Toxicon 107 (2015) 327–334

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Comparative proteomic analysis of male and female venoms from the Cuban scorpion *Rhopalurus junceus*

Rodolfo Rodríguez-Ravelo [a, b, 1], Cesar V.F. Batista [c, 1], Fredy I.V. Coronas [a], Fernando Z. Zamudio [a], Lorena Hernández-Orihuela [c], Georgina Espinosa-López [d], Ariel Ruiz-Urquiola [e], Lourival D. Possani [a, *]

[a] Department of Molecular Medicine and Bioprocesses, Institute of Biotechnology, National Autonomous University of Mexico, Avenida Universidad, 2001, Colonia Chamilpa, Apartado Postal 510-3, Cuernavaca 62210, Mexico
[b] Center for Mountain Development, Ministry of Science, Technology and Environment, Limonar de Monte Roux, El Salvador, Guantánamo, Cuba
[c] Proteomics Laboratory, Departament of Developmental Genetics, Institute of Biotechnology, National Autonomous University of Mexico, Avenida Universidad, 2001, Colonia Chamilpa, Apartado Postal 510-3, Cuernavaca 62210, Mexico
[d] Department of Biochemistry, School of Biology, University of Havana, Havana, Cuba
[e] Center of Marine Research, University of Havana, Havana, Cuba

ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form
24 June 2015
Accepted 30 June 2015
Available online 10 July 2015

Keywords:
Amino acid sequence
Gender differences
Ion-channel
Mass spectrometry
Proteomic analysis
Venom components

ABSTRACT

A complete mass spectrometry analysis of venom components from male and female scorpions of the species *Rhophalurus junceus* of Cuba is reported. In the order of 200 individual molecular masses were identified in both venoms, from which 63 are identical in male and females genders. It means that a significant difference of venom components exists between individuals of different sexes, but the most abundant components are present in both sexes. The relative abundance of identical components is different among the genders. Three well defined groups of different peptides were separated and identified. The first group corresponds to peptides with molecular masses of 1000–2000 Da; the second to peptides with 3500–4500 Da molecular weight, and the third with 6500–8000 Da molecular weights. A total of 86 peptides rich in disulfide bridges were found in the venoms, 27 with three disulfide bridges and 59 with four disulfide bridges. LC-MS/MS analysis allowed the identification and amino acid sequence determination of 31 novel peptides in male venom. Two new putative K^+-channel peptides were sequences by Edman degradation. They contain 37 amino acid residues, packed by three disulfide bridges and were assigned the systematic numbers: α-KTx 1.18 and α-KTx 2.15.

Biological significance

Venom secretions of animals contain a substantial amount of pharmacologically active agents that are used to subjugate their preys or to defend themselves from predators. The mechanisms for selection and establishment of these biodiversity of components are under intense investigation. Natural selection and the ecological environment where these animals live are considered fundamental facts that should define the structure and function of the venom components. Male and female arthropods are known to contain pharmacologically active venoms components, but little information is available on possible gender differences that might exist, within the same species. Here we have conducted an extended proteomic analysis of venoms from male and female scorpions. To the best of our knowledge this is the most complete proteomic analysis available showing the differences of molecular masses of venoms components from different genders of scorpions.

1. Introduction

Scorpion venoms are known to contain a great diversity of pharmacologically active compounds, varying from toxic peptides

* Corresponding author.
E-mail address: possani@ibt.unam.mx (L.D. Possani).
[1] Contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.toxicon.2015.06.026
0041-0101/

that recognize ion-channels to antimicrobial peptides and different types of enzymes, reviewed in (Rodríguez de la Vega et al., 2013 and Ortiz et al., 2015). The best known components found in scorpion venoms are short and long chain peptides that recognize ion-channels: sodium, potassium, calcium and chloride (see review by Possani et al., 1999). Short chain peptides containing from 29 to 41 amino acid residues, cross-linked by three or four disulfide bridges, are known to block K^+-channels (Rodríguez de la Vega and Possani, 2004), and peptides with 59–72 amino acid residues, mostly cross-linked by four disulfide bridges, are considered to be modifiers of the gating mechanism of Na^+-channels (Rodríguez de la Vega and Possani, 2005). Two sub-types of Na^+-channel specific toxins are described: α-toxins, originally obtained from the venom of North African scorpions, modify the closing mechanism of the channel, reviewed in Gurevitz (2012) and β-toxins, initially shown to exist in American scorpions modify the open mechanism of the channels, reviewed in Pedraza-Escalona and Possani (2013). The K^+-channel blocking peptides are classified into four sub-groups: α, β, γ and κ (Tytgat et al., 1999), see also review Rodríguez de la Vega et al. (2013). The venoms of the scorpions belonging to the family Buthidae are the best studied; probably because they might cause human lethality (Possani et al., 1999). Recently, scorpions belonging both to Buthidae and non-Buthidae families have been studied intensively by means of transcriptomic and proteomic analysis (Ma et al., 2009; Rendon-Anaya et al., 2012; Ruiming et al., 2010; Schwartz et al., 2008 and Xu et al., 2014).

