J. Biosci., Vol. 3 Number 1, March 1981, pp. 29-31. © Printed in India.

Structure of presynaptic toxins: Crystallization and preliminary x-ray diffraction data on Notechis II-5, a presynaptic toxin phospholipase

K. K. KANNAN, S. SINH, M. RAMANADHAM and D. EAKER*

Neutron Physics Division, Bhabha Atomic Research Centre, Bombay 400 085

* Department of Biochemistry, Uppsala University, Uppsala, Sweden

MS received 3 December 1980

Abstract. The presynaptic neutrotoxin-phospholipase, Notechis II-5 from the venom of Notechis *Scutatus scutatus* (Australian tiger snake) has been crystallized in a form suited for x-ray diffraction analysis. The crystals belong to the orthorhombic space group $P2_1\ 2_1,2_1$, with unit cell dimensions, a=146.1, b =43.5 and c=39.0 A. There are two molecules of Notechis II-5 in the asymmetric unit. The molecular weight is about 13,500. Notechis II-5 is highly homologous to Notexin, another presynaptic toxin from the venom of the Australian tiger snake, to bovine and porcine pancreatic phospholipases A and other venom phospholipases.

Keywords. Notechis II-5; Australian tiger snake; presynaptic toxin; crystallization; x-ray diffraction.

Introduction

The characteristic paralysis caused after snake bite is due to the blocking of the nerve transmission across the cholinergic neuromuscular junction by protein toxins present in the snake venom (Eaker *et al.,* 1976). Two categories of neurotoxins have been characterized on the basis of their site of activity. Post-synaptic toxins show curare-like action and the nicotinic acetylcholine receptors of the muscle motor end plate. These proteins consist of about 70 amino acids and the three-dimensional structures of some of this group of neurotoxins have been well established and their function has been well characterized (Low *et al.,* 1976; Tsernoglou and Petsko, 1976). Presynaptic toxins interfere with the release of acetylcholine from the motor nerve terminals. The presynaptic toxins fall into two classes (a) multimeric protein complex and (b) monomeric protein. All these toxins exhibit pronounced phospholipase activity. In the case of the multimeric toxins, the phospholipase activity is usually associated with one of the sub-units and is an essential prerequisite for the toxicity. The nature of the presynaptic toxicity and the need for the phospholipase activity are not well understood.

We have investigated the structure-function of the monomeric presynaptic toxins by x-ray diffraction work. Earlier we had successfully reported the crystalization and x-ray data on Notexin isolated from the venom of the Australian tiger

snake, Notechis *Scutatus scutatus* (Kannan *et al.,* 1977). In this paper we report the crystalization and the crystalldgraphic data on another presynaptic toxin Notechis II-5 isolated from the same Australian tiger snake venom (Halpert and Eaker, 1976).

Notechis II-5 is highly homologous to Notexin and to porcine, bovine and venom phospholipases. It shows moderate to high phospholipase activity and is 20 times less neurotoxic compared to Notexin. These toxins consist of about 119 amino acids and have a molecular weight of about 13,500. The homologous phospholipases do not exhibit any toxicity. It would thus be useful to correlate the tertiary structure of these proteins with their toxicity and phospholipase activity as compared to the non-toxic phospholipases from different species.

Crystallization

The purification of Notechis II-5 has been described by Eaker and co-workers (Halpert and Eaker, 1976). The purified and lyophilized powder was suspended in 0.05 M Tris sulphate buffer (pH 8.5) and a few drops of 1 M ammonium acetate were added to the suspension until the precipitate just dissolved. The solution was left at 4°C and crystals were obtained within a short period. The crystals are rectangular plates having dimensions $0.3\times0.3\times0.5$ mm^3, diffract well and are stable to x-rays.

