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Stereospecific peptide folds. A rationally designed molecular bracelet†

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Received (in Cambridge, UK) 13th July 2004, Accepted 9th August 2004
First published as an Advance Article on the web 20th September 2004

A canonical planar β-hairpin peptide, stereochemically reengineered into a semicircular bracelet type motif by L-to-D stereochemical inversion in two pairs of its cross-strand neighbor residues, displays protein like ordering including two-state behavior in H_2O, which is unusual for a small peptide of this size.

Stereochemistry is a powerful tool to control the nature of molecular conformational folds. The α-helix and β-sheet[1] are peptide molecular motifs of poly-L stereochemical nature. The lifting of stereochemical degeneracy in the biological building block alphabet could furnish peptide molecular motifs of morphologically diverse but stereochemically definable nature. Stereochemically controlled design of supramolecular systems[2] may thus be accomplished by the biochemical route of sequentially programmed hetero-polymer folding. Illustrating the design concept a bracelet-type peptide molecular fold is described here as an example of stereospecific supramolecular design.

The β-hairpin features a pair of mutually hydrogen-bonded β-strands joined antiparallel across a central β-turn.[3] The typical extended pleated sheet like morphology of the canonical β-hairpin, with the side-chains alternating along its opposite faces, is a consequence of poly-L configurational nature of this stereospecific poly peptide motif. Stereochemical inversion of its cross-strand neighbor residues, interchanging the main-chain and side-chain directions, will transform the molecular morphology of the β-hairpin in a stereochemically definable manner. Implementing such a two point side-by-side inversion in a small canonical planar β-hairpin, a crescent shaped molecular motif is approached as a bracelet type molecular fold shown in Fig. 1. The molecule [Ac-Tyr(1)-Lys(2)-D-Val(3)-Phe(4)-D-Asn(5)-Glu(6)-D-Pro(7)-Gly-(8)-Lys(9)-D-Ala(10)-Ile(11)-D-Val(12)-Glu(13)-Ala(14)–NH_2] is designed as a standard β-hairpin invoking a well-documented stereochemical recipe, using D-Pro(7)-Gly(8) segment in type II′ β-turn conformation as a β-hairpin nucleator.[4] The cross-strand residues Val(3)-Val(12) and Asn(5)-Ala(10) are inverted stereochemically to create a crescent shaped molecular morphology for the targeted bracelet type β-hairpin. Some of the cross-strand residues are so chosen as to promote interstrand association based on ion pair [Lys(2)-Glu(13) and Glu(6)-Lys(9)] and hydrophobic [Val(3)-Val(12) and Phe(4)-Ile(11)] interaction.

The peptide made by solid phase synthesis was characterized by MALDI-MS and NMR. NMR analysis[5] in H_2O and DMSO established an inter-strand NOE signature, illustrated in Fig. 2 for H_2O (see ESI,† Fig. S4 for DMSO), expected of a canonical hairpin. In addition, a flagpole type NOE pattern was also observed due to the bracelet type curvature of the hairpin in both H_2O and DMSO. The inter-strand NH-C^{α}H NOEs between Ile(11)-Val(3), Ile(11)-Phe(4) and $CONH_2$–Tyr(1) in DMSO and between Phe(4)-Ala(10), Ile(11)-Val(3), Ile(11)-Asn(5), and Glu(13)-Tyr(1) in H_2O, established a well ordered nature of the hairpin through its length in both solvents. The inter and intra-strand NH-C^{α}H NOEs between Gly(8)-Val(12), Val(12)-Gly(8), Phe(4)-Gly(8), Glu(6)-Val(12), Lys(2)-Pro(7), Lys(2)-Gly(8), and Ala(14)-Pro(7) in H_2O and between Gly(8)-Val(3), Gly(8)-Asn(5), Glu(6)-Val(3), Lys(9)-Val(12), and Lys(9)-Glu(13) in DMSO, indicated the residues to be in close spatial proximity as expected in the crescent shaped hairpin. All observable $^3J_{NH}$ values were greater than 7 Hz suggesting a general bias towards β-sheet type ϕ torsional angles[6] in both H_2O and DMSO. With the $^3J_{NH}$ values $\geqslant$ 8.89 Hz, the bias was appreciable in Ile(11), Glu(13) in H_2O and in Tyr(1), D-Val(3), Phe(4), Lys(9), D-Val(12) in DMSO. The peptide was submitted to NMR based DYANA[7] modeling in both H_2O and DMSO. Torsion angle dynamics was performed using 25 selected distance restraints calibrated according to relative NOE intensities in each solvent. In addition, considering the $^3J_{NH}$ values, the ϕ dihedrals in Ile(11), Glu(13) in H_2O and in Tyr(1), D-Val(3), Phe(4), Lys(9), D-Val(12) in DMSO were restrained between $-120^\circ \leqslant \phi \leqslant -145^\circ$ or $145^\circ \leqslant \phi \leqslant 120^\circ$ depending upon the residue chirality.[6] The mean global backbone root mean square deviation (RMSD) calculated by MOLMOL[8] over ten lowest energy DYANA structures, shown in Fig. S5 (ESI) was 0.47 ± 0.21 Å in H_2O, and 0.80 ± 0.34 Å in DMSO. The backbone RMSD between the mean DYANA structures in H_2O and DMSO (Fig. 1a and 1b) was 1.74 Å, indicating a similar type of conformational fold in both solvents.

