IUBMB *Life*, 49: 207–210, 2000

1521-6543/00 $12.00 + .00

Original Research Article

Cloning and Characterization of a Novel cDNA Sequence Encoding the Precursor of a Novel Venom Peptide (BmKbpp) Related to a Bradykinin-Potentiating Peptide from Chinese Scorpion *Buthus martensii* Karsch

Xian-Chun Zeng, Wen-Xin Li, Fang Peng, and Zhi-Hui Zhu

Department of Virology and Molecular Biology, School of life Science, Wuhan University, Wuhan 430072, People's Republic of China

Summary

Based on the amino acid sequence of a bradykinin-potentiating peptide (Bpp) (peptide K-12) from scorpion *Buthus occitanus*, a full-length cDNA sequence encoding the precursor of a novel venom peptide (named BmKbpp) related to this Bpp, has been isolated and analyzed. The cDNA encodes a precursor of 72 amino acid residues, including a signal peptide of 22 residues and an extra Arg-Arg-Arg tail at the C-terminal end of the precursor, which have to be removed in the processing step. The C-terminal region (21 residues) of the precursor is homologous (57% identical) with the sequence of peptide K-12. Thus, according to the primary structure of the BmKbpp precursor, there may be a propeptide between the signal peptide and the putative mature BmKbpp at the C-terminal region of the precursor.

IUBMB *Life*, 49: 207–210, 2000

Keywords BmKbpp; bradykinin-potentiating peptide; cDNA; scorpion; venom.

INTRODUCTION

Venoms from scorpions contain a series of small polypeptides of ~31–70 amino acid residues with three or four disulfide bridges, which act selectively on different types of ion channels (such as Na^+, K^+, or Cl^- channels) and display various degrees of toxicity towards mammals, insects, or crustaceans, respectively (*1–5*). Scorpion toxins that interact with Na^+ channels contain 60–70 residues cross-linked by four disulfide bridges. These long-chain toxins can be divided into two major groups, α and β, according to their physiological effects and binding properties (*2*, *3*). The toxins that act on the K^+ channel are mainly composed of 30–40 residues stabilized by three or four disulfide bonds (*4*). Chlorotoxin, specific for the Cl^- channel, has only 36 amino acid residues with four disulfide bridges (*5*).

In addition, some scorpion venoms contain a different class of polypeptides with no disulfide bridges, which show the activity of a bradykinin-potentiating peptide (Bpp)[1]. For example, Nassar et al. (*6*) isolated Bpps from *Buthus occitanus* and *Leiurus quinquestriatus* scorpion venoms, which showed an activity similar to that of the synthetic bradykinin-potentiating factor B and C; Ferreira et al. (*7*) isolated a bradykinin-potentiating factor from scorpion *Tityus serrulatus* venom. More recently, Meki et al. (*8*) isolated a Bpp (peptide K-12) from the Egyptian scorpion *B. occitanus*, the activity of which may result from the inhibition of angiotensin-converting enzyme. However, so far, the precursor of any venom peptide from scorpion with no disulfide bridges has not been reported yet.

B. martensii Karsch (BmK) is a representative species in China. Recently, some toxins from these scorpions, which are active on Na^+ channels or K^+ channels, have been described in some aspects, including their amino acid sequence determination, molecular cloning, function, and three-dimensional structural analysis (*9–12*). In this paper, we describe the cloning and characterization of a cDNA sequence encoding the precursor of a novel Bpp-related venom peptide (named BmKbpp) with no disulfide bridges. To our knowledge, this is the first report of this kind.

Received 23 November 1999; accepted 25 November 1999.

Address correspondence to Wen-Xin Li. Fax: 86-027-87882661; E-mail: xianchun_zeng@hotmail.com

[1]Abbreviations: BmKbpp, Bpp-related venom peptide from *Buthus martensii* Karsch; Bpp, bradykinin-potentiating peptide; PCR, polymerase chain reaction.

