PRESENCE OF AN IDENTICAL AMINO ACID SEQUENCE IN THE NEUROTOXINS OF Androctonus australis hector.

Hervé Rochat, Catherine Rochat, François Miranda et Serge Lissitzky

Laboratoire de Biochimie Médicale, Faculté de Médecine et de Pharmacie, Bd d'Alès, Marseille 5, France.

Received August 29, 1967

Two low-molecular weight, basic neurotoxins (I and II) have been purified from the venom of the scorpion Androctonus australis (Rochat et al., 1967). The pure proteins consist of 64 amino acid residues arranged in a single peptide chain cross-linked by 4 disulfide bridges. The amino acid composition of the toxins is quite different but they show an identical biological activity. Preliminary studies on the primary structure of these proteins are reported in this communication. They show a common sequence of at least nine amino acid residues near the N-terminal end :

Tox.I : NH_2-Lys-Arg-Asp-Gly-Tyr-Ile-Val-Asp-Cys-Val-Asn-Pro-Tyr-

Tox.II: NH_2-Val-Lys-Asp-Gly-Tyr-Ile-Val-Asp-Cys-Val-Asn-Thr-Tyr-

Methods and Material

Performic acid oxidations were carried out according to Hirs (1956). Performic acid was prepared by adding 1 ml of 30 % H_2O_2 to 15 ml of 99 % formic acid. The solution was allowed to stand 2h at 20°. 4 ml of methanol were then added and the mixture was cooled to -15°. Toxin I (44mg) and toxin II (20mg) dissolved in 1.2 ml and 0.6ml of 99 % formic acid were added to 7.6 ml and 3.8 ml of the oxidizing mixture respectively and allowed to stand for 2.5h at -15°. After dilution with ice water (100 and 50 ml) the solution was lyophilized twice.

Amino acid sequences were determined by the Edman procedure (Blombäck et al., 1966). The PTH-amino acids were identified by paper chromatography (Edman and Sjöquist, 1956). Confirmation was obtained by amino acid analysis before and after each degradation step (substractive method).

Tryptic and chymotryptic digestions of the performic acid oxidized toxins (0.4 % in 0.1M ammonium bicarbonate pH 8.6) were carried out for 6h at 38° with an enzyme to protein ratio of 2 % by weight. All the tryptic and chymotryptic peptides have been separated either by Sephadex filtration or by fingerprinting (Rochat et al. to be published). Amino acid analysis were performed by ion-exchange chromatography (Piez and Morris, 1960) using the Autoanalyzer Technicon. Hydrolysis were carried out in 6.0 N HCl at 110° for 20 and 70h.

Dimethylallylamine was from K and K (Plainview, N.Y., USA) phenylisothiocyanate from Fluka (Buchs, Switzerland) and trypsin 2 x cryst. and chymotrypsin 3 x cryst. from Worthington (Freehold, N.J., USA).

Results

Performic acid oxidized and native toxins showed the same amino acid composition except that cysteic acid replaced cystine residues and that tryptophan was destroyed in the oxidized toxins. 0.9 tyrosine residue was lacking in the case of oxidized toxin II.

The Edman procedure performed on native toxin I (1.5 µmole) gave the following sequence : H_2N-Lys-Arg-Asp-Gly-Tyr-Ile-Val- . One of the tryptic peptides (T2) of oxidized toxin I had the following amino acid composition : $(CySO_3H)_4$, Asp_4, Ser_1, Pro_3, Gly_2, Val_2, Ile_2, Leu_1, Tyr_3, Lys_1, His_1 .

Peptide T2 (0.8 µmole) was submitted to the Edman procedure including the " substractive " approach, up to the 6th cycle. Due to inadequate residual material, the nature of the subsequent amino acid residues was ascertained only by identification of the PTH-derivatives. The N-terminal sequence of peptide T2 was : Asp-Gly-Tyr-Ile-Val-Asp-$CySO_3H$-Val-Asn-

The Edman method performed on native toxin II (1.3 µmole) gave the following sequence : NH_2-Val-Lys-Asp-Gly-Tyr-Ile-Val-Asp-X_1- Val-Asn-Thr-Tyr-X_2 -Phe- . X_1 and X_2 corresponded to steps that gave no free PTH-amino acid. Amino acid compositions of the residual peptides before and after these degradation steps were identical.

