Purification, Characterization and ^{1}H NMR Resonance Assignment of an α-Like Neurotoxin BmK 16 from the Venom of Chinese Scorpion *Buthus martensii* Karsch

ZHANG, Nai-Xia(张乃霞)　　WU, Gong(吴宫)　　WANG, Zhong-Hua(王中华)
WU, Hou-Ming*(吴厚铭)
State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China

A natural scorpion toxin BmK 16 was purified for the first time from the venom of the Chinese scorpion *Buthus martensii* Karsch (BmK) by using combined gel-filtration, ion exchange and reversed phase chromatography. The sequence of the *N*-terminal 8 amino acid residues was determined by Edman degradation. Using the *N*-terminal sequence as a tag, the database searching revealed a hit in the scorpion cDNA Bank. The sequence for *N*-terminal 8 amino acid residues, molecular weight and amino acid compositions of BmK 16 were identical with the calculated values according to the first 64 residues' sequence of the precursor peptide alpha-neurotoxin TX16 derived from the sequence of the cDNA AF156597 (EMBL). The sequence-specific resonance assignment of BmK 16 was achieved and the intact sequence of BmK 16 was determined as followings: VRDAY IAKPH NCVYE CARNE YCNDL CTKNG AKSGY CQWVG KYGNG CWCKE LPDNV PIRVP GKCH. Furthermore, the results from the sequence homology analysis and the toxicity assays indicated that BmK 16 was an α-like scorpion neurotoxin.

Keywords scorpion toxin, *Buthus martensii* Karsch, BmK 16, α-like scorpion neurotoxin, amino acid sequence, resonance assignment

Introduction

The scorpion venoms are rich source of fascinating peptides, which usually composed of 28—70 amino acid residues. They interact specifically with the Na^+, K^+, Ca^{2+} or Cl^- ion channels and are widely served as useful tools in probing the protein mapping of ion channels and clarifying the molecular mechanism involved in the signal transmission and channel gating. The long-chain neurotoxin polypeptides (62—67 amino acid residues) isolated from scorpions can be divided into two classes, α- and β-toxins, on the basis of their effects on the sodium channels.[1] They bind to different receptor sites on the sodium channels and induce distinct electro-physiological effects. The β-toxins shift the voltage of activation toward more negative potentials causing the membrane to fire spontaneously and repetitively. The α-toxins slow, or make incomplete the inactivation of the sodium ion permeability in the channel.[2] At the same time, the α-toxins display a wide array of preferences on interaction with sodium channels of different animal phyla.[3] Accordingly, scorpion α-toxins are divided into the following three categories: (1) Classic α-toxins, which are highly active on mammals, *e.g.*, α-toxin II from the venom of the scorpion *Androctonus australis hector* (AaH II), α-toxin from the scorpion *Leiurus quinquestriatus hebraeus* (Lqh II); (2) insect α-toxins, which are highly toxic to insects and show weak activity in mammalian CNS, *e.g.*, α-toxin from *Leiurus quinquestriatus hebraeus* (Lqh αIT), α-toxin III from *L. quinquestriatus quinquestriatus* (Lqq III) and α-toxin from *Buthus occitanus tunetanus* (BotIT1); (3) α-like toxins, which are considerable toxic to both mammals and insects, *e.g.*, α-like toxin from *Leiurus quinquestriatus hebraeus* (Lqh III), α-like toxin from *Buthus occitanus mardochei* (Bom III), α-like toxin from Chinese scorpion *Buthus martensii* Karsch (BmK I).[4-7]

α-Like toxin displays special electro-physiological actions atypical to other α-toxins. For example, Lqh III inhibits the sodium current inactivation in the cockroach axon, but induces in addition a resting depolarization due to a slowly decaying tail current. The specific binding site of α-toxins and α-like toxins on the extracellular surface of sodium channels has been identified as the receptor 3 using competitive binding studies.[4,8] It has been demonstrated that receptor 3 on insect sodium channels is homologues but not identical to its mammalian counterpart.[8] In addition, the α-like toxin is identified binding to different sodium channel subtypes other than those identified by the classic α-toxins.[9] Recently, it has been discovered that α-like toxins have ability to specifically target a somatic Na channel subtype in the mammalian brain.[10] This discovery boosts the interest in α-like toxins.