MATERIALS AND METHODS

Construction of the cDNA Library

Standard recombinant DNA techniques were used (*13*). The *B. martensii* Karsch scorpions were killed 2–3 days after extraction of their venom to allow the toxin-producing cells of the venom glands to enter the secretory phase. Total RNA was extracted from homogenized venom gland tissues by using a modified one-step extraction method (*14*). Poly(A)$^+$ mRNA was purified by using a PolyATract mRNA lsolation System (Promega). The SuperScript plasmid system (from GIBCO/BRL) was used to generate the library. Double-stranded cDNA was synthesized from 5 μg of telson mRNA. The Nucleon QC system (Amersham) was used to remove the residual adapters and *Not*l fragments from double-stranded cDNA before ligation of cDNA to the *Not*I–*Sal*I–cut plasmid pSPORT 1. Electroporation was used to introduce ligated cDNA into *Escherichia coli* 10B. From 1 μg of cDNA $\sim 1.8 \times 10^6$ *E. coli* 10B transformants were generated.

Screening the cDNA Library by PCR

The polymerase chain reaction (PCR) was used to screen the library. The degenerate forward primer was 5′-T(TCAG)AG-(AG)GA(TC)TA(TC)GC(ACTG)AA-3′, corresponding to residues 1 to 6 of the N-terminal partial amino acid residue sequence of peptide K-12, a Bpp from scorpion *B. occitanus* (*8*); the reverse primer was 5′-AGCGGCCGCCCT$_{(15)}$-3′, corresponding to a partial sequence of the pSPORT I vector and the 3′-terminal poly(A) sequence of a cDNA. The PCR was carried out by two steps. The first step used only forward primers and consisted of 10 cycles at 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min. In the second step, reverse primers were added and the reaction was run for 30 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min with a final extension phase of 5 min. The purified PCR products were then cloned into a pGEM-T vector and sequenced. Next, we amplified the 5′ region of the BmKbpp cDNA by a second PCR step, using primer 3 as the forward primer (5′-CTCACTATAGGGAAAGCTGC-3′, corresponding to the T7 promoter of the pSPORT1 vector) and primer 4 as the reverse primer (5′-TCTTCTTCGTTCGGCTGGAGC-3′, corresponding to the 3′-terminal part of the encoding region of BmKbpp cDNA determined by first PCR). The second PCR was carried out as follows: 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, with a final extension phase of 5 min. The purified PCR product was cloned and sequenced.

Screening the cDNA Library by Specific Probe Hybridization

A specific cDNA probe used to screen the library was synthesized by PCR using primers 1 and 4 as the forward and reverse primers, respectively. Probes were ^{32}P-labeled by nick-translation (Boehringer Mannheim, Germany) and were used to screen 20,000 clones from the cDNA library as described elsewhere (*13*).

DNA Sequencing

Nucleotide sequence was determined by using the PRISM 377 DNA sequencer (ABI) with a universal M13 forward primer or T7 promoter primer according to the method of Sanger et al. (*15*).

RESULTS AND DISCUSSION

The PCR strategy was prepared for obtaining the full-length cDNA sequence of BmKbpp as described in Fig. 1. The first PCR obtained one DNA band of 180 bp. This DNA was inserted into pGEM-T vector, and DNA sequence analysis demonstrated that it encoded a peptide of 21 residues (plus 3 additional residues R-R-R), which was homologous with the sequence of peptide K-12. The second PCR obtained a fragment of 350 bp, which encompassed the 5′ untranslated region and the encoding region of BmKbpp cDNA. The full-length cDNA sequence of BmKbpp was then completed by overlapping these two PCR fragments. This combination matched the gene encoding the BmKbpp precursor of 72 amino acid residues, including a signal peptide of 22 residues in the N-terminal part and an extra Arg-Arg-Arg tail at the C-terminal end of the precursor that had to be removed in the processing step (Fig. 2).

To investigate whether or not the BmKbpp precursor has homologs, the partial coding region of BmKbpp cDNA was used as a probe to screen the same library by specific probe hybridization. The results indicated that 2% of the clones were positive. Of these, 10 clones were analyzed; all were found to have an identical sequence and to encode the same protein as BmKbpp. This suggests BmKbpp may have no any homolog. The nucleotide sequence of BmKbpp cDNA showed that the 5′ and 3′ untranslated region are 43 and 86 bp long, respectively. The upstream lateral sequence of the initial code (ATG) of the cDNA of BmKbpp precursor is CAAA, which is identical with that