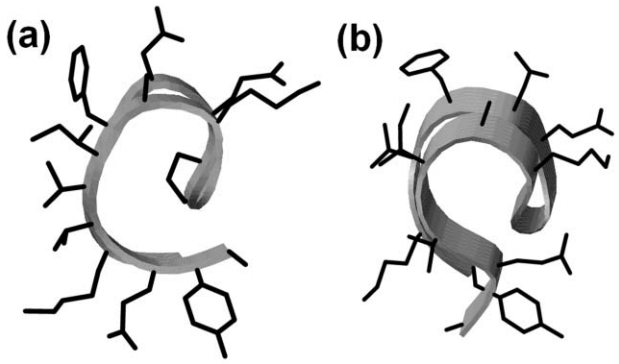

Fig. 1 Canonical β-hairpin stereochemically reprogrammed into a bracelet-shaped molecular motif. Ribbon representation of the minimum energy DYANA structure in (a) H_2O, (b) DMSO.

Uncertainty of NOE buildup and possibility of conformational averaging militate against judgment of absolute degree of conformational ordering in peptides based on NMR. The NMR chemical shift index[9] (CSI) method helps but is unsuitable for the present purpose due to the atypical nature of both the stereochemical and the conformational structure of our peptide. Indeed the C^{α}H shifts in H_2O did not resemble the canonical β-sheet type CSI patterns reported in the literature.[10] The peptide displayed positive CD bands of modest intensity at 200 nm and 218 nm in H_2O (Fig. 3), which does not resemble the standard β-sheet CD signature, characterized by a minimum in the range 210–225 nm.[11] The qualitative nature of the band and its intensity remained more or less unchanged in trifluoroethanol and methanol, the solvents known for their conformation inducing effects in many small peptides.[12,13] The positive CD band at 218 nm (Fig. 3) in H_2O displayed an apparent sigmoidal dependence on temperature in the range 280–360 K. With the mid point of the thermal CD transition (T_M) at 318 K (Fig. 3, inset) the peptide

† Electronic supplementary information (ESI) available: All experimental procedures, HPLC, MALDI-MS, CD, NMR and MD analysis in H_2O and DMSO. See http://www.rsc.org/suppdata/cc/b4/b410532j/

DOI: 10.1039/b410532j

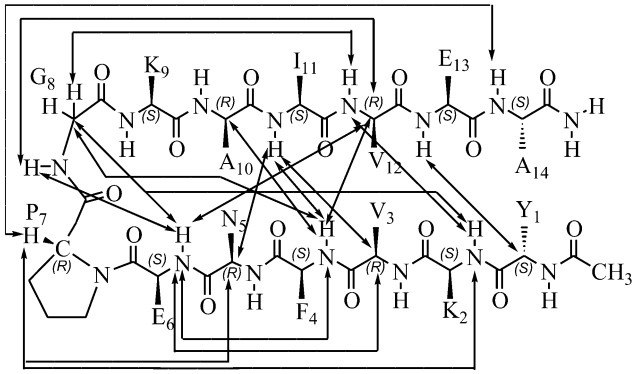

Fig. 2 Summary of long-range backbone NOEs observed in H_2O.

