SNAKE VENOM TOXINS

THE COMPLETE AMINO ACID SEQUENCE OF CYTOTOXIN $V^{II}4$ FROM THE VENOM OF NAJA MOSSAMBICA MOSSAMBICA

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SUMMARY

The primary structure of cytotoxin $V^{II}4$, consisting of 60 amino acid residues cross-linked by four disulphide bridges, was determined. Cytotoxin $V^{II}4$ differs in altogether 17 residue positions from the other cytotoxins of the same snake. Comparing the amino acid sequence of cytotoxin $V^{II}4$ from Naja mossambica mossambica venom to the known sequences of cytotoxins from the genera Naja and Haemachatus, 33 residues, including 8 half cystines, are identical. The amino acid sequence of cytotoxins as a group has 11 residue positions in common with the amino acid sequence of neurotoxins and based on this observation, 8 residues in the primary structure of cytotoxins could be implicated as probably being functionally important.

In a previous communication (1) the amino acid sequences of three cytotoxins (cardiotoxins), isolated (2) from the venom of Naja mossambica mossambica have been described. It was found that these cytotoxins comprise more than 50% of the whole venom and also that the LD_{50} values show an inverse relationship to the quantities of each present in the whole venom. An unique situation was thus presented where determination of the primary structure of these cytotoxins could reveal the significance of these observations as well as aid in the identification of the functionally important amino acid positions.

Cytotoxin $V^{II}4$ being the fourth member of the cytotoxins isolated from N. m. mossambica venom was the minor component and also the least toxic (2). Compared to the other cytotoxins of the same snake, the amino acid composition of cytotoxin $V^{II}4$ was unusual in that it was

devoid of tryptophan and phenylalanine. This paper reports the amino acid sequence of cytotoxin $V^{II}4$.

MATERIALS AND METHODS

Cytotoxin $V^{II}4$ was prepared from the venom of N. m. mossambica as described (2). The preparation of reduced and S-carboxymethylated cytotoxin, digestion with trypsin and aminopeptidase M, amino acid analyses and structure determination of the whole protein and nomenclature of peptides have been described previously (1). Chymotrypsin digestion was carried out according to the method by Botes and Strydom (3) in the presence of trypsin inhibitor (1 mg for each mg of chymotrypsin). Initial purification of peptides consisted of chromatographic fractionation on DEAE- and CM-cellulose columns. Peptides were purified using high-voltage electrophoresis, paper chromatography and Sephadex G-25 chromatography (1). Sequence determination of peptides was by a modified Edman and Begg procedure (4) utilising three extractions with n-hexane:benzene (5:1, v/v) (5) after the coupling stage and three extractions with diethyl ether (4) after the cleavage reaction.

RESULTS AND DISCUSSION

The chromatograms for the fractionation of tryptic and chymotryptic digests of cytotoxin $V^{II}4$ on DEAE-cellulose are given in Figs. 1 and 2 respectively. Fraction A (Fig. 1) contained peptides that could not be purified in sufficient purity and quantity by the techniques employed and was therefore subjected to rechromatography on CM-cellulose (Fig. 1, inset) followed by the usual peptide purification procedures. The amino acid composition of the tryptic and chymotryptic peptides is given in Tables I and II respectively. No S-carboxymethylcysteine was found in hydrolysates of unreduced S-carboxymethylated toxin. Employing the Beckman sequenator the first 43 amino acid residues (Fig. 3) could be established. The alignment of peptides T-1 to T-7 and C-1 to C-3 was obvious from their amino acid compositions and the known NH_2-