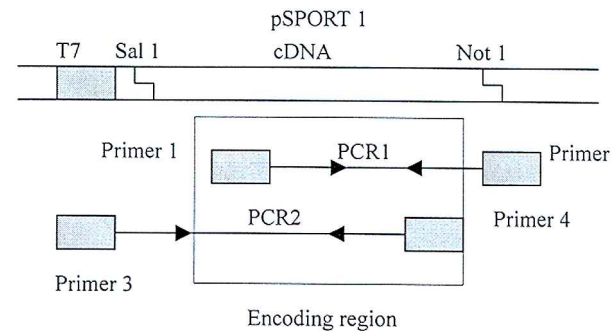

Figure 1. Strategy for screening of BmKbpp full-length cDNA from the cDNA library by PCR. Primer 1 was complementary to a portion of the N-terminal part of peptide K-12, a Bpp from scorpion *B. occitanus* (*10*). Primer 2 corresponded to a partial sequence of *Not*I adapter and 3′-poly(A) sequence of the cDNA. Primer 3 corresponded to the T7 promoter sequence. Primer 4 was synthesized according to the 3′ region of the BmKbpp cDNA sequence determined by the first PCR.

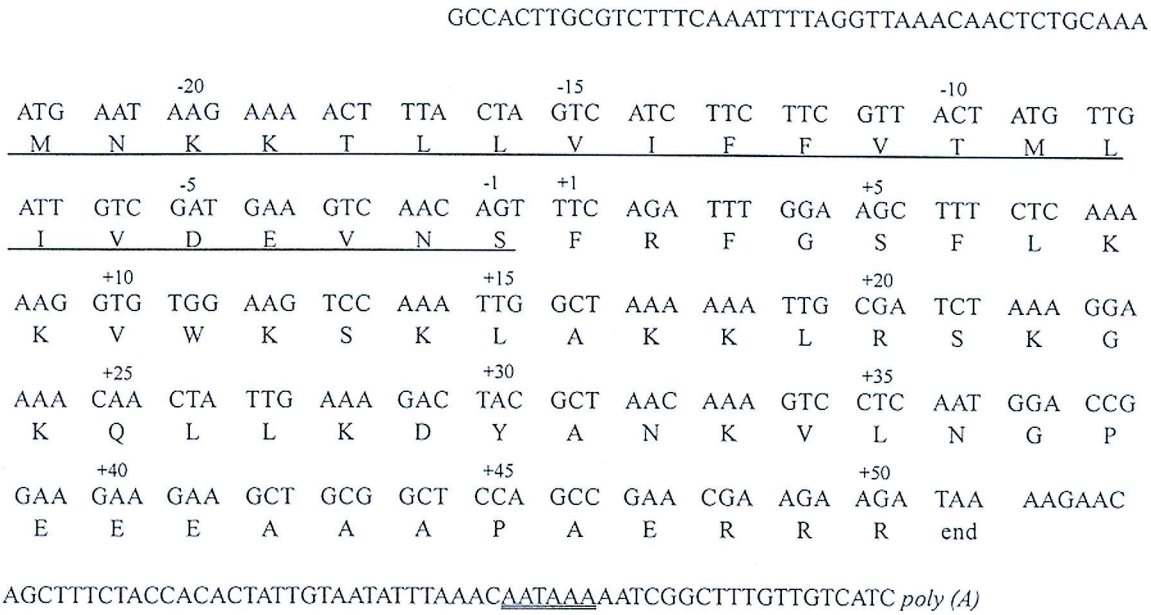

Figure 2. Nucleotide sequence of a full-length cDNA encoding the BmKbpp precursor. The predicted protein sequence is given below the nucleotide sequence and is numbered starting from the NH_2-terminal amino acid residue of the peptide. The signal peptide is underlined; a potential polyadenylation signal (AATAAA) is underlined twice.

of most other toxin cDNAs from BmK described previously; also, a single AATAAA polyadenylation signal was found 19 nucleotides upstream of the poly(A)-tail.