In this paper, we report the purification, the charac-

* E-mail: hmwu@mail.sioc.ac.cn; Tel.: 86-21-64163300; Fax: 86-21-64166128
Received December 19, 2002; revised March 25, 2003; accepted June 27, 2003.
Project supported by the National Natural Science Foundation of China (No. 20132030).

terization and the sequence determination of an α-like toxin named BmK 16 from the venom of Chinese scorpion *Buthus martensii* Karsch. The isolation and purification of this peptide were carried out by a hybrid chromatographic procedure consisted of gel-filtration, ion-exchange chromatography and reversed-phase HPLC. Edman degradation sequencing and 2D NMR techniques were employed to investigate the primary structure of BmK 16.

Materials and methods

Materials

Scorpions of the species *Buthus martensii* Karsch were collected from local culture farms in Henan province, China. The crude venom was obtained by electrically stimulating of the telson (the last caudal segments containing the venom gland) of scorpions. The crude venom was preserved after it had been lyophilized. Acetonitrile was of spectroscopic grade. Other chemical reagents were of analytical reagent grade. Trifluoroacetic acid (TFA) was purchased from Merck. D_2O and DCl were obtained from Sigma. NaOD was available from Aldrich Chemical Company.

Sample preparation and chemical characterization

The peptide BmK 16 was isolated and purified from the venom of Chinese scorpion *Buthus martensii* Karsch as described previously.[11] The peptide purification was carried out by a hybrid chromatographic procedure consisted of gel-filtration, ion-exchange FPLC and reversed-phase HPLC. The crude venom was dissolved in ammonium bicarbonate buffer (50 mmol/L, pH 8.5) and centrifuged at 4000 g for 15 min. The supernatant was applied to a Sephadex G-50 column (2.5 cm × 150 cm, Pharmacia Fine Chemicals), which was equilibrated and eluted with ammonium bicarbonate buffer (50 mmol/L, pH 8.5). The further separation of fraction IV from the Sephadex G-50 column was performed on a Mono S column (HR 5/5, Pharmacia), with a step gradient of solution A (20 mmol/L sodium acetate buffer, pH 5.0) to solution B (20 mmol/L sodium acetate buffer containing 1 mol/L NaCl) (see Fig. 1B). Fraction 12 from the Mono S column was further purified by HPLC using a C_{18} column (5 μm, 4.6 mm × 250 mm, Alltech), eluted at a flow of 1 mL/min with a linear gradient from 75% solution A (water containing 10% acetonitrile and 0.1% trifluoroacetic acid) to 40% solution B (acetonitrile containing 20% water and 0.1% trifluoroacetic acid). The peptide BmK 16 was obtained after the HPLC chromatography.

The molecular weight of BmK 16 was determined using a triple-stage quadruple mass spectrometer (Quattro VG, Micromass, UK). Amino acid analysis was performed on a Beckman 6300 apparatus (Beckman, USA) after hydrolysis of the sample in 6 mol/L HCl under vacuum at 110 ℃ for 20 h. The *N*-terminal sequence of BmK 16 was achieved by Edman degradation using a Beckman LF3200 Protein-Peptide sequencer.