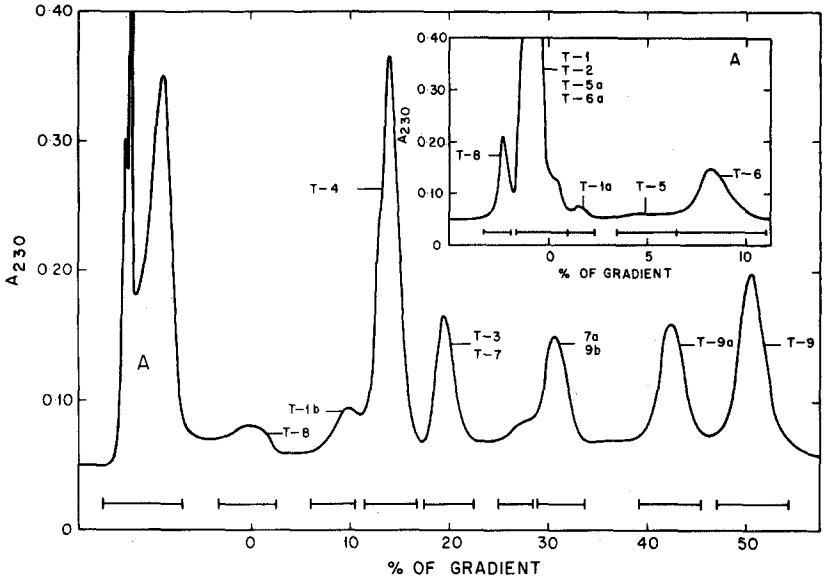

Fig. 1 Chromatography of tryptic digest of reduced and S-carboxymethylated $V^{II}4$ on DEAE-cellulose (1.9 cm x 50 cm). Gradient elution was from 0.05 to 0.6M NH_4HCO_3 over 2 liters. Inset: Rechromatography of Fraction A on CM-cellulose (0.9 cm x 150 cm) and gradient elution from 0.025 to 0.5M NH_4HCO_3 over 2 liters.

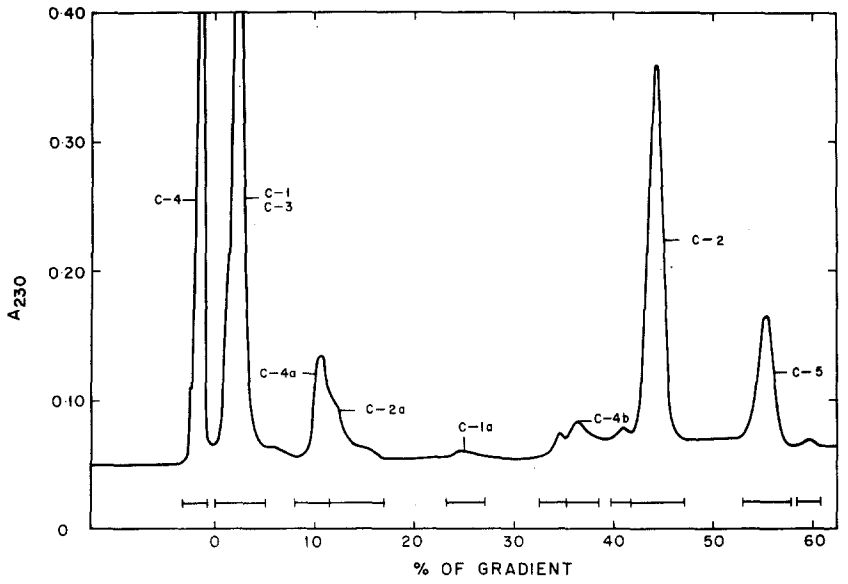

Fig. 2 Fractionation of chymotryptic digest of reduced and S-carboxymethylated $V^{II}4$ on DEAE-cellulose (0.9 cm x 150 cm). Gradient elution was from 0.025 to 0.6M NH_4HCO_3 over 2 liters.

TABLE I

AMINO ACID COMPOSITION OF TRYPTIC PEPTIDES OF REDUCED AND S-CARBOXY-METHYLATED CYTOTOXIN V^{II}_4

Values are given in residues per molecule. The numbers in parentheses are the assumed number of residues. Analytical values lower than 0.1 residue are not reported.