A search for amino acid sequence homology indicated that the C-terminal region of BmKbpp precursor showed 57% identity with the sequence of peptide K-12 from scorpion *B. occitanus*; three additional positions showed conservative replacement between them, for a total result of 71% homology (Fig. 3). Three extra Arg residues were present at the N-terminus of the precursor, which may be removed in the processing steps as some scorpion toxins do (*16*, *17*). As with the precursors of most BmK mammalian toxins (*9*, *18*), the signal peptide cleavage of the BmKbpp precursor occurred at a small neutral residue (Ser); moreover, the residue at position −3 is a Val. The signal peptide of BmKbpp precursor has two properties that are different from those of most other scorpion toxins: (*a*) It has two positively charged amino acid residues (Lys-Lys) in the −19 and −20 positions versus only one Lys residue for other scorpion toxin precursors, a change known to improve the export efficiency of most eukaryotic signal peptides. (*b*) It also has two negatively charged residues at the C-terminal part (Asp-Glu) versus one Asp or Glu residue for most toxin precursors of scorpions.

The high sequence identity between the C-terminal region of BmKbpp precursor and peptide K-12 suggests that there may be a propeptide between the signal peptide and putative mature BmKbpp at the C-terminal region of the precursor, and that the BmKbpp may be structurally and functionally related to peptide K12. Although the precursor contains two typical processing signals, the dibasic pairs Lys-8/Lys-9 and Lys-17/Lys-18, separated from each other by seven residues, the cleavage site of the propeptide could not be determined because of lack of information about the processing mechanism for propeptides in scorpions. The similar phenomenon was observed in a precursor of seven Bpps and a C-type natriuretic peptide from the snake *Bothrops jararaca* (*19*). In that precursor, the seven Bpps are aligned tandemly after the signal peptide, followed by a

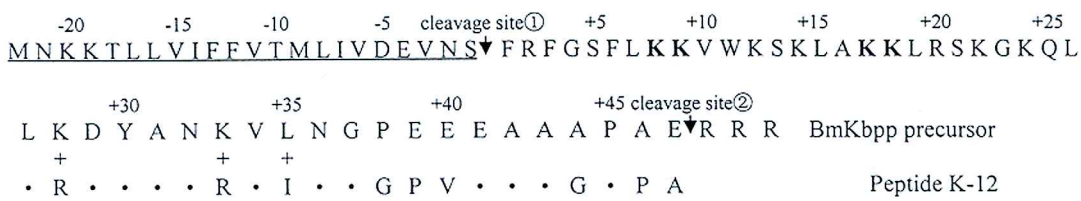

Figure 3. Sequence analysis of BmKbpp precursor, and sequence comparison of BmKbpp precursor with that of peptide K-12 from scorpion *B. occitanus* (*10*). Amino acids common to the two peptides are indicated by centered dots; and three conservative replacement positions are indicated by +. The signal peptide is underlined. The dibasic pairs of residues at amino acid positions 8–9 and 17–18 are in boldface type.

propeptide (linker) sequence with unknown function and a CNP at the C terminus.

In conclusion, we have described the precursor of a novel venom peptide related to a Bpp. We believe this is the first report of its kind. The discovery of a putative propeptide in the precursor sequence suggests that the activation and functioning of BmKbpp may be dependent on other factors. Our result may provide a new way studying the mechanism of BmKbpp and its pharmacological effects.

ACKNOWLEDGEMENTS

This work has been sponsored by the National Medicine Creation Doctor-Foundation of China to X.-C. Z. and W.-X. L. (no. 96-901-033), by the Wuhan Chenguang Project of Science and Technology to X.-C. Z. (no. 965001037-24), and by the National Natural Science Foundation of China to W.-X. L. (no. 39970897). The nucleotide sequence data presented here were submitted to the GenBank database; the accession number is AF 145952.

