

Characterization of two different peptides from the venom of the scorpion *Buthus indicus*

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Received 6 September 1989

Two disulfide-rich, low-molecular mass peptides (~3 kDa and ~4 kDa) have been isolated from *Buthus indicus* venom using ion-exchange and reverse-phase HPLC. Peptide I has 35 residues with 8 half-cystine residues and is clearly related to four-disulfide core proteins of the neurophysin type and to toxins of other scorpion species (55–63% residue identity). Peptide II, present in low yield, has 28 residues with 6 half-cystine residues and a structure largely dissimilar from that of peptide I and other characterized toxins, although probably still a member of the disulfide core peptide type. Consequently, scorpion venom contains, in addition to toxins characterized before, toxin-like compounds with distant relationships.

Scorpion venom; Half cystine; Peptide characterization; Amino acid sequence; Homology

1. INTRODUCTION

Scorpion venom is a mixture of largely basic toxic polypeptides, containing 28–78 amino acid residues in single chains [1–4]. Several of these toxic polypeptides have been used for neurobiological studies [4] and for distinguishing ion channels [5]. Of the known scorpion families, toxins have been best characterized from Buthidae, where two main size groups have been found, one with 67–71 residues [6,7], the other with 36–39 residues [8]. During investigation of the smaller group in the venom of *Buthus indicus* from Sind, Pakistan, we purified two toxin-like peptides, one with 35 residues and related to the previously characterized toxin from other species, the other with 28 residues and apparently not closely related.

2. MATERIALS AND METHODS

Venom was extracted from scorpion (*Buthus indicus*) by electrical stimulation in deionized water, centrifuged at 10000 rpm, and lyophilized. Peptide I, constituting a major fraction, was isolated by direct HPLC of the crude venom on Vydac C18 (Phenomenex, New York) in 0.1% trifluoroacetic acid with a linear gradient of acetonitrile. The peptide was recovered from peak Bus II of the elution curve as shown in [12]. Peptide II was isolated by direct HPLC

of the venom on a cation-exchange column (TSK CM-5PW, LKB) in 20 mM sodium acetate, pH 4.5, with a linear gradient of 0–0.5 M NaCl. It constitutes a minor fraction as shown in fig.1, and was rechromatographed on Vydac C18 to give the pure component. SDS-polyacrylamide gel electrophoresis utilized 15% gels with Tris-glycine, pH 8.3, 0.1% SDS.

The two peptides were reduced with dithioerythritol and S-carboxymethylated with ¹⁴C-labelled iodoacetate in 0.4 M Tris-HCl [9]. Amino acid sequences were determined manually by the dimethylaminoazobenzene/phenylisothiocyanate double coupling method [10], and by degradations with Beckman 890C and Applied Biosystems 470A sequencers. Phenylthiohydantoin derivatives were identified by liquid-phase chromatography [11]. Amino acid compositions were determined with a Beckman 121M amino acid analyzer after hydrolysis with 6 M HCl/0.5% phenol at 110°C for 24 h.

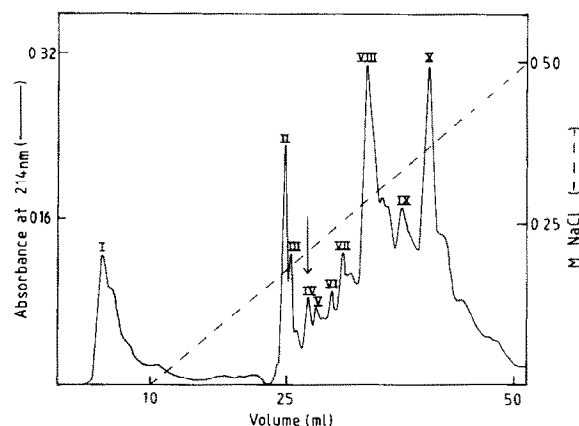


Fig.1. Purification of peptide II from scorpion crude venom. Column: TSK CM-5PW in 20 mM sodium acetate, pH 4.5, and elution with a gradient (dashed line) of 0–0.5 M NaCl. The arrow indicates the elution position of peptide II.

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The sequences presented here have been submitted to the EMBL/GenBank database under accession number Y07514



Fig.2. Primary structures of scorpion peptides I and II. Amino acid sequences were determined by direct degradation of the reduced and carboxymethylated polypeptides.

3. RESULTS

3.1. Peptide purification

Crude venom was fractionated by cation-exchange HPLC and reverse-phase HPLC as given in section 2, to produce several components [12]. Two of these peptides give a single band on SDS-polyacrylamide gel electrophoresis, with molecular mass ranges of 4 kDa for peptide I (purified as in [12]) and 3 kDa for peptide II (purified as shown in fig.1). Yields from venom were about 60 nmol for peptide I and 20 nmol of peptide II. Both peptides were pure as judged by N-terminal sequence analysis, showing one structure in each case.

