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Identification of insect-selective and mammal-selective toxins from *Parabuthus leiosoma* venom ☆

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Abstract

Venoms were collected from two scorpion species: *Parabuthus leiosoma* and *Parabuthus pallidus* from Kenya. Subcutaneous injection and oral toxicity tests of crude and pure fractions of scorpion venoms were done in *Mus musculus* (mice), *Chilo partellus* and *Busseola fusca*. The highest activity against *C. partellus* was found in *P. leiosoma* venom (LC_{50} 0.689 mg/50 mg body weight). Bioassay-guided purification by a combination of cation-exchange (CE) and reverse-phase high-performance liquid chromatography (RP-HPLC) led to the isolation of three toxic peptides. A lepidopteran-selective toxin (*P. leiosoma* insect toxin, Plit) was isolated, and the partial N-terminal amino acid sequence (-KDGYPVDNANCK-YE-) plus the molecular weight (6688.5 Da) determined. A peptide with significant insect toxicity coupled with mild effects on mice (*P. leiosoma* toxin, Plt) was isolated, and the partial N-terminal amino acid sequence (-LCEKFKVQRL-VELNCVD-) plus the molecular weight (6742.5 Da) was determined. Another toxin with anti-mammalian activity (*P. leisoma* mammal-selective toxin, Plmt), and N-terminal partial amino acid sequence of ADVPGNYPLDKNGNRYY- plus a molecular weight of 7145.5 Da was also isolated. Comparison of the partial N-terminal amino acid sequences with other toxins revealed that Plit shows high homology to other known insect toxins. Similarly, Plmt shows high homology with several birtoxin-like anti-mammalian toxins. Plt does not exhibit homology with any known scorpion toxin with combined anti-insect and anti-mammalian activity.

Keywords: Arthropod; Scorpions; Buthidae; Insecticidal; Anti-mammal; Toxins; Peptides; *Parabuthus leiosoma*

1. Introduction

Scorpions are of considerable importance in ecological food webs since they feed on insect pests, vectors and nuisance species such as biting

☆ *Ethical statement*: The research was done observing good laboratory practices.

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flies, spiders and other scorpions (http://www.nature.nps.gov). The scorpion venom is used to subdue the prey, for offence and as a defense against predators. The active principles in scorpion venoms constitute polypeptides (61–76 amino acid residues) that demonstrate animal group specificity with regard to their toxicity (Possani et al., 1999; Gurevitz et al., 2007). Scorpion venoms cause significant morbidity and mortality in many parts of the world (Rajarajesward et al., 1979). Previously, the majority of chemical and pharmacological studies of scorpion venoms were performed primarily for medical importance as poisons (Lazarovici et al., 1982). Currently, the studies are motivated by the ability of scorpion venoms to serve as pharmacological tools for the excitation of biological systems (Catterall, 1980) and models for target-specific insecticides. The diversity of insect-selective scorpion toxin sequences, with spatially conserved scaffold cross-linked by four disulfide bridges due to evolutionary advantages like high stability and ability to withstand mutations, has renewed the interest in their identification for the development of recombinant biopesticides as safer alternatives to broad-spectrum chemical insecticides (Possani et al., 1999; Martin-Eauclaire and Courad, 1995; Gurevitz et al., 2001). It has been established that venoms of some scorpion species contain several peptides that are selectively toxic to insects thus offering potential alternative, biodegradable and environmentally safe pest control tools (Minton, 1974; Lazarovici et al., 1982).

Several insect-selective toxins have been purified and identified from scorpions collected from different parts of the world (Becerril et al., 1997; Possani et al., 1999; Tytgat et al., 1999; Fajloun et al., 2001; Gurevitz et al., 2007). The toxins in the venoms of scorpions from several genera in Buthidae have been identified (Zlotkin et al., 1978). However, from *Parabuthus* sp. only *Parabuthus transvaalicus* toxin has been identified (Inceoglu et al., 2001, 2002, 2003). This paper reports the extraction and evaluation of venoms from *Parabuthus leiosoma* and *Parabuthus pallidus* collected from Kenya; the isolation, and N-terminal amino acid sequence determination of new insect and mammal specific toxins (Plit and Plmt, respectively) from *P. leiosoma* venom. The isolation of a non-specific toxin (Plt) is also reported. This knowledge may shed more light on toxin diversification within Buthidae.

2. Materials and methods

2.1. Materials and reagents

P. leiosoma and *P. pallidus* were collected from Nguruman, Nanyuki and Mbololo. Scorpions were fed on *Chilo partellus* and *Busseola fusca* and maintained on a 12:12 h light:dark cycle photo period at 28 °C and 40% humidity. Third-instar *C. partellus* and *B. fusca* larvae, raised at the Animal Rearing and Quarantine Unit (ARQU) in ICIPE, were used in insecticidal assays. Larval colonies were maintained at 25 °C on a 12:12 h photoperiod at 45% relative humidity, and fed on artificial diet. Swiss albino laboratory mice (*Mus musculus*), reared at ARQU in ICIPE, were used for mammalian toxicity assays. Mice colonies were maintained at 20–24 °C on a 14:10 h light:dark cycle photo period, and fed on 5% fat Agway Prolab 3000 rat and mouse chow.

2.2. Extraction of scorpion venom

Manual stimulation was used for collecting scorpion venoms (Froy et al., 1999). Briefly, a live scorpion was released in an arena (tray) and provoked, by tapping on the tail, to sting a 50 ml open centrifuge tube wrapped with parafilm. The venom deposited on the surface of the parafilm was reconstituted in double-distilled water, transferred using a micropipette into a vial placed on ice bath and frozen at −20 °C.

The venoms collected from each of the scorpion species from the same locality were pooled, separately lyophilized in an Eppendorf tube (1.5 ml), weighed, re-suspended in de-ionized water (2000 μl) and homogenized using a Potter–Elvehjam machine. The insoluble material was removed by centrifugation (26,000*g* or 15,000 rpm, 20 min, 4 °C), de-ionized water (1000 μl) added to the pellet, homogenization repeated, the contents centrifuged and the supernatant collected. The procedure was repeated two to three times to maximize the yield of the peptide toxins from the venom. The supernatants were pooled, lyophilized and stored at −20 °C for bioassay and purification.

2.3. Purification of toxin

Lyophilized venom of the scorpion species that exhibited the highest activity was sequentially subjected to cation-exchange (CE) and reverse-phase