REFERENCES

1. Possani, L. D. (1984) Structure of scorpion toxins. In *Handbook of Natural Toxins* (Tu, A.T., ed.), Vol. 3, pp. 513–550, Marcel Dekker, New York.
2. Gordon, D., Savarin, P., Gurevitz, M., and Zinn-Justin, S. (1998) Functional anatomy of scorpion toxins affecting sodium channels. *J. Toxicol. Toxin Rev*. **17**, 131–159.
3. Jablonsky, M. J., Jackson, P. L., Trent, J. O., Watt, D. D., and Krishna, N. R. (1999) Solution structure of a β-neurotoxin from the New World scorpion *Centruroides sculpturatus* Ewing. *Biochem. Biophys. Res. Commun*. **95**, 1607–1614.
4. Legros, C., Ceard, B., Bougis, P. E., and Martin-Eauclaire, M. F. (1998) Evidence for a new class of scorpion toxins active against K^+ channels. *FEBS Lett*. **431**, 375–380.
5. Debin, J. A., Maggio, J. E., and Strichartz, G. R. (1993) Purification and characterization of chlorotoxin, a chloride channel ligand from venom of scorpion. *Am. J. Physiol. Soc*. **264**, C364–369.
6. Nassar, A. Y., Abu-Sinna, G., and Abu-Amra, S. (1989) Isolated fractions from toxins of Egyptian scorpions and cobra, activated smooth muscle contraction and glomerular filtration [Abstract]. *Toxicon* **27**, 65–71.
7. Ferreira, L. A., Alves, E. W., and Henriques, O. B. (1993) Peptide T, a novel bradykinin potentiator isolated from *Tityus serrulatus* scorpion venom. *Toxicon* **31**, 941–947.
8. Meki, A. R., Nassar, A. Y., and Rochat, I. I. (1995) A bradykinin-potentiating peptide (peptide K12) isolated from the venom of Egyptian scorpion *Buthus occitanus. Peptides* **16**, 1359–1365.
9. Xiong, Y. M., Ling, M. H., Lan, Z. D., Wang, D. C., and Chi, C. W. (1999) The cDNA sequence of an excitatory insect selective neurotoxin from the scorpion *Buthus martensii* Karsch. *Toxicon* **37**, 335–341.
10. Gong, J., Kini, R. M., Guee, M. C., Gopalakrishnakone, P., and Chung, M. C. (1997) Makatoxin I, a novel toxin isolated from the venom of the scorpion *Buthus martensii* Karsch, exhibits nitrergic actions. *J. Biol. Chem*. **272**, 8320–8324.
11. Romi-Lebrun, R., Lebrun, B., Martin-Eauclaire, M. F., Ishiguro, M., Escoubas, P., Wu, F. Q., Hisada, M., Pongs, O., and Nakajima, T. (1997) Purification, characterization and synthesis of three novel toxins from the Chinese scorpion *Buthus martensii* Karsch, which act on K^+ channels. *Biochemistry* **36**, 13473–13482.
12. Li, H. M., Wang, D. C., Zeng, A. H., Jin, L., and Hu, R. Q. (1996) Crystal structure of an acidic neurotoxin from scorpion *Buthus martensii* Karsch at 1.85 Å resolution. *J. Mol. Biol*. **261**, 415–431.
13. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
14. Chomezynski, P., and Sacchi, N. (1987) Single step method of RNA isolation by acid guanidinium thiocyanate phenochloroform extraction. *Anal. Biochem*. **162**, 157–165.
15. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) DNA sequencing with chain-termination inhibitors. *Proc. Natl. Acad. Sci. U.S.A*. **74**, 5463–5467.
16. Martin-Eauclaire, M. F., Ceard, B., Ribeiro, A. M., Diniz, C. R., Rochat, H., and Bougis, P. E. (1992) Molecular cloning and nucleotide sequence analysis of a cDNA encoding the main neurotoxin from the venom of the South American scorpion *Tityus serrulatus. FEBS Lett*. **302**, 220–222.
17. Becerril, B., Marangoni, S., and Possani, L. D. (1997) Toxins and genes isolated from scorpions of the genus *Tityus. Toxicon* **35**, 821–835.
18. Xiong, Y. M., Ling, M. H., Wang, D. C., and Chi, W. (1997) The cDNA and genomic DNA sequence of a mammalian neurotoxin from the scorpion *Buthus martensii* Karsch. *Toxicon* **35**, 1025–1031.
19. Murayama, N., Hayashi, M. A. F., Ohi, H., Ferreira, L. A. F., Hermann, V. V., Saito, H., Fujita, Y., Higuchi, S., Fernandes, B. L., Yamane, T., and Camargo, A. C. M. (1997) Cloning and sequence analysis of a *Bothrops jararaca* cDNA encoding a precursor of seven bradykinin-potentiating peptides and a C-type natriuretic peptide. *Proc. Nat1. Acad. Sci. U.S.A*. **94**, 1189–1193.