AMINO ACID SEQUENCE OF NEUROTOXIN I FROM *NAJA NAJA OXIANA* VENOM

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Received 24 May 1974

1. Introduction

Neurotoxins from snake venoms can be used as fairly adequate tools for studying synaptic transmission at the molecular level. Venoms of some snakes contain toxins affecting post-synaptic membranes, while other snake venoms affect the pre-synaptic membranes. To understand the mechanism of blocking of neuromuscular transmission it would help to have more knowledge of the neurotoxin structure. At present somewhat better known are the neurotoxins from the venoms of Elapidae and Hydrophidae. The neurotoxins generally are classified into two groups: the so-called 'long' and 'short' toxins. The primary structures of 13 long neurotoxins [1–7] and 16 short ones [7–16] have been reported in the literature. Recently we have determined the amino acid sequence of neurotoxin II from the cobra *Naja naja oxiana* [16]. Nearly at the same time Ruden in his review [17] also gives the primary structure of this toxin as found by Arnberg et al. The present paper deals with the determination of the total amino acid sequence of neurotoxin I from the venom of *Naja naja oxiana.*

2. Methods

Crude venom of the Middle Asia cobra *Naja naja oxiana* was obtained from the Kirghiz Zoocombinate. Neurotoxin I was isolated from the venom by the procedure described by Turakulov et al. [18]. Reduction and carboxymethylation was carried out according to Crestfield et al. [19]. Amino acid composition was determined on a Bio Cal BC 201 analyzer. The number of Trp residues was obtained from UV absorption and alkaline hydrolysis data. The isoelectric point for neurotoxin I was measured on an analytical electrofocusing column, LKB-8100. Tryptic and chymotryptic hydrolysis of the carboxymethylated toxin was carried out at 1:50 enzyme to substrate ratio in 0.1 M NH_4HCO_3 at pH 8.3 (37°C, 4 hr). The peptides were separated by paper electrophoresis and paper chromatography. The N-terminal amino acid sequence of carboxymethylated toxin was determined conventionally according to Edman, and the C-terminal sequence was found by digesting with carboxypeptidase C. The peptide structures were established by described procedures [20].

3. Results and discussion

Neurotoxin I from *Naja naja oxiana* is more acidic (pI = 9.05) than, for example, neurotoxin II. Its content in the crude venom is approximately 2% by weight. According to Turakulov et al. [18] LD_{50} for neurotoxin I for intraperitoneal injection in mice is

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Table 1
Amino acid composition of carboxymethylated neurotoxin I and its tryptic peptides

Amino acid	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	Toxin I
Cm-Cys					0.8 (1)	1.7 (2)	1.7 (2)	2.6 (3)	9.8 (10)
Asp						0.9 (1)	0.9 (1)	2.6 (3)	5.9 (6)
Thr					1.0 (1)	4.1 (4)	1.0 (1)	1.0 (1)	8.6 (9)
Ser			0.2			1.2 (1)	1.0 (1)	1.0 (1)	4.1 (4)
Glu	1.0 (1)		1.0 (1)	0.2		1.8 (2)	0.2	0.2	5.9 (6)
Pro	1.0 (1)			0.9 (1)		2.9 (3)		2.0 (2)	6.6 (7)
Gly		1.0 (1)	0.2		0.2	1.0 (1)	1.0 (1)		4.0 (4)
Ala						1.1 (1)	1.0 (1)		4.0 (4)
Val									2.0 (2)
Ile					1.0 (1)	1.6 (2)			4.8 (5)
Leu						0.9 (1)			1.9 (2)
Tyr					0.8 (1)	0.8 (1)			2.8 (3)
His								0.7 (1)	0.8 (1)
Lys	1.0 (1)	1.0 (1)	0.9 (1)	0.2	1.1 (1)	1.1 (1)		1.0 (1)	6.0 (6)
Arg	0.9 (1)			1.0 (1)			0.8 (1)		2.1 (2)
Trp							(2)		(2)
Total	4	2	2	2	5	20	10	12	73
Yield (%)	35	44	21	26	84	39	24	48	

0.56 mg per kg. The amino acid composition data show that the toxin molecule has 73 amino acid residues. Analysis of the alkaline hydrolyzate of the toxin showed it to contain two Trp-residues. The molar extinction of carboxymethylated neurotoxin in 10% acetic acid at 280 nm was 16 900, also indicative of two Trp-residues.

The N-terminal sequence, H–Ile–Thr–Cys–Tyr–Lys–Thr–Phe–Ile . . ., was established by Edman degradation of carboxymethylated neurotoxin, while the C-terminal sequence . . . Arg–Pro was determined by disgestion with carboxypeptidase C.

