AMINO ACID SEQUENCE OF α-BUNGAROTOXIN FROM THE VENOM OF BUNGARUS MULTICINCTUS

D. Mebs
Institut für Rechtsmedizin, University of Frankfurt a.M., Germany

K. Narita*, S. Iwanaga and Y. Samejima
Institute for Protein Research, Osaka University, Osaka, Japan

C.Y. Lee
Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, China

Received June 16, 1971

SUMMARY: The primary structure of α-bungarotoxin isolated from the venom of Bungarus multicinctus was determined. It composes of 74 amino acid residues including ten half-cystines. Comparing the sequence with those of cobras and sea snake species, striking similarities can be found especially between α-bungarotoxin and Naja nivea α-toxin, indicating 50 % sequence homology.

Polypeptides are the toxic principles of snake venoms of the families Hydrophiidae (sea snakes) and Elapidae (cobras, Kraits, etc.). Some of the so-called neurotoxins showing curare-like action were isolated mainly from venoms of cobras (Naja and Hemachatus) and sea snakes (Laticauda) and their amino acid sequences have been elucidated (1-6). Moreover a pharmacologically well characterized toxin deriving from the venom of the Elapidae-snake Bungarus multicinctus, called "α-bungarotoxin" producing a neuromuscular block by combining irreversibly with the cholinergic receptor at the motor endplate (7,8) has been obtained in chemically homogeneous state recently In this preliminary report the amino acid sequence is presented.

*To whom requests should be addressed.

MATERIALS AND METHODS

The venom of Bungarus multicinctus either obtained from Miami Serpentarium Laboratories, Miami, USA, or freshly collected in the laboratory of one (Lee) of us was used in this study. The toxin (α-bungarotoxin) was isolated by chromatography and rechromatography on a column of CM-Sephadex C-25 using a linear buffer gradient of ammonium acetate 0.05 M, pH 5.8 to 0.5 M, pH 7.0. It proved homogeneous in polyacrylamide gel electrophoresis, sedimentation velocity, amino acid and end group analyses.

Reduced and aminoethylated (RAE-) α-bungarotoxin was prepared according to the method of RAFTERY and COLE (9). Cleavage of the methionyl linkage by cyanogen bromide was carried out in 70 per cent formic acid for 24 hr at 25°C. The two large fragments were separated by chromatography on a CM-Sephadex C-25 column (Fig. 1) and digested with α-chymotrypsin (enzyme: substrate ratio 1:20, 6 hr, 37°C, 0.2 M NH_4HCO_3 buffer pH 8.0), thermolysin (1:50, 6 hr, 40°C, pH 8.0) or trypsin (1:50, 4 hr, 37°C, pH 8.0). After separation of these chymotryptic, tryptic and thermolytic peptides by column chromatography on Sephadex G-15 (fine grade), Dowex 50-X2 and by paper chromatography or electrophoresis, their sequences were fully or partially determined by the direct Edman degradation (cf. 10) and by digestion with carboxypeptidases A and B.

RESULTS AND DISCUSSION

The molecular weight of α-bungarotoxin was estimated to be 8,000 by sedimentation equilibrium method and to be about 7,800 by thin-layer gel chromatography on Sephadex G-50 (superfine), respectively. It consists of 74 amino acid residues: Lys_6, His_2, Arg_3, Trp_1, Asp_4, Thr_7, Ser_6, Glu_5, Pro_8, Gly_4, Ala_5,

Cys_{10}, Val_5, Met_1, Ile_2, Leu_2, Tyr_2, and Phe_1.

Edman degradation of the RAE-toxin could be followed up to the 14th residue (Fig. 2). The C-terminal determination with carboxypeptidases A and B was unsuccessful and only hydrazinolysis yielded glycine as the C-terminus.

