AMINO ACID SEQUENCE OF TOXIN A FROM THE VENOM OF THE INDIAN COBRA (*NAJA NAJA*)*

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SUMMARY. Toxin A, isolated from the venom of the Indian cobra by column chromatography on Sephadex G-75, G-50 and carboxymethyl (CM)-cellulose, was homogeneous by the criteria of end group analysis and polyacrylamide gel electrophoresis. The complete amino acid sequence of the toxin which contains 71 amino acid residues (no methionine) was determined.

Snake venoms are mixtures of proteins with enzyme character, and of strongly basic polypeptides which are toxins or neurotoxins. Snakes belonging to the family *Elapidae* (numerous cobra species, tiger snake, etc.) and *Hydropheidae* (many species of sea snakes) contain neurotoxins which produce flaccid paralysis and death by respiratory failure (1). Much has been found out on the mode of action of neurotoxin from the venom of the Indian cobra (2,3) which blocks neuromuscular transmission both pre- and postsynaptically, and decreases the sensitivity of the end-plate to the depolarizing action of acetylcholine (4-6). Unlike the crude venom, purified cobra neurotoxins act like d-tubocurarine, except for slower onset and lesser reversibility of the paralysis (1).

A chemically homogeneous major neurotoxin "toxin A" has been isolated from the venom of the Indian cobra (*Naja naja*) and the amino acid composition has been studied (7). Recently, the amino acid sequences of several neurotoxins from *Naja nigricollis* (8), from *Naja haje haje* (9), of cobratoxin (*Naja naja atra* (10,11), of toxins II and IV from *Hemachatus haemachatus* (12) and

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of erabutoxins a and b from Laticauda semifasciata (13,14) have been determined. In this paper we report the complete amino acid sequence of toxin A isolated from the venom of the Indian cobra.

MATERIALS AND METHODS

Toxin A was obtained by column chromatography on Sephadex G-75, G-50 and CM-cellulose (7), and purified further by repeated chromatography on a CM-cellulose column in a similar buffer system. The amino acid analyses of toxin A and its peptide fragments were performed on a Hitachi Model KLA-3B automatic amino acid analyzer after hydrolysis with 6 N HCl for 20 to 24 hrs at 105°. Sequential degradations were performed by the modified Edman procedure (15,16). The PTH derivatives were determined by tlc in five different solvent systems. The carboxy(C)-terminal sequence was determined by hydrazinolysis (17) or by the use of carboxypeptidases A and B. The tryptic peptides were separated by paper chromatography (n-butanol-acetic acid-water, 4:1:5, v/v, or n-butanol-acetic acid-pyridine-water, 15:3:10:12, v/v), and by high voltage paper electrophoresis at pH 3.6.

RESULTS AND DICSUSSION

Toxin A which shows a single band on polyacrylamide gel electrophoresis was obtained in a yield of 10% from the crude venom (7). Toxin A had an LD_{50} of 0.15 μg/g in mice. The molecular weight of toxin A was estimated to be 6,300 by the method of sedimentation equilibrium. Based on this value and on the amino acid analysis, one molecule of toxin A was considered to have the following 71 amino acid residues: Lys_4, His_1, Arg_6, Trp_1, Asp_9, Thr_9, Ser_3, Glu_1, Pro_6, Gly_5, Ala_2, $1/2Cys_{10}$, Val_4, Ile_5, Leu_1, Phe_3, Tyr_1.

A twenty-nine step Edman degradation of toxin A revealed the amino terminal sequence to be H_2N-Ile·Arg·Cys·Phe·Ile·Thr·Pro·Asp·Ile·Thr·Ser·Lys·Asp·Cys·Pro·Asn·Gly·His·Val·Cys·Tyr·Thr·Thr·Lys·Thr·Trp·Cys·Asp·Gly·Phe..... The C-terminal sequence was examined by the use of carboxypeptidases A and B. No amino acids were released from reduced and carboxymethylated(RCM)-toxin A by carboxypeptidases A and B, an observation suggestive of proline at or near the

C-terminus. However, when the C-terminus of toxin A was investigated by the tritiation method described by Matsuo et al. (18), the radioactive amino acid, examined by paper chromatography and high voltage paper electrophoresis, was deduced to be C-terminal alanine. However, in the sequential studies of the peptide fragments obtained by enzymatic digestion of toxin A, we were unable to isolate a peptide with alanine as the C-terminal amino acid and instead obtained a peptide C-terminal proline. In addition, proline was identified as the C-terminal amino acid by hydrazinolysis (17) of RCM-toxin A. Furthermore, two alanine residues existing in the toxin A molecule were localized in positions $^{42}_{43}$-Ala-Ala-. The C-terminal amino acid must therefore be proline. The appearance of alanine as C-terminal amino acid by the method of Matsuo (18) defies an explanation at this time.