Results and discussion

The crystals were mounted in the usual way in Lindemann capillaries and 12° precession photographs (figures 1 and 2) were collected on a 600 watt Philips fine focus tube using Ni filtered CuK_a radiation. The photographs exhibited systematic absences along the h00, 0k0 and 001 directions. The unit cell parameters derived

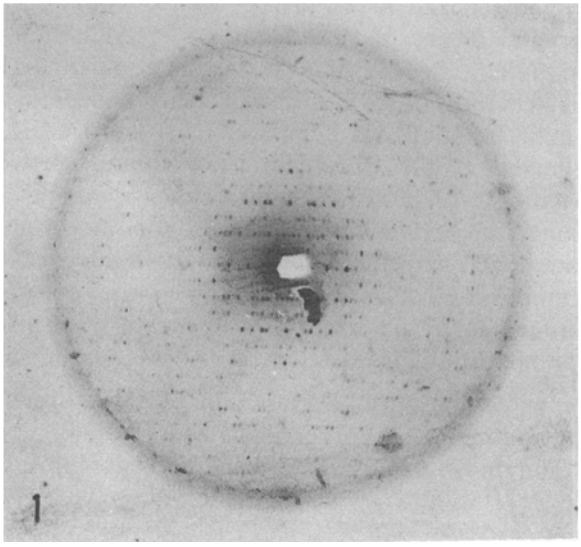

Figure 1. HKO photograph of Notechis II-5. Precession angle 12°.

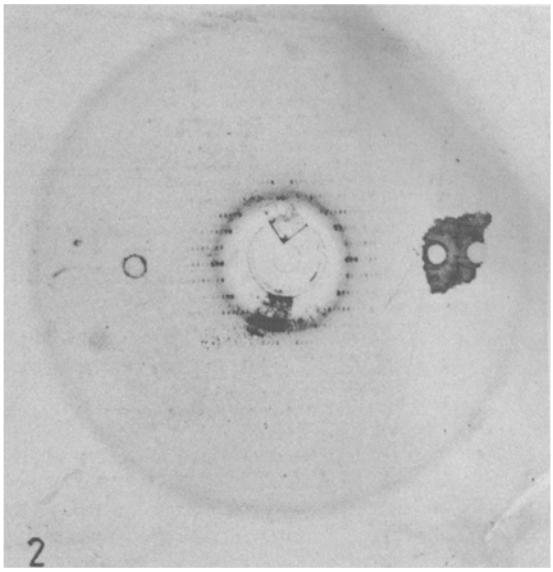

Figure.2. HK1 photograph of Notechis II-5. Precession angle 12°.

from the diffraction photographs are a= 146.1, b=43.5 and c=39.0 Å^{3}and the space group is $P2_12_12_1$. The volume is 247,643 Å^3 giving a Mathews number (Mathews, 1968) V_M= 4.5 Å^3/VDalton. This value is very much higher than the normally observed one of 2.3 Å^3/Dalton for one Notechis II-5 molecule per asymmetric unit. The homologous Notexin crystals gave a Mathews number of V_M = 3.05. It is thus evident that the unit cell of Notechis II-5 crystals consists of a dimer of molecular weight 27,000 in the asymmetric unit, which would give a Mathews number of 2.25 Å^3/Dalton, a more acceptable value for globular proteins.

The homologous phospholipase A2 crystallized from *Crotalus adamanteus* and *C. atrox* have a Mathews number of 2.28 and 2.21 respectively, for a dimer of molecular weight 30,000 in the asymmetric unit (Pasek *et al.,* 1975). This is analogous to Notechis II-5 reported here. Further work is in progress to get heavy atom derivatives.

Acknowledgements

We would like to thank Dr R. Chidambaram, Dr P. K. Iyengar, Shri C. V. Sundaram and Dr R. Krishnan for their interest and encouragement.

References

Eaker, D., Halpert, J., Fohlman, J. and Karlsson, E. (1976), in *Animal, plant and microbial toxins,* eds. A. Ohsaka, K. Hayashi and Y. Sawai (New York::Plenum) Vol. 2, p. 27.

Halpert, J. and Eaker, D. (1976) *J. Biol. Chem.,* **251,** 7343.

Kannan, K. K., Lougren, S., Cid-Dresdner, H., Petef, M., and Eaker, D. (1977) *Toxicon,* **15,** 434.

Low, B. W., Preston, H. S., Sato, A., Rosen, L. S., Searl, J. E., Rudko, A. D., and Richardson, J. J. (1976) *Proc. Natl. Acad. Sci. USA,* **73,** 2991.

Mathews, B. W., (1968) *J. Mol. Biol.,* **33,** 491.

Pasek, M., Keith, C., Feldman, D. and Sigler, P. D. (1975) *J. Mol. Biol.,* **97,** 395.

Tsernoglou, D. and Petsko, G. A., (1976) *FEBS Lett.,* **68,** 1.