# NMR evidence for the nucleation of a β-hairpin peptide conformation in water by an Asn-Gly type I′ β-turn sequence

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**The contribution of the β-turn sequence to the folding and stability of a peptide β-hairpin in water has been analysed from studies of a truncated peptide lacking one β-strand and hence the majority of the interstrand hydrophobic interactions; NMR analysis shows that the Asn-Gly type I′ β-turn conformation is significantly populated, suggesting that the intrinsic conformation preference of the turn sequence may play an important role in nucleating hairpin folding.**

Hydrogen bonding,[1] hydrophobic interactions[2,3] and conformational preferences[4,5] (largely associated with the β-turn sequence) have been variously proposed to play key roles in the folding of peptide and protein fragments into β-hairpin structures. Given the prominent position of anti-parallel β-sheets in the architecture of globular proteins, autonomously folding β-hairpin peptides provide useful model systems for probing β-sheet stabilisation in solution, for assessing their possible role in nucleating protein folding and as novel motifs in molecular recognition.[6–8]

We have recently described a 16-residue model peptide that folds into a β-hairpin in water (50% populated at 303 K) without the need for cross-links or incorporation of non-natural amino acids [Fig. 1(*a*)].[8] Our sequence was designed, and subsequently shown, to adopt a β-hairpin with a type I′ two-residue β-turn forming at the Asn-Gly sequence. This choice of β-turn sequence was based upon statistical analyses of two-residue turns in the Protein Data Bank which are predominantly of the type I′ variety (and to a lesser extent type II′ variety) with the Asn-Gly sequence having a high abundance.[3,9,10] To understand further the origin of the stability of the hairpin in water we have investigated the conformational properties of several shorter peptide fragments to assess the role of the turn sequence in nucleating the folding and in stabilising the β-hairpin conformation. Thermodynamic analysis of the intact β-hairpin has shown that the folded conformation in water is stabilised by cross-strand hydrophobic interactions between the residue side chains of the two β-strands.[8] Such interactions give rise to an entropy-driven folding process which is associated with a large negative change in the heat capacity ($\Delta C_p^o$) between the folded and unfolded states. Both of these features are hallmarks of the hydrophobic effect playing a key role in the self-assembly process.[11] Here we assess whether these hydrophobic contacts are pivotal to the formation of the adopted conformation or whether they act to stabilise an intrinsic conformational preference nucleated by the residues in the turn sequence.

In the peptide shown in Fig. 1(*b*), residues 1–5 of the N-terminal β-stand have been deleted leaving only residues immediately adjacent to the turn sequence on the N-terminal strand and all residues of the C-terminal strand. The majority of interstrand hydrophobic contacts previously identified[8] (involving $Y^3$, $T^4$ and $V^5$) have now been removed such that any tendency for the β-turn to form must now reflect, in large part, the intrinsic conformational preference of the turn sequence and the immediately flanking residues. The residues flanking the Asn-Gly turn sequence on the N-terminal strand (namely $S^6$ and $I^7$) were retained in the sequence of the truncated peptide (**1b**) to provide convenient NMR 'handles' for detecting turn formation. In the hairpin (**1a**), cross-strand Hα–Hα ($S^6$–$K^{11}$) and NH–NH ($I^7$–$K^{10}$) medium range NOEs are detected that are consistent with this conformation.[8]

Analysis of the 200 ms ROESY spectrum of **1b** at 278 K reveals the presence of both of these cross-strand interactions [$S^6$ Hα–$K^{11}$ Hα, Fig. 2(*a*); $I^7$ NH–$K^{10}$ NH, Fig. 2(*b*)], providing convincing evidence that the two-residue turn conformation is populated in water in the absence of the interstrand hydrophobic interactions that appear to stabilise the intact hairpin. While these medium range ROEs provide evidence for secondary structure formation, the intensity of the ROEs between protons

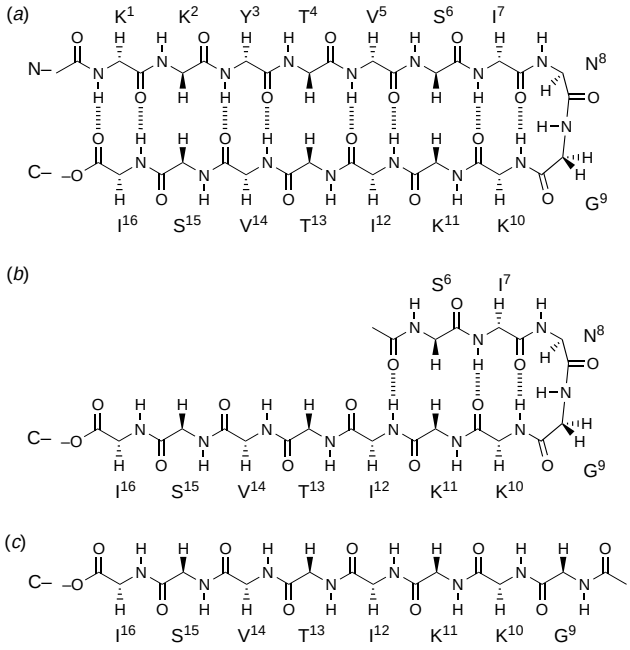


**Fig. 1** Amino acid sequences (one letter code, numbering from the N-terminus) and the proposed folded conformations. Side chains are excluded for clarity.