Next RCM-toxin A was digested by trypsin or chymotrypsin, and the

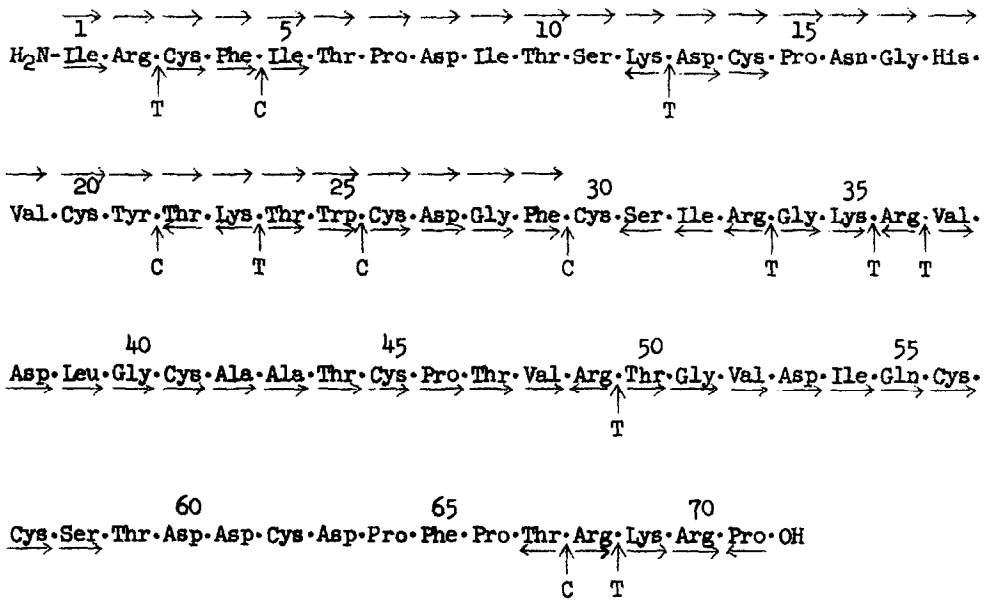

Fig. 1. Amino acid sequence of toxin A isolated from the venom of the Indian cobra (Naja naja). Horizontal arrows above and below amino acid residues denote that the sequence was determined in RCM-toxin A, and its tryptic or chymotryptic peptides, respectively. Right- and left-pointed arrows show that the sequence was elucidated, respectively, by Edman degradation, by the action of carboxypeptidases A and B, and by hydrazinolysis. Vertical arrows in the sequence show peptide bonds cleaved by trypsin (T) and chymotrypsin (C), respectively.

resulting peptides were separated by paper chromatography and high voltage paper electrophoresis at pH 3.6.

Amino acid sequences of the peptides obtained were elucidated by Edman degradation and by the use of carboxypeptidases A and B. Fig. 1 summarizes these results (19) and presents the primary structure of toxin A. There is a remarkable degree of similarity between the neurotoxins having 61-62 amino acid residues (8-12), including the neurotoxins of the sea snake (13). By contrast, the amino acid sequence and composition of toxin A is quite different. However, some resemblances can be found in the amino acid sequence between toxin A and other neurotoxins. The sequence of toxin A is very similar to that of a neurotoxin from Naja nivea (19).

Interestingly enough, except for the two cysteine residues 26 and 30 of toxin A, the remaining 8 cysteine residues are in analogous but not identical locations. If one assumes the four disulfide bonds in the neurotoxins to be formed from identical cysteine residues, an additional disulfide bond in toxin A may be formed from cysteine residues 26 and 30, which differ from those of the other neurotoxins.

CD and ORD spectra of toxin A resemble closely those of cobratoxin (20, 21) and erabutoxins a and b (22). The tertiary structure of toxin A probably resembles that of the other neurotoxins, with the disulfide bonds as the major determinants for preservation of the active conformation.

Editors note: Additional data to support the sequence shown were made available to the referee by the authors.

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