaged to fold against a pre-ordered hairpin, such that the entropic cost of folding is reduced. In addition, this pre-organisation could lead to stronger hydrogen bonds through a restriction of backbone motions allowing shorter range interactions.[13,14] A further possibility is that electrostatic effects, mediated through the extended hydrogen bonding network, might further strengthen the interactions at the second interface through polarisation of amide bonds. Such a mechanism is supported by *ab initio* calculations on solvated hydrogen bonded amide dimers.[15] The effect of organic solvents in promoting intramolecular hydrogen bonding is generally regarded to have an electrostatic origin by weakening competing interactions between solute and solvent.[16–19] The experimental conditions described have proved ideal for monitoring this cooperative effect between the strands of a designed β-sheet; both the hairpin and sheet structures have only marginal stability, allowing even small perturbations to conformational equilibria between folded and unfolded states to be readily detected by measurable changes in NMR and CD parameters.

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Footnote and References

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