Treatment of the RAE-toxin with cyanogen bromide yielded two peptide fragments (CB-I and CB-II), which are separable on a CM-Sephadex C-25 column (Fig. 1), and their amino acid

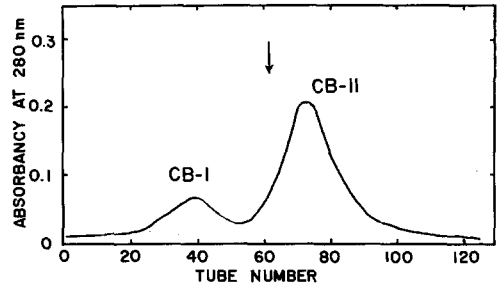

Fig. 1. Separation of the CNBr-cleaved peptides from RAE-α-bungarotoxin on a CM-Sephadex C-25 column. The material (50 mg) was applied to the column (1.5 x 9 cm), which had been equilibrated with 0.1 M ammonium acetate buffer, pH 5.8. Linear gradient elution was performed with 200 ml of the equilibration buffer in the mixing vessel and 200 ml of 0.5 M ammonium acetate buffer, pH 7.0, in the reservoir. At the point indicated by the arrow, the elution was continued with a linear gradient of ammonium acetate 0.5 M to 1.0 M, pH 7.0. The flow rate was 60 ml per hr and 6 ml fractions were collected.

compositions are shown in Table I. Amino acid sequence analysis of ten residues from the N-terminal end of CB-I showed sequence identical with that in the parental RAE-toxin. CB-I was digested with α-chymotrypsin and the resulting peptides were fractionated by gel filtration on a Sephadex G-15 column and further purified by paper electrophoresis. The major peptide (positions 5-22) was cleaved subsequently by thermolysin into two smaller peptides. All sequences were elucidated by the Edman degradation and three residues of the C-terminus by carboxypeptidases A and B treatment (Fig. 2).

Table I

Amino Acid Composition of the CNBr-Cleaved Peptides from RAE-α-bungarotoxin

Amino acid	CB-I (N-terminal peptide)	CB-II (C-terminal peptide)
	Residues/mole	
Aspartic acid	1.12 (1)	2.95 (3)
Threonine	3.61 (4)	3.04 (3)
Serine	1.98 (2)	3.45 (4)
Homoserine + lactone	0.22 + (1)	–
Glutamic acid	1.27 (1)	4.04 (4)
Proline	2.70 (3)	5.14 (5)
Glycine	1.29 (1)	3.00 (3)
Alanine	2.17 (2)	2.82 (3)
Valine	1.82 (2)	2.72 (3)
Isoleucine	1.73 (2)	0.22 (0)
Leucine	1.10 (1)	1.05 (1)
Tyrosine	0.87 (1)	1.04 (1)
Phenylalanine	–	0.82 (1)
Lysine	1.22 (1)	5.24 (5)
Aminoethylcysteine	3.17 (3)	6.96 (7)
Histidine	1.00 (1)	1.00 (1)
Arginine	1.02 (1)	2.16 (2)
Tryptophan	–	+
Total	27	47

The amino acid analysis was performed with an amino acid analyzer, Beckman/Spinco, Model MS, and Model JLC-5AH, Japan Electron Optics Lab. Ltd., by the method of Spackman *et al.* (11). The values in parentheses indicate the most probable number of residues.

CB-II, which contains tryptophan and C-terminal glycine, was digested with α-chymotrypsin and the resulting peptides were separated by chromatography on a Dowex 50-X2 column and purified by paper chromatography. Overlapping peptides obtained after thermolysin digestion of the CB-II were fractionated electrophoretically. Tryptic peptides deriving from digestion

