Toxicon 137 (2017) 19–26

Contents lists available at ScienceDirect

Toxicon

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

Comparative proteomics reveals recruitment patterns of some protein families in the venoms of Cnidaria

Adrian Jaimes-Becerra [a, *], Ray Chung [b, 1], André C. Morandini [a], Andrew J. Weston [c], Gabriel Padilla [d], Ranko Gacesa [e], Malcolm Ward [b, 2], Paul F. Long [e, f, g], Antonio C. Marques [a, h]

[a] Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua Matão, Trav. 14, 101, 05508-090 São Paulo, SP, Brazil
[b] Proteomics Facility, Institute of Psychiatry, Psychology & Neuroscience, King's College London, 16 De Crespigny Park, London SE5 8AF, United Kingdom
[c] Mass Spectrometry Laboratory, UCL School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, United Kingdom
[d] Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Professor Lineu Prestes 1374, 05508-000 Sao Paulo, SP, Brazil
[e] Faculty of Life Sciences & Medicine, King's College London, 150 Stamford Street, London SE1 9NH, United Kingdom
[f] Brazil Institute, King's College London, Strand, London WC2R 2LS, United Kingdom
[g] Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, B16, 05508-000 São Paulo, SP, Brazil
[h] Centro de Biologia Marinha, Universidade de São Paulo, Rodovia Manoel Hypólito do Rego, km. 131,5, 11600-000 São Sebastião, Brazil

ARTICLE INFO

Article history:
Received 17 April 2017
Received in revised form
7 July 2017
Accepted 10 July 2017
Available online 13 July 2017

Keywords:
Evolution
Venom
Cnidaria
Nematocysts
Proteomics

ABSTRACT

Cnidarians are probably the oldest group of animals to be venomous, yet our current picture of cnidarian venom evolution is highly imbalanced due to limited taxon sampling. High-throughput tandem mass spectrometry was used to determine venom composition of the scyphozoan *Chrysaora lactea* and two cubozoans *Tamoya haplonema* and *Chiropsalmus quadrumanus*. Protein recruitment patterns were then compared against 5 other cnidarian venom proteomes taken from the literature. A total of 28 putative toxin protein families were identified, many for the first time in Cnidaria. Character mapping analysis revealed that 17 toxin protein families with predominantly cytolytic biological activities were likely recruited into the cnidarian venom proteome before the lineage split between Anthozoa and Medusozoa. Thereafter, venoms of Medusozoa and Anthozoa differed during subsequent divergence of cnidarian classes. Recruitment and loss of toxin protein families did not correlate with accepted phylogenetic patterns of Cnidaria. Selective pressures that drive toxin diversification independent of taxonomic positioning have yet to be identified in Cnidaria and now warrant experimental consideration.

1. Introduction

Cnidaria is believed to be the most basal of the extant Metazoa to be venomous, having evolved since Neoproterozoic times, ~650 million years ago, long before the Cambrian radiation (Van Iten et al., 2014). Cnidaria is a diverse phylum comprising over 13,500 free living or parasitic marine, freshwater and terrestrial species (Daly et al., 2007 plus myxozoans by Okamura et al., 2015a). Cnidaria has two major subphyla: Anthozoa and Medusozoa. Anthozoa include sea anemones and both hard and soft corals (Bridge et al., 1992; Marques and Collins, 2004). Medusozoa comprise the classes Staurozoa (e.g. stalked jellyfish), Cubozoa (e.g. box jellyfish), Scyphozoa (e.g. 'true' jellyfish) and Hydrozoa (e.g. *Hydra* and relatives including several species of smaller jellyfish) (Marques and Collins, 2004; Collins et al., 2006; Van Iten et al., 2014). Recent molecular phylogenetic analyses have corroborated the cnidarian nature of Myxozoa, with strong support as a sister-group to Medusozoa (reviewed in Okamura et al., 2015b).

