Oxidative Folding Catalyst of Cysteine rich Conotoxins and Conformation analysis of Cysteine Disulfides of ICK motif

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SYNOPSIS

The two novel redox conopeptides were derived from venom duct transcriptome of Indian marine cone snails. The redox conopeptides Fr874 from *C. frigidus* and Am1038 derived from *C. amadis* have high density of tryptophan residues. The putative mature peptides Fr874, Fr890[P1O], Fr890[P2O], Fr906, Am1038 and Am1054 incorporating the possibility of post-translational modification of proline to 4-trans hydroxyl proline have been chemically synthesized and characterized by mass spectrometry. The estimated reduction potential of synthetic peptides varies from -298 mV to -328 mV that are similar to redox family of proteins. Evaluation of catalytical activity on oxidative folding of α-conotoxin ImI indicates that they have increased the yield of natively folded globular form of α-conotoxin ImI. The maximum catalytical activity has been observed for the peptides containing Hyp residues Fr906 and Am1054. The positional specific effects of Hyp residue have been observed in the catalytical activity of *C. frigidus* redox conopeptides. The optimization of 3D structures of redox conopeptides has revealed that *C. frigidus* peptides adopt N-terminal helical fold and *C. amadis* peptides adopt distinct structures based on the configuration of Phe4-Pro/Hyp5 peptide bond. The current report confirms the diversity of redox conopeptides and their tendency to assist the oxidative folding of conotoxins.

The inhibitory cystine knot (ICK) motif is an evolutionarily optimized disulfide-rich peptide motif widely present in diverse phyla with distinct biological functions. Cysteine disulfides are highly conserved in the ICK motif with C_1-C_4 (Disulfide-I), C_2-C_5(Disulfide-II), and C_3-C_6(Disulfide-III) connectivity's in a sequence. Disulfide-I and disulfide-II form a loop and the disulfide-III tethers through the loop forming a knotted fold. The current report has analyzed the conformation of disulfides in the ICK motif using the side-chain torsional angles of cysteine disulfide. In crystal structures: 88% of Disulfide-I have (+,-)SynRHHook, 92% of Disulfide-II have (+,-)RHSpiral, and 100% of Disulfide-III have (-,-)LHSpiral conformations. In NMR structures, conformational diversity has been observed for each of the cysteine disulfides of the ICK motif. The highest percentage occurrence in NMR structures: 27% of Disulfide-I have (+,-)SynRHHook, 36% of Disulfide-II have (+,-)RHSpiral, and 50% of Disulfide-III have(-,-)LHSpiral conformations. In the view of the method of identification of disulfides between cysteine residues using NMR spectroscopy, the NMR structure representsan ensemble of conformations of disulfides instead of specific disulfide conformation. The retention of the conformation in both X-ray and NMR structures supports the conservation of conformation of disulfides in the ICK motif. The tendency to exhibit specific conformation of disulfide even with variations in 3D structures supports the evolutionarily optimized nature of the ICK motif.