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Biochemical and molecular characterisation of cubozoan protein toxins

Diane L. Brinkman [a,*], James N. Burnell [b]

[a] Australian Institute of Marine Science, P.M.B. No 3, Townsville Mail Centre, Townsville, Qld 4810, Australia
[b] Department of Biochemistry and Molecular Biology, School of Pharmacy and Molecular Sciences, James Cook University, Townsville, Qld 4814, Australia

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ABSTRACT

Class Cubozoa includes several species of box jellyfish that are harmful to humans. The venoms of box jellyfish are stored and discharged by nematocysts and contain a variety of bioactive proteins that are cytolytic, cytotoxic, inflammatory or lethal. Although cubozoan venoms generally share similar biological activities, the diverse range and severity of effects caused by different species indicate that their venoms vary in protein composition, activity and potency. To date, few individual venom proteins have been thoroughly characterised, however, accumulating evidence suggests that cubozoan jellyfish produce at least one group of homologous bioactive proteins that are labile, basic, haemolytic and similar in molecular mass (42–46 kDa). The novel box jellyfish toxins are also potentially lethal and the cause of cutaneous pain, inflammation and necrosis, similar to that observed in envenomed humans. Secondary structure analysis and remote protein homology predictions suggest that the box jellyfish toxins may act as α-pore-forming toxins. However, more research is required to elucidate their structures and investigate their mechanism(s) of action. The biological, biochemical and molecular characteristics of cubozoan venoms and their bioactive protein components are reviewed, with particular focus on cubozoan cytolysins and the newly emerging family of box jellyfish toxins.

1. Introduction

Cubozoan jellyfish produce an array of toxic proteins which are stored and delivered by their nematocysts. Although these toxins are primarily involved in prey capture and possibly the deterrence of predators, they are also clinically important due to their deleterious effects in envenomed humans. Consequently, significant research effort has focussed on the characterisation of venom proteins from the large and extremely venomous box jellyfish, *Chironex fleckeri* (Fig. 1), and to a lesser extent, from species such as *Chiropsalmus quadrigatus* and *Carybdea marsupialis*. However, despite several decades of biochemical, toxicological and pharmacological research, few toxic cubozoan proteins have been isolated and characterised, and their mechanisms of action remain unclear. In particular, progress in the biochemical characterisation of bioactive box jellyfish proteins has been hindered by issues relating to protein instability and hydrophobicity, as well as variability in protein sources, extraction methods and analytical techniques.

Nonetheless, there is emerging evidence that cubozoans produce at least one unique group of bioactive proteins. Typically, these box jellyfish toxins are labile, basic cytolysins (42–46 kDa) that are not known to occur in other cnidarians (Nagai et al., 2000a, b, 2002; Brinkman and Burnell, 2007). Some studies have also demonstrated that the toxins are lethal and cause pain, inflammation and necrosis of the skin in experimental animals, suggesting that the proteins may be the primary cause of similar effects in envenomed humans (Nagai et al., 2000a, 2002; Nagai, 2003). Recent advances in the molecular characterisation of cubozoan toxins include the cDNA cloning and

* Corresponding author. Tel.: +61 7 4753 4402; fax: +61 7 4772 5852.
E-mail address: d.brinkman@aims.gov.au (D.L. Brinkman).

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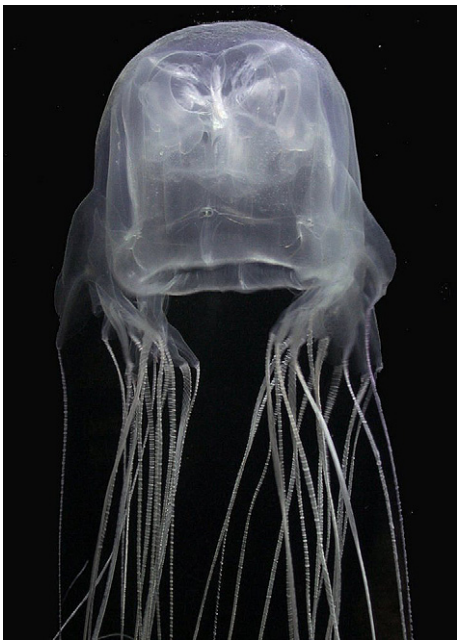

Fig. 1. The large, multi-tentacled chirodropid, *Chironex fleckeri*. Mature specimens can grow to a bell height of ~20–30 cm and weigh more than 6 kg. Up to 15 tentacles are attached to a single pedalium located at each lower corner of the bell. Fully extended, each tentacle can measure more than 3 m in length. Nematocysts are arranged radially in discrete bands along the length of each tentacle. Photograph © Dr. Lisa-Ann Gershwin.

