02.1-01 STRUCTURE AND FUNCTION OF PRESYNAPTIC NEUROTOXINS: NOTEXIN AND NOTECHIS II-5. By K.K. Kannan*, Hilda Cid**, M. Ramanadham*, S. Sinh* and S. Ramakumar+, *Neutron Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India, **Wallenberg Laboratory, Uppsala University, Uppsala, Sweden, +Indian Institute of Science, Bangalore, India.

Venom from snake, spider and other insects usually contains neurotoxins which affect the nerve transmission around the neuromuscular region of different organisms. They are normally classified as postsynaptic toxins which block cholinergic receptor site and presynaptic toxins which obstruct the release of acetyl choline across the neuromuscular junction.

We have crystallized two presynaptic toxins purified from Australian tiger snake (notechis scutatus scutatus) venom, kindly supplied to us by Dr. D. Eaker, Uppsala University, Sweden. They are notexin and Notechis II-5. These toxins are highly homologous among themselves and also exhibit a high degree of homology to porcine, bovine and other insect and snake venom phospholipases. Notexin and Notechis II-5 also show moderate phospholipase activity. They have a molecular weight of about 13,500 and consist of about 120 amino-acid residues.

Notexin has been crystallized from 1.5M ammonium sulphate, Tris HCl at pH 8.5. The crystals belong to the trigonal space group $P3_121$ or $P3_221$ (Toxicon (1977) 15, P435). The unit cell dimensions are; a = b = 75.03, c = 49.04A and $\gamma = 120°$.

Notechis II-5 has been crystallized from buffer solution containing Tris HCl, at pH 8.5 and ammonium acetate (J. Biosciences (1981), in press). The crystals are orthorhombic $P2_12_12_1$ with unit cell dimensions; a = 146.1 b = 43.5, c = 39.0A.

One heavy-atom derivative has been prepared by iodinating Notexin crystals and another one by soaking Notexin crystals with $Sr(NO_3)_2$. The structure investigation by the molecular replacement method using the porcine phospholipase coordinates kindly supplied by Dr. Drenth and coworkers, Groningen, Holland and the isomorphous replacement method, is in progress with data collected to 2A resolution. The results will be presented.

02.1-02 THE CRYSTAL AND MOLECULAR STRUCTURE OF NEUROTOXINS FROM SCORPION VENOM. S. E. Ealick, R. J. Almassy, J. C. Fontecilla, F. L. Suddath and C. E. Bugg, Department of Biochemistry and Comprehensive Cancer Center, University of Alabama in Birmingham, Birmingham, Alabama, 35294.

The general effects of scorpion venoms or purified toxins injected in mammals are those expected from massive release of neurotransmitter induced by depolarization of the nerve endings. Several of the toxins have been shown to prolong the action potential of excitable membranes by blocking inactivation of the sodium channels. Therefore, scorpion toxins are of considerable interest as probes for studying the properties of sodium channels.

The structure of a neurotoxin from the venom of **Centruroides sculpturatus** Ewing, a scorpion native to the Arizona desert, has been determined at 3 A resolution.[1] Subsequently, the model has been extended to 1.8 A resolution and finally to the current resolution of 1.3 A. The small basic protein contains 65 amino acid residues and 4 disulfide bridges. The space group is $P2_12_12_1$ with a = 52.07 A, b = 41.94 A and c = 28.32 A. At 1.8 A resolution the model includes 75 water molecules and one molecule of 2-methyl-2,4-pentanediol (MPD). At 1.3 A resolution additional solvent has been added and two regions are being examined for possible disorder. The first region is the C-terminal disulfide bridge (12-65) in which a second site is described as a 1.0 A displacement of the disulfide bridge in the direction of the S-S bond. The second region involves two residues LYS 32 and ASN 33 which are part of an exposed beta turn. The fit of this region compared to other fragments of the molecule is poor and alternative sites are being explored.

The position of protein H atoms have been calculated and their contributions have been included in the calculated structure factors. Refinement is proceeding using two independent methods. The first is a conventional method in which shifts calculated from difference Fourier maps by the gradient-curvature method are applied and followed by idealization of molecular geometry. The second method is a free-atom block-diagonal least-squares refinement. For each case there are approximately 2400 parameters and 16,000 data (12,000 above 2σ). The current R value for data above 2σ is 22% at the present stage of refinement.

The most significant secondary structural features are two and one-half turns of α-helix and three strands of antiparallel β-sheet. The α-helix is joined to the β-sheet by two disulfide bridges. Most of the conserved residues occur on one surface in the three-dimensional structure.

In addition to the variant-3 scorpion toxin described above we have collected native data for Centruroides sculpturatus Ewing variant-2 toxin and toxin-V to 2.5 and 2.2 A resolution, respectively. Variant-2 is different from variant-3 at only 5 amino acid residues while the sequence changes for toxin V are much more extensive. The space group for variant-2 is $P3_121$ or $P3_221$ with a = 48.87 A and c = 43.50 A. A good solution has been obtained for the rotation function calculated by fitting a variant-3 model structure the variant-2 data. The calculation of the translational function is currently underway. The space group for toxin V is also $P3_121$ or $P3_221$ with a = 51.22 A and c = 67.29 A. The method of molecular replacement is also being applied to toxin V.

1 J. C. Fontecilla-Camps, R. J. Almassy, F. L. Suddath, D. D. Watt and C. E. Bugg (1980). Proc. Natl. Acad. Sci. 77, 6496-6500.