

Phospholipase A2 in Cnidaria

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Abstract

Phospholipase A2 (PLA2) is an enzyme present in snake and other venoms and body fluids. We measured PLA2 catalytic activity in tissue homogenates of 22 species representing the classes Anthozoa, Hydrozoa, Scyphozoa and Cubozoa of the phylum Cnidaria. High PLA2 levels were found in the hydrozoan fire coral *Millepora* sp. (median 735 U/g protein) and the stony coral *Pocillopora damicornis* (693 U/g) that cause skin irritation upon contact. High levels of PLA2 activity were also found in the acontia of the sea anemone *Adamsia carciniopados* (293 U/g). Acontia are long threads containing nematocysts and are used in defense and aggression by the animal. Tentacles of scyphozoan and cubozoan species had high PLA2 activity levels: those of the multitentacled box jellyfish *Chironex fleckeri* contained 184 U/g PLA2 activity. The functions of cnidarian PLA2 may include roles in the capture and digestion of prey and defense of the animal. The current observations support the idea that cnidarian PLA2 may participate in the sting site irritation and systemic envenomation syndrome resulting from contact with cnidarians.

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1. Introduction

Cnidaria are among the earliest evolved metazoan animal phyla with representatives found in fossils from the Precambrian 550 million years ago (Chen et al., 2002; Wood et al., 2002). The phylum Cnidaria comprises benthic and pelagic aquatic animals including the classes Anthozoa (hard corals, soft corals, sea pens, sea anemones), Hydrozoa (hydroids, fire corals), Scyphozoa (jellyfish) and Cubozoa (box jellyfish). The body plan of Cnidaria (earlier called Coelenterata) is diploblastic, i.e. the body consists of two cell layers, the ectoderm (epidermis) covering the outer surface of the body and the endoderm (gastrodermis) lining

the body cavity. There is homogeneous elastic material (mesoglea) between these layers. This gelatinous material is a distinct feature of the bell of jellyfish.

Nematocysts are stinging capsules characteristic of Cnidaria. Nematocysts contain and fire harpoon-like microscopic structures (cnida) that penetrate the surface layer of the victim and deliver a mixture of highly toxic substances. The composition of cnidarian venoms is not known in detail.

Phospholipase A2 (PLA2) cleaves glycerophospholipids at the *sn*-2 position. The reaction products are lysophospholipid and fatty acid, e.g. arachidonic acid (Murakami and Kudo, 2002). The metabolites derived from arachidonic acid (prostaglandins, thromboxanes and leukotrienes) control a wide variety of cellular functions including inflammation (Smith et al., 2000). There is a family of secreted PLA2s comprising low molecular weight (13–15 kDa) disulphide-

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linked proteins that depend on Ca^{2+} -ion for enzymatic activity. PLA2 secreted by the pancreas functions as a digestive enzyme, and other secreted PLA2s are active components of snake and other venoms. In addition to secreted PLA2s, there are cytosolic Ca^{2+} -dependent and independent PLA2-species. Based on molecular structure, PLA2s are classified into various groups numbered from I to XIV and numerous subgroups. The number of identified forms of PLA2 has increased rapidly (Six and Dennis, 2000; Balsinde et al., 2002). Recent investigations indicate that secreted PLA2s are important defensive molecules of innate immunity capable of killing bacteria (Laine et al., 1999; Koduri et al., 2002).

Besides vertebrates, PLA2s have been identified in a number of other phyla including marine invertebrates (McIntosh et al., 1995; Shiomi et al., 1998; MacPherson and Jacobs, 2000; Kishimura et al., 2000; Talvinen and Nevalainen, 2002; Nevalainen et al., 2004). High PLA2 activities have been reported in hard corals, fire coral, crown-of-thorns starfish, sea cucumber and marine sponges (Nevalainen et al., 2001, 2004). The purpose of the current study was to investigate the occurrence of PLA2 activity in representatives of the four classes of Cnidaria.

