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Characterization of two peptides isolated from the venom of social wasp *Chartergellus communis* (Hymenoptera: Vespidae): Influence of multiple alanine residues and C-terminal amidation on biological effects

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ABSTRACT

Chatergellus communis is a wasp species endemic to the neotropical region and its venom constituents have never been described. In this study, two peptides from *C. communis* venom, denominated Communis and Communis-AAAA, were chemically and biologically characterized. In respect to the chemical characterization, the following amino acid sequences and molecular masses were identified:

Communis: Ile-Asn-Trp-Lys-Ala-Ile-Leu-Gly-Lys-Ile-Gly-Lys-COOH (1340.9 Da)

Communis-AAAA: Ile-Asn-Trp-Lys-Ala-Ile-Leu-Gly-Lys-Ile-Gly-Lys-Ala-Ala-Ala-Ala-Val-Xle-NH_2 (1836.3 Da).

Furthermore, their biological effects were compared, accounting for the differences in structural characteristics between the two peptides. To this end, three biological assays were performed in order to evaluate the hyperalgesic, edematogenic and hemolytic effects of these molecules. Communis-AAAA, unlike Communis, showed a potent hemolytic activity with EC_{50} = 142.6 μM. Moreover, the highest dose of Communis-AAAA (2nmol/animal) induced hyperalgesia in mice. On the other hand, Communis (10nmol/animal) was able to induce edema but did not present hemolytic or hyperalgesic activity. Although both peptides have similarities in linear structures, we demonstrated the distinct biological effects of Communis and Communis-AAAA. This is the first study with *Chartegellus communis* venom, and both Communis and Communis-AAAA are unpublished peptides.

1. Introduction

The insects belonging to the Hymenoptera order are included in the families Apidae (bees), Vespidae (wasps, hornets and yellow jackets) and Formicidae (ants), and all of them are capable of producing and injecting their venoms using a inoculator apparatus [1,2].

Venoms from wasps are known to contain a cocktail of diversified compounds, which are able to cause a variety of biological manifestations in the human organism after stinging [3]. The main molecules that were identified are proteic compounds, such as allergens, enzymes and bioactive peptides, and other compounds with low molecular masses, such as biogenic amines and amino acids.

These components have been linked directly and indirectly to several responses, such as failure of kidney and liver, myotoxicity, neurotoxicity and vasoactive properties, which may cause intravascular hemolysis, rhabdomyolysis and death [4,5].

In respect to peptides, the mastoparans are among the better characterized compounds in this venom. They were first described in the wasp *Vespula lewisii,* and the name refers to the first target description, mastocytes [6,7]. A remarkable feature of these molecules is their size, since they have an average of 14 amino acids, and the presence of residues of lysine [8]. They are able to exert many effects on biological

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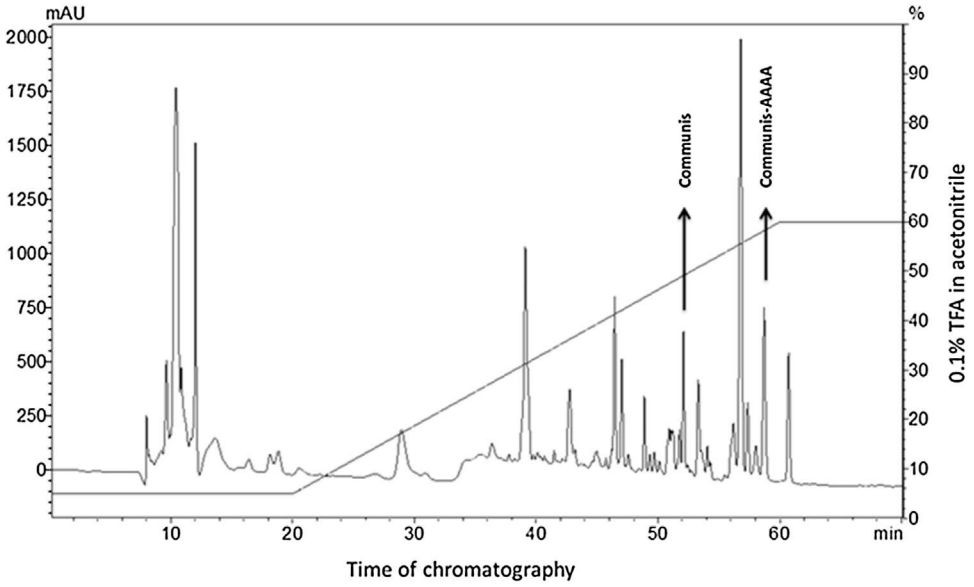

Fig. 1. Chromatogram of the compounds with low molecular masses present in the venom of *C. communis* using a semi-preparative C18 column. They were reconstituted in water and 5% of acetonitrile. Absorbance was monitored at 216 and 280 nm.

systems, such as the activation of G-protein receptors [9,10], stimulation of phospholipases A2 and C, and the modulation of Ca^{2+} [11]. Moreover, mastoparans possess cytolytic activity that can be associated with the induction of apoptosis, necrosis [10] and antimicrobial effects [12].

