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Do interstrand hydrogen bonds contribute to β-hairpin peptide stability in solution? IR analysis of peptide folding in water

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The amide I carbonyl stretch in the IR spectrum, together with ^{1}H NMR Hα chemical shifts, have been used to investigate the folding of a 16-residue β-hairpin peptide in water: while Hα shifts are consistent with a significant population of the folded state (*ca.* 40%), we see no features in the IR spectrum in the amide I region to suggest a significant contribution from interstrand hydrogen bonds, although at high peptide concentration (≥10 mM) the appearance of a new band at 1616 cm^{-1} is consistent with the onset of irreversible peptide aggregation.

The question of how the compact, biologically active state of a protein can assemble reversibly from a relatively disordered polypeptide chain remains a cornerstone to our understanding of the relationship between amino acid sequence and three dimensional structure. One possible mechanism for the folding process proposes that short range interactions within the polypeptide chain are responsible for transient formation of elements of secondary structure (α-helix or β-sheet) which can subsequently act as nucleation sites for further collapse to the native folded state.[1] Protein fragments or designed peptides have proved useful in modelling these nucleation events. While α-helical peptides have been well studied,[2] short peptides that form β-structures, in particular β-hairpins, have been described only relatively recently.[3] There is still some debate as to which are the dominant factors in stabilising these model β-sheet structures, with contrasting opinions on the relative importance of interstrand hydrogen bonding interactions, hydrophobic burial of sides chains and amino acid conformational preferences arising from local steric interactions.[4] Although it is widely regarded that the hydrophobic effect contributes significantly to the folding of globular proteins,[5] thermodynamic evidence from simpler model systems is more difficult to obtain.

In several recent studies, we have investigated in detail the reversible temperature-dependent changes in the ^{1}H NMR Hα chemical shifts that accompany the folding of β-hairpin peptide **1** (Fig. 1) in aqueous solution.[4,6,7] Changes in Hα chemical shift deviations from random coil values indicate that the β-hairpin unfolds at temperatures both above and below *ca.* 298 K. A thermodynamic analysis of temperature-dependent effects on Hα chemical shifts shows that folding is entropy-driven at 298 K with a corresponding negative change in heat capacity.[4,6] Both of these thermodynamic signatures, including the observation of 'cold denaturation', point to the hydrophobic effect providing a key driving force for folding, at least in this model system. However, the contribution interstrand hydrogen bonding interactions make to the stability of the folded state is unclear.

The sensitivity of vibrational spectroscopy to protein structure is well documented, particularly the use of the amide I carbonyl stretch to monitor hydrogen bonding and secondary structure formation.[8–10] Variable temperature IR spectroscopy has been used to probe protein secondary structure in a model α-helix,[11] in β-sheet rich proteins,[12] other β-hairpin systems,[10] in intermolecular β-sheet formation,[13] and even in monitoring peptide aggregation and the onset of amyloid formation.[14,15] For the model α-helix, temperature-dependent changes in the IR spectrum show evidence for unfolding above 298 K with loss of intramolecular hydrogen bonding. Previous studies of β-hairpin peptides[10] have reported a characteristic amide I band at *ca.* 1617 cm^{-1} in aqueous solution at a significantly lower wavenumber than corresponding bands for unstructured random coil peptides.

We have investigated by FTIR spectroscopy the conformation in aqueous solution of the β-hairpin peptide **1**, and a truncated eight residue analogue **2** (Fig. 1). The latter represents the isolated C-terminal β-strand of the hairpin which we use as a reference state. FTIR spectra of peptide **2** at 2 mM concentration show an envelope of strong amide I bands centred around 1640 and 1670 cm^{-1} [Fig. 2(a)], which exhibit little temperature-dependence in the range 278–330 K, consistent with a monomeric, disordered 'random coil' conformation.[10]

Surprisingly, a 2 mM solution of β-hairpin peptide **1** gives a remarkably similar FTIR spectrum to peptide **2** [Fig. 2(b)]. These spectral features again show little temperature-dependence. Resolution enhancement techniques (derivative spectroscopy and Fourier self-deconvolution) reveal a very weak band at 1616 cm^{-1}, representing only a few percent of the total intensity of the amide I envelope. The temperature-dependent changes in the IR spectrum are very small and reversible, which we interpret in terms of temperature variation in band shape and position. To check the extent to which the hairpin is folded in aqueous solution we have repeated ^{1}H NMR experiments on the *exact same* sample used for FTIR analysis. Deviations of Hα chemical shifts from random coil values (ΔδHα values) are shown in Fig. 3 for both peptides **1** and **2**. While ΔδHα values for peptide **2** are very small, again supporting a random coil conformation, reasonably large deviations are apparent for peptide **1**, showing the characteristic pattern for a folded β-hairpin peptide, and ΔδHα values virtually identical to those previously described.[4]

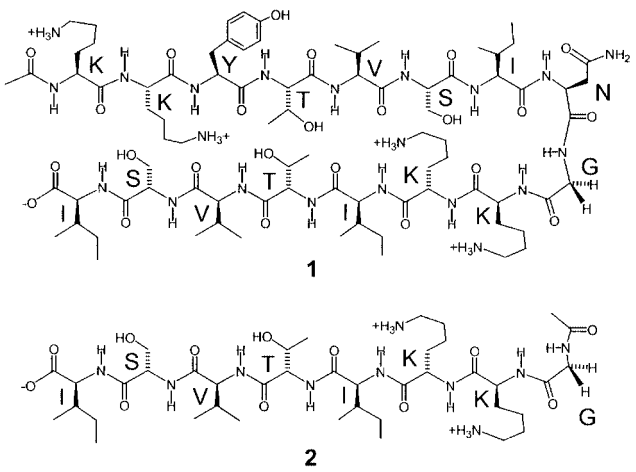

Fig. 1 Schematic representation of the amino acid sequences of peptides **1** and **2**, using the one letter amino acid code. The backbone alignment of the two strands of the β-hairpin peptide are shown to indicate the position of putative interstrand hydrogen bonding interactions.

