THE N- AND C-TERMINAL AMINO ACID SEQUENCES OF COBRATOXIN FROM FORMOSAN COBRA VENOM

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Summary

Cobratoxin from the venom of the Formosan cobra consists of a single chain of 62 amino acids of which the first 24 and the C-terminal 5 have now been elucidated and in part correlated with the known sequences of other venoms from snakes and scorpions which differ significantly.

Cobratoxin was first isolated and crystallized from the venom of the Formosan cobra (Naja naja atra) (1). Related neurotoxins have also been isolated from several other snakes and scorpions (2-4).

Cobratoxin consists of a single peptide chain of 62 amino acid residues cross-linked by four disulfide bridges. The N-terminal is leucine and the C-terminal asparagine (5). The optical rotatory dispersion (ORD) of cobratoxin between 230-300 mμ (6) has a large positive peak at 233 mμ and differs significantly from the ORD of usual proteins with a right-handed α-helical structure, a finding which suggests a β-structure (7). In this paper we report on the N- and C-terminal amino acid sequences of cobratoxin.

Experimental and Results

Reduced and carboxymethylated "RCM-cobratoxin" was prepared by the method of Crestfield (8): 300 mg of cobratoxin was dissolved in 10 ml of

0.2 M Tris-HCl buffer, pH 8.2, containing 8 M urea, and 0.3 ml of β-mercaptoethanol was added. The tube was flushed with nitrogen gas and left at room temperature for 4 hours. For alkylation 900 mg of iodoacetic acid in 2.3 ml of 2.0 N NaOH was added with constant stirring and the pH was maintained at 8.6. The solution was desalted by passage through a column of Sephadex G-25 and the void volume containing RCM-cobratoxin was lyophilized.

The N-terminal amino acid sequence was determined by the Edman procedure (9). RCM-cobratoxin (2 μmoles) was dissolved in 0.2 ml of pyridine-water (3:2, v/v) containing 0.4 M dimethylallylamine. The solution was adjusted to pH 9 with trifluoroacetic acid. After the addition of 20 μl of phenylisothiocyanate, the tube was flushed with nitrogen gas, stoppered and left at 40° for 1 hour. The solution was then extracted five times with 1.2 ml of benzene and the aqueous phase was freeze-dried. Cleavage was performed in 10-20 μl of trifluoroacetic acid for 20 minutes at 40°. The residual peptide was precipitated with 0.6 ml of ethylene chloride and the residue washed with 0.6 ml of ethylene chloride. The precipitate was dried over P_2O_5 and KOH in the cold overnight in vacuo, and was used for the next degradation cycle. The ethylene chloride extract containing the intermediate thiazolidone was evaporated by a jet of nitrogen and converted to the corresponding thiohydantoin by incubating at 80° for 10 minutes with 0.3 ml of 1 N HCl. The phenylthiohydantoin (PTH) amino acids formed were extracted with 0.8 ml of ethyl acetate four times. After removal of ethyl acetate the PTH amino acids were identified by thin layer chromatography. RCM-cobratoxin was taken through 24 cycles of successive degradations to yield the following sequence: NH_2-Leu-Glu-Cys-His-Asn-Gln-Gln-Ser-Ser-Gln-Thr-Pro-Thr-Thr-Thr-Gly-Cys-Gly-Gly-Gly-Gln-Asn-Cys-Tyr----.

The C-terminal amino acid sequence was deduced from the relative rates of liberation of new amino acids by digestion with carboxypeptidase. Cobratoxin was incubated at pH 8.0-8.5 and 25° for 3 hours with carboxypeptidase B, pretreated with DFP at pH 8.0 for 15 minutes. The reaction was terminated

by adjusting the solution to pH 4.0 by addition of 0.1 N HCl. After lyophilization the liberated amino acids were quantitatively assayed in an automatic amino acid analyzer. Asparagine was the main product with significant amounts of carboxymethylcysteine and arginine, and traces of aspartic acid, presumably due to stepwise degradation of the peptide chain. The liberation of asparagine was complete after 3 hours and attained a plateau value corresponding to two residues of asparagine per mole of cobratoxin. Carboxymethylcysteine and arginine are released much more slowly after 3 hours of digestion. Their amounts correspond to approximately one mole per mole of protein. Thus the C-terminal amino acid sequence of cobratoxin may be written as: -----Arg-Cys-Asn-Asn·COOH.

Table 1

Amino Acid Composition of Neurotoxins from the Venoms of Snakes and Scorpions

Amino Acid	Source of Neurotoxin					
	Naja naja atra	*Naja nigricollis* (Toxin α)	*Laticauda semifasciata* Erabutoxin a	*Laticauda semifasciata* Erabutoxin b	*Androctonus australis* Toxin I	*Androctonus australis* II
Aspartic acid	8	7	5	4	9	8
Threonine	8	8	5	5	2	3
Serine	4	2	8	8	6	2
Glutamic acid	7	6	8	8	0	4
Proline	2	5	4	4	6	3
Glycine	7	5	5	5	6	7
Alanine	0	0	0	0	1	3
Half-cystine	8	8	8	8	8	8
Valine	1	2	2	2	4	4
Methionine	0	0	0	0	0	0
Isoleucine	2	3	4	4	3	1
Tyrosine	2	1	1	1	3	7
Leucine	1	2	1	1	4	2
Phenylalanine	0	0	2	2	1	1
Lysine	3	6	4	4	6	5
Histidine	2	2	1	2	1	2
Arginine	6	3	3	3	2	3
Tryptophan	1	1	1	1	1	1
TOTAL	62	61	62	62	63	64