Tryptic hydrolysis of carboxymethylated neurotoxin proceeded specifically enough, without atypical rupture of bond. The Arg–Pro bond (72–73) did not break up. Eight different peptides were isolated from the tryptic hydrolyzate (table 1). Paper electrophoresis and paper chromatography did not provide the 20-membered tryptic peptide in quantities sufficient for analysis. However the chymotryptic hydrolysis of carboxymethylated toxin gave us 9 peptides which provided sufficient information for establishing the structure of neurotoxin I. The bonds Leu–Gly (41–42) and Lys–Gln (69–70) did not undergo complete hydrolysis under the action of chymotrypsin.

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                                                  10                                  20
Ile-Thr-Cys-Tyr-Lys-Thr-Pro-Ile-Pro-Ile-Thr-Ser-Glu-Thr-Cys-Ala-Pro-Gly-Gln-Asn-

                                                  30                                  40
Leu-Cys-Tyr-Thr-Lys-Thr-Trp-Cys-Asp-Ala-Trp-Cys-Gly-Ser-Arg-Gly-Lys-Val-Ile-Glu-

                                                  50                                  60
Leu-Gly-Cys-Ala-Ala-Thr-Cys-Pro-Thr-Val-Glu-Ser-Tyr-Gln-Asp-Ile-Lys-Cys-Cys-Ser-

                                                  70
Thr-Asp-Asp-Cys-Asn-Pro-His-Pro-Lys-Gln-Lys-Arg-Pro.
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Fig. 1. The primary structure of neurotoxin I from the *Naja naja oxiana* venom.

Table 2
Amino acid composition of chymotryptic peptides from carboxymethylated neurotoxin I

Amino acid	Ch-1	Ch-2	Ch-3	Ch-4	Ch-5	Ch-6	Ch-7	Ch-8	Ch-9
Cm-Cys			2.6 (3)	1.9 (2)	3.0 (3)	1.0 (1)	2.7 (3)	1.0 (1)	1.8 (2)
Asp			3.8 (4)	1.1 (1)			4.0 (4)	1.1 (1)	
Thr		1.7 (2)	1.0 (1)	2.7 (3)	1.8 (2)	0.9 (1)	1.0 (1)		1.9 (2)
Ser	0.1		1.2 (1)	1.2 (1)	2.1 (2)		1.2 (1)		1.3 (1)
Glu	1.0 (1)		2.0 (2)	2.1 (2)	2.0 (2)		1.1 (1)		1.1 (1)
Pro	0.9 (1)		2.7 (3)	2.7 (3)	1.0 (1)		1.9 (2)		0.8 (1)
Gly	0.1		0.1	1.1 (1)	3.1 (3)			0.1	1.3 (1)
Ala				0.9 (1)	1.9 (2)			1.0 (1)	2.0 (2)
Val					1.6 (2)				0.8 (1)
Ile			0.9 (1)	1.7 (2)	0.8 (1)	1.0 (1)	1.0 (1)		
Leu				1.0 (1)	1.0 (1)				
Tyr				0.9 (1)	0.8 (1)	0.9 (1)			1.0 (1)
His			0.7 (1)				1.0 (1)		
Lys	0.9 (1)	1.0 (1)	2.8 (3)	1.0 (1)	0.9 (1)		1.9 (2)		
Arg	1.0 (1)		0.8 (1)		1.0 (1)				
Trp		(1)						(1)	
Total	4	4	20	19	22	4	16	4	12
Yield (%)	10	28	13	14	12	10	9	12	14

Neurotoxin I from *Naja naja oxiana* venom belongs to the group of long neurotoxins and has 5 intramolecular disulfide bonds. It differs from other known toxins of the group by the complete absence of Phe-residues and low content of basic amino acids. The sequence between Cys (3) and Cys (15) is peculiar to neurotoxin I only, whereas Thr (6), Tyr (23), Lys (25), Trp–Cys–Asp (27–29), Arg–Gly–Lys (35–37), Leu–Gly–Cys–Ala–Ala–Thr–Cys–Pro (41–48) and Cys–Cys–Ser (58–60) constitute a framework similar to that of other long toxins. However in position 51, where all the toxins have a basic amino acid –Lys or Arg–, neurotoxin I from *Naja naja oxiana* has Glu. It is quite feasible that these structural features result in a certain physiological specificity of neurotoxin I.

Neurotoxins are promising materials for making structure–function correlations about biologically active peptides on the physicochemical and immunochemical levels. Also it is noteworthy that neurotoxins are good models for observing the evolutional changes in protein molecules.

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