The most evident synapomorphy of Cnidaria is the presence of cnidae, organelles produced by the Golgi apparatus of specialised cells called cnidoblasts (Marques and Collins, 2004; Fautin, 2009; Beckmann and Özbek, 2012). Cnidae are found in various parts of the body of a cnidarian and are classified into three morphological types: nematocysts, spirocysts and ptychocysts (Östman, 2000;

* Corresponding author.
E-mail address: adrianjaimesb@usp.br (A. Jaimes-Becerra).
[1] Present addresses: Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, De Crespigny Park, London, SE5 8AF, United Kingdom.
[2] Present addresses: Aulesa Biosciences Limited, 44, Elgar Drive, Shefford, Bedfordshire, SG17 5RZ, United Kingdom.

http://dx.doi.org/10.1016/j.toxicon.2017.07.012
0041-0101/

Özbek et al., 2009). The nematocysts discharge venom and are found in all cnidarians, but are morphologically and functionally heterogeneous (David et al., 2008; Fautin, 2009). In addition to prey capture and defence against predation, the venom of nematocysts may also mediate spatial intraspecific and interspecific competition (Bigger, 1980; Kass-Simon and Scappaticci, 2002).

There has been resurgence in interest surrounding the nature and evolutionary origins of cnidarian venom toxins, since the first application of high throughput tandem mass spectrometry realised high sequence homology between cnidarian toxins and those of other venomous animals (Weston et al., 2012, 2013). Many studies using genomic, transcriptomic or proteomic approaches have also realised these astonishing similarities (Balasubramanian et al., 2012; Brinkman et al., 2012, 2015; Li et al., 2012, 2014, 2016; Gacesa et al., 2015; Jouiaei et al., 2015a; Macrander et al., 2015, 2016; Lewis-Ames et al., 2016; Ponce et al., 2016, Huang et al., 2016), leading to the recognition that understanding the mechanisms underpinning toxin diversification in Cnidaria could provide a platform from which the evolution of this trait in higher animals might be more fully explored (Starcevic and Long, 2013; Starcevic et al., 2015; Jouiaei et al., 2015b). For this to be achieved, a comprehensive inventory of toxins must first be undertaken and then mapped against different taxonomic levels from established cnidarian phylogeny. To date, studies attempting to infer evolutionary aspects of toxin recruitment in Cnidaria have suffered limited taxon sampling, but when taken together these studies have demonstrated a degree of functional recruitment of certain toxin protein families at different taxonomic levels (Rachamim et al., 2014; Brinkman et al., 2015; Jouiaei et al., 2015b). Here, the number of venom proteomes is expanded and used with data from the literature for character mapping analysis, to establish a more complete venom assembly hypothesis between the major taxonomic classes of Cnidaria.

2. Material & methods

Nematocyst proteomics: The scyphozoan *Chrysaora lactea* and two cubozoans *Tamoya haplonema* and *Chiropsalmus quadrumanus* (Fig. 1) were collected with permission (SISBIO license 15031-2) on May 7th, 2012 by bottom shrimp trawls (2 cm mesh size) dragged at 10 m depth along Enseada beach (Guarujá County, São Paulo State, ca. 23°43′20″S 43°23′40W). Animals were identified based on morphological characters (Morandini et al., 2005; Morandini and Marques, 2010; Collins et al., 2011) and intact nematocysts were isolated from excised tentacles as previously described (Weston et al., 2013). To extract solubilised proteins, 1 mL of protein extraction buffer (50 mM TEAB, 0.04% (w/v) SDS, Roche protease and phosphatase inhibitor cocktail) was added to freeze dried nematocysts. The reconstituted material was disrupted in a sonic bath (VWR, Lutterworth, UK) for 15 min. The debris was removed by centrifugation (10,000 × g for 10 min at 4 °C). The supernatant was decanted and the soluble protein concentration determined by Bradford assay. A volume equivalent to 15 μg of protein was made up to 15 μL in extraction buffer and added to 15 μL 2 × Laemmli sample buffer, heated for 10 min at 95 °C and loaded onto a 4–12% (w/v) NuPAGE gel (Life Technologies) and separated by 1D SDS-PAGE. Electrophoresis was performed in MES buffer (Life technologies) at 150 V for approximately 100 min. The entire gel lane was then divided into 15 equal sections, excised and cut into 2 mm pieces. In-gel reduction, alkylation, and proteolytic digestion with trypsin were performed as follows: Cysteine residues were reduced with 10 mM dithiothreitol and alkylated with 55 mM iodoacetamide in 100 mM ammonium bicarbonate to form stable carbamidomethyl derivatives. Trypsin digestion was carried out overnight at 37 °C in 50 mM ammonium bicarbonate buffer and the supernatant was retained. Peptides were extracted from the gel pieces by two washes with 50 mM ammonium bicarbonate and acetonitrile. Each wash involved shaking the gel pieces for 10 min. The extracts were pooled with the initial digestion supernatant and then lyophilised. Lyophilised extract was reconstituted in 30 μL of 50 mM ammonium bicarbonate buffer for LC-MS/MS.