Some studies have reported that the presence of multiple hydrophobic residues, such as alanine, reinforces the interaction with the phospholipid membrane, thus contributing to increasing the positive charge [13,14] and then contributing to biological effects related to the interaction in biological membranes. For example, polyalanine, a rationally designed peptide based on a peptide originally isolated from the polar fish *Pleuronectes americanus*, demonstrated promising activity against several bacteria by improving some characteristics. These included the amphipathicity of the molecule and an increased probability of presenting a helical structure [15]. However, mastoparans with multiple alanine residues have not been observed.

The wasps of the *Chartergellus communis* species were first described by Richards in 1978 [16] and belong to one of the less abundant neotropical genera of wasps [17]. In Brazil, they are predominant in savannah-type vegetation classified as cerrado stricto-senso or “cerradão” [18].

The aim of this work was to identify and analyze two peptides isolated from *Chartergellus communis* venom, called Communis and Communis-AAAA. The study was conducted with the intention of evaluating the relationship between their structural characteristics and biological actions, in particular, their hemolytic activity, hyperalgesic potential and effects on edema formation.

2. Material and methods

2.1. Wasp collection and venom extraction

Two nests containing approximately 370 social wasps of the species *Chartergellus communis* were collected in the Parque Municipal do Itiquira, a green area located near the town of Formosa (Goiás state, Brazil, geographic coordinates: 15°22′4“S/47°27′20“W). The procedures for collection, access and transport of genetic material were carried out with the authorization and licenses of the appropriate government agencies of Brazil (license ICMBio number 21723-2/process CNPq number 010476/2013-0). All wasps were initially euthanized by freezing at −20 °C. After their death, manual extraction of glands and venom reservoirs was carried out in female wasps. The material obtained was submitted to successive ultrafiltration procedures, by using a filter of 10 KDa (Microcon, Millipore), in order to acquire only compounds with low molecular masses (LMM). Finally, the LMM samples were lyophilized, quantified and stocked at −80 °C until use.

2.2. Isolation of peptide fractions

The isolation of the two peptide fractions present in the *Chartergellus communis* LMM venom fraction was performed by the technique of High Performance Liquid Chromatography (HPLC) in reverse phase, using a semi-preparative column (C18 Luna, 10 µm, 250 × 10 mm, Phenomenex®, Torrence, CA, USA). For this, the samples were re-suspended in deionized water containing 5% of acetonitrile. Solvent A consisted of 0.1% trifluoroacetic acid (TFA) in deionized water (v/v) and solvent B was a solution of 0.1% TFA in acetonitrile (ACN). The absorbances were monitored at 216 and 280 nm, and the total time of chromatography was 100 min. To obtain peptides with the high purity necessary for sequencing steps and biological assays, the process of rechomatography had to be followed, using an analytical column (C18 Synergi 4 µm, Fusion-RP, 250 × 4.6 mm, Phenomenex®, Torrance, CA, USA).

2.3. Sequencing and identification of peptides

The peptide fractions isolated after HPLC were submitted to mass spectrometry using a Matrix-Assisted Laser Desorption Ionization time of flight (MALDI TOF/TOF) Autoflex speed (Bruker Daltonics®, Germany), to inspect molecular masses, to identify the primary amino acid sequences and to verify the degree of sample purity. This step was carried out in two distinct operation modes: the positive reflective mode, to determine molecular masses, and the LIFT mode, to proceed with the *de novo* sequencing. The matrix used was alpha-cyano-4-hydroxycinnamic acid and the detection range consisted of a mass/charge (m/z) ratio of 100–10,000 Da. To obtain the data in this analysis the following software was utilized: FlexControl 3.4 (Bruker Daltonics®, Germany), for the acquisition of the mass spectra, and FlexAnalysis 3.4 (Bruker Daltonics®, Germany), for interpretation and *de novo* sequencing. As a complementary sequencing method, the technique of Automated Edman degradation was performed using a PPSQ-31A Protein Sequencer from Shimadzu Scientific Instruments, Inc. (Columbia, MD, USA) (data shown in Supplementary Results) (Supplementary data 1 and 2).