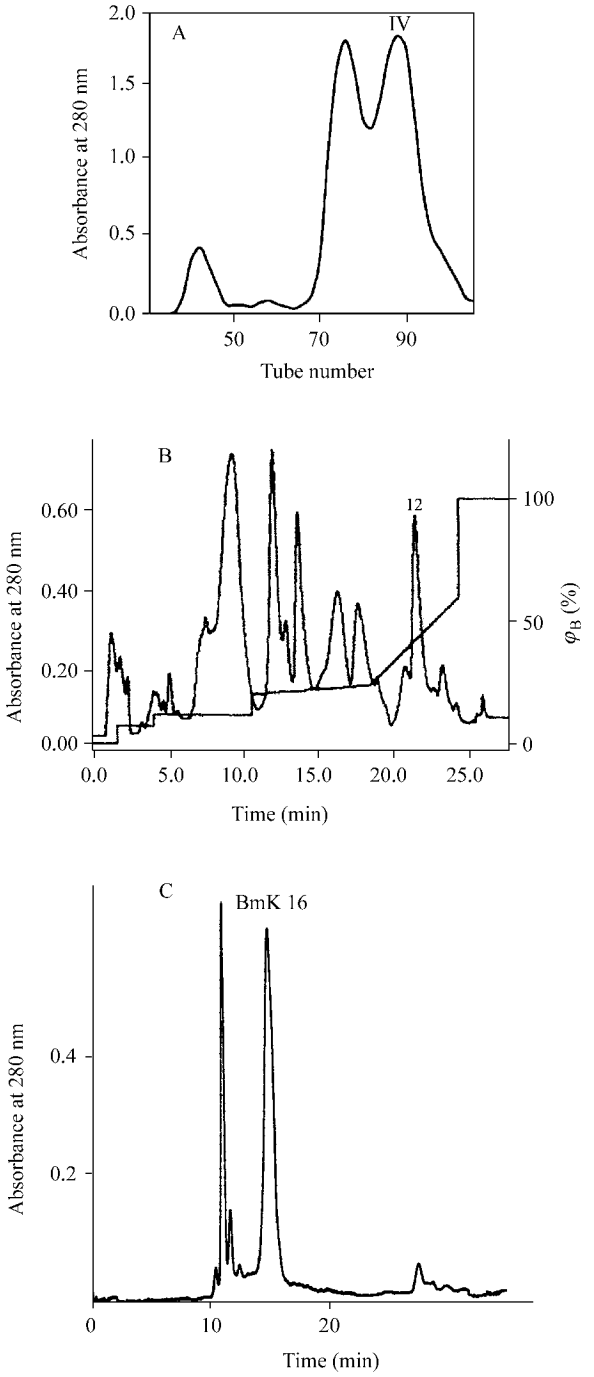

Fig. 1 Isolation and purification of BmK 16. (A) Gel-filtration chromatography of crude venom on a Sephadex G-50 column. (B) FPLC fractionation of the fraction IV from the Sephadex G-50 column on a Mono S column. (C) RP-HPLC fractionation of the fraction 12 from the Mono S column on a C_{18} column.

Toxicity tests

Toxicity on mammals was recorded after subcutaneous injection into mice (Kunming strain, male, 18—20 g), and toxicity to insects was monitored by injection into the ventral intersegmental membranes of the cockroach [Blat-

tella germanica, (60 ± 3) mg]. The lyophilized sample was weighted and dissolved in a solution of 9 mg/mL NaCl and 10 mg/mL BSA (Sigma) and injected into each mouse (50 μL) and cockroach (2 μL).

NMR *experiments*

The toxin BmK 16 was dissolved in 20 mmol/L NaH_2PO_4-H_3PO_4 buffer containing 20 mg/L NaN_3 and 0.1 mmol/L EDTA. The solvents were either 100% D_2O or the mixture of 90% H_2O and 10% D_2O (V/V). The pH was adjusted to 5.0 by adding microliter of dilute DCl or NaOD, and the final concentration was 2.8 mmol/L.

All ^1H NMR experiments of BmK 16 were carried out on a Unity Inova600 spetrometer. Two-dimensional double-quantum-filtered correlation spectroscopy (DQF-COSY),[12] total correlation spectroscopy (TOCSY),[13,14] and nuclear Overhauser enhancement spectroscopy (NOESY)[15] spectra were performed in phase sensitive mode using TPPI (the time-proportional phase incrementation) method. The quadrature detection was employed for both F2 and F1 dimension acquisition with the carrier frequency always maintained at the solvent resonance. An MLEV-17 composite pulse was used for spin lock in TOCSY experiments. Water suppression was achieved using presaturation during the relaxation delay (1.5 s). All the two dimensional spectra were measured with 4096 data points in t_2 dimension and 512 points in t_1 dimension. Shifted sine window function and zero-filling was applied prior to Fourier transformation. All NMR data were recorded at 300 K. TOCSY spectra were performed in various mixing periods (30, 80 and 120 ms in H_2O; 80 ms in D_2O). NOESY spectra were obtained with different mixing times (200 and 300 ms in H_2O; 250 ms in D_2O) respectively. For determination of slowly exchanging protons at pH = 5.0, a series of TOCSY spectra (τ_m = 80 ms) were recorded at 300 K after the sample being redissolved in D_2O (100%). Those amide protons still having correlation peaks in the TOCSY after standing for 24 h at 300 K were considered to be slowly exchangeable.