Data analysis: Data analysis was performed as previously described (Weston et al., 2013; Gacesa et al., 2015) but with minor modifications. Briefly, a one search matching strategy of rawfile MS/MS data against the Tox-Prot UniProtKB/Swiss-Prot database (Jungo et al., 2012) using the MASCOT search engine was first executed (Perkins et al., 1999). Methionine oxidation, phosphorylation on S/T/Y, deamidation on N/D and carbamidomethyl cysteine were selected as fixed modifications. Digestion with trypsin allowed up to three missed cleavages. The data were searched with a parent ion tolerance of 5 ppm and a fragment ion tolerance of 0.5 Da. The MASCOT result files were next uploaded into Scaffold v4.3.4 (Proteome Software, Portland, Oregon, USA) (Searle, 2010) and spectra corresponding to likely venom toxin peptides were manually validated for unbroken series of overlapping b-type and y-type sequence specific fragments ions, where losses consistent with the sequence were acceptable. Validated spectra (Figs. S1–S3) corresponding to peptides with predicted venom toxin functions were next distinguished from peptides with likely other non-toxic physiological functions using 'ToxClassifier' (Gacesa et al., 2016). This is a suite of machine learning based classifiers that provide consistent discrimination of toxins from non-toxin peptide

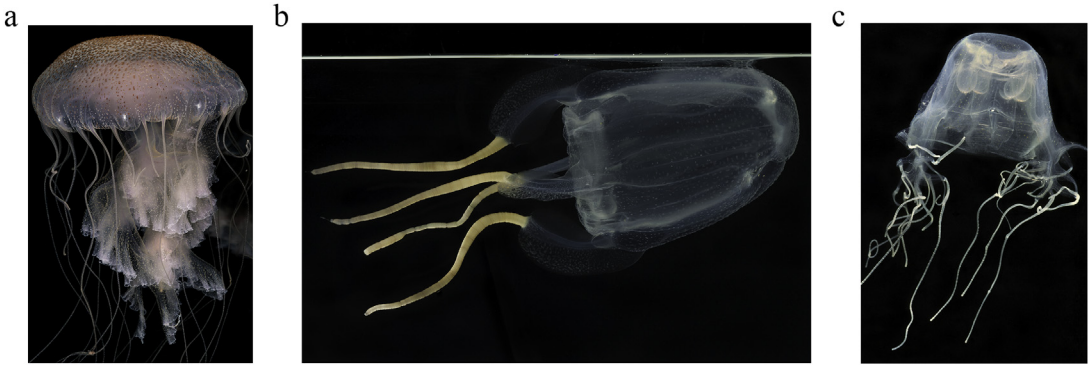

Fig. 1. A) *Chrysaora lactea*, B) *Tamoya haplonema* and C) *Chiropsalmus quadrumanus*. Medusa adult stages of Cnidaria from which the venom proteomes of isolated nematocysts were acquired for this study. (Photos courtesy of Dr Alvaro Migotto, Centro de Biologia Marinha, Universidade de São Paulo São Sebastião, Brasil).