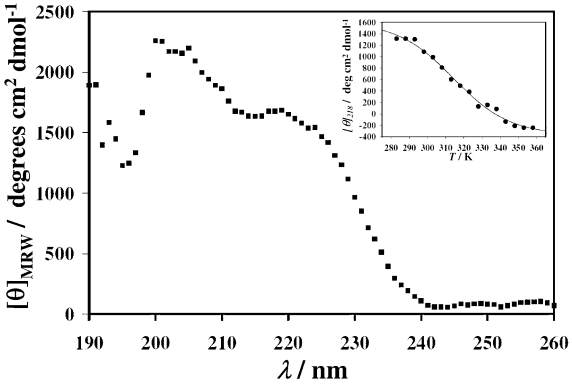

Fig. 3 Far UV CD spectrum of the peptide in H_2O at 298 K. (Inset) CD-monitored thermal melt of the molecular bracelet at 218 nm ($[\theta]_{218}$ = mean residue ellipticity *versus* temperature/K).

seems to be well ordered in H_2O at ambient temperature (298 K). A two state type behavior is implied which is unusual for a small peptide of this size.

Molecular Dynamics (MD) in presence of explicit or implicit solvent has emerged as a reliable diagnostic of peptide conformational equilibria reflecting molecular interactions involved in stabilization of specific folds.[14] The average DYANA structures of the peptide were submitted to MD at 300 K in a solvent box with gromos-96 force field in GROMACS software.[15] Simulations over 5 ns period revealed a well preserved network of hydrogen bonds in both DMSO and H_2O and somewhat better ordered nature of the β-turn[16] in H_2O than in DMSO (see ESI). Twenty periodically sampled conformers from the MD trajectory displayed mean global backbone RMSD of 1.77 ± 0.82 Å in H_2O and 2.20 ± 0.93 Å in DMSO. Thus both NMR and MD evidence reflect better-ordered nature of the peptide in H_2O than in DMSO. The observation of fewer inter-strand NH-NH NOEs in H_2O than in DMSO (Fig. 2), on the other hand, implies that either there was an inadequate NOE buildup in H_2O or the inter-strand hydrogen bonds are only partially populated in this more polar solvent. The apparently stronger ordering of the peptide in H_2O could thus have a different origin.

Though there was no strong evidence of a salt bridge formation from NMR (except one NOE between $C^{\gamma}H$–$C^{\beta}H$ and one NOE between $C^{\delta}H$–$C^{\gamma}H$ of Lys(9)-Glu(6) in H_2O and DMSO, respectively), 10 best energy minimized structures from DYANA modeling indicated the existence of hydrogen bonds between the ionized side chains in H_2O, but between only Lys(2)-Glu(13) side-chains in DMSO. MD data confirmed that an effective salt bridging [4 Å $\leqslant N_{\varepsilon}$–$C_{\delta} \leqslant$ 6 Å][13] was operative between Lys(9)-Glu(6) and Lys(2)-Glu(13) in H_2O but only between Lys(9)-Glu(6) in DMSO (see ESI). Time dependent proximity plots of laterally related side-chain C_{β}-C_{β} atoms of Phe(4)-Ile(11) and Val(3)-Val(12) in both solvents indicate that the side chains may be in solvophobic contact[17] ($\leqslant$6 Å) in H_2O but not in DMSO (see ESI). Several lateral and diagonal NOEs supportive of the hydrophobic association appeared in H_2O [Val(3)$C^{\alpha}H$–Ile(11)$C^{\beta}H$, Val(3)-$C^{\alpha}H$–Ile(11)$C^{\gamma}H$, Val(3)$C^{\alpha}H$–Ile(11)$C^{\delta}H$, Val(3)$C^{\gamma}H$–Val(12)-$C^{\gamma}H$, Phe(4)$C^{\zeta}H$–Ala(10)$C^{\beta}H$ and Tyr(1)$C^{\delta}H$–Ala(14)$C^{\beta}H$] but there were few such NOEs in DMSO [Phe(4)$C^{\delta}H$–Ile(11)$C^{\delta}H$ and a weak interaction between Asn(5)$C^{\beta}H$–Ile(11)$C^{\delta}H$] beside the adjacent side-chain NOEs observed in both solvents. Thus synergistic effects of hydrophobic association[18] and ion pair interaction,[13,19] reported in β-hairpin peptides in H_2O based on both NMR and MD simulations, could be responsible for the better ordered nature of the bracelet-shaped peptide fold in H_2O than in DMSO. Even considering these interactions of somewhat canonical nature observed in many β-hairpin models reported in literature, the extent of ordering reflected in our peptide in H_2O is exceptional for its length. Thus there could be other non-canonical features like electrostatic interactions underlying its stability that need to be investigated.

This work was supported by a research grant from the Council of Scientific and Industrial Research, New Delhi, India. The use of NMR at National High Field NMR Facility, TIFR, Mumbai is highly acknowledged.

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