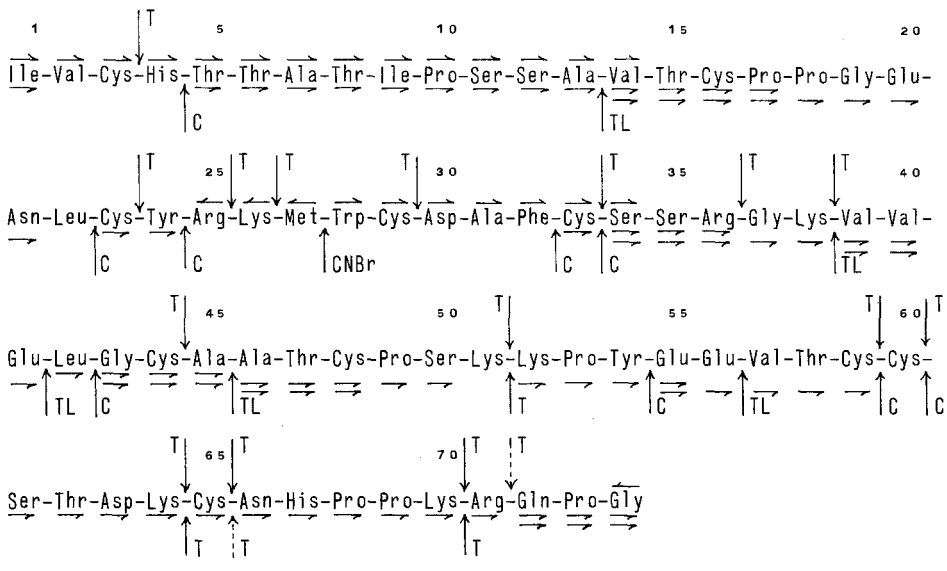

Fig. 2. Amino acid sequence of α-bungarotoxin from Bungarus multicinctus venom. Horizontal arrows above and below of amino acid residues denote, that the sequence was analyzed using RAE-toxin or CNBr-fragments and peptides of enzymatic digest, respectively. Right- and left-pointed arrows show that the sequence was determined, respectively, by the Edman degradation, carboxypeptidases A and B treatment and hydrazinolysis. The cleavage sites by cyanogen bromide (CNBr), α-chymotrypsin (C), thermolysin (TL) and trypsin (T, arrows from above: whole RAE-toxin was used) are indicated by vertical arrows.

---

of the whole RAE-α-bungarotoxin gave further information for the peptide alignment and confirmed the primary structure as presented in Fig. 2.

Comparing the sequence with those of cobra-neurotoxins (1, 2,5,6) and of sea snake-toxins (3,4) striking similarities can be found especially between Naja nivea α-toxin consisting of 71 residues and α-bungarotoxin. If deletions of the two residues at the positions 14 and 15 and of the one residue at the position 22 in the primary structure of α-bungarotoxin are considered, 37 residues including the location of the ten half-cystines are identical between Naja nivea α-toxin and α-bungarotoxin. Similar homologies among the other neurotoxins mentioned above (61-62 residues per molecule) and α-bungarotoxin are also

observed: 24 to 27 residues are identical if deletions concerning the half-cystine residues are made. Moreover, in all neurotoxins, especially the locations of tyrosine-24, lysine-26 and tryptophan-28 are exactly the same, which seem to be closely related to their biological activity and tertiary structure.

ACKNOWLEDGEMENT: This work was supported in part by the Deutsche Forschungsgemeinschaft. The authors are indebted to Professor Tomoji Suzuki for his support and encouragement, to Dr. K. Kakiuchi for molecular weight determination by sedimentation equilibrium method and to Miss Y. Hirano and Miss Y. Yagi for assistance in performing the peptide fractionation and amino acid analyses.

REFERENCES

(1) Eaker, D.L. and Porath, J., 7th Internat. Congr. Biochem., Tokyo, 1967, Abstr. III, p.499.
(2) Yang, C.C., Yang, H.J. and Huang, J.S., Biochim. Biophys. Acta, 188, 65 (1969).
(3) Sato, S. and Tamiya, N., Biochem. J., 122, 453 (1971).
(4) Endo, Y., Sato, S., Ishii, S. and Tamiya, Y., Biochem. J., 122, 463 (1971)
(5) Botes, D.P. and Strydom, D.J., J. Biol. Chem., 244, 4147 (1969).
(6) Botes, D.P., Communication at the 2nd Internat. Symposium on Animal and Plant Toxins, Tel-Aviv, Israel, 1970.
(7) Chang, C.C. and Lee, C.Y., Arch. Intern. Pharmacody., 144, 241 (1963).
(8) Lee, C.Y. and Chang, C.C., Mem. Inst. Butantan, Int. Symp. on Animal Venoms, 33, 555 (1966).
(9) Raftery, M.A. and Cole, R.D., Biochem. Biophys. Res. Commun., 10, 467 (1963).
(10) Iwanaga, S., Wallén, P., Gröndahl, N.J., Henschen, A. and Blomback, B., Eur. J. Biochem., 8, 189 (1969).
(11) Spackman, D.H., Stein, W.H. and Moore, S., Anal. Chem., 30, 1190 (1958).