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PRIMARY STRUCTURE OF INSECTOTOXIN BucaIT FROM THE SUBSPECIES *Mesobuthus caucasicus*

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Scorpion venoms have been used for millennia to treat many diseases [1]. Their compositions display a high degree of heterogeneity [2]. Whole scorpion venom affects both invertebrates and vertebrates owing to the presence in it of a large amount of polypeptide toxins with 3–4 disulfide bonds. Scorpion toxins are subdivided into two classes. These are short ones with molecular weights up to 4,000 Da and long ones, up to 7,200 Da. Within these two large groups, they are classified according to their physiological action on potential-dependent Na^+-channels, K^+-channels, and Ca^{2+}-channels in addition to volume-dependent Cl channels. Scorpion toxins are widely used in medicine and the economy, in particular, to create transgenic varieties of agricultural plants (wheat, soy, rice, etc.) that exhibit high resistance to various types of parasites and are broadly represented on the market [3].

Special attention has recently been paid to the identification of insect-selective toxins that can be used to create recombinant biopesticides as an alternative to highly toxic chemical agents for battling insect pests. The goal of our work was to isolate and establish the structure of insectotoxin from venom of the subspecies *Mesobuthus caucasicus*.

According to disk electrophoresis in 15% PAAG and isoelectric focusing, whole venom from *M. caucasicus* is a multi-component mixture containing at least 20–22 different protein-peptide components. Difficultly soluble nontoxic mucoproteides (~10% of whole venom mass) were separated by centrifugation. Whole venom was separated by gel chromatography over a column of Toyo Pearl HW 55f gel and cation-exchange chromatography over CM-Toyo Pearl 650M. The chromatographic separations produced 17 peaks. Each fraction was preliminarily desalted and then tested for biological activity. Fraction Mc-4-2 had the greatest activity against mealy worm larvae. This was separated by reversed-phase HPLC over a Nucleosil $5C_{18}$ column (4.6 mm × 250 mm) using a Mashrey-Nagel (USA) HPLC and gradient elution. The buffer systems were A (0.1% TFA) and B (CH_3CN). Detection was carried out at 226 nm at sensitivity 0.2 AUFS.

During the course of physiological tests at the Institute of Physiology and Biophysics, Academy of Sciences, Republic of Uzbekistan, in collaboration with Prof. P. B. Usmanov, it was found that fraction Mc-4-2-7 at a dose of 5 μg/g caused instantaneous paralysis of cockroaches, mealy worms, and bollworm. Therefore, the insectotoxin from *M. caucasicus* scorpion venom was designated BucaIT.

The next step was to perform structural studies of the isolated polypeptide toxins. Fraction 4-2-7 was rechromatographed by HPLC over a Nucleosil $5C_{18}$ column under the aforementioned conditions and lyophilized. Mass spectroscopy determined the molecular weight (MW) of the toxin as 3830.16 Da. The MW after reduction and carboxymethylation was 4232.75 Da. The difference of 402.59 Da led to the conclusion that BucaIT contained eight cysteine residues.

The following sequence was found by establishing the structural features of BucaIT using automated determination of the amino-acid sequence by the Edman method:

				5					10				
Met-	Cys-	Met-	Pro-	Cys-	Phe-	Thr-	Thr-	Asp-	Ala-	Asn-	Met-	Ala-	Arg-
15					20					25			
Lys-	Cys-	Ser-	Asp-	Cys-	Cys-	Gly-	Gly-	Asn-	Gly-	Lys-	Cys-	Phe-	Gly-
	30					35							
Pro-	Gln-	Cys-	Leu-	Cys-	Asn-	Arg-	Ala.						

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The C-terminus Ala was determined from the difference of the MW of total amino acids 1–35 and the total MW of BucaIT.

Comparative structural studies of the BucaIT sequence using the program BLAST [4] showed that BucaIT was similar to short scorpion toxins. The best match was observed for Insectotoxin-I5A (94%), Insectotoxin-I4, Neurotoxin MeuClTx, Neutotoxin Bm 12-b (94%), Neurotoxin BmKCT (91%), Neurotoxin MeuClTx-1 (88%), Insectotoxin-I5 (85%), and Chlorotoxin (CTX)(ClTx) (82%).

A comparison of the structural features of BucaIT with other short toxins isolated from scorpion venom showed that a detailed study of the pharmacological and physiological properties of this venom was needed.

Considering the high homology of the amino-acid sequence, similar 3D structures of BucaIT and other short toxins were proposed [5]. A structural model of insectotoxin BucaIT that was generated using the Swiss-model protein modeling server [6] and was based on structural features of Chlorotoxin and Insectotoxin I5A showed that BucaIT had a typical structure for scorpion toxins consisting of one α-spiral and three antiparallel β-sheets with four disulfide bonds formed between residues Cys16–Cys31, Cys5–Cys26, Cys20–Cys33, and Cys2–Cys19.

REFERENCES

1. T. Junghanss and M. Bodio, *Clin. Infect. Dis.*, **43**, No. 10, 1309 (2006).
2. C. Goudet, C. W. Chi, and J. Tytgat, *Toxicon*, **40**, No. 9, 1239 (2002).
3. P. Christou, T. Capell, A. Kohli, and J. A. Gatehouse, *Trends Plant Sci.*, **11**, No. 6, 302 (2006).
4. S. F. Altschul, T. L. Madden, A. A. Schaffer, and J. Zhang, *Nucleic Acid Res.*, **25**, No. 17, 3389 (1997).
5. I. Huys, E. Waelkens, and J. Tytgat, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **803**, No. 1, 67 (2004).
6. K. Arnold, L. Bordoli, J. Kopp, and T. Schwede, *Bioinformatics*, **22**, No. 2, 195 (2006).