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Pore-blocking and Pore-forming Toxins : Structure and Mechanism by NMR

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Polypeptide and protein toxins are generally soluble and stable and adopt well-defined structures, all of which make them eminently suitable for NMR-based structure determination. They nevertheless range in size from small peptides to large proteins, with those at the high extreme presenting a considerable challenge to current NMR methods. This talk describes our studies of the structure and function of toxins ranging from polypeptides that block ion pores to larger proteins that create pores in membranes.

Polypeptide toxins that block voltage-gated ion channels, usually with high potency and sub-type selectivity, are promising leads for the development of novel therapeutic agents. **ShK toxin,** a 35-residue polypeptide toxin containing three disulfide bonds, is a potent blocker of the voltage-gated potassium channel, Kv1.3. It is a compact molecule containing two short -helices and a number of reverse turns [1]. The essential residues for K-channel binding cluster on one face of the molecule to form a well-defined channel-binding surface. The compactness of this binding surface makes it feasible to design truncated analogues. Being much cheaper to make and more likely to be bioavailable in vivo, these analogues are more attractive lead compounds than the parent polypeptide. The design of truncated and stabilised peptide analogues of ShK toxin and their conformational evaluation by NMR [2] will be described in the first part of this talk. The structures of other polypeptide toxin determined recently will also be described briefly.

The second part of the talk will focus on the structure of equinatoxin II, a 20-kDa sea anemone toxin that is a potent cytolysin. The structure, determined on $^{13}C/^{15}N$-labelled protein, consists of two short helices packed against opposite faces of a β-sandwich structure formed by two five-stranded β-sheets [3], and provides valuable clues as to how it might lyse cell membranes. We have also investigated its interaction with sphingomyelin, which appears to be an important constituent of membranes that are susceptible to lysis by this toxin. In addition, ^{2}H and ^{31}P solid-state NMR have been used to study the effect of EqT II on the structure and dynamics of membrane lipids in bilayers both in the presence and absence of sphingomyelin [4]. NMR strategies for determining the structure of the active pore of this toxin (probably a tetramer) in a membrane bilayer will be discussed.

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3. Hinds, M.G., Zhang, W., Anderluh, G., Hansen, P.E. & Norton, R.S. (2002) *JMol Biol* 315, 1219-1229.

4. Bonev, B.B., Lam, Y-H., Anderluh G., Watts, A., Norton, R.S. & Separovic, F. (2002) submitted.