THE AMINO ACID SEQUENCE OF CARDIOTOXIN FROM FORMOSAN COBRA (Naja naja atra) VENOM

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Summary

The primary structure of the purified cardiotoxin from Naja naja atra venom, containing 60 amino acid residues, was determined. Comparing the amino acid sequence of cardiotoxin to that of cobrotoxin, a neurotoxin containing 62 residues obtained from the same snake venom, 20 residues including 8 half cystines are identical, assuming that there are 3 residues' deletion and 2 residues' insertion in the cardiotoxin molecule.

Several pharmacological active components such as neurotoxin (najatoxin, cobrotoxin, toxin α), cardiotoxin, direct lytic factor (DLF), cobramines, toxin γ, cytotoxin, and phospholipase A have been separated from cobra venom (see Meldrum, 1965; Lee, 1970). While the neurotoxin is considered as the major toxic component of cobra venom, causing peripheral respiratory paralysis, cardiotoxin produces cardiovascular changes and is responsible for the local necrotic lesions. A chemically homogeneous cardiotoxin has been isolated from the venom of Naja naja atra and its pharmacological properties have been studied at length (Lee et al., 1968). It has recently been shown that cardiotoxin is pharmacologically indistinguishable from DLF or cobramine B (Slotta and Vick, 1969; Lee, Lin and Wei, 1970). It is of interest to compare the primary structure of cadiotoxin with that of cobrotoxin containing 62 amino acid residues of the known sequence (Yang, Yang and Huang, 1969), since both toxins are obtained from the same snake venom but their pharmacological actions are quite different (Chang and Lee, 1966; Lee et al., 1968). The elucidation

of the primary structure of cardiotoxin has thus been explored and the results are preliminarily presented in this communication.

Materials and Methods

Cardiotoxin used in this study was prepared from Formosan cobra (Naja naja atra) venom by chromatography on a CM-Sephadex (C-50) column as previously described (Lee et al., 1968) and purified further by repeated rechromatography on a CM-cellulose column, using similar buffer system. Homogeneity was verified by acrylamide gel electrophoresis, sedimentation velocity, amino acid analysis and end group analysis.

Amino acid analyses of the toxin and peptides derived by fragmentation techniques, cyanogen bromide cleavage and tryptic digestion of the reduced-aminoethylated (RAE) or reduced-carboxymethylated (RCM) toxin, were carried out as usual. Amino acid sequences of large peptide fragments were determined mainly by the Edman degradation method modified by Iwanaga et al. (1969). For the elucidation of the structures of smaller peptides, further modification in the degradation was made (Narita, 1969).

Results and Discussion

Molecular weight of cardiotoxin was estimated to be about 6,000 by sedimentation equilibrium method. Based on this value one molecule of the toxin was composed of 60 amino acid residues: Lys 9, His 0, Arg 2, Trp 0, Asp 6, Thr 3, Ser 2, Glu 0, Pro 5, Gly 2, Ala 2, Cys 8, Val 7, Met 2, Ile 1, Leu 6, Tyr 3 and Phe 2.

The stepwise degradation of RAE-toxin was achieved up to the 12th residue from the N-terminus and carboxypeptidase A released asparagine and S-aminoethylcysteine from the C-terminus as shown in Fig. 1. Two methionyl linkages in RAE-toxin were cleaved by cyanogen bromide (in 70 % formic acid for 24 hours at room temperature) and three major fragments were fractionated by successive gel filtrations on Bio-gel P-10 and P-30 columns with 1 M formic acid. The

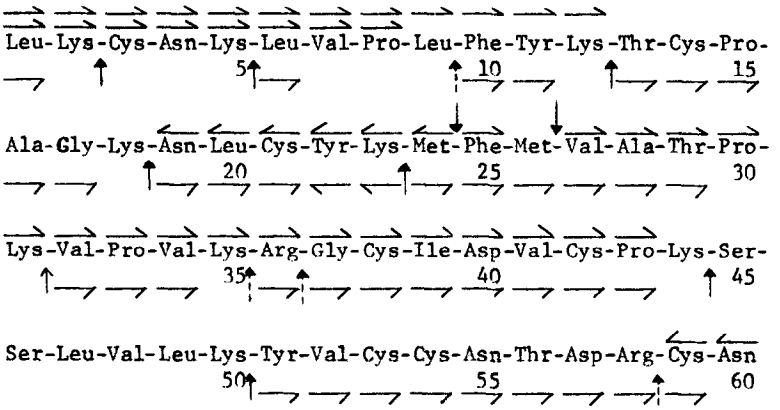

Fig. 1. Amino acid sequence of cardiotoxin from Formosan cobra (Naja naja atra) venom. Horizontal arrows above and below of amino acid residues denote that the sequence was determined in RAE-toxin or BrCN-fragments derived from RAE-toxin and the tryptic peptides, respectively. Right- and left-pointed arrows show that the sequence was elucidated, respectively, by the Edman degradation and by the action of carboxypeptidase A. Vertical arrows above and below the amino acid sequence show peptide bonds cleaved by cyanogen bromide and trypsin, respectively.

N-terminal fragment contained 24 residues and the successive 8 residues from the N-terminus were characterized by the Edman method and 6 residues from the C-terminus (homoserine) were elucidated by the use of carboxypeptidase A. The C-terminal fragment containing no homoserine was composed of 34 residues and 18 residues from the N-terminus were determined by the Edman method. The internal fragment between the N- and the C-terminal fragments was a dipeptide, phenylalanyl-homoserine.

Next, RCM-toxin was digested by trypsin and resulting peptides were separated by chromatography on a Dowex 50-X2 column and further fractionation or purification of heterogeneous fractions were made by paper chromatography or paper electrophoresis. Amino acid sequences of the tryptic peptides were elucidated mainly by the Edman degradation. The structure of a hexapeptide, Ser-Ser-Leu-Val-Leu-Lys, was elucidated by the use of thermolysine and carboxypeptidase A, since the Edman method was not effective. Summarizing these results the primary structure of the toxin shown in Fig. 1 could be deduced.

Location of the 4 disulfide bonds remains to be determined.

The amino acid composition of cardiotoxin is quite different from that of cobrotoxin. Cardiotoxin is a strongly basic polypeptide, characterized by the high lysine and low arginine contents, and also by the lack of tryptophan, histidine and glutamic acid, whereas cobrotoxin is devoid of alanine, methionine and phenylalanine.

Despite the dissimilarity in their amino acid compositions, however, some resemblance can be found in the amino acid sequences between cardiotoxin and cobrotoxin, assuming that 3 residues are deleted between the 13th and 14th residues and 2 residues are inserted between the 38th and 41st residues (namely $\overset{39}{\text{Ile}}$ - $\overset{40}{\text{Asp}}$) in the molecule of cardiotoxin. Twenty residues in the two toxins are identical, while 20 residues can be explained by one base change and 18 by two base change in their genetic codons, without considering the deletion and insertion as described above. The most remarkable feature is that the location of the 8 half cystine residues in cardiotoxin is identical with those in the known primary structures of several snake venom neurotoxins. Therefore it seems that the tertiary structure of cardiotoxin is closely homologous to those of the neurotoxins.

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