The spectra of BmK 16 were acquired and processed using Vnmr 6.1B software. The processed data of the toxin were analyzed with XEASY (26) on a Silicon Graphics Indigo 2 computer.

Results

Purification and characterization

The crude venom was initially separated by gel-filtration chromatography on a Sephadex G-50 column to give rise 4 fractions (Fig. 1A). The toxin-containing fraction IV from the Sephadex G-50 column was separated by FPLC into 12 sub-fractions (Fig. 1B) on a Mono S cation exchange column. Fraction 12 from the Mono S column was purified on a C18 column to afford the peptide BmK 16 (Fig. 1C).

The ESI MS spectrum of BmK 16 was shown in Fig. 2, which indicated the homogeneity and molecular mass of 7231.8 Da. The result of amino acid analysis of the toxin BmK 16 was listed in Table 1. The *N*-terminal sequence of BmK 16 determined by Edman degradation was VADAYIAK.

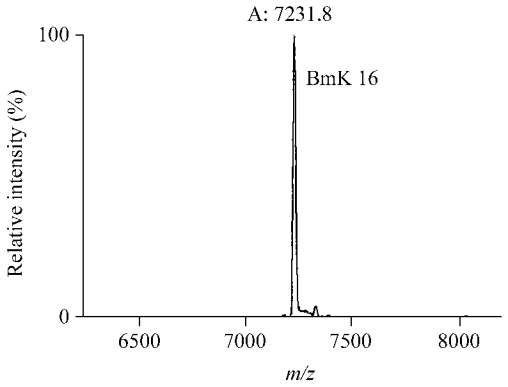

Fig. 2 ESI-MS spectrum of BmK 16.

Table 1 Amino acid compositions of BmK 16

Amino acid	Number of residues
Gly	5.7 (6)
Ala	4.2 (4)
Val	4.7 (5)
Ile	2.0 (2)
Leu	2.1 (2)
Asx	8.0 (9)
Thr	0.7 (1)
Ser	0.8 (1)
Glx	3.5 (4)
Pro	3.5 (4)
Tyr	4.8 (5)
Lys	6.2 (6)
His	1.9 (2)
Arg	3.0 (3)
Cys-Cys	3.5 (4)
Trp	2
MW (calculated)	7231.5 Da
MW (experimental)	7231.8 Da

Toxicity assay

The toxicity of the toxins BmK I and BmK 16 on mammals was tested using mice by subcutaneous injection. LD_{50}s of BmK I and BmK 16 were 0.63 and 0.24 mg/kg, respectively. The toxicity of two toxins on insect was tested using cockroach and PU_{50}s of BmK I and BmK 16 were 54 and 29 μg/kg, respectively. Compared with BmK I, BmK 16 showed higher toxicities on both mammals and insects.

Resonance assignment

The sequence-specific resonance assignment of the toxin BmK 16 was achieved according to the general procedure developed by Wüthrich.[16] The spin systems of BmK 16 were identified by their scalar connectivities on the basis of the DQF-COSY and the TOCSY spectra. The fingerprint region of the DQF-COSY spectrum recorded in H_2O showed most of the HN-H_α cross peaks expected. The TOCSY spectra were used to correlate the side-chain spin-systems with the HN-H_α cross-peaks. Intraresidue NOESY cross-peaks between γ-NH_2 of Asn or δ-NH_2 of Gln and β-H of Asn or r-H of Gln were used to discriminate between Asp/Asn and Glu/Gln, respectively.

The spin systems of the toxin BmK 16 were connected in sequence by virtue of sequential HN-H_α, HN-HN and HN-H_β connectivities in well-dispersed NOESY spectra.