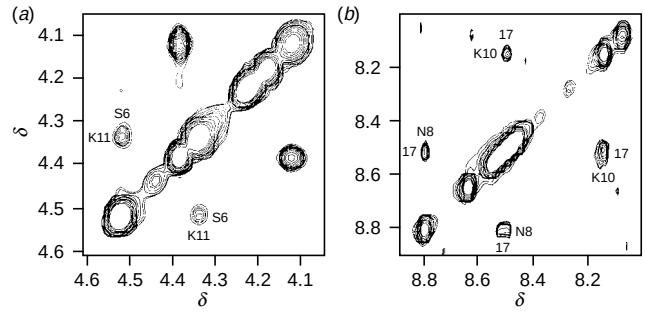


**Fig. 2** Portions of the 200 ms ROESY spectrum of peptide **1b** at 278 K recorded at 500 MHz on a Bruker DRX500 spectrometer: (*a*) Hα–Hα and (*b*) NH–NH cross-strand interactions are highlighted that are consistent with the folded conformation shown in Fig. 1(*b*)

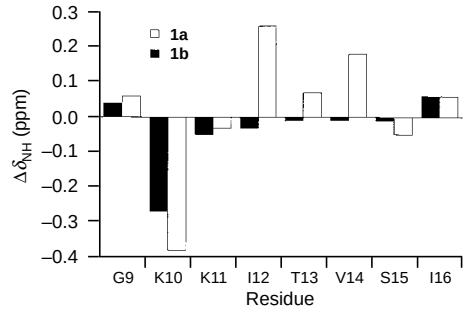


**Fig. 3** Plot of difference in NH chemical shift ($\Delta\delta_{NH}$) for residues 9–16 of the folded hairpin peptide **1a** and the truncated analogue **1b** with respect to reference peptide **1c**

of neighbouring residues provide additional support for local order in the peptide backbone, consistent with the population of a type I′ turn conformation. For example, a significant NH–NH interaction is detected for $I^7$–$N^8$, while all other sequential NH–NH ROEs are too weak to observe [Fig. 2(*b*)]. In general, sequential Hα–NH ROEs are stronger than the equivalent intraresidue Hα–NH interaction (ratio of intensities > 4),[12] but for $N^8$ and $G^9$ the ratio of intensities is very close to 1, again characteristic of the reverse turn conformation found for the hairpin (**1a**).[8]

To what extent is the turn formed under these conditions? Perturbations to the Hα and NH chemical shifts are characteristic of secondary structure formation and can provide a method of estimating the population of folded structures.[8] To this end we have examined perturbations to the chemical shifts of residues on the C-terminal strand of the hairpin and peptide **1b** to estimate relative populations. As a reference state we have used the corresponding Hα and NH chemical shifts of the isolated C-terminal strand (**1c**) to fully account for any sequence-dependent effects on chemical shifts. Thus, differences in chemical shifts between **1a** and **1c**, and between **1b** and **1c**, are expected, in large part, to reflect perturbations due to secondary structure formation. The data in Fig. 3 show the difference in NH chemical shifts ($\Delta\delta_{NH}$) at 278 K for residues 9–16 of the hairpin (**1a**) and truncated peptide (**1b**) with respect to the reference state **1c**, and illustrate convincingly the folding pattern of the two peptides. The NH of $K^{10}$ shifts upfield for both peptides reflecting the anisotropic effect from the carbonyl group of $G^9$ in the folded structure. $T^{12}$, $V^{14}$ and $I^{16}$ of the hairpin are proposed to form hydrogen bonds to the opposite strand and significant downfield shifts are observed for the NHs of these residues that are consistent with this model. In contrast, only the NH of $I^{12}$ of the truncated peptide (**1b**) has the opportunity to form a hydrogen bond, but no significant perturbation is observed suggesting a very weak interaction that probably reflects the more dynamic nature of its hydrogen bonding partner, the *N*-acetyl carbonyl of $S^6$ at the N-terminus.

Since both peptides are able to form the same β-turn conformation, as is evident from the ROE data described above, we interpret the larger change in shift for $K^{10}$ NH of the hairpin (**1a**) as reflecting a greater population of the folded conformation than is present for the truncated peptide (**1b**). We estimate the population of turn conformation of **1b** to be *ca*. 70% of that of the hairpin under the same conditions (278 K). We have previously estimated the folded population of the hairpin at this temperature to be *ca*. 32% *versus* 68% 'random coil';[8] on this basis the population of the folded conformation of **1b** is *ca*. 22%. Quantitative estimates of the intensity of the cross-strand ROEs shown in Fig. 2 similarly suggest a folded population in the range 20–30%, indicating some agreement between the different methods of estimation.

The data show convincingly that the sequence INGK at the core of the β-hairpin peptide used in these studies has a natural propensity to form a two-residue β-turn conformation in water *alone*, in the absence of significant structure stabilising hydrophobic interactions or a conformationally restricted proline residue. The INGK turn sequence is likely to play an important role in nucleating hairpin formation through association of the two β-strands.

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## Notes and References

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