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NMR STRUCTRUE DETERMINATION OF A NOVEL CONOTOXIN, [Pro 7,13] αA-conotoxin P_{IVA}

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High-resolution solution conformation of a novel conotoxin, [Pro 7,13] αA-conotoxin P_{IVA}, GCCGSYPNAACHPCSCKDROSYCGQ-NH_2, has been determined by two-dimensional ^1H nmr methods in combination with distance geometry calculation to rmsd values of 0.90 Å and 1.16 Å for the backbone and heavy atoms, respectively. Total of 324 NOE-derived interproton distance restraints including 33 long-range NOE restraints as well as 11 ϕ and 7 χ^1 torsion angle restraints were used for computation of structures. Back calculation of experimental NOE spectrum has provided 49 new NOE restraints and yielded the final R-factors of R_a=0.641 and R_b=0.157. The overall shape of the [Pro 7,13] αA-conotoxin P_{IVA} resembles an "iron". The residues 15S-19R form a loop that protrudes out of the " bottom plate" formed by the rest of the protein and constitute the "handle" portion of the iron. The N-terminus of the molecule is not mobile due to the attractive electrostatic interactions between the γ-hydroxyl group of 20 Hyp and between the phenolic hydroxyl group of 22Y. The flexible 3-11 disulfide loop consists mostly of hydrophobic residues, while the best-defined 14-23 disulfide loop contains the highly charged hydrophilic 15S-19R "handle" domain exposed to the exterior of the protein. Binding to nicotinic acetylcholine receptor would be mediated through two different types of interactions: one involving the aromatic hydrophobic residues 6Y and 12H and the other involving the positively charged hydrophilic sidechains of the 15S-19R loop.