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Soluble expression, purification and functional identification of the framework XV conotoxins derived from different *Conus* species

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ABSTRACT

The conotoxin cysteine framework XV (-C-C-CC-C-C-C-C-), which was named Lt15a, was firstly identified from the cDNA library of *Conus litteratus*. After that, 18 new framework XV conotoxin sequences were cloned from nine *Conus* species. Like other conopeptides, the XV-conotoxins have the conserved signal peptide and propeptide, and there are also some conserved residues in their mature peptide. All the framework XV conotoxins were apparently converged into two branches, because of the indel and point mutations occurred in their mature peptides. By fused with thioredoxin and 6 × His tag, six XV-conotoxins were successfully expressed in *Escherichia coli* and purified. Different framework XV conotoxins have distinct biological activities on mice and frogs, and that may be related to the diversity of the toxin sequences. All the six XV-conotoxins had no obvious effects on the sodium currents of DRG neuron cells of Sprague–Dawley (SD) rats. The identification of this framework of conotoxins enriches our understanding of the structural and functional diversity of conotoxin.

1. Introduction

Conotoxins are regarded as the most potential drug leads and diversified molecules in the world. As we know, there are about 500–700 *Conus* species around the world and the venom of each species contains 100–2000 small highly structured venom peptides [2,13,14]. It is estimated that there are about 500,000 natural conotoxins in the world. Conotoxins are also functionally versatile molecules by selectively targeting different subtypes of neurotransmitter receptors or voltage-gated ion channels [21]. Therefore, conotoxins are usually used as molecular tools in neuroscience research and therapeutic drugs in the clinic [15].

Conotoxins are initially translated as prepropeptide precursors which composed of three regions: a highly conserved signal peptide at the N-terminal end, an intermediate pro-region and the hypervaried mature toxin with conserved cysteine arrangement at C-terminal end [14]. The biologically active conotoxin is produced by proteolytic cleavage from the prepropeptide, an essential post-translational step in conotoxin maturation [4]. The mature toxins are a class of small disulfide-rich peptides containing 12–50 amino acids with a high frequency of posttranslational modifications. And the disulfide bridges make the peptides maintain a stable and conserved tertiary structure. It was reported that the hypervaried mature peptide and the posttranslational modifications may result in the molecular diversity of conotoxins [9].

At present, all the conotoxins can be divided into 26 superfamilies based on their conserved signal peptide, or into 26 cysteine frameworks based on the cysteine number, arrangement and disulfide connectivity of the mature peptide [10]. Previously, conotoxins from the same superfamily usually share a characterized cysteine arrangement in their mature peptides and each cysteine arrangement usually corresponds to the specific disulfide connectivity [6,14]. However, since more conotoxins were found afterwards, some new superfamilies and frameworks were reported, this rule has been severely broken. As M superfamily, a superfamily may contain multiple frameworks [25,28]; while the same framework may also exist in more than one superfamily, like framework VI/VII [10,25].

In this paper, we reported the cysteine framework XV (-C-C-CC-C-C-C-C-) conotoxins, which were first identified in the venom of

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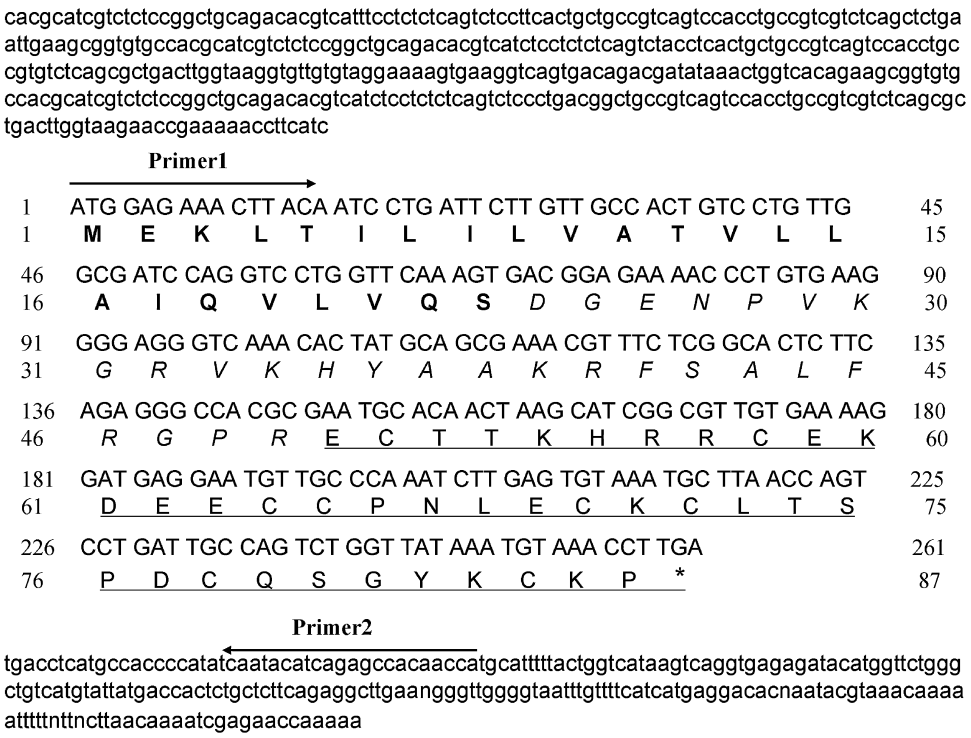

