JOURNAL OF PROTEOMICS 106 (2014) 17–29

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Jellyfish venomics and venom gland transcriptomics analysis of *Stomolophus meleagris* to reveal the toxins associated with sting

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ARTICLE INFO

Article history:
Received 21 November 2013
Accepted 5 April 2014

Keywords:
Jellyfish
Stomolophus meleagris
Venomics
Transcriptomics
Toxin
Sting

ABSTRACT

Jellyfish *Stomolophus meleagris* is a very dangerous animal because of its strong toxicity. However, the composition of the venom is still unclear. Both proteomics and transcriptomics approaches were applied in present study to investigate the major components and their possible relationships to the sting. The proteomics of the venom from *S. meleagris* was conducted by tryptic digestion of the crude venom followed by RP-HPLC separation and MS/MS analysis of the tryptic peptides. The venom gland transcriptome was analyzed using a high-throughput Illumina sequencing platform HiSeq™ 2000 with de novo assembly. A total of 218 toxins were identified including C-type lectin, phospholipase A_2 (PLA_2), potassium channel inhibitor, protease inhibitor, metalloprotease, hemolysin and other toxins, most of which should be responsible for the sting. Among them, serine protease inhibitor, PLA_2, potassium channel inhibitor and metalloprotease are predominant, representing 28.44%, 21.56%, 16.06% and 15.14% of the identified venom proteins, respectively. Overall, our combined proteomics and transcriptomics approach provides a systematic overview of the toxins in the venom of jellyfish *S. meleagris* and it will be significant to understand the mechanism of the sting.

Biological significance

Jellyfish *Stomolophus meleagris* is a very dangerous animal because of its strong toxicity. It often bloomed in the coast of China in recent years and caused thousands of people stung and even deaths every year. However, the components which caused sting are still unknown yet. In addition, no study about the venomics of jellyfish *S. meleagris* has been reported. In the present study, both proteomics and transcriptomics approaches were applied to investigate the major components related to the sting. The result showed that major component included C-type lectin, phospholipase A_2, potassium channel inhibitor, protease inhibitor, metalloprotease, hemolysin and other toxins, which should be responsible for the effect of sting. This is the first research about the venomics of jellyfish *S. meleagris*. It will be significant to understand the mechanism of the biological effects and helpful to develop ways to deal with the sting.

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http://dx.doi.org/10.1016/j.jprot.2014.04.011
1874-3919/

1. Introduction

Historically, human beings have suffered from the venomous animals like snake, spider, bee, pufferfish, sea anemone and jellyfish. Especially, with a massive increase in the jellyfish blooms worldwide in recent years [1–4], numerous victims have been stung and even caused many deaths every year.

Jellyfish *Stomolophus meleagris*, a cnidarian of the phylum *Coelenterata*, the class *Scyphomedusae*, the order *Rhizostomeae*, the family *Stomolophidae*, and the genus *Stomolophus*, is one of the most dangerous jellyfish in China. It is a large jellyfish with a bell ranging from 180 mm to 980 mm in diameter maturely and often abundant in late summer to early autumn from the Bohai Sea to the Yellow Sea, and in the South China Sea (Fig. 1). Thousands of people including swimmers, fishermen and seamen got stung every year and the victims would suffer itch, edema, myalgia, dyspnea, hypotension, shock, and even death after being stung (Fig. 2). The venom, secreted by the nematocysts of tentacles and used for predation or defense by injecting various toxins into the body of prey or predator, should be responsible for those serious symptoms. However, what's the composition of the venom? How do those toxins perform the sting? These questions are not clear yet. Some researchers have tried to clarify those questions by isolating and characterizing the toxins from the jellyfish venom individually [5–11]. But, the isolation and identification is a difficult and time-consuming process and the composition of the venom is so complex that it is almost impossible to isolate all the toxins from it within a short time. In addition, the sting is a synthetical action, which needs the cooperation of all the toxins in the venom. Therefore, an overall method is urgently needed to investigate the composition of jellyfish venom and the mechanism of sting.