Two amino acids that are uniquely presence in the BmK 16 were used as the starting points for sequential assignments: Ala4-Tyr5, Val13-Tyr14, Glu20-Tyr21 and Tyr42-Gly43. Starting from the above dipeptide segments the assignment proceeded smoothly both in forth and reverse directions via HN-H_α (Fig. 3), HN-HN (Fig. 4) and HN-H_β (data not shown), and the sequential connectivities were observed throughout except Cys16-Ala17. The sequential assignments of four proline residues were obtained on the basis of strong NOE cross peaks observed between the δ-protons of each proline and the α-protons of the proceeding residues, and between the α-protons of prolines and the backbone amide protons of the next residues. The chemical shifts of the toxin BmK 16 were listed in Table 2.

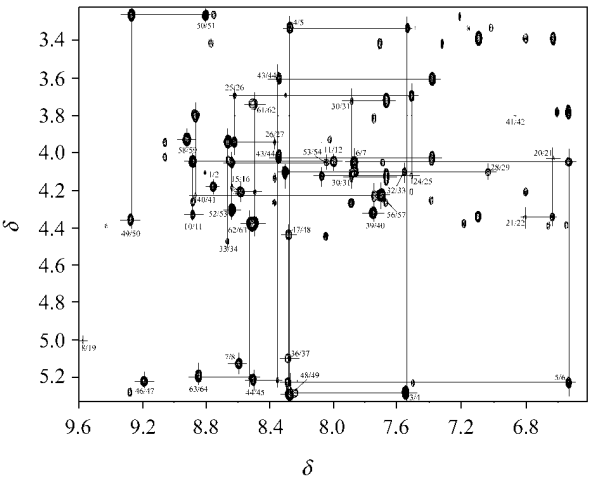

Fig. 3 Sequential HN-H_α NOE correlations of BmK 16 (H_2O, 300 K, pH = 5.0).

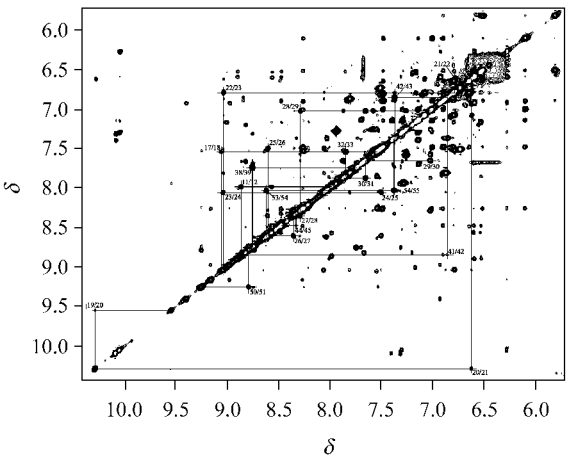

Fig. 4 Sequential HN-HN NOE correlations of BmK 16 (H_2O, 300 K, pH = 5.0).

Table 2 ^1H chemical shifts of BmK 16 (300 K, pH = 5.0)

Residue	NH	α-H	β-H	Others
Val1		4.20	1.56	γ-CH_3 0.79, 0.90
Arg2	8.77	4.70	1.67, 1.93	γ-CH_2 1.31, 1.44; δ-CH_2 2.99, 3.09; NH 7.70
Asp3	8.24	5.26	2.73	
Ala4	7.54	3.34	1.12	
Tyr5	8.28	5.21	3.03, 2.79	2,6-H 7.50; 3,5-H 6.91
Ile6	6.53	4.06	1.76	γ-CH_2 1.65, 1.25; γ-CH_3 0.75; δ-CH_3 0.66
Ala7	7.88	5.10	0.82	
Lys8	8.60	4.42	0.62	γ-CH_2 0.61, 0.65; δ-CH_2 0.90, 1.05; ε-CH_2 2.55, 2.66
Pro9		4.29	1.17, 1.32	γ-CH_2 1.78,1.90; δ-CH_2 3.32, 3.70
His10	7.68	4.36	2.23, 2.71	2-H 8.55; 4-H 7.32
Asn11	8.89	4.06	3.80, 2.15	γ-NH_2 6.54, 6.62
Cys12	8.00	4.83	2.37, 3.17	
Val13	7.81	4.65	2.53	γ-CH_3 0.53, 0.89
Tyr14	9.04	4.41	2.88, 2.69	2,6-H 7.19; 3,5-H 6.55
Glu15	8.50	4.23	1.92, 2.00	γ-CH_2 2.30

