SYNOPSIS

Aromatic Interactions in Peptides : Designed Helices and β-Hairpins

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The folding of a linear polypeptide chain into precise secondary and supersecondary structures, followed by their subsequent assembly into unique three-dimensional protein folds, is a central problem of structural biology. The design and construction of complex folds using first principles relies on a complete understanding of the factors that facilitate formation of protein secondary structural elements, namely, helices, strands and turns (Venkatraman et al, 2001). The employment of stereochemically constrained non-protein amino acids as nucleating agents of predefined structure scaffolds has now opened new avenues for the study of peptide folding and stability. ^{D}Pro-Xxx sequences have been successfully utilized for the nucleation of turn segments and Aib for helix stabilization (Venkatraman et al, 2001).

The availability of pre-formed secondary structure modules permits examination of forces that drive and stabilize the folded structure of proteins. The key players that stabilize the protein interior are networks of hydrogen bonds and hydrophobic interactions (Burley and Petsko, 1985). Examination of hydrophobic interactions has gained impetus only recently, with reports on the utility of aromatic pairs as structure stabilizing agents (Searle, 2004; Waters, 2004). This thesis reports studies conducted on designed secondary structure scaffolds in which preferred modes of interactions between aromatic amino acids, systematically positioned on secondary structure scaffolds, have been examined. The influence of aromatic amino acids on peptide folding and stability has been investigated extensively using solution NMR methods. **Chapter 1** introduces the reader to the general concept of peptide design, followed by a summary of the current knowledge of aromatic interactions in proteins and peptides. **Chapters 2-7** describe results obtained from the various studies carried out on peptide hairpins and helices. The presented results emphasize the role of

aromatic interactions in hairpin stabilization, the utility of Trp residues in obtaining well-folded, crystallizable helices and the influence of Phe-Pro and Pro-Trp interactions on secondary structure.

Chapter 1 describes approaches towards secondary structure design by employing specific nucleating elements such as ^{D}Pro-Xxx segments for hairpins and Aib for helices. This is followed by a perspective of aromatic interactions in proteins (mutational studies and database analyses) and peptides, which also serves to define the framework for the current studies.

Chapter 2 discusses the role of aromatic (Trp/Tyr) interactions in stabilizing ^{D}Pro-Gly nucleated peptide hairpin structures. The sequences of peptides were designed to examine cross-strand aromatic interactions at the non-hydrogen bonding position of strand segments (**Ac-L-Y-V-^{D}P-G-L-Y/W-V-OMe; 2.1/2.2**), diagonal interactions with aromatic residues placed at the hydrogen bonding position (**Boc-Y/W-L-V-^{D}P-G-W-L-V-OMe; 2.3/2.6**) and lateral interactions with aromatics at alternating positions in strands (**Boc-L-L-V-^{D}P-G-Y-L-W-OMe; 2.4**). **Boc-L-L-V-^{D}P-G-Y-W-V-OMe** (**2.5**) and **Boc-L-Y-V-^{D}P-G-L-L-V-OMe** (**2.7**) served as controls for aromatic interactions. Presence of well-folded hairpin conformations in all the peptides was confirmed by the observation of characteristic hairpin NOEs. Strong edge-to-face interactions were obtained in the case of peptides **2.1** and **2.2**, as was evident from the NOEs between aromatic rings, temperature dependence of ring proton chemical shifts and the anomalous upfield shifted resonances, due to shielding effects of aromatic rings on spatially proximal protons. The aromatic rings were also seen to show a preferential orientation towards the amide plane of the succeeding residue with $\chi 1$ values adopting a *trans* conformation, forming favorable aromatic-amide interactions.

Chapter 3 discusses the incorporation of tryptophan residues in helical scaffolds. This work was based on the observation that crystals of Boc-Leu-Trp-Val-OMe formed a pseudo-helical arrangement with the tripeptide packing into a supramolecular helix with strong aromatic interactions (Sengupta et al, 2005). Longer helices with bridging segments between the L-W-V tripeptide units were examined by

solution NMR and in the solid using X-ray crystallography. Well-folded helical structures were obtained in the case of peptides with two-residue (**Boc-L-W-V-A-U-L-W-V-OMe; 3.1**) and three-residue (**Boc-L-W-$^{L/D}$V-U-A-U-L-W-V-OMe; 3.2/3.3**) bridging units. The intermolecular hydrogen bonding pattern, involving the indole NH, observed for the tripeptide in crystals was found to be conserved in the octapeptide (**3.1**), both in crystals and in solution. The results suggest that L-W-V segments can indeed be accommodated in helices formed using α-amino acids. Higher homologs of α-amino acids, such as γAbu (**Boc-L-W-V-γAbu-L-W-V-OMe; 3.4**) and δAva (**Boc-L-W-V-δAva-L-W-V-OMe; 3.5**), however, did not form well-folded helices in solution. These sequences were largely unstructured, with very few long-range NOEs.