2. Materials and methods

Specimens were collected in the Great Barrier Reef, Queensland, Australia from tidal pools and by snorkeling and scuba diving at the depth of 2–20 m, and from Trondheim fjord of the Norwegian Atlantic coast by scuba diving at the depth of 4–25 m, bottom trawling at 500 m and by scraping the fjord slope at 20–100 m. *Carukia barnesi* jellyfish, a cause of the Irukandji syndrome (Flecker, 1952; Barnes, 1964), were captured immediately off coastal beaches between Cairns and Port Douglas, north Queensland. Specimens were immediately placed into vessels containing seawater pending light microscopic examination. Those specimens identified as *C. barnesi* by gross morphology (Southcott, 1967) were placed in plastic bags, frozen in liquid nitrogen, examined in the laboratory by light microscopy and formally identified, prior to processing, as *C. barnesi* according to Southcott's description of bell, tentacle and nematocyst morphology. Specimens of *Chironex fleckeri* were collected offshore Darwin, Northern Territory, Australia and formally identified by Dr. Phil Alderslade of the Northern Territory Art Gallery and Museum. The jumble jellyfish *Carybdea rastonii* (Haacke, 1887) were collected offshore Adelaide, South Australia and formally identified by Dr. James Doube, Flinders Medical School. The tentacles of both the latter two species were removed and immediately frozen. The samples were stored frozen at $-20\text{ }^{\circ}\text{C}$ until use. For PLA2 assay, the tissues were thawed, homogenized in chilled 0.9% sodium chloride and centrifuged. The *C. barnesi* samples were obtained from 20–30 pooled whole specimens homogenized and centri-

fuged in phosphate buffered saline at $5\text{ }^{\circ}\text{C}$. The supernatants were assayed for PLA2 catalytic activity as described (Schädlich et al., 1987), with slight modifications. Briefly, L-alpha1-palmitoyl phosphatidylethanolamine, containing ^{14}C -labelled arachidonic acid in the *sn*-2 position (NEN Life Science Products, Boston, MA, USA), was used as a substrate in a buffer containing 20 mM glycine HCl, pH 8.0, 6 mM sodium deoxycholate and 2 mM CaCl_2 . The activity is expressed in units. The unit of PLA2 activity is defined as the amount of activity that releases $1\text{ }\mu\text{M}$ fatty acid/min. The protein concentration of tissue extracts was measured as described (Bradford, 1976) by using commercial reagents and bovine serum albumin as a standard (Sigma). The results are expressed as units per gram of protein (U/g).

3. Results

Phospholipase A2 catalytic activity in tissue homogenates is given in Table 1. There were high PLA2 levels in all classes of Cnidaria with marked variation between the species as well as between different specimens representing the same species.

High PLA2 levels were measured in the fire coral *Millepora* sp. (median 735 U/g protein) and the stony coral *Pocillopora damicornis* (693 U/g) that are known to cause skin irritation upon contact. Sea anemones contained varying levels of PLA2 activity. High levels of activity were found in the acontia of the sea anemone *Adamsia carciniopados* (cloak anemone) (293 U/g). Acontia are long slender threads containing nematocysts and used in defense and aggression by the animal. There were high levels of PLA2 activity in the scyphozoan *Cyanea capillata* (lion's mane jellyfish) (110 U/g) and the cubozoan *C. fleckeri* (box jellyfish) (184 U/g) tentacles and lower levels in the cubozoans *C. rastonii* (jumble) (91 U/g) and *C. barnesi* (irukandji) (130 U/g).

4. Discussion

Toxicity is a common feature of cnidarians (Gunthorpe and Cameron, 1990), and a large number of toxins have been identified in cnidarian nematocysts (Macek, 1992). Cnidaria are specialized subcellular organelles of diverse structure and function that are plentiful amongst cnidarians (Yanagihara et al., 2002). They can be classified into three main types, nematocysts, spirocysts, and ptychocysts, based on structural and functional features. The major function of nematocysts is the delivery of venom through the skin, whereas spirocysts are adhesive and ptychocysts are involved in protection (Rifkin, 1996). The latter two structures are found in anthozoans, whereas the jellyfish (scyphozoans and cubozoans) contain only nematocysts. The biological roles of toxins delivered by nematocysts include the capture and killing of prey, digestion, repelling

Table 1
Phospholipase A2 activity in tissues homogenates of cnidarians expressed as units per g protein (U/g); median (range); n, number of specimens