---

* In a previous paper (Rochat et al., 1967) lysine was given as the N-terminal amino acid. This confusion was probably due to incomplete hydrolysis of the DNP-protein which gave rise to DNP-Val-εDNP-Lys, the migration of which in the solvent system used for paper chromatography was identical to that of di-DNP-Lys .

Table I. Studies on the N-terminal end of toxin I

Material	Method	Results
Native toxin	Edman	NH_2-Lys-Arg-Asp-Gly-Tyr-Ile-Val-
Peptide T2*	Edman	Asp-Gly-Tyr-Ile-Val-Asp-Cy(SO_3H)-Val-Asn-(15 amino acid residues)
Peptide T1*	HCl**	Lys,Arg
Peptide C7*	"	Lys,Arg,Asp,Gly,Tyr
Peptide C13*	"	Ile,Val,Asp,Cy(SO_3H),Val,Asp,Pro,Tyr
N-terminal sequence :		1 2 3 4 5 6 7 8 9 10 11 12 13 NH_2-Lys-Arg-Asp-Gly-Tyr-Ile-Val-Asp-Cys-Val-Asn-Pro-Tyr-

* T and C refer to tryptic and chymotryptic peptides of the performic acid oxidized toxin.

** Hydrolysis by 6.0 N HCl.

Table II. Studies on the N-terminal end of toxin II

Material	Method	Results
Native toxin	Edman	NH_2-Val-Lys-Asp-Gly-Tyr-Ile-Val-Asp-X_1-Val-Asn-Thr-Tyr-X_2-Phe
Peptide T6 *	HCl**	Val, Lys
Peptide T9 *	"	Asp, Gly, Tyr, Ile, Val, Asp, Cy(SO_3H), Val, Asp, Thr, Tyr, Cy(SO_3H), Phe, Asp, Gly, Arg

N-terminal sequence :

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
NH_2-Val	Lys	Asp	Gly	Tyr	Ile	Val	Asp	Cys	Val	Asn	Thr	Tyr	Cys	Phe	(Asp,	Gly)-	Arg

* T refers to tryptic peptides of the performic acid oxidized toxin

** Hydrolysis by 6.0 N HCl

Discussion

Table I summarizes the results relevant to the N-terminal sequence of toxin I. Peptide T2 contains the 3 tyrosine residues of the protein. As it shares a common sequence of 5 amino acid residues with the N-terminal sequence of the native toxin (one of which is a tyrosine residue) it necessarily links to the N-terminal dipeptide T1. When compared to the amino acid sequence determined by the Edman method on native toxine I, peptide C7 has an amino acid composition corresponding to the first 5 N-terminal amino acid residues. Peptide C13 which contains one tyrosine residue is a fragment of peptide T2. As it contains the same number of valine residues as peptide T2, it should continue peptide C7. Finally the position of residues 12 and 13 are infered from the bond specificity of chymotrypsin. From these observations, the N-terminal sequence of the first 13 amino acids residues of toxin I has been established (table I).

Table II summarizes results concerning toxin II. The amino acid composition of peptides T6 and T9 is given. As compared with the results of Edman degradation on native toxin II, the position of T9 which contains the single isoleucine and phenylalanine residues of the protein, is unambiguous. Moreover it contains 2 cysteic acid residues which likely correspond in the degradation of native toxin to steps 9 and 14 (X_1 and X_2) which gave no PTH-amino acid. The N-terminal sequence of the 15 first residues of toxin II is given in table II.

To sum up, toxins I and II of Androctonus australis show a common sequence extended from the aspartic acid residue in position 3 to the tyrosine residue in position 13 with the substitution of proline in position 12 (toxin I) by threonine (toxin II). Further studies are in progress to show whether this analogy extends further along the chains and how it could be correlated with the identical biological activity of the toxins.

Acknowledgements : We thank Prof. Rolland and Mrs Vinet for the amino acid analysis.

REFERENCES

Blombäck, B., Blombäck, M., Edman, P. and Hessel, B., Biochim. Biophys. Acta, 115, 371 (1966).
Edman, P. and Sjöquist, J., Acta chem. Scand., 10, 1507 (1956).
Hirs, C.H.W., J. Biol. Chem., 219, 611 (1956)
Piez, K.A. and Morris, L., Analyt. Biochem., 1, 187 (1960).
Rochat, C., Rochat, H., Miranda, F. and Lissitzky, S., Biochemistry 6, 578 (1967).