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Sequence Analysis of Txk from the Scorpion *Mesobuthus eupeus* Venom Glands Using Semi-Nested RT-PCR

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Abstract: Natural toxins are useful probes for evaluating the involvement of K+ channels in cell activity and for investigating K+ channel structure and localization. In recent years, peptide toxins that block various K+ channels with high affinity have been purified from diverse animal venoms. One polypeptide beta neurotoxin named Txk was isolated from the venom of scorpion *Mesobuthus eupeus* of Khuzestan. This toxin consists of 91 amino acid residues which modulate voltage-gated sodium channels gating. In this study, cDNA of Txk β-toxin was amplified and sequence of beta neurotoxin compared with *M. martensii occitanus Israelis* and *Tityus costatus* however, the comparison suggests that the length of the peptide is close to the long-chain potassium ion channel blocker peptide family.

Key words: Sequence analysis, K+ channels, beta neurotoxin, scorpion venom, amino acid, Armenia

INTRODUCTION

Scorpion venom contains a complex mixture of bioactive peptides, non-disulfide-bridge peptides that exhibit potentiating activity, anti-microbial action, hemolytic and immune-modulatory functions among which are peptides responsible for the neurotoxin effects observed during envenomation caused by scorpion stings. A subset of arthropod venoms are complex mixtures of highly evolved peptide libraries with toxin activities that include anti-microbial pore forming and ion channel (Ma *et al*., 2009). Peptides with high affinity and specificity, targeted to ion-channels were reported to exist in these venoms. Many of these peptides have been widely used for identification, isolation and physiological characterization of ion-channel proteins. The best known are those that recognize K+ and Na+ channels (Elgar *et al*., 2006; Gandhe *et al*., 2007) which are usually rich disulfide-containing peptides. Scorpion toxins that modulate voltage-gated sodium channels gating are divided into α and β classes according to their mode of action and binding features to distinct receptor sites (Elgar *et al*., 2006; Wei-Jun and Chao-Qun, 2009). β-toxins are further classified into depressant and excitatory toxins. β-toxins shift the voltage dependence of channel activation to more negative membrane potentials upon binding to receptor site 4, assigned mainly to external loops in domain 2 of mammalian and insect voltage-gated sodium channels (Elgar *et al*., 2006). These peptides have the potential to combat cancer tumors and a variety of bacterial and fungal infections (Elgar *et al*., 2006).

At present, the research is mainly focused on the isolation and identification of Txk β-toxin proteins from scorpion *Mesobuthus eupeus* from Khuzestan province. *Mesobuthus eupeus* is one of the most frequent scorpions from Mesobuthus species and is belong to Buthidae family. This scorpion is reported from the most area of Iran, especially Khuzestan (Dehghani *et al*., 2009). In the present study, cDNA of Txk β-toxin was amplified and characterized from scorpion *Mesobuthus eupeus* from Khuzestan province.

MATERIALS AND METHODS

Scorpion samples: Scorpion Buthida *Mesobuthus eupeus* Khuzestan species were collected from Khuzestan province (Iran) and transported to the laboratory reference of the Razi Institute where they were killed 2 days after manual extraction of their venom to allow the toxin-producing cells of the venom glands to enter the secretory phase. Twenty separated venom glands are used for total RNA extraction.

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Total RNA extraction: Total RNA was extracted from the venom glands of scorpions using RNA™ (Cinagene, Iran) according to the manufacture procedure. RNA pellet was dissolved in DEPC-ddH_2O and used for cDNA synthesis immediately.

cDNA library synthesis: Thev cDNA was synthesized with the extracted total RNA as template and modT (modified oligodT) (5-cgcggatccatgcaaaggaatctggtcgt -3′) as primer. ModT was added to extracted RNA and incubated in 70°C for 5 min and then immediately on ice for 2 min. Then, 5X buffer, dNTP, Ribolock, Reverce transcriptase enzyme and ddH_2O were added to samples and incubated for 60 min in 42°C. Samples were incubated 10 min in 70°C and immediately on ice.

Semi-nested RT-PCR amplification: Amplification of Txk cDNA was performed using Semi-nested RT-PCR strategy. The 1st round of PCR was performed using modT-R (5′-cccagatctcgagctcagtg-3′), Txk-F (5′-gcgcgga tccaagatggctttcaagttttcatt-3′) primers and synthesized cDNA as template. Second round of PCR was performed using Txk-F and Txk-R (5′-gcgcaagctttacagttgttatca ttgataaattg-3′) primers. PCR products of initial amplification were used as template for the 2nd round of amplification. The PCR conditions for both rounds were 35 cycles with denaturation at 94°C (40 sec), anealing at 56°C (90 sec) and extention at 72°C (1 min) with a initial denaturation at 95°C (5 min) and final extention at 72°C (10 min). Amplification products were separated by 1% agarose gel electrophoresis and visualized by UV transilluminator.