Class/species	PLA2 activity (U/g), median (range)	n	A/N
<i>Anthozoa</i>			
Soft coral			
<i>Alcyonium digitatum</i>	47 (8–118)	7	N
<i>Simularia flexibilis</i>	22 (17–26)	2	A
<i>Sarcophyton elegans</i>	39 (33–219)	4	A
<i>Dendronephthya</i> sp.	63 (48–87)	10	A
Sea fan			
<i>Paramuricea</i> sp.	114	1	N
Sea pen			
<i>Virgularia nidularis</i>	47	1	N
Sea anemone			
<i>Metridium senile</i> , whole animal	34 (5–78)	7	N
<i>Metridium senile</i> , tentacles	9 (4–13)	2	N
<i>Metridium senile</i> , acontia	70 (10–129)	2	N
<i>Adamsia cariniopados</i> , whole animal	66 (57–169)	4	N
<i>Adamsia cariniopados</i> , tentacles	13	1	N
<i>Adamsia cariniopados</i> , acontia	293 (177–304)	3	N
<i>Sagartia elegans</i>	59 (2–110)	2	N
<i>Boleocera tuedia</i>	21 (18–34)	4	N
<i>Stoichactis</i> sp.	207 (182–232)	2	A
<i>Actinia australis</i>	73	1	A
Stony coral			
<i>Acropora</i> sp.	267	1	A
<i>Pocillopora damicornis</i>	693 (482–2200)	10	A
<i>Hydrozoa</i>			
<i>Obelia geniculata</i>	2	1	N
<i>Tubularia larynx</i>	63	1	N
<i>Millepora</i> sp.	735	1	A
<i>Scyphozoa</i>			
<i>Aurelia aurita</i>	40 (35–44)	2	N
<i>Cyanea capillata</i> , bell	81 (80–118)	3	N
<i>Cyanea capillata</i> , tentacles	110 (77–197)	6	N
<i>Cubozoa</i>			
<i>Chironex fleckeri</i> , tentacles	184	1	A
<i>Carybdea rastonii</i> , tentacles	91	1 ^a	A
<i>Carukia barnesi</i> , whole animal	130	1 ^a	A

A, specimens collected in Australia; N, specimens collected in Norway.

^a From a pool of 20 specimens.

of predators and intraspecies spatial competition (Macek, 1992).

We detected varying levels of PLA2 catalytic activity in the tissue extracts of the representatives the cnidarian classes Anthozoa, Hydrozoa, Scyphozoa, and Cubozoa. High levels were found the tentacles and acontia that are known rich sources of nematocysts containing toxic substances. However, it must be emphasized that high levels of PLA2 activity were measured in whole animal extracts. Therefore, the current assays for PLA2 activity on whole tissue extracts do not necessarily indicate that the measured

PLA2 activity represents a toxic component of nematocysts. Besides being a component of at least some nematocyst venoms, PLA2 may also be a constituent of digestive tissue (gastoderms).

Phospholipase A2 was first isolated from snake venom where the various forms of the enzyme have haemolytic, myotoxic, neurotoxic, procoagulant and anticoagulant functions (Davidson and Dennis, 1990; Balsinde et al., 2002). Snake venom PLA2s have been studied extensively, but there is only limited information on the molecular structure of invertebrate PLA2s. Some marine invertebrate PLA2 sequences, e.g. those of the echinoderm starfish *Asteria pectinifera* (Kishimura et al., 2000) and crown-of-thorn starfish *Acanthaster planci* (Shiomi et al., 1998), can be grouped together with secreted group I and II PLA2s (Six and Dennis, 2000) based on sequence homology with snake venom and mammalian PLA2s. PLA2 of the marine snail *Conus magus* venom belongs to group IX (McIntosh et al., 1995). We have recently detected considerable PLA2 activity in marine sponges (Porifera) including *Cymbastela coralliophila*, *Spirastrella vagabunda* and *Theonella swinhoei* (Nevalainen et al., 2004) but these PLA2s have not been sequenced so far.

