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A Novel Peptide from *Buthus martensii* Karch

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Abstrate: A novel peptide was purified and characterized from *Buthus martensii* Karch. The peptide, named BmK M6, is a single-chain polypeptide cross-linked by four intramolecular disulfide bridges. The molecular weight of the peptide was determined by MOLDI-TOF-MS as 7034 Da. The partial amino acid sequence of BmK M6 from N-terminal is VRDAYIAKPEN CVYECGITQDCNKLCTENG.

Keywords: *Buthus martensii* Karch, peptide, amino acid sequence.

To date more than 100 toxins have been identified from the scorpion venoms[1,2]. The previous studies of scorpion toxins showed that long-chain peptides had a high affinity for the sodium channel with different species specificity thus were categorized as mammalian and/or insect toxins[3, 4]. The Asia scorpion *Buthus martensii* Karch (BmK) is a species belonging to the Buthidae family. In Chinese traditional medicine, the whole scorpion, especially the tail, is used to treat neurological symptoms, such as incomplete paralysis and mimetic paralysis. Till now, several long-chain toxins were purified from the BmK venom. Some of them were mammalian toxins represented by BmK M Series[5], others were insect toxins represented by BmK IT series[6]. In this paper, we report the purification and identification of a novel peptide with little mammalian toxicity from the *Buthus martensii* Karch venom.

Experimental

The crude venom of BmK was obtained from Hebei Province. Sephadex G-50 and CM-Sephadex C-50 were purchased from Phamacia Fine Chemicals and iodoacetamide DTT, TFA from Fluka. Protein Pak^{TM} 60 column was from Waters and ZORBAX 300 SB C-18 column from Agilent.

Purification

About 3 g of BmK venom was dissolved in 100 mL distilled water and centrifuged at 1000 g for 10 min. The supernatant was first separated on a Sephadex G-50 column

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(2.6×100 cm) using distilled water as eluting solvent at 2 mL/min flow rate. Seven fractions were divided. The fifth fraction was lyophilized to obtained 2 g and dissolved in 0.01 mol/L phosphate buffer (pH 6.8), then subjected to a CM-Sephadex-C50 ion-exchange column (2.6×50 cm) previously equilibrated with the same buffer, eluting stepwise with 0.01, 0.02, 0.05, 0.1 and 0.2 mol/L of phosphate buffer, pH 6.8. Collection of the elution by 0.02 mol/L phosphate buffer was dialyzed against water and lyophilized then further purified by ZORBAX 300 SB C-18 column (9.2×250 mm) using SHIMADZU SPD-10ATVP HPLC system, elution was carried out with 15% B in 5 min and a linear gradient from 15 to 55% B in another 40 min at 2 mL/min flow rate. (Eluting solvent A: 0.1% TFA, B: 0.085% TFA in 70% acetonitrile). Three fractions were obtained. The third fraction was pooled and loaded on Protein Pak^{TM}60 column (7.8×300 mm) repeatedly, eluted with 0.2% NaCL solution at 1 mL/min flow rate with UV-Monitor at 220 nm. The second protein peak collected was pure BmK M6 with yield of 50 mg (1.6% in crude venom).

Characterization and partial sequence determination

The purity of the BmK M6 was proved by MALDI-TOF-MS, RP-HPLC and Size-exclusion HPLC. The molecular weight of BmK M6 was determined by Bruker BAFLEX^{TM} III MALDI-TOF-MS using CCA as matrix. To determine the amino acid composition, the peptide was hydrolyzed with 6 mol/L HCL at 110 °C for 24 h in sealed evacuated tube. Then loaded on the HITACHI L-8500 amino acid analysis instrument. The amino acid sequence of BmK M6 from N-terminal was determined on Applied Biosystems Procise protein sequencing system.

Reduction and S-carboxymethylation

A solution of BmK M6 (1 mg) in 0.5 mL denaturing solution of 6 mol/L guanidine-HCL, 0.01 mol/L Tris buffer (pH 8.5) was incubated at 37 °C for 2 h. Then 6 mg DTT was added and kept at 37 °C for another 8 h flushed with N_2. 10 mg of iodoacetamide was added and the mixture was kept in dark for 45 min flushed with N_2 at room temperature. Finally the mixture was dialysis with water using Dialysis Cassettes (3500MW cut off) and the S-carboxymethylated toxin (Rcam-peptide) was pooled and lyophilized.