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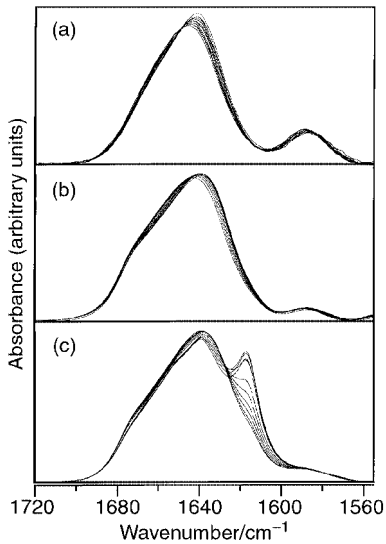

Fig. 2 Variable temperature FTIR spectra of (a) 2 mM aqueous solution of peptide **2**; (b) 2 mM solution of β-hairpin peptide **1**; (c) 10 mM solution of peptide **1**, all in the temperature range 278–330 K. FTIR spectra were collected using a Nicolet Nexus 670 FTIR spectrometer equipped with MCT detector. Circulating water bath controlled temperatures were measured with a J-type thermocouple inside the cell. All peptide solutions were made up in D_2O and the pH (uncorrected for the deuterium isotope effect) adjusted to 5.0 using D_2O solutions of NaOD and DCl. Typically IR spectra were collected as 1000 scans with a resolution of 2 cm^{-1} using a 100 μm cell with calcium fluoride windows. In (a) and (b), the signal intensity at 1640 cm^{-1} increases with increasing temperature, but in (c) decreases with increasing temperature in the range 278–330 K.

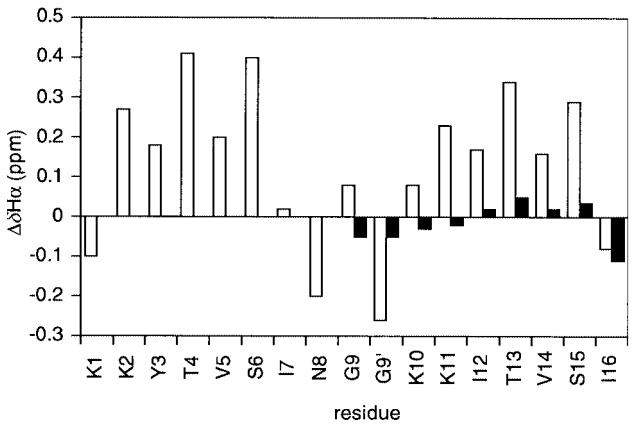

Fig. 3 Deviations of Hα chemical shifts from random coil values[18] (ΔδHα values) for β-hairpin peptide **1** and 8-mer peptide **2** (black bars) at 298 K. NMR data were collected at 500 MHz using reported procedures.[4]

Increasing the concentration of β-hairpin peptide **1** from 2 to 10 mM results in the appearance of a new band in the FTIR spectrum at *ca.* 1616 cm^{-1} [Fig. 2(c)], which is highly and irreversibly temperature-dependent, increasing in intensity initially as the temperature is increased. Integration of this band suggests that at its maximum intensity it corresponds to *ca.* 20% of the total concentration of the peptide. We readily attribute these concentration-dependent changes to irreversible peptide aggregation as reported for a number of model peptides,[10] and partially unfolded proteins that undergo amyloid formation.[14]

Despite previous IR studies of isolated β-hairpin peptides, that identify and assign an amide I band at *ca.* 1617 cm^{-1} to hydrogen bonding across the β-sheet,[10] such features are not a characteristic of the current system. The thermodynamic profile for the folding of peptide **1** has already indicated that hydrophobic contributions from the burial of non-polar side chains play an important part in stabilising the folded conformation.[4,6] The results presented in this study from FTIR analysis in aqueous solution suggest that direct interstrand hydrogen bonding plays, at best, a rather minor role in hairpin stabilisation, and that water molecules appear to compete effectively for hydrogen bonding sites. Significant effects on the amide I band only become apparent in the aggregated state at high concentrations, where arrays of intermolecular hydrogen bonds are co-operatively stabilised. There is, however, still some disagreement regarding the relative importance of the various contributions to the observed changes in the amide I band; hydrogen bonding interactions and other mechanisms involving adjacent oscillators coupled through the covalent framework, have already been discussed.[8,13,16,17]

Although hydrophobic side chains appear to be able to interact sufficiently to populate the folded state of the monomeric peptide, as evident from extensive NOE measurements,[4,6] the hydrophilic peptide backbone appears to remain sufficiently solvated that we can detect no significant changes in the amide I band between the single stranded reference peptide **2** and the folded hairpin **1**. Precise details of the nature of the 'folded state' remain unclear,[6] but explicit consideration of the role of solvent molecules in such models appears to be a necessity, with the possibility of water intercalated between the amide groups of opposing strands. Weakly stabilised β-hairpins ($\Delta G \approx 0$) undoubtedly adopt dynamic conformations in solution. Molecular dynamics simulations show that hydrophobic contacts between strands can be achieved without greatly restricting the orientation of the peptide backbone, while retaining some degree of solvent accessibility.[6] Measurements of backbone $^3J_{NH-H\alpha}$ values for peptides **1** and **2** demonstrate that the peptide backbone is no more ordered in the folded hairpin than in the reference peptide,[4] suggesting that the amino acid sequence predisposes the β-strand residues to adopt an extended conformation, with β-hairpin folding arising from coalescence of the two β-strand arms.

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