With the progress in the omics technologies and bioinformatics, it is convenient to analyze the whole components from the cell of organism instead of individual analysis. Global analysis of cDNA and proteins are being widely applied in the venomics field for the purpose of exploiting and comparing the total venom transcriptome or proteome as well as to analyze specific populations of toxins, such as snake venomics [12–19], spider venomics [20,21], scorpion venomics [22–25], snail venomics [26,27], ant venomics [28], centipede venomics [29] and so on. However, no jellyfish genome and large sequence data set is available to help with venom protein identification by mass spectrometric analysis or to index venom genes. Therefore, in the present study, we combined proteomic and transcriptomic approaches with de novo assembly to characterize the venom profile of jellyfish *S. meleagris*, aiming to identify the major components and the possible relationship with sting.

2. Materials and methods

2.1. Animals and venom

Jellyfish *S. meleagris* were collected from the coast of Qingdao, China in August 2012. The fresh tentacles were excised manually from living specimens as soon as possible after being captured and were then snap-frozen immediately in liquid nitrogen and stored at −80 °C for use. The venom extraction method was almost the same as we described before [11]. Briefly, after autolysis overnight at 4 °C, the tentacles were stirred for 10 min and filtered through a plankton net to remove most of the tentacle debris. The filtrate was then centrifuged at 10,000 *g* for 15 min at 4 °C, and the sediment was collected and sonicated in cold extraction buffer (20 mM Tris–HCl, pH 7.8, 0.15 M NaCl, 1 mM EDTA, 5 μg/mL Pepstatin A and 0.5 mM PMSF). The venom was then lyophilized and stored at −80 °C for proteomic analysis. Then, a combination strategy of proteomics and transcriptomics approach was employed for the high-throughput identification of sting-related toxins in the venom of jellyfish *S. meleagris*.

2.2. Sample digestion

The method of *S. meleagris* venomics was conducted almost the same as we described before [30]. Briefly, the lyophilized venom was dissolved in 6 M guanidine hydrochloride, 100 mM, pH 8.3, Tris–HCl and then reacted at 37 °C for 2.5 h with 10 mM DTT. Subsequently, 50 mM iodoacetic acid was added to the terminal concentration of 50 mM and reacted at room temperature for 40 min in dark. 200 μL, 100 mM NH_4HCO_3 was added and then centrifuged at 4 °C, 10,000 *g* for 2 h with an ultrafiltration device (MWCO 3 kDa). The sample was then adjusted to pH 8.0–8.5 and digested with trypsin (trypsin:proteins = 1:20) for 20 h at 37 °C. Finally, the digested proteins were lyophilized and stored at −80 °C for further use.

2.3. HPLC separation and mass spectrometric analysis

The digested proteins were desalted on reversed phase (RP) columns (Zorbax 300 SB C_{18}, Agilent Technologies) and then separated on an analytical RP column (150 μm i.d., 100 mm length, Column technology Inc., Fremont, CA) using Ettan MDLC system (GE Healthcare). In the process of separation, the column was equilibrated with 95% buffer A (0.1% formic acid in HPLC grade water) for 15 min. 20 μg of tryptic peptide mixtures was loaded onto the columns with a flow rate of 2 μL/min by using a linear gradient of 4–50% buffer B (0.1% formic acid in acetonitrile) for 120 min. A Finnigan LTQ linear ion trap MS (Thermo Electron) equipped with an electrospray interface was connected to the LC setup for eluted peptide detection. The positive ion mode was employed with the spray voltage of the mass spectrometer at 3.0 kV and the spray temperature of 200 °C for peptides. Normalized collision energy was set to 35% and the full scan ranged from m/z 300 to 1800. Data-dependent MS/MS spectra were obtained simultaneously and each scan cycle consisted of one full MS scan in profile mode followed by five MS/MS scans in centroid mode with the following dynamic exclusion settings: repeat count 2, repeat duration 30 s, and exclusion duration 90 s.

2.4. Identification of the venom proteins

BioworksBrowser rev.3.1 (Thermo Electron, San Jose, CA) was employed to search against the SWISS-PROT toxin database