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Mass spectrometry analysis, amino acid sequence and biological activity of venom components from the Brazilian scorpion *Opisthacanthus cayaporum* ☆

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ABSTRACT

This communication reports the separation of 80 fractions from the venom of the Ischnuridae scorpion *Opisthacanthus cayaporum* by high-performance liquid chromatography (HPLC). From these, 93 distinct components were identified by liquid chromatography/electrospray mass spectrometry (LC/ESI-MS) analysis, with molecular weights varying from 229.2 to 61,144.0 atomic mass units. Additionally, the HPLC fractions were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) which resulted in 221 distinct components, among which were 52 of the 93 obtained by LC/ESI-MS. The entire set of different molecular species found (total of 262 molecular masses) has a trimodal molecular weight distribution, with 42% of the components possessing 229.2–2985.3 Da, 37% within the range of 3045.0–7258.6 Da and 12% within the range 7458.4–9429 Da. Seventeen peptides/proteins were isolated and were sequenced by Edman degradation, among which were a scorpine-like peptide (8315 Da), presenting antimicrobial activity, and two phospholipase A2 with a molecular weight around 14 kDa. The pharmacological effects of the venom were tested on isolated rat and insect (cockroach) nerves using the single sucrose-gap assay. The ED50 of the venom was 1.1 mg/ml in insect nerves. Venom concentrations in the order of 3 mg/ml causes only 9% reduction of compound action potentials (APs) of rat nerves, suggesting that this venom is rather specific for insects. Comparative analysis of venom from male and female *O. cayaporum* was performed by HPLC and MALDI-TOF-MS showing no qualitative variations, but rather quantitative differences among both samples.

1. Introduction

Many organisms produce and secrete venom for defense and/or capture of preys. These secretions are a rich source of pharmacologically active compounds such as enzymes, toxic peptides, low molecular weight substances and proteins of unknown functions. Toxins isolated from venoms affect with high selectivity and affinity a large number of targets, among which are ion channels, acetylcholine receptors, enzymes, cell

☆ *Ethical statement*: The scorpions are milked after CO2 anesthesia. No direct assays were performed with other animals alive. The physiological experiments were performed with tissues recovered from animals according to standard procedures approved by the ethical committee of our Institutions.

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membrane polarity and various hemostatic pathways. Toxic peptides isolated from venomous animals are usually of small sizes, ranging from 8 to 70 amino acids, which are highly compacted and stabilized by either disulfide bonds or by hydrogen bonding interactions. Some toxic peptides show post-translational-modified amino acids such as those from cone snails and amphibians (reviewed in Buczek et al., 2005; Auvynet et al., 2006, respectively).

Among the venomous animals are the scorpions. During about 400 millions years, they have successfully developed a large variety of bioactive peptides. These peptides contain 13–76 amino acids which can be classified into two types: disulfide-bridged or non-disulfide-bridged containing peptides. Scorpion toxins with disulfide bridges specifically interact with membrane-bound ionic channels including Na^+, K^+, Cl^- and Ca^{2+} channels (reviewed in Catterall et al., 2007; Possani et al., 1999). The Na^+-channel-specific toxins (Na^+-toxins) are composed of 58–76 amino acid residues linked by four disulfide bridges, whereas those specific for K^+ channels (K^+-toxins) and Cl^- channels are short-chain peptides composed of 20–41 amino acid residues and stabilized by three or four disulfide bridges (Possani and Rodríguez de la Vega, 2006). Recently, some "long-chain" K^+-channel toxins and Na^+-channel toxins that are constrained by only three disulfide bridges have also been reported (Diego-García et al., 2007). Toxins affecting Ca^{2+} channels possess a more variable amino acid length (Zhu et al., 2004a; Gurrola et al., 2005; Zhijian et al., 2006). Thus far, the best-known groups are those acting on Na^+ and K^+ channels. Currently, about 190 Na^+-channel toxins, 130 K^+-channel toxins, 17 Cl^--channel toxins and 5 Ca^{2+}-channel toxins have been described (Scorpion Toxin Database, see: http://sdmc.i2r.a-star.edu.sg/scorpion/). These ion channel blockers or modulators have been widely used as tools for the identification, isolation and characterization of ion channel proteins, including their pharmacological and physiological functions. Fewer non-disulfide-bridged components have been isolated and characterized from scorpion venoms, among which are peptides showing bradykinin-potentiating, antimicrobial, hemolytic and immune-modulating activities (Zeng et al., 2005). Proteomic approach was conducted with nine scorpion species documenting the overall composition of their venom gland secretion. All of these studies were done with scorpions from the family Buthidae and most of them belonging to the *Tityus* genus (Pimenta et al., 2001; Batista et al., 2004, 2006; Diego-García et al., 2005; Barona et al., 2006; Borges et al., 2006; Caliskan et al., 2006; Nascimento et al., 2006; Favreau et al., 2006).

