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Proteomic characterisation of toxins isolated from nematocysts of the South Atlantic jellyfish *Olindias sambaquiensis*

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ABSTRACT

Surprisingly little is known of the toxic arsenal of cnidarian nematocysts compared to other venomous animals. Here we investigate the toxins of nematocysts isolated from the jellyfish *Olindias sambaquiensis*. A total of 29 unique ms/ms events were annotated as potential toxins homologous to the toxic proteins from diverse animal phyla, including cone-snails, snakes, spiders, scorpions, wasp, bee, parasitic worm and other Cnidaria. Biological activities of these potential toxins include cytolysins, neurotoxins, phospholipases and toxic peptidases. The presence of several toxic enzymes is intriguing, such as sphingomyelin phosphodiesterase B (SMase B) that has only been described in certain spider venoms, and a prepro-haystatin P-IIId snake venom metalloproteinase (SVMP) that activates coagulation factor X, which is very rare even in snake venoms. Our annotation reveals sequence orthologs to many representatives of the most important superfamilies of peptide venoms suggesting that their origins in higher organisms arise from deep eumetazoan innovations. Accordingly, cnidarian venoms may possess unique biological properties that might generate new leads in the discovery of novel pharmacologically active drugs.

1. Introduction

Cnidaria is a diverse phylum of basal metazoans comprising over 10,000 species, predominately marine organisms (Daly et al., 2007; Zhang, 2011). The phylum has two major lineages: Anthozoa (sea anemones and corals) which live as sessile polyps, and the Medusozoa (jellyfishes and *Hydra*), comprising the classes Cubozoa, Hydrozoa, Scyphozoa and Staurozoa. Species of these four classes have either a free-swimming or attached medusa stage and many retain the ancestral stage of sessile polyps during their life cycles. Cnidarians have external radial symmetry, although many species are either asymmetric or bilateral in their internal anatomy (Marques and Collins, 2004). Cnidarian polyps and medusae have a single body opening

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that acts as both mouth and anus and is generally surrounded by tentacles bearing stinging cells. The nematocytes (stinging cells), sometimes called cnidocytes, are a special type of cnidae and constitutes the defining synapomorphic trait of the phylum Cnidaria (Marques and Collins, 2004). Cnidarians are toxin-producing animals and, having existed since the Cambrian (Cartwright et al., 2007) or even Pre-Cambrian (Van Iten et al., 2013), is possibly the oldest lineage of animals to have evolved means to inject toxins into prey. While they do not have the macromorphological apparatus such as the fangs of snakes to deliver its venomous substances, cnidarians have unique secretory organelles (nematocysts) within their stinging cells. There have been numerous studies characterising the venoms and toxins of many poisonous animals such as cone-snails, scorpions, snakes and spiders, but by comparison very few cnidarian venoms and toxins have been examined in detail (Turk and Kem, 2009). This is surprising because, although most cnidarians do not have harmful nematocysts that are able to penetrate human skin, contact with certain cubozoans, such as *Chironex fleckeri* (the Pacific Sea Wasp), *Carukia barnesi* and *Malo kingi* (the latter two are both commonly called Irukandji Jellyfish) can be fatal (Fenner and Harrisson, 2000). Other medusozoan taxa, species of both planktonic (Haddad et al., 2002) and benthic animals (Marques et al., 2002), are also involved in human envenomation.

Characterisation of the lethal components of Cubozoa and Scyphozoa venoms has been elusive because it was thought that these toxins are large polypeptides that are unstable. Two haemolysin toxins designated cfTX-1 and cfTX-2 from *C. fleckeri* has been examined by cDNA cloning, protein expression and recent proteomic analysis (Brinkman and Burnell, 2009; Brinkman et al., 2012). Tentacle extracts of *C. fleckeri* have phospholipase A2 (PLA2) activity, as demonstrated in tissue extracts in species from four classes of Cnidaria that include hydrozoan fire corals (*Millepora* spp.), the scleractinian coral *Pocillopora damicornis* and the sea anemones *Adamsia carciniopados* (Nevalainen et al., 2004) and *Urticina crassicornis* (Razpotnik et al., 2010).

