ABSTRACT

Title of the thesis: Stability of cis peptide bonds in short peptides and proteins

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The peptide bond normally assumes a trans configuration. However, cis peptide bonds become important in protein structures when proline appears in the sequence. The neighboring residues also control the stability of such cis peptidyl-prolyl bonds.

In this work, short peptides with general sequence motif Ac-Pro-Pro-Xaa-NH₂ were synthesized and it was seen that when aromatic residues (Xaa = Phe, Tyr, Trp, His (pH >8)) occupied the position Xaa, the cis conformation of the diproline motif is stabilized considerably. The stability was provided via enthalpy driven CH⁻⁻π interaction, between the aromatic side chain and the N-terminal proline H(α)-atom. This establishes Pro-Pro-Aro as a new sequence motif capable of stabilizing cis prolyl-prolyl bonds. The evolution of this Pro-cisPro-Aro motif among homologous proteins showed that the aromatic residue and the central cis-proline residue are co-conserved strongly.

Using host-guest approach, the effect of different amino acid substitutions (at position Xaa in the motif Xaa-Pro-Tyr) on the cis/trans isomerization of the Xaa-Pro peptidyl-prolyl bond was also investigated. Two peptide series was synthesized, XPA (control) and XPY, were used for this study. It was seen that for Ala, Gly and Pro, Xaa-Pro showed the maximum population increment of the cis-isomer (compared to XPA) while Ile, Thr, Ser and Asp showed the least enhancement. ¹H NMR analysis showed that the χ¹ occupancy of g(-) rotamer of the Tyr (Y) residue bear a 1:1
correlation with the residence time that the cis conformer spends in CH—π interaction state.

We also investigated the effect of perturbing a conserved cisPro residue present in the 71Gly-Pro-Tyr motif in glutamyl-tRNA synthetase (GluRS) from E. coli. Both the wild type and the P72A mutant protein were cloned, expresses and purified. The P72A mutation altered the stability and the unfolding profile of GluRS. It was also found that while the wt-GluRS refolding was associated with a slow process, possibly due to peptidyl-prolyl cis/trans isomerization; the slow step almost disappeared in the P72A mutant. Although the experimental study is too preliminary, it lays the foundation to a more elaborative experimental work on the 71Gly-Pro-Tyr motif in GluRS, where not only Pro but also the Tyr can be mutated to study the effect of mutating the aromatic residue in a cisPro-Aro motif in a protein.