Chapter 4 outlines the influence of a large number of Trp residues on secondary structure. The Trp-Leu-Trp sequence was used in strand regions of a hairpin nucleated by the ^{D}Pro-Gly segment, such that interstrand Trp-Trp interactions stabilize the peptide in a manner analogous to the Trpzip sequences (Cochran et al, 2001). Solution NMR studies revealed that the peptide (**Boc-W-L-W-^{D}P-G-W-L-W-OMe; 4.1**) formed a frayed hairpin with a type I' turn. NOEs between the indole ring of W6 and the pyrrolidine ring of ^{D}P4 were suggestive of a local indole-pyrrolidine interaction as the turn destabilizing agent. Conversely, Trp-rich peptides examined with one (**Boc-W-L-W-U-W-L-W-OMe; 4.3**) and two (**Boc-U-W-L-W-U-W-L-W-OMe; 4.4**) helix nucleating Aib residues adopted well-folded helical conformations in chloroform. Helix-to-hairpin equilibria can be probed using model sequences with a centrally positioned Aib-Gly turn nucleating segment (Awasthi et al, 2001). An Aib-Gly nucleated Trp-rich peptide (**Boc-W-L-W-U-G-W-L-W-OMe; 4.2**), designed to form a hairpin, showed a helical conformation even in methanol. Solution NMR and crystal structure analysis of a tetrapeptide analog of **4.2** (**Boc-W-U-G-W-OMe; 4.5**) also revealed the presence of a helical turn. The results suggest that alternating Trp residues are preferentially accommodated in helices and destabilize peptide hairpins by possible aromatic-amide interactions.

Chapter 5 addresses the issue of accommodating bulky Trp residues in turn sequences. Analysis of peptides (**Boc-L-V-$^{D/L}$P-$^{L/D}$W-L-V-OMe; 5.1/5.2**) (**Boc-L-V-$^{D/L}$P-**

$^{D/L}$W-L-V-OMe; 5.3/5.4) for their ability to accommodate Trp residues at the i+2 position of Pro-Trp turns, using solution NMR methods, revealed that tryptophan was not favored in the turn region. Structural characterization of an HIV-inhibitory peptide analog with a ^{D}Pro-^{L}Trp turn (**H_2N-R-I-N-N-I-^{D}P-W-S-E-A-L-L-$CONH_2$; 5.5**) indicated that the Pro-Trp segment stabilized a local helical structure. This observation was further supported by structural studies of short 7-residue staphylococcal RNA III inhibitory peptides (Balaban et al, 2000) (**H_2N-Y-S-P-W-T-N-F-$CONH_2$; 5.6**) (**H_2N-Y-K-P-I-T-N-F-$CONH_2$; 5.7**) (**H_2N-Y-K-P-I-T-N-W-$CONH_2$; 5.8**). NMR data revealed the presence of a helix-like structure in solution with evidence for a single helical turn, despite their small size.

Chapter 6 details the solution structures of the *Conus monile* peptide Mo1659 (**H_2N-F-H-G-G-S-W-Y-R-F-P-W-G-Y-$CONH_2$; 6.1**) (Sudarslal et al, 2000) and its analogs (**H_2N-F-H-G-G-S-W-Y-R-F-P-W-G-Y-COOH; 6.2**) (**H_2N-F-H-G-G-S-W-Y-R-F-L-W-G-Y-$CONH_2$; 6.3**), determined by solution NMR methods. The sequence of **6.1** was interesting, not only due to its high aromatic content, but also the presence of a Pro-Trp segment. Solution NMR studies in methanol revealed that *cis-trans* isomerization of the Xxx-Pro bond gave rise to two equally populated conformers in solution for **6.1** and **6.2**, with a Pro→Leu mutation (**6.3**) abolishing conformational interconversion. Although sequential d_{NN} NOEs were present, the absence of long-range NOEs indicated that the peptides were largely unstructured, with the residues adopting a local helical conformation. The populations of the isomers were drastically altered (9:1 *trans*:*cis*) in water, with the *trans* conformer showing several long-range NOEs between the side chains of aromatic residues to the backbone. A strong aromatic-amide interaction between Phe9 and the Phe-Pro amide bond stabilizes the *cis* peptide unit in the minor conformer, as revealed by NOEs in the ROESY spectra and a Protein Data Bank (PDB) analysis.

Chapter 7 explores the utility of diproline templates for hairpin nucleation in water soluble peptides. Additionally, the ability of a Cys-His *vs* Tyr-His interaction in stabilizing a hairpin scaffold in polar solvents, has also been investigated. Both peptides (**H_2N-R-F-Y/C(*t*Bu)-C(*t*Bu)/Y-V-^{D}P-^{L}P-L-H-T-F-K-$CONH_2$; 7.1/7.2**) form well-

folded hairpin conformations in methanol, as inferred from NOEs obtained in ROESY experiments. A comparison of the NOE data for the two peptides revealed that the peptide with a Cys-His interaction (**7.1**) was better structured compared to the peptide with a Tyr-His interaction (**7.2**). This result is also supported by analyses of chemical shifts and their temperature dependence. CD spectra recorded for **7.1** showed transitions at two wavelengths being affected on addition of water to a methanol sample. The results indicate that interactions between the terminal Phe residues in **7.1** are strong at low percentages of water; increasing amounts of water disrupts secondary structure by invading backbone hydrogen bonds. In the case of **7.2**, a linear reduction in the 217nm band was seen, due to water invading the interstrand hydrogen bonds. These observations suggest that a Cys-His interaction is more stabilizing than a Tyr-His interaction.

Chapter 8 summarizes the important findings of the previous chapters and discusses future prospects of the present research.

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