Fig. 1. The full-length cDNA and putative amino acid sequence of Lt15.1. The signal peptide sequence is in boldface, propeptide sequence is in italics, and mature toxin sequence is underlined. The coding sequence is in uppercase while untranslated region in lowercase letters. Primer1 and Primer2 were designed according to the sequences indicated by the arrow.

C. litteratus at 2006 [18], and were classified into O2 superfamily according to the signal peptide. We described the cDNA cloning, identification, recombinant expression, functional assay and evolutional study of these conotoxins. These results could expand our understanding of the diversity and function of conotoxins.

2. Materials and methods

2.1. Sample collection and cDNA library construction

Specimens of *Conus litteratus*, *Conus betulinus*, *Conus emaciatus*, *Conus captitaneus*, *Conus caracteristicus*, *Conus rattus*, *Conus vitulinus*, *Conus varius*, *Conus vexillum* and *Conus miles* were collected from reef flats in West Island near Sanya, China. Venom ducts of cone snails were dissected and immediately preserved in liquid nitrogen respectively. Total RNAs were isolated from the homogenized venom duct by using TRIZOL (Invitrogen, Carlsbad, USA) according to the previously described steps [18].

2.2. Identification of Lt15.1

A cDNA library of the venom duct from *C. litteratus* was constructed and sequenced as described previously [18]. The full-length cDNA clone of conotoxin gene was isolated based on the sequences analysis with the BLAST algorithm available at the National Center for Biotechnology Information (NCBI). Five ESTs were found encoding a conotoxin with the cysteine arrangement (-C-C-CC-C-C-C-C-). The full-length cDNA clone was named Lt15.1 and this scaffold was named framework XV (Fig. 1).

2.3. Cloning and sequencing of cDNAs encoding framework XV conotoxins

In order to investigate the expression of framework XV conotoxins in different *Conus* species, the cDNA libraries of the nine *Conus* species were used respectively as the templates to screen the cDNAs encoding conotoxins with XV scaffold (-C-C-CC-C-C-C-C-) as Lt15.1. Because of the highly conserved 5′UTR, signal peptide and 3′UTR of the conotoxins in the same superfamily, two primers, 5′ primer (Primer1, 5′-CCTTCATCATGGAGAAACTTAC-3′) designed corresponding to the boundary region between 5′UTR and the signal region of Lt15.1, 3′ primer (Primer2, 5′-GGTTGTGGCTCTGATGTATTG-3′) designed according to the 3′UTR region around the stop codon of Lt15.1, were used in the PCR reactions to amplify the XV conotoxin sequences (Fig. 1). The PCR conditions were: 95 °C for 3 min, followed by 30 cycles of 95 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min, the final step was 72 °C for 7 min. The PCR fragments were cloned into pGEM-T Easy Vector (Promega Inc., USA). Ligated vectors were transformed to *Escherichia coli* DH5α competent cells by heat shock. Ampicillin (100 mg/mL) was used for antibiotic resistance selection. Blue/white colony screening was done on LB agar plates using 80 mg/mL X-gal and 0.5 mL/L of 100 mM IPTG to select the positive colonies. The clones were subjected to DNA sequencing (ABI 3730 automatic sequencer, Applied Biosystems, USA) using T7 forward and SP6 reverse primer.

2.4. Sequencing analysis

The signal peptide sequences and cleavage sites of conotoxins were predicted using the ConoPrec tool in ConoServer (http://www.conoserver.org) and the SignalP algorithm (http://www.cbs.dtu.dk/services/SignalP). Nucleotide and amino acid multiple alignments were generated using the ClustalX or GENEDOC, and the alignments were refined manually. Phylogenetic trees were constructed using the neighbor-joining method with MEGA5.

2.5. Recombinant expression of framework XV conotoxins

In order to express the XV-conotoxins soluble in *E. coli*, the fusion expressed vector pTRX-XV-conotoxin was constructed according to