Continued

Residue	NH	α-H	β-H	Others
Cys16	8.59	4.80	3.03, 3.11	
Ala17	9.08	4.81	1.48	
Arg18	7.56	4.98	2.25, 1.93	γ-CH_2 1.74; δ-CH_2 3.35; NH 7.45
Asn19	9.58	4.43	2.98, 3.20	γ-NH_2 7.96, 7.30
Glu20	10.32	4.04	2.09	γ-CH_2 2.45, 2.54
Tyr21	6.64	4.37	3.06, 3.40	2,6-H 7.10; 3,5-H 6.81
Cys22	6.80	4.23	2.39, 2.63	
Asn23	9.07	3.96	2.82	γ-NH_2 7.83, 6.89
Asp24	8.08	4.15	2.73, 2.56	
Leu25	7.52	3.71	0.97, 1.29	γ-H 0.84; δ-CH_3 0.20, −0.14
Cys26	8.63	3.96	2.55	
Thr27	8.37	4.29	4.17	γ-CH_3 1.12
Lys28	8.32	4.11	1.94, 1.79	γ-CH_2 1.43; δ-CH_2 1.53; ε-CH_2 2.88; ε-NH_3^+ 7.52
Asn29	7.04	4.60	2.35, 2.94	γ-NH_2 7.28, 7.60
Gly30	7.68	3.73, 4.16		
Ala31	7.89	4.69	1.21	
Lys32	7.87	4.11	1.71	γ-CH_2 1.44; δ-CH_2 1.58, 1.65; ε-CH_2 2.97
Ser33	7.56	4.51	3.95, 4.06	
Gly34	8.68	4.27, 4.83		
Tyr35	9.00	4.82	3.01, 3.12	2,6-H 6.95; 3,5-H 6.61
Cys36	9.43	5.07	2.70, 2.82	
Gln37	8.30	4.62	1.82, 2.32	γ-CH_2 2.08, 2.14; δ-NH_2 5.83, 6.53
Trp38	8.78	4.47	3.43, 3.09	2-H 7.33; 4-H 7.72; 5-H 7.15; 6-H 7.16; 7-H 7.42; NH 10.13
Val39	7.77	4.35	2.07	γ-CH_3 0.86
Gly40	7.75	3.83, 4.25		
Lys41	8.87	3.81	1.33, 1.65	γ-CH_2 0.76,0.90; δ-CH_2 1.54; ε-CH_2 2.87, 2.92
Tyr42	6.87	4.65	1.20, 2.88	2,6-H 6.81; 3,5-H 6.67
Gly43	7.39	3.61, 4.03		
Asn44	8.35	5.20	2.51, 2.82	γ-NH_2 5.58, 6.62
Gly45	8.51	4.70, 3.87		
Cys46	8.23	5.22	2.66	
Trp47	9.19	4.47	2.23, 2.39	2-H 6.31; 4-H 6.12; 5-H 7.03; 6-H 7.16; 7-H 7.32; NH 10.09
Cys48	8.29	5.28	2.32, 2.81	
Lys49	8.27	4.40	1.62	γ-CH_2 1.43, 1.30; δ-CH_2 1.73; ε-CH_2 3.06
Glu50	9.28	3.27	1.95, 2.10	γ-CH_2 2.15
Leu51	8.81	4.13	1.76, 1.12	γ-CH_2 1.56; δ-CH_3 0.60, 0.76
Pro52		4.33	2.44, 2.01	γ-CH_2 2.23, 1.97; δ-CH_2 3.75, 3.51
Asp53	8.64	4.07	2.43, 2.60	
Asn54	8.05	4.48	2.64, 2.96	γ-NH_2 6.76, 7.53
Val55	7.40	4.28	1.89	γ-CH_3 1.09
Pro56		4.25	2.20, 1.87	γ-CH_2 2.12, 1.90; δ-CH_2 3.70, 4.06
Ile57	7.71	4.87	1.75	γ-CH_2 0.81, 1.08; γ-CH_3 0.55; δ-CH_3 0.64
Arg58	8.04	3.95	1.58, 0.84	γ-CH_2 0.16, 0.20; δ-CH_2 1.99, 2.66; NH 6.46
Val59	8.93	4.76	2.39	γ-CH_3 0.73, 0.87
Pro60		4.42	1.81, 2.35	γ-CH_2 1.98, 2.16; δ-CH_2 3.63, 3.80
Gly61	8.51	4.54, 3.75		
Lys62	8.50	4.40	1.70	γ-CH_2 1.43; δ-CH_2 1.64; ε-CH_2 2.95
Cys63	8.53	5.18	3.75, 2.54	
His64	8.85	4.67	3.00, 3.28	2-H 8.44; 4-H 7.22