The venom components from the Cuban scorpion *Rhopalurus junceus*, here after abbreviated *R. junceus*, is scarcely studied (García-Gómez et al., 2011 and Rodriguez-Ravelo et al., 2013).

It is known that venom components can vary, depending on sex. Although there is a limited knowledge on the venom composition of male and female scorpions belonging to a given species (De Sousa et al., 2010 and Yamaji et al., 2004), in this study we decided to explore in depth the variation existing in the venom composition of both genders of the Cuban scorpion *R. junceus*. Here we describe an extended proteomic analysis conducted with both female and male scorpions of this species. Since many scorpion components are rich-cysteine containing peptides, the proteomic analysis was performed with native and reduced peptides. Additional information on amino acid sequence of peptides from this species is also reported for the first time here. In our opinion, this is the most complete study conducted until now on venom components of scorpion based on sexual differences.

2. Material and methods

2.1. Source of animals and chemicals

Scorpions of the species *R. junceus* were collected in Baracoense phytogeographical district, belonging to Nipe-Sagua-Baracoa massif (situated East of the Quibiján and Toa Rivers), with official permission of the "Environment Unit, CITMA-Guantánamo" of Cuba for the collection and management of flora and fauna. Twenty male and twenty female adult scorpions were collected and kept separately. Each one of the animals was individually milked for venom in separated tubes, using electrical stimulation. The venom from male and female scorpions were pooled separately at the Finlay Institute in Habana, lyophilized and kept at −20 °C. Once in Mexico, the pooled crude venom of each gender was solubilized in water, centrifuged at 10,000 × g for 15 min. The whole soluble venom content was estimated by absorbance at 280 nm (assuming one unit of absorbance is equivalent to 1 mg/mL of venom concentration). Samples of each gender were fractionated by chromatographic separation prior spectrometric analysis. The solvents and chemicals used were of analytical grade and double-distilled water was used throughout, as earlier described by our group (García-Gómez et al., 2011 and Batista et al., 2004).

2.2. Isolation procedures

The soluble venom of male and female scorpion was separately applied into the high performance liquid chromatographic (HPLC) system. A total of 1 mg protein content in 100 μl solution of each was applied independently. An analytical C18 reverse-phase column (dimensions of 250 × 10 mm) obtained from Vydac (USA) was used for HPLC fractionation. Components were purified using a linear gradient starting from solution A [0.12% trifluoroacetic acid (TFA) in water] to 60% solution B (0.10% TFA in acetonitrile), run for 60 min. The detection was monitored by absorbance at 230 nm with full scale sensitivity and eluted at 1 mL/min flow-rate. All HPLC fractions were collected manually divided into two equal aliquots and then dried using a Savant SpeedVac dryer.

2.3. Sequence by Edman degradation

A venom component that elutes from HPLC at 24.78 min was selected. This fraction contained two different molecular mass peptides. The sample was reduced and alkylated according to procedure described (García-Gómez et al., 2011). After separation by HPLC into a C18 reverse column, both peptides were subjected to Edman degradation, using a PPSQ-31A Protein Sequencer from Shimadzu Scientific Instruments, Inc. (Columbia, Maryland, USA), and the full amino acid sequence was determined. The completion of the amino acid sequence determination was confirmed by mass spectrometry analysis of the native peptides. The protein sequence data reported in this paper will appear in the UniProt Knowledgebase under the accession numbers C0HJS9 for a-KTx 1.18 and C0HJT0 for a-KTx 2.15.

2.4. Reduction of HPLC fractions

Half the SpeedVac lyophilized aliquots were solubilized in 50 μL of 50 mM DTT at pH 6.5. The reduction of disulfide bridges was processed at 56 °C for 1 h. After that, the aliquots were desalting with ZipTip C18 using 0.1% acetic acid to stabilize the tip. The venom components were eluted with 70% acetonitrile containing 0.1% acetic acid and subjected to MS analysis.

2.5. Mass spectrometry analysis

All samples analyzed by mass spectrometry followed the same work flow. First, 0.5 μg of soluble venom from male and female scorpions were individually analyzed by liquid chromatography–electrospray tandem mass spectrometry LC-MS/MS. The full comparative analysis was conducted with the same venom of each gender of scorpion. Different aliquots from the same chromatographic separation were used. A C18-capillary column packed in house was coupled to a mass spectrometer Orbitrap from Thermo Scientific (San Jose, CA). Mobile phase A used was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. A flow rate of 400 nL/min was used. Following equilibration of the column in 5% solvent B, an aliquot of each total venom (5 μl corresponding to 0.5 μg of venom) was injected, then the organic content of the mobile phase was increased linearly to 60% over 150 min, and then to 70% over 1 min. The effluent was sprayed by a nanoelectrospray ion source into the hybrid lineal ion trap–Orbitrap mass spectrometer. MS spectra were acquired in positive and profile mode using the Orbitrap analyzer in the *m/z* range between 350 and 1600 at 60,000 resolution. For each MS/MS spectrum, the 10 most intense multiple charged ions over a threshold of 1000