3.2. Primary structure

The amino acid sequences of both peptides were determined (fig.2). The two peptides were degraded to the C-termini by liquid-phase sequencer analysis for peptide I and gas-phase sequencer analysis for peptide II. Both structures are in excellent agreement with the compositions from acid hydrolysis (table 1). Positions of cysteine derivatives were confirmed by radioactivity of the extracts from degradation of the ¹⁴C-carboxymethylated parent peptides.

Table 1

Amino acid composition of the scorpion venom peptides I and II

Residues	Peptide I	Peptide II
Cys(Cm)	7.7 (8)	6.0 (6)
Asx	2.1 (2)	4.2 (4)
Thr	2.5 (3)	1.0 (1)
Ser	1.0 (1)	—
Glx	2.0 (2)	3.0 (3)
Pro	2.8 (3)	2.0 (2)
Gly	4.8 (5)	3.0 (3)
Ala	0.9 (1)	1.0 (1)
Val	—	2.8 (3)
Met	0.9 (1)	1.2 (1)
Leu	1.0 (1)	—
Tyr	0.9 (1)	—
Phe	1.0 (1)	—
Lys	5.0 (5)	2.9 (3)
His	—	0.9 (1)
Arg	1.0 (1)	—
Sum	35	28

Values are molar ratios after acid hydrolysis, and, within parentheses, the sums from the sequence analysis. Cys was determined as Cys(Cm) after reduction and carboxymethylation

4. DISCUSSION

Peptides I and II are not closely related, the smaller peptide II is not a fragment of the larger peptide I, and their half-cystine spacings are different. Thus, although both peptides belong to the smaller of the two peptide groups in scorpion venom [6], they differ substantially.

Peptide I is a form related to the small toxic peptides previously characterized in scorpion venom [13]. As shown in fig.3, it is similar to the venom peptide characterized from *Buthus eupeus* (Central Asian scorpion), 21 of 35 residues are identical, and to that of *Androctonus mauretanicus*, showing 24 identities. In all three cases, the half-cystine distributions are identical, suggesting conservation of folding patterns. However, considering the extensive conservation of other, small disulfide-linked toxins [9], the many residue replacements now found, with exchanges at close to half of all positions, is noteworthy. Apparently, functional requirements on the structure are not strict, allowing rapid evolutionary changes of the corresponding genes.

Peptide I, and two previously characterized scorpion peptides, are related to neurophysins, agglutinins [14,15] and other peptides of the four-disulfide core protein family with the toxin/agglutinin fold. The common pattern is shown in fig.4, showing similar half-cystine arrangements. Peptide II may also belong to this structural type although the end half-cystine residues appear to be missing as in other structures of this family (cf. fig.4). Consequently, although possibly a member of this group, peptide II is not closely related to the other members of the family and differs greatly in primary structure. Instead, judging from size and half-cystine content, it appears possible that peptide II correlates more closely with peptides isolated from *Chactoid* scorpions [1]. However, those peptides have apparently not been further analyzed, and relationships, if any, cannot yet be judged. Consequently, peptide II is thus far a peptide with unknown details of family affiliation. It should be noted that both peptides I and II are extremely rich in half-cystine residues and therefore tightly folded. This is also a typical property of other toxins, like for example the unrelated heat-stable bacterial toxins [9]. In all these cases, close to 25% of all residues are half-cystine, presumably allowing extremely tight and stable folds.



Fig.3. Comparison of the peptide I structure with those of related peptides from other scorpion species [8,13]. Gaps are indicated by dashes and identical residues are boxed.

	2	5	16	1920	28	33	35	
Scorpion venom Peptide I	C -2- C	-10- C	-2- C	CC -7- C	-4- C	-1- C	C	
	10	13	21	2728	34	44	54	
Neurophysin Domain I	C -2- C	-7- C	-5- C	CC -5- C	-9- C	-9- C	C	
		61	67	7374	79	85		
Domain II		C -5- C	-5- C	CC -4- C	-5- C	C		
		3	10	1920	24	26		
Scorpion venom Peptide II		C -6- C	-8- C	CC -3- C	-1- C	C		
		5	12	1718	24	31	35	40
Wheat Germ Agglutinin		C -6- C	-4- C	CC -5- C	-6- C	-3- C	-4- C	C
		3	12	1718	24	31	37	41
Hevein (rubber tree)		C -8- C	-4- C	CC -5- C	-6- C	-5- C	-3- C	C

Fig.4. Similarities in half-cystine patterns of peptides I and II with those of other proteins of the four-disulfide core protein family [15]. In the case of peptide I, the homology is clear and the family assignment well supported. In the case of peptide II, alignment of primary structures provides few residue identities and little evidence beyond the half-cystine patterns shown. Numbers above Cys positions give numerical positions, while numbers between Cys positions show the number of intervening residues in each case. The peptide I structure is from fig.1, the other structures from [6].

Acknowledgements: This work was supported by grants from the Swedish Medical Research Council (03X-3532), and the Magn. Bergvall Foundation. Fellowships to O.U.B. from the International Seminar in Chemistry (Uppsala, Sweden), and to J.S. from the Swedish Institute allowed research visits to Sweden.

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