Amino acid sequence of BmK 16

The results of amino acid analysis, *N*-terminal sequence analysis (eight residues) and molecular weight determination are consistent with the calculated values according to the amino acid sequence of the mature peptide from the precursor peptide alpha-neurotoxin TX16 derived from the sequence of the cDNA AF156597 (EMBL). These data suggested that BmK 16 shared the same primary structure with the mature peptide from the precursor peptide alpha-neurotoxin TX16. The sequence-specific resonance assignment of BmK 16 was achieved and the results supported above conclusion. Therefore, the intact sequence of BmK 16 is as followings: VRDAY IAKPH NCVYE CARNE YCNDL CTKNG AKSGY CQWVG KYGNG CWCKE LPDNV PIRVP GKCH.

Discussion

Recently, the cDNA sequence library of BmK neurotoxins has been available.[17,18] This afforded a new approach for the determination of primary sequences of BmK neurotoxins by the database searching on the basis of the partial sequence of the unknown peptide within the library of precursor peptides derived from the cDNA sequences. In this study the sequence of BmK 16 was determined by Edman degradation sequencing of the *N*-terminal 8 amino acid residues of the toxin and the following database searching. The amino acid sequence of BmK 16 was further verified by the sequential assignment of the proton resonance of the peptide.

The sequence alignment of BmK 16 with other representative α- and α-like toxins (Fig. 5) shows that BmK 16 and BmK I share high homology of the primary structure, only having difference at the position 49. Meanwhile, the toxicity tests of BmK 16 showed that it was high toxic to both mammals and insects, suggesting the character of the α-like toxin. On the other hand, although the sequences of the toxins BmK 16 and BmK I share high homology, the toxicity of BmK 16 towards mammal and insect is about two times higher than that of BmK I. At the same time, it is fascinating that the sequence of BmK 16 has a distinct lower homology with α-like neurotoxins Lqh III and Bom III than with insect α-toxins LqhαIT, Lqq III and BotIT1. Therefore, the facts mentioned above indicate that BmK 16 should be a new member of the α-like neurotoxin subfamily.

The work reported in this paper is a good starting point for the further study of the solution structure of BmK 16 to gain an insight the pattern of the interactions between the α-like toxins and the sodium ion channel.

Acknowledgement

The authors thank the Institute of Molecular Biology and Biophysics, ETH-Hönggerberg Zürich, Switzerland, for the program XEASY.

```
                1         10        20        30        40        50        60   64
BmK 16          VRDAYIAKPHNCVYECA-RNEYCNDLCTKNGAKSGYCQWVGKYGNGCWCKELPDNVPIRVPG-KCH-
BmK I           ................-.............................I............-...-
Lqh III         ...G...Q.E....H.FPGSSG.DT..KEK.GT..H.GFKVGH.LA...NA.....G.I.E.E...S*
Bom III         G..G...Q.E....H.FPGSSG.DT..KEK..T..H.GFLPGS.VA...DN..NK...V.G.E...-
LqhαIT          ........NY......F-.DA...E.......S......A.....A...YA...........-...-
Lqq III         ........NY......F-.DS...........S......A.....A...YA...........-...-
BotIT1          .......QNY....F.M-KDD...........S......A.....A...YA........I..-...S
AaH II          .K.G..VDDV..T.F.G-..A...EE...LKGE......ASP...A.Y.YK...H.RTKG..-R..-*
Lqh II          IK.G..VDDV..T.F.G-..A...EE...LKGE......ASP...A.Y.YK...H.RTKG..-R.R-
```