DNA sequencing: The amplified cDNA fragments were purified from agarose gel by QIAquick agarose gel extraction kit (www.fermentas.com) and then sent to Kawsar Biotech Company for nucleotide sequencing.

Sequence analysis: Sequence was compared with GenBank database using the BLAST software from NCBI site (http://www.ncbi.nlm.nih.gov). The tool software available at the Expasy website (http://ca.expasy.org/ tools/pi_tool.html) was used to convert nucleotide sequence to amino acid. The molecular weight and isoelectric point was estimated using ProtParam tool (http://www.expasy.org/tools/protparam.html). The signal peptide was predicted by SignalP (http://www.cbs.dtu.dk/ services/SignalP/). Multiple sequence alignments were done using the CLUSTAL_W program and edited with the BOXSHADE software (http://www.ch.embnet.org/ software/BOX_form.html). The SBASE online software (http://hydra.icgeb.trieste.it/sbase/) was used to determine the conserved domains.

RESULTS AND DISCUSSION

Characterization and semi-nested RT-PCR amplification of the *Txk β-toxin* gene cDNA: In this research, we starting with 4 μg of total RNA from 0.5 g of tissues were obtained using RNX™ regent according to the standard protocol. To characterize and assay the mRNAs, single strand cDNAs were synthesized and electrophoresis on a 1% agarose gel cDNA fragments of about 273 bp encoding a beta neurotoxin from *M. mupeus* were amplified using RT-PCR technique. The size of the coding region was 273 bp. We used PCR strategy to obtain a cDNA probe for the screening of the cDNA library (Fig. 1).

M-Marker: A putative 19-amino-acids length signal peptide was identified and glycine at the position 20 was assumed to represent the start of the mature protein. According to the sequencing results, the peptide coding sequence was 273 bp in length, encoding for 91 aa residue peptide with molecular weight 10.207 kDa and theoretical isoelectric point of 9/06.

The cDNA homology search against the GenBank database revealed that the amino acid sequence of beta neurotoxin Txk shared high sequence similarity with beta neurotoxins of other scorpions. Particularly, the deduced amino acid sequence exhibited 96, 94 and 67% similarities with the homologous Long-chain potassium ion channel blocker from *M. martensii*, *Buthus occitanus Israelis* and *Tityus costatus*, respectively.

In this study, beta-neurotoxin Txk from venom glands of *M. eupeus* scorpion from Khuzestan province was identified. The amino acid sequence of beta-neurotoxin Txk was compared to beta neurotoxins from several scorpions which revealed higher homology within these species. *M. martensii* beta-neurotoxin Txk has

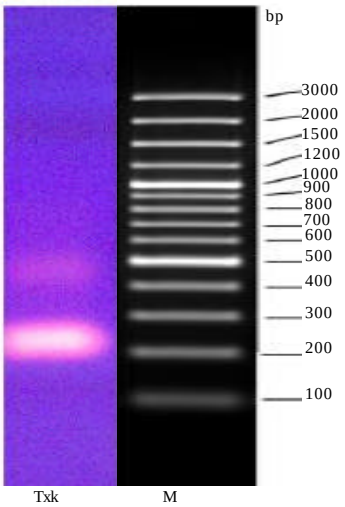

Fig. 1: PCR amplification of beta neurotoxin Txk cDNA

demonstrated the highest homology with beta-neurotoxin Txk determined in this study whereas, the least similarity was found with *Tityus costatus* beta-neurotoxin and shows homology to the published leader peptides of KTx, Ktx2 (Legros *et al*., 1996) and Tsk (Wei-Jun and Chao-Qun, 2009), K+ channel blockers from scorpion venoms.

CONCLUSION

According to the differences between *M. martensii* and *Tityus costatus*, it is concluded that *M. eupeus* of Khuzestan and *Tityus costatus*, *M. eupeus* belong to the different subspecies. The beta neurotoxin from *M. eupeus* belongs to the Toxin_3 superfamily. The length of the peptide is close to the long-chain potassium ion channel blocker peptide family. The largest group of K+ channel peptide inhibitors is the family of neurotoxic peptides found in scorpion venoms. These peptides block in nanomolar concentrations both voltagegated and Ca^{2+} activated K+ channels in a wide variety of cell types and generally contain 3,140 amino acid residues crosslinked by three or four disulfide bridges (Whetstone and Hammock, 2007; Ma *et al*., 2009).

RECOMMENDATION

The comparison suggests that the length of the peptide is close to the Long-chain potassium ion channel blocker peptide family.

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