Further insights into scorpion venom compositions were achieved by gene cloning using PCR-based methods conducted with cDNA libraries as template. Recently, the first transcriptome analysis of genes transcribed by the venomous gland of a scorpion was reported (Schwartz et al., 2007a).

Nothing or very little is known (a congress report, Schwartz et al., 2007b) about the venom from the Brazilian scorpion *Opisthacanthus cayaporum*, from the Ischnuridae family, which is the object of this communication. This family includes the genera *Opisthacanthus*, *Hadogenes* and *Cheloctonus* which are not venomous to man, and a sting from one of these genera should cause no more damage than that of a bee sting. Due to its lower toxicity, there have been few reports on the venom of scorpions from the Ischnuridae family. This family is distributed through Africa, South-East Asia, Australia and South America and associated islands. *Opisthacanthus* occurs in the Caribbean, Central and South Americas, Africa and Madagascar. The genus *Opisthacanthus* presents a gondwanian pattern of distribution. In Brazil the family Ischnuridae is represented by two species. *O. cayaporum* is endemic to open savannas in the eastern Amazonian (South of the State of Pará and State of Tocantins). From the venom of the species *Opisthacanthus madagascariensis*, endemic of Madagascar, cytotoxic linear peptides (IsCT and IsCT2, Dai et al., 2001, 2002) and K^+-channel blockers (IsTX and Om-toxins, Yamaji et al., 2004; Chagot et al., 2005) have been identified and studied.

In this communication we report the results of proteomic, biochemical and pharmacological characterizations of the soluble venom extracted from the scorpion *O. cayaporum*. The molecular masses of the venom components were identified after high-performance liquid chromatographic separation (HPLC) of the venom. Several of the main HPLC components were sequenced. Pharmacological assays were performed using both insects and rat nerve cells. Finally, a comparative MALDI-TOF-MS analysis was performed using samples from male and female *O. cayaporum* in order to verify the existence of possible sex-linked venom variations.

2. Material and methods

2.1. Venom source and purification procedures

Scorpions of the species *O. cayaporum* were captured in Palmas (State of Tocantins, Brazil) under the Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis (IBAMA) license number 048/2007-CGFAU. The crude venom from 15 adult scorpions of both sexes was obtained by electrical stimulation and dried. The material was dissolved in water and centrifuged at 10,000*g* for 10 min. The soluble supernatant was stored at −20 °C. When needed a sample was separated by HPLC in a C18 reverse-phase analytical column (Vydac, Hisperia, CA), using a linear gradient from 0% solvent A (0.12% trifluoroacetic acid, TFA, in water) to 60% solvent B (0.10% TFA in acetonitrile) run for 60 min, at a flow rate of 1 ml/min. In this conditions some peptides were obtained in homogeneous form; others required a second HPLC procedure, using an analytical C18 reverse-phase column, run with slightly modified gradients to improve separation, as described in the figure legends.

In order to compare the chromatographic profiles of male and female venoms, the venom of scorpions of each sex were separately obtained and submitted to HPLC fractioning as described above.