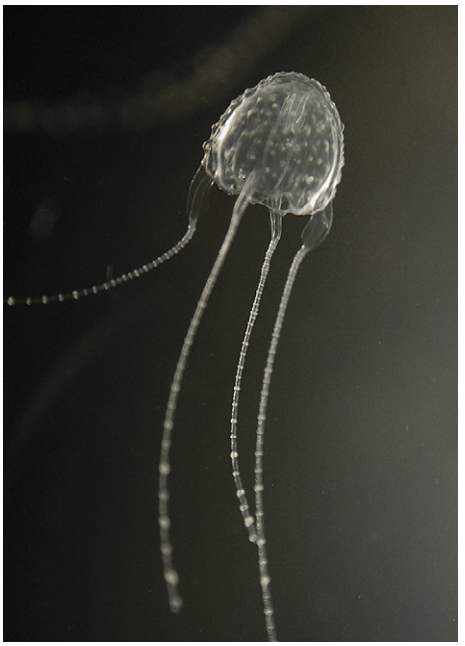

Fig. 2. The small, four-tentacled carybdeid, *Carukia barnesi* (Irukandji). Clumps of nematocysts are distributed across the surface of the bell. Nematocysts are also localised in discrete bands along each tentacle. The bell height of this specimen is ~12 mm and each tentacle can extend to ~35 cm. Photograph by Dr. Jamie Seymour.

sequencing of five homologous proteins (from four species) that belong to this novel family of box jellyfish toxins. Undoubtedly, as research in this field progresses, additional medically important cubozoan toxins will be discovered and characterised.

This review provides a summary of our current knowledge of the toxic proteins produced by cubozoan jellyfish, with particular emphasis on the research achievements of the last decade. In preface, the effects of box jellyfish stings on human health and the biological activities associated with cubozoan venoms are described and compared.

2. Effects of cubozoan jellyfish stings in humans

Orders Carybdeida and Chirodropida both contain species of box jellyfish that are either relatively harmless to humans or potentially life-threatening, and the spectrum of symptoms that manifest in envenomed humans varies significantly. Contact with the tentacles of carybdeids, such as *Carybdea rastoni*, *Carybdea alata* and *C. marsupialis*, commonly results in mild to moderate cutaneous pain, inflammation, erythematous vesicular eruptions and persistent cutaneous hypersensitivity reaction (Nagai, 2003; Ozaki et al., 1986; Tamanaha and Izumi, 1996; Sánchez-Rodríguez et al., 2006). In contrast, stings by the Australian carybdeid, *Carukia barnesi* (Fig. 2), are associated with the delayed systemic effects of Irukandji syndrome (Barnes, 1964). Although immediate sharp cutaneous pain may be experienced, the local effects of *C. barnesi* stings are often relatively mild. More severe symptoms usually develop 5–120 min after the sting and may include lower back and abdominal pain, cramps in the limbs, profuse sweating, nausea, vomiting, hypertension, tachycardia, pulmonary oedema, anxiety and distress (Barnes, 1964; Bailey et al., 2003; Tibballs, 2006; Burnett et al., 1996b). Other carybdeid species have also been implicated in causing Irukandji or milder Irukandji-like syndromes (e.g. Burnett et al., 1996b; Grady and Burnett, 2003; Huynh et al., 2003; Gershwin, 2005a, 2007; Little et al., 2006; de Pender et al., 2006; Winter et al., 2008) and occasional fatalities have been reported (Fenner and Hadok, 2002).

The chirodropid, *C. fleckeri*, is considered to be the most dangerous jellyfish to humans in the world (Wiltshire et al., 2000) and whilst the vast majority of human envenoming by *C. fleckeri* are not lethal, ~70 fatalities have been reported in Australia alone (Fenner and Harrison, 2000; Ramasamy et al., 2004). Children are particularly vulnerable. The most recent fatalities in Australia include a 7-year-old girl near Bamaga (northern Queensland, January, 2006; ABC News, 2006) and a 6-year-old boy at the Tiwi Islands (Northern Territory, November, 2007; ABC News, 2007). Clinical manifestations of major or life-threatening human envenoming include excruciating pain, impaired consciousness, dyspnoea, cardiac dysfunction, pulmonary oedema, shock, hypertension, hypotension, rapid acute cutaneous inflammation, dermonecrosis and permanent scarring (Lumley et al., 1988; Beadnell et al., 1992; Williamson et al., 1984). In the most severe cases, respiratory and cardiac failure can occur within several minutes (Lumley et al., 1988).

In contrast, other chirodropid species such as *Chiropsella bronzie* (formally known as *Chiropsalmus* sp.; Gershwin,