

## AMINO ACID SEQUENCE OF NEUROTOXIN II FROM *NAJA NAJA OXIANA* VENOM

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### 1. Introduction

The neurotoxins of snake venom are known to specifically bind the cholinergic receptor site on the postsynaptic membrane, thus inhibiting nerve impulses.

At present the amino acid sequences of 12 neurotoxins [1–8], each of about 60 amino acid residues and 5 longer toxins containing some 70 residues [6–10], have been elucidated.

The venom of *Naja naja oxiana*, a cobra from Middle Asia, in addition to cardiotoxin and number of enzymes, contains at least three neurotoxins. Karlsson and Eaker [11] have determined the composition and N-terminal sequence of the principal neurotoxin of *Naja naja oxiana* venom. In the present paper we describe the determination of the total amino acid sequence of this neurotoxin.

### 2. Methods

Neurotoxin II was isolated from *Naja naja oxiana* venom by means of a procedure described earlier by Turakulov et al. [12]. Reduction and carboxymethylation was carried out according to Crestfield et al. [13]. The amino acid composition was determined on a Bio

Cal BC 201 amino acid analyzer. The tryptic digestion products of the carboxymethylated toxin were separated by chromatography on a Chromo-Beads type P cation exchange resin (Technicon). The effluent was analyzed with a Technicon II analyzer. Further separation of the peptides was carried out by paper electrophoresis and paper chromatography. Chymotryptic and thermolytic peptides were separated only by paper electrophoresis and paper chromatography.

### 3. Results and discussion

According to the amino acid composition of the carboxymethylated toxin tryptic digest one could have expected it to contain 11 fairly short peptides. The use of a 22  $\mu$  grain cation exchanger permitted the resolution of our chromatographic column to be augmented to the extent that most of the peptides were concentrated in well defined zones. All together nine different peptides were isolated from the tryptic hydrolysate. Moreover elution of the column with 0.5 M NaOH allowed us to detect another peptide viz. Ser–Asp–His–Arg. Apparently such peptides have a tendency to nonspecific adsorption on cation exchange resins.

To obtain overlapping fragments we subjected the

carboxymethylated neurotoxin to chymotryptic digestion. Analysis of the chymotryptic peptides furnished sufficient information for reassembling the entire polypeptide chain of neurotoxin II.

The tryptophan containing thermolytic peptide was isolated in order to confirm the structure of an important fragment of the neurotoxin molecule. Determination of the sequence of peptide showed the

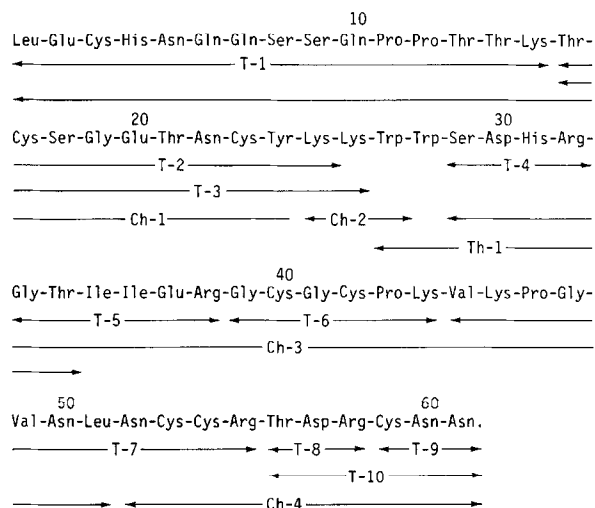


Fig. 1. The amino acid sequence of neurotoxin II from *Naja naja oxiana* venom: T, tryptic peptides; Ch, chymotryptic peptides; Th, thermolytic peptide.

presence of a Trp-Trp sequence in the neurotoxin.

Neurotoxin II from *Naja naja oxiana* is homologous with curari-form neurotoxins from the cobra and sea snake venoms. A peculiarity of this neurotoxin is the Trp-Trp sequence in positions 27 and 28. Consequently the elucidation of the functional role of the tryptophan (27) residue is of considerable interest.

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