Discussion

Recently, several kinds of homogeneous neurotoxins have been isolated. Table 1 shows the amino acid compositions of those from Naja naja atra, Naja nigricollis (2), Laticauda semifasciata (4), and Androctonus australis (3). Their molecular weights range between 5,000-7,000. They are all strongly

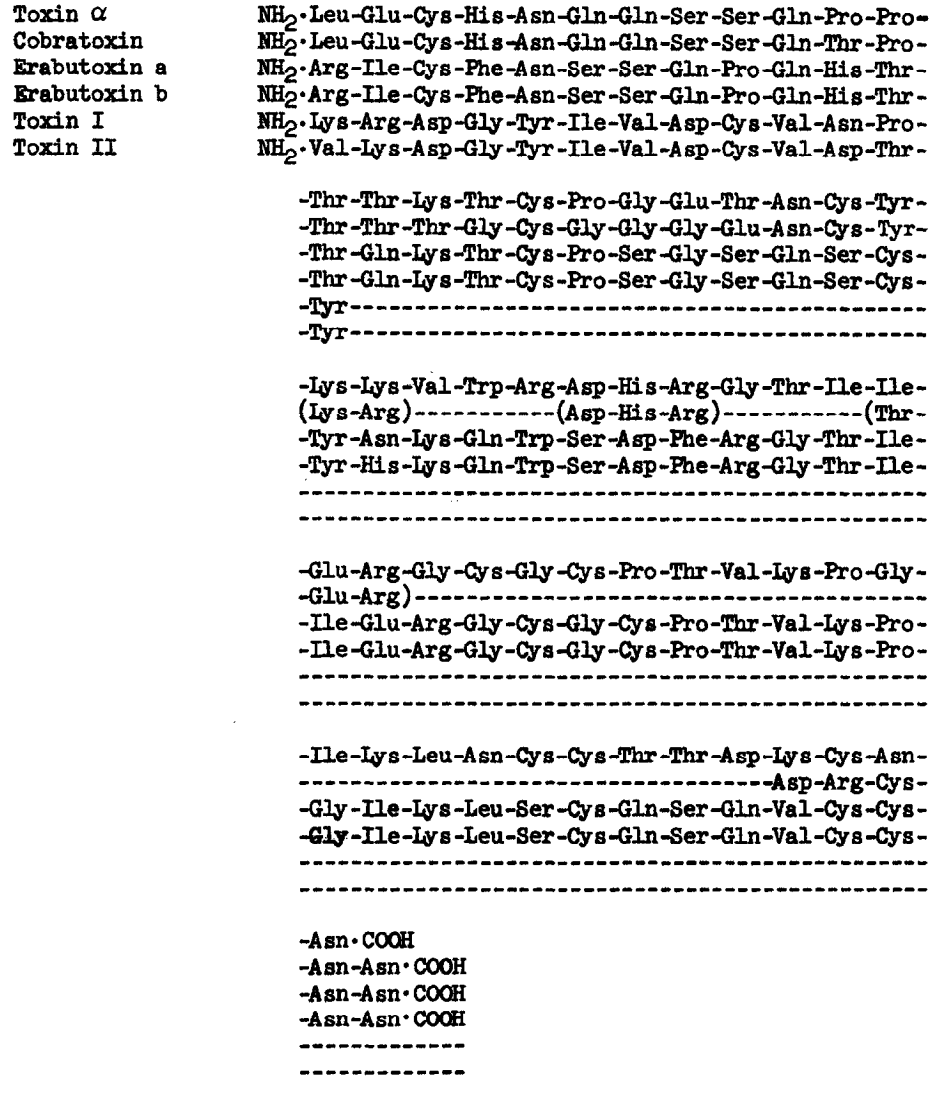

Fig. 1. Structures of Naja nigricollis neurotoxin (Toxin α), Naja, naja atra neurotoxin (Cobratoxin), Laticauda semifasciata neurotoxin (Erabutoxins a and b), and scorpion neurotoxins (Toxin I and II). Dotten lines indicate parts where the sequence has not been determined. The amino acid sequence in parentheses was determined with the peptides obtained from the trypsin hydrolysate of cobratoxin.

basic and contain 2-4 cystine residues. Moreover, two to four amino acids are completely absent. The neurotoxins from snake venoms have a curare-like action different from that of scorpion neurotoxin which acts both on the peripheral as well as on the central nervous system. Comparison of sequences, when completed, may give clues for these different pharmacological actions. The amino acid sequence of Naja nigricollis neurotoxin (2) is very similar to that of Naja naja atra, especially in the terminal sequence (Fig. 1). On the other hand, the N-terminal amino acid sequence of scorpion neurotoxin differs considerably from those of cobra neurotoxins.

Further studies are in progress to elucidate the complete amino acid sequence of cobratoxin and to correlate its structural features with the biological activity of the toxin.

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