Toxicity assay

The mammalian toxicity was tested on Kunming mice (18-22 g) as described by Meier and Theakston[7].

Results and Discussion

Through four steps combined with Sephadex-G50 chromatography, CM-Sephadex C-50 ion-exchange chromatography, RP-C-18 HPLC and Size-exclusion HPLC, 50 mg of BmK M6 was purified to homogeneity from 3g crude scorpion venom. The yield of BmK M6 was about 1.6%. The mobility of BmK M6 on the ion-exchange column

suggested it was an acid peptide. The mass spectrum showed the molecular weight of the Rcam-peptide was 7498 Da (**Figure 1**) in excess of 464 Da to that of the native peptide (**Figure 2**). This difference of 464 Da was attributed to the arrangement of four disulfide bridges into eight carboxymethylated cysteines.

Figure 1 MALDI-TOF-MS of Rcam-BmK M6

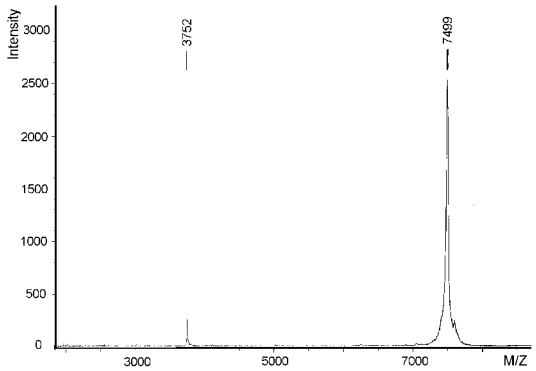
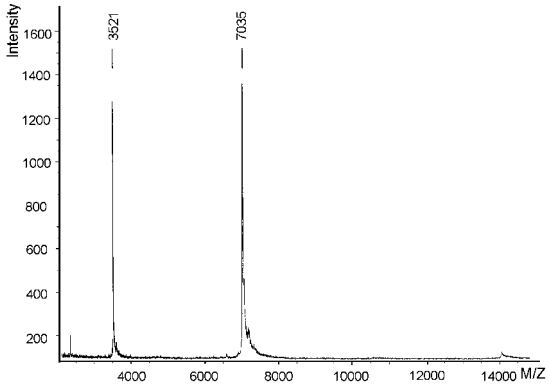


Figure 2 MALDI-TOF-MS of native BmK M6



Amino acid composition analysis and mass spectrum indicated BmK M6 was composed of 64 residues except for tryptophan as listed in **Table 1**. The automated protein sequencer gave the sequence from N-terminal with ambiguities of positions 12, 16, 22 and 26. Comparison of the sequence between BmK M6 and other BmK toxins showed that the four positions were cysteines located. In BmK mammalian toxins the cysteines were in highly conserved positions. Thus the result of the partial sequence of BmK M6 was VRDAYIAKPENCVYECGITQDCNKLCTENG. And it was found that BmK M6 was belonging to the mammalian toxin group, showing 70%, 80%, 65% sequence homologies with BmK M1[8], BmK M4[5] and BmK M8[9] respectively. The toxicity assay showed the mammalian toxicity of BmK M6 was low. In the dose of 50

mg/kg, the mice appeared symptoms of being poisoned and recovered a minute later. Chi [3] indicated the toxicity on mammalian is related to the pI of the toxin. The basic toxin was the most poisonous, the neutral was less toxic, while the acidic toxins had the lowest toxicity. BmK M6, an acid peptide, had little toxicity on mammalian. This fact also proved above couclusion.

Table 1 The amino acid composition of the BmK M6

amino acid	residue number	amino acid	residue number
Asp	6.4 (7)	Ile	3.3 (3)
Thr	1.9 (2)	Leu	2.0 (2)
Ser	2.7 (3)	Tyr	3.2 (3)
Glu	7.8 (8)	Lys	4.5 (5)
Gly	7.1 (7)	Arg	3.0 (3)
Ala	3.8 (4)	Pro	3.7 (4)
Val	3.4 (3)	His	2.0 (2)
Cys	(8[*])	Trp	ND

[a]Cysteine was determined by the molecular weight difference between native and Rcam-BmK M6.
ND: not determined

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