Most anthozoan toxins are neurotoxic, ion-channel modulating peptides, for examples the potassium ion channel blocking toxin AeK isolated from *Actinia equina* (Minagawa et al., 1998) and the toxin ShK isolated from *Stichodactyla helianthus* (Castañeda et al., 1995). Sea anemone neurotoxins and cytolytic enzymes have also been examined for cardiotonic properties (Suput et al., 2001), which includes the peptide hK2a isolated from *Anthopleura* sp. (Ouyang et al., 2005) and the actinoporins such as equinatoxin isolated from *A. equina* (reviewed in Kem, 1988; Macek et al., 1994). The paucity of data on unequivocal identification of cnidarian toxins stems from early studies, which had been limited by the use of low throughput, bioassay-guided chromatography (reviewed in Suput, 2009). Nevertheless, several novel peptides with ion-channel blocking activity have been identified in venom released from the sea anemone *Bunodosoma cangicum* using liquid chromatography coupled to mass spectrometry (Zaharenko et al., 2008). The first comprehensive proteome expression profile of toxins from a cnidarian nematocyst using high-resolution, high throughput protein analysis has been reported (Balasubramanian et al., 2012). Although the primary objective of this study was to examine the structural and mechanistic properties of nematocysts of *Hydra magnipapillata*, the putative venoms were described in Supplemental data. These toxins were similar to the ion-channel blockers and pore-forming toxins of sea anemones.

Using a similar high throughput proteomics technique, we recently described the metaproteome of a symbiont enriched fraction of the coral *Stylophora pistillata* (Weston et al., 2012). Surprisingly, a more complex mixture of toxins was revealed than was expected, and the presence of non-dinoflagellate toxins was attributed to the venom content of contaminating nematocysts. It is intriguing that so many of the toxins from the venom of *S. pistillata* were found to be related to such diverse toxins from such dissimilar organisms (e.g. bacteria, fungi, invertebrates and vertebrates) compared to that reported from previous cnidarian studies (Zaharenko et al., 2008; Brinkman et al., 2012). Therefore, to further examine the provenance, biological complement and evolutionary significance of early metazoan toxins, we now report a high throughput proteomic profile of putative toxins of the venom from the isolated nematocysts of *Olindias sambaquiensis,* a common hydrozoan jellyfish endemic to the South Atlantic coastal waters of Brazil, Uruguay and Argentina.

2. Materials and methods

2.1. Nematocyst isolation

Animals were collected during a jellyfish monitoring project along the central coast of São Paulo state (Guarujá County). Animals were captured through bottom shrimp trawls (2 cm mesh size) dragged at Enseada beach (24°59′52″S 46°13′26″W) at 10 m depth, with each trawl lasting 10 min on May 7th 2012. The collected animals measured 4–6 cm in bell diameter. After capture, the animals were transferred into plastic buckets containing seawater and transported live to the marine laboratory at the Instituto de Biociências, Universidade de São Paulo. The animals were identified as *O. sambaquiensis* (Fig. 1) based on general morphological characters (gonads on radial canals; number of radial and centripetal canals per quadrant; tentacles of two types with and without adhesive pads) according to the descriptions of Vannucci (1951) and Bouillon (1999). After 2 days held in aquarium conditions (filtered seawater, natural light and at a controlled temperature of 20 °C), two animals had their tentacles excised. Intact nematocysts were isolated by modification of the method of Weber et al. (1987). The tentacles were gently homogenized in a pestle and mortar in cold SuFi solution (300 mM sucrose containing 50% v/v Ficoll-Paque Plus, GE Healthcare). This material was kept at 4 °C for 30 min and then passed through a 2 mm diameter sieve. The sample was centrifuged for 10 min at 3000 *g* at 4 °C. The supernatant containing debris and cell fragments was removed. The pellet containing intact nematocysts was carefully suspended and washed three times in cold SuFi