Fig. 5 Sequences alignment of BmK 16, BmK I and also other representative α- and α-like toxins. The amino acid sequences are aligned according to their cysteine residues. An asterisk indicates amidation at the C terminus. Gaps are presented as dashes. Numbers are labeled according to the sequence of BmK 16. BmK I,[17] Lqh III and Bom III are α-like toxins;[19] LqhαIT, Lqq III and BotIT1 are α-toxins highly active on insects.[19,20]

References

1 Jover, E.; Couraud, F.; Rochat, H. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 111.

2 Simard, J. M.; Meves, H.; Watt, D. D. In *Natural Toxins: Toxicology, Chemistry and Safety*, Eds.: Keeler, R. F.; Mandava, N. B.; Tu, A. T., Alaken Inc., Fort Collins, Co, **1992**, pp. 236—263.

3 Martin-eauclaire, M. F.; Couraud, F. In *Handbook of Neurotoxicology*, Eds.: Chang, L. W.; Dyer, R. S., Marcel Dekker, New York, **1995**, pp. 683—716.

4 Gordon, D.; Martin-Eauclaire, M. F.; Cestele, S.; Kopeyan, C.; Carlier, E.; Khalifa, R. B.; Pelhate, M.; Rochat, H. *J. Biol. Chem.* **1996**, *271*, 8034.

5 Gordon, D.; Savarin, P.; Gorevitz, M.; Zinn-Justin, S. *J. Toxicol., Toxin Rev.* **1998**, *17*, 131.

6 Ji, Y. H.; Mansuelle, P.; Terakawa, S.; Kopeyan, C.; Yanaihara, N.; Hsu, K.; Rochat, H. *Toxicon* **1996**, *34*, 987.

7 Goudet, C.; Huys, I.; Clynen, E.; Schoofs, L.; Wang, D. C.; Waelkens, E.; Tytgat, J. *FEBS Lett.* **2001**, *495*, 61.

8 Cestele, S.; Stankiewicz, M.; Mansuelle, P.; De Waard, M.; Dargent, B.; Gilles, N.; Pelhate, M.; Rochat, H.; Martin-Eauclaire, M. F.; Gordon, D. *Eur. J. Neurosci.* **1999**, *11*, 975.

9 Gilles, N.; Blanchet, C.; Shichor, I.; Zaninetti, M.; Lotan, I.; Bertrand, D.; Gordon, D. *J. Neurosci.* **1999**, *19*, 8730.

10 Hansel, A.; Lu, S. Q.; Leipold, E.; Heinemann, S. H. *Pflügers Arch.* **2002**, *443/S02*, P02.

11 Wu, H. M.; Wu, G.; Huang, X. L.; He, F. H.; Jiang, S. K. *Pure Appl. Chem.* **1999**, *71*, 1157.

12 Piantini, U.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800.

13 Braunschweilier, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521.

14 Griesinger, C.; Otting, G.; Wüthrich, K.; Ernst, R. R. *J. Am. Chem. Soc.* **1988**, *110*, 7870.

15 Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546.

16 Wüthrich, K. *NMR of Proteins and Nucleic Acids*, John Wiley & Sons, New York, **1986**, pp. 117—175.

17 Goudet, C.; Chi, C.-W.; Tytgat, J. *Toxicon* **2002**, *40*, 1239.

18 Zhu, S.-Y.; Li, W.-X.; Zeng, X.-C.; Liu, H.; Jiang, D.-H.; Mao, X. *Toxicon* **2000**, *38*, 1653.

19 Hamon, A.; Gilles, N.; Sautière, P.; Martinage, A.; Kopeyan, C.; Ulens, C.; Tytgat, J.; Lancelin, J.-M.; Gordon, D. *Eur. J. Biochem.* **2002**, *269*, 3920.

20 Borchani, L.; Stankiewicz, M.; Kopeyan, C.; Mansuelle, P.; Kharrat, R.; Cestele, S.; Karoui, H.; Rochat, H.; Pelhate, M.; El Ayeb, M. *Toxicon* **1997**, *35*, 365.

(E0212195 LI, L. T.; FAN, Y. Y.)