Amino acid	V^{II}_4	T-1	T-1a	T-1b	T-2	T-3	T-4	T-5	T-5a	T-6	T-6a	T-7	T-7a	T-8	T-9	T-9a	T-9b
Lysine	9.82(10)	2.07(2)	1.06(1)	0.99(1)	1.10(1)	0.99(1)	0.97(1)	1.97(2)	1.04(1)	1.00(1)	1.00(1)	1.05(1)	0.98(1)	0.95(1)			
Arginine	1.97(2)									0.76(1)		0.91(1)			1.04(1)	1.06(1)	
S-carboxy-methyl cysteine	7.44(8)	0.80(1)		0.90(1)		1.01(1)	0.96(1)					1.84(2)	1.79(2)		3.16(3)	1.92(2)	0.88(1)
Aspartic acid	5.94(6)	0.99(1)		1.03(1)			1.00(1)					1.04(1)	1.08(1)	0.73(1)	1.79(2)	1.20(1)	1.00(1)
Threonine	2.02(2)					0.90(1)									1.13(1)	1.08(1)	
Serine	2.68(3)							1.11(1)	0.85(1)					0.94(1)	0.96(1)	0.94(1)	
Glutamic acid	1.05(1)					0.98(1)											
Proline	4.29(4)				1.02(1)	1.08(1)				0.84(1)	0.91(1)	1.08(1)	1.04(1)				
Glycine	1.94(2)					1.04(1)						1.09(1)	0.93(1)	0.20			
Alanine	2.85(3)			0.20	0.95(1)			1.30(1)	0.96(1)					1.06(1)			
Valine	5.02(5)									1.65(2)	1.59(2)	0.73(1)	1.06(1)	0.84(1)	1.10(1)	0.99(1)	
Methionine	2.94(3)							1.56(2)	1.63(2)	0.81(1)	0.92(1)			0.23			
Isoleucine	2.78(3)			0.39	1.93(2)							0.93(1)	1.00(1)				
Leucine	4.82(5)	0.93(1)	0.94(1)	0.24	0.86(1)		1.05(1)	1.06(1)	1.01(1)					1.02(1)			
Tyrosine	2.94(3)			0.20	0.90(1)		0.98(1)								0.93(1)	0.90(1)	
Total	60	5	2	3	7	6	5	7	6	6	5	9	8	6	10	8	2
Yield (%)		28	24	30	64	81	94	0.3	49	50	33	9	44	34	78	28	21

TABLE II

AMINO ACID COMPOSITION OF CHYMOTRYPTIC PEPTIDES OF REDUCED AND S-CARBOXYMETHYLATED CYTOTOXIN $V^{II}4$

Values are given in residues per molecule. The numbers in parentheses are the assumed number of residues. Analytical values lower than 0.1 residue are not reported.

Amino acid	C-1	C-1a	C-2	C-2a	C-3	C-4	C-4a	C-4b	C-5
Lysine	2.00(2)	0.98(1)	2.01(2)	2.04(2)	1.03(1)	4.29(5)	2.85(3)	1.76(2)	
Arginine		0.11				0.94(1)	0.96(1)	0.89(1)	0.92(1)
S-carboxymethyl cysteine	1.08(1)	1.00(1)	2.18(2)	0.58(1)		1.96(2)	1.72(2)	1.06(2)	2.99(3)
Aspartic acid	1.04(1)	1.06(1)	1.29(1)	1.33(1)		2.00(2)	1.98(2)	2.06(2)	2.08(2)
Threonine			0.83(1)	1.03(1)					0.92(1)
Serine		0.15				1.67(2)	0.89(1)	1.03(1)	0.92(1)
Glutamic acid			1.08(1)	1.21(1)					
Proline	0.98(1)	0.22	0.97(1)	1.00(1)		1.80(2)	1.61(2)	1.51(2)	
Glycine		0.20	1.10(1)	1.32(1)		0.99(1)	1.10(1)	1.25(1)	
Alanine	0.95(1)	0.25				1.77(2)	1.07(1)	1.05(1)	
Valine		0.27				3.43(4)	4.05(4)	2.78(3)	0.94(1)
Methionine		0.24			1.97(2)	0.96(1)			
Isoleucine	1.81(2)	0.30				0.91(1)	1.02(1)	0.88(1)	
Leucine	1.91(2)	0.96(1)	1.11(1)	1.21(1)		1.40(2)	1.14(1)	1.01(1)	
Tyrosine	0.76(1)	0.16	0.92(1)			0.82(1)	0.87(1)		
Total	11	4	11	9	3	26	20	17	9
Yield (%)	41	29	47	3	34	51	24	5	95

