PHOSPHOLIPASE A2 IN MARINE INVERTEBRATES

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Abstract

Phospholipase A2 (PLA2) catalytic activity was determined by a radiochemical assay in tissue extracts from 21 species belonging to five phyla of marine invertebrates that were collected from the Great Barrier Reef, Queensland, Australia. The highest PLA2 activities were found in hard coral, fire coral, crown-of thorns starfish and sea cucumber. High PLA2 activities were found in a number of echinoderms and sea anemones, whereas annelids, crustaceans and molluscs contained relatively low amounts of PLA2 activity. The results demonstrate the presence of PLA2 activity in a number of marine invertebrates. The molecular structure, classification and physiological functions of these PLA2s remain to be studied.

Key words : Cnidaria, echinodermata, mollusca, toxins

Introduction

Phospholipases A2 (PLA2) form a large family of lipolytic enzymes (1). PLA2 is a major component of snake and other venoms, digestive secretions of the gastrointestinal tract, as well as secretions of various mucous surfaces including tears and seminal fluid. In addition to toxic and digestive functions, PLA2 has effective bactericidal properties and participates in the regulation of inflammation by releasing arachidonic acids from cellular membrane phospholipids for eicosanoid synthesis. The purpose of the current study was to investigate the occurrence of PLA2 in marine invertebrate tissues.

Material and methods

Specimens were collected at 0-20 m depth from the Great Barrier Reef, Northern Queensland, Australia. Samples were immersed in 50 mM acetate buffer, pH 5, containing protease inhibitors, and frozen at -18°C. After thawing, the specimens were homogenised by Ultra Turrax or shaken vigorously in a glass container (coral specimens) and centrifuged at 5 000 g. The supernatants were assayed for PLA2 activity by using 14C-labelled phosphatidylcholine in mixed micelles as a substrate. Protein was determined by a standard dye-binding assay.

Results and discussion

The highest PLA2 activities were found in the extracts of hard coral, fire coral, crown-of thorns starfish and sea cucumber (Table 1). The high PLA2 content in the puffer fish intestine is similar to mammalian intestine where the enzyme is expressed in mucosal Paneth cells (2). High PLA2 activities were found in a number of echinoderms and sea anemones, whereas the annelids, crustaceans and molluscs tested had relatively low PLA2 activity. The functions of PLA2 in invertebrates are not well known. The action of PLA2 on phospholipids initiates the synthesis of eicosanoids present in most animal species including invertebrates (3). Insect immune response to bacteria is mediated by eicosanoids (4). PLA2 activity has been reported in the granular amebocytes, important immunocompetent cells of the horseshoe crab, *Limulus polyphemus* (5). PLA2 is a well-characterised digestive enzyme in mammals (1). Digestive PLA2s have been found in the tiger beetle *Cicindella circumpicta* (6) and the starfish *Asterina pectinifera* (7). The presence of PLA2 in snake venoms has been known since the 1890' (1) and has been reported in numerous invertebrate venoms including that of the marine snail *Conus magus* (8), the scorpion *Pandinus imperator* (9), the ant *Pseudomyrmex triplarinus* (10), the sea anemone *Aiptasia pallida* (11) and the jellyfish *Rhopilema nomadica* (12). It is pertinent that the highest levels of PLA2 observed in echinoderms in the present study were found in tissues that are associated with toxins; **E.G.** the spines of the crown-of-thorns starfish have a powerful neurotoxin. Pyloric cecae of echinoderms contain PLA2 that may function as a digestive enzyme.

The current results demonstrate the presence of PLA2 activity in a number of marine invertebrates. The molecular structure, classification and physiological functions of these PLA2s remain to be studied.

Table 1. Phospholipase A2 activity concentration (U/g protein) in tissue extracts. Where a specific tissue is not indicated the assay was undertaken on whole animal extract.

	U/g (SD)	n
Cnidaria		
Soft coral *Sinularia flexibilis*	22 (6)	2
Soft coral *Sarcophyton elegans*	82 (91)	4
Soft coral *Dendronephthya sp.*	64 (12)	10
Hard coral *Acropora* sp.	267	1
Hard coral *Pocillopora damicornis*	945 (540)	10
Hydrozoan fire coral *Millepora* sp.	735	1
Sea anemone *Stoichactis* sp.	207 (35)	2
Sea anemone *Actinia australis*	90 (24)	2
Annelida		
Worm *Phyllodoce novaehollandiae*	29 (32)	4
Crustacea		
Ghost crab *Ocypode cordimana*	3 (2)	7
Prawn *Panaeus monodon*		
Hepatopancreas	52 (79)	3
Muscle	0.5 (0.2)	2
Mollusca		
Rock-oyster *Saccostrea cucullata*	1	1
Clam *Donax cuneatus*	2 (1)	6
Nudibanch *Phyllida* sp.	65	1
Echinodermata		
Crinoid *Colobometra perspinosa*	124	1
Brittle star *Ophiocoma erinaceus*		
Disc	33	1
Arm	29	1
Starfish *Gomophia* sp.	253	1
Starfish *Fromia* sp.	288	1
Starfish *Linckia laevigata*	244	1
Crown-of-thorns starfish *Acanthaster planci*		
Skin	97	1
Spines	1625	1
Body wall	117	1
Pyloric ceca	498	1
Sea cucumber *Stichopus chloronotus*		
Body wall	5423	1
Intestine	235	1
Rete mirabile	614	1
Vertebrata		
Pufferfish *Arothron manilensis*		
Skin	177 (87)	5
Muscle	4 (2)	5
Liver	78 (73)	5
Intestine	2753 (1058)	5

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