The partial N-terminal amino acid sequence of PLA2 of the jellyfish *R. nomadica* is modestly homologous to *Apis mellifera* (bee) venom group III PLA2 (Lotan et al., 1995). The PLA2 from the sea anemone *A. cariniopados* is the first cloned and sequenced cnidarian PLA2 (Talvinen and Nevalainen, 2002). On the one hand, *A. cariniopados* PLA2 has common features with other known secreted PLA2s such as N-terminal signal for secretion, 12 cysteines for putative disulphide formation and conserved residues in the sites of catalytic activity and Ca²⁺-binding. On the other hand, this PLA2 has only 30–40% sequence similarity with other secreted PLA2s, and the place *A. cariniopados* PLA2 (Talvinen and Nevalainen, 2002) in the currently established groups of secreted PLA2s is uncertain (Balsinde et al., 2002).

Phospholipase A2 activity has been detected in cytolytic toxin preparations from sea anemones (Hessinger and Lenhoff, 1976; Galettis and Norton, 1990). Hemolytic PLA2 isolated from the nematocysts of the sea anemone *Aiptasia pallida* had pH optimum of activity at pH 7.7 and required mM Ca²⁺-concentration for catalytic activity in common with venom and other secreted PLA2s (Grotendorst and Hessinger, 2000). The PLA2 isolated from *A. pallida* had two isoforms with molecular sizes of 45 and 43 kDa, respectively, and the latter protein had an isoelectric point of 8.8 (Grotendorst and Hessinger, 1999). The PLA2 of the jellyfish *Rhopilema nomadica* shares the property of heat stability (Gusmani et al., 1997) with pancreatic group IB PLA2. In the current study, considerable PLA2 activities were found in the tissue extracts of sea anemones and other anthozoan species including soft corals, sea fan and sea pen. Especially high PLA2 activities were measured in the hydrozoan fire coral *Millepora* sp. and the stony coral *P.*

damicornis. PLA2 was earlier isolated from acontial nematocyst preparations of the sea anemone *A. pallida* (Hessinger and Lenhoff, 1976). Acontia are a rich source of nematocysts, and in the current study, higher PLA2 activities were measured in acontia than in the whole animal extracts of the sea anemones *M. senile* and *A. carciniopados*.

The toxins within the nematocysts of the box jellyfish *C. fleckeri* combine to represent one of the most potent lethal animal venoms in that they can cause human death within minutes (Burnett et al., 1996). Toxic fractions of *C. fleckeri* venom have been reported to contain components of molecular sizes from 10 to 600 kDa, although the larger ones may represent macromolecular aggregates (Bloom et al., 1998). Earlier studies using less sensitive methods, reported the absence of PLA2 activity from *C. fleckeri* venom (Baxter and Marr, 1969; Keen and Crone, 1969; Crone, 1976). In the current study, we measured relative high levels of PLA2 activity in the extracts of tentacles of the highly toxic cubozoan *C. fleckeri* and lower levels in the less toxic *C. rastonii*, and *C. barnesi*. The latter two jellyfish belong to the order Carybdea, characterized by having only one tentacle at each of the four corners of the bell (Burnett et al., 1996). Contact with *C. fleckeri* tentacles results in severe skin irritation and dermatonecrosis, whereas *C. rastonii* can cause a transient papular wheal with erythema (Fenner and Williamson, 1987). An even less significant sting site reaction is typical of *C. barnesi* (Barnes, 1964). PLA2 activity found in the current small series of cubozoans is consistent with the hypothesis that the venom PLA2 may be involved in the sting site response. The severe systemic effects caused by cubozoan stings are attributed to the potent cardiotoxin components of the nematocyst venom (Galettis and Norton, 1990; Wiltshire et al., 2000). The role of PLA2 in the systemic effects of cnidarian venom is unknown, and the molecular structure and the pharmacological/toxicological properties of cnidarian PLA2 remain to be studied.

In summary, we have identified various levels of PLA2 activity in all classes of the phylum Cnidaria. The functions of cnidarian PLA2s remain to be elucidated, but they may include roles in the capture and digestion of prey, as well as defense, since high PLA2 activities are present in both the tentacles and acontia of these animals. It is feasible to hypothesize a toxic role to PLA2 in Cnidaria.

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