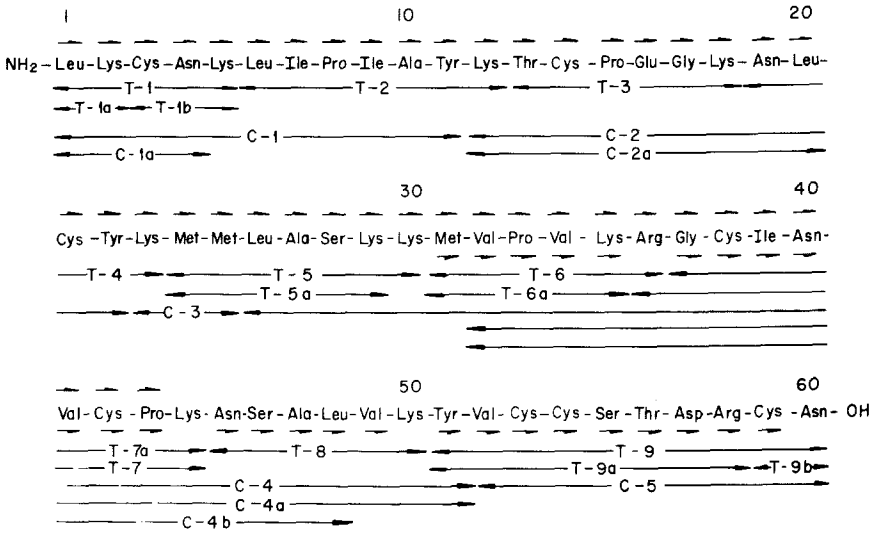

Fig. 3 Amino acid sequence of cytotoxin $V^{II}4$. Tryptic and chymotryptic peptides which were found, are indicated. Upper half-arrows indicate the extent of the automatic sequence determination while the lower half-arrows indicate the extent of manual Edman degradation.

terminal sequence. The amino acid sequences of peptides T-6, T-7a, T-8, T-9 and T-9a, were obtained by the manual operation of the modified Edman and Begg procedure (4,5). When peptides T-6, T-7 and T-8 are aligned in the sequence as shown in Figure 3, the resultant peptide has the amino acid composition of peptide C-4b minus a NH_2-terminal methionine and COOH-terminal valine and lysine. This information in conjunction with the amino acid composition of peptides C-4 and C-4a and the data obtained from the Beckman sequenator, allows peptide T-8 to be placed COOH-terminal to peptide T-7. An overlap of peptides C-4 and C-4a with the unique NH_2-terminal tyrosine of peptide T-9 allows the latter peptide to be placed COOH-terminal to peptide T-8 thereby furnishing the complete amino acid sequence of cytotoxin $V^{II}4$. Aminopeptidase M digestion established an asparaginyl residue to be the COOH-terminal of peptide T-9b and hence the whole protein. The complete sequence of cytotoxin $V^{II}4$ is presented in Fig. 3.

Comparing the sequence of cytotoxin $V^{II}4$ with those of cytotoxins $V^{II}1$, $V^{II}2$ and $V^{II}3$ from the same snake (1), seventeen dissimilarities (residue positions 5, 9-11, 25-30, 40, 45, 47, 49, 52, 55 and 58) are noted. Four of these amino acid positions are also different when the primary structure of cytotoxin $V^{II}4$ is compared to the known primary structures of other cytotoxins from genera Naja (1, 6, 7, 8, 9, 10) and Haemachatus (11) (see alignment chart in Ref. 1). Foremost is the substitution of an invariant methionine residue at position 26 by leucine. Although hydrophobicity is conserved at this position, a potentially functional thio-ether group in methionine was replaced by a gem-dimethyl group in leucine. Position 40 in cytotoxin $V^{II}4$ was identified as an asparaginyl residue in contrast to the invariant aspartyl residue found for the other cytotoxins. As noted previously for position 57 in cytotoxins $V^{II}1$ and $V^{II}3$ from N. m. mossambica venom (1), an anomaly exists at an amino acid position identified as aspartate since it could arise by deamidation of an asparaginyl residue. It is imperative, therefore, that position 40 of the cytotoxins should be considered as invariable until more information about this position becomes available. The other two substitutions (Ala 47 and Ser 55) in cytotoxin $V^{II}4$ are identical to the substitutions found in only one other cytotoxin namely, $V^{II}1$ of N. haje annulifera (9). From the comparison of all cytotoxin structures, 33 residues including 8 half cystines are identical. Eleven of these (Cys 3, 14, 21, 38, 42, 53, 54, 59, Tyr 22, Gly 37 and Pro 43) are identical in all snake venom toxins and are believed to be essential for the general folding of the peptide chain (12). Eight (Leu 6, Lys 18, Leu 20, Met 24, Pro 33, Lys 35, Asp/Asn 40 and Ser 46) of the remaining residues emerge as exclusively invariant for cytotoxins when their sequences are compared to the sequences of the short and long neurotoxins (see Ref. 12 for alignment of sequences where the corresponding positions are Leu 9,

Lys 21, Leu 23, Met 27, Pro 40, Lys 42, Asp 47 and Ser 53). The fact that these amino acid positions have been conserved suggests that they are important for the biological activity of cytotoxins.

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