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Characterization of phospholipase A_2 from the pyloric ceca of two species of starfish, *Coscinasterias acutispina* and *Plazaster borealis*

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Abstract

Phospholipase A (PLA) activities in the pyloric ceca and viscera from seven species of marine invertebrates (four starfish, one sea urchin, and two shellfish) were determined. Relatively high PLA specific activities were found in the pyloric ceca of two species of starfish (*Coscinasterias acutispina* and *Plazaster borealis*). Phospholipase A_2s (PLA_2s) were partially purified from the pyloric ceca of the starfish, *C. acutispina* PLA_2 (C-PLA_2) and *P. borealis* PLA_2 (P-PLA_2). The C-PLA_2 and P-PLA_2 mainly released oleic acid from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. Temperature optima of the C-PLA_2 and P-PLA_2 were at around 60 °C and 50 °C, respectively, and pH optima of the C-PLA_2 and P-PLA_2 were both at around pH 10.0. The activities of the C-PLA_2 and P-PLA_2 were enhanced by sodium deoxycholate and 1 mM or higher concentration of Ca^{2+}. The C-PLA_2 and P-PLA_2 did not show the fatty acid specificity for hydrolysis of phosphatidylcholine. Unlike porcine pancreatic PLA_2, the C-PLA_2 and P-PLA_2 hydrolyzed phosphatidylcholine more effectively than phosphatidylethanolamine.

Keywords: Phospholipase A_2; *Coscinasterias acutispina*; *Plazaster borealis*; Starfish; Marine invertebrate; Pyloric cecum; Polar group specificity

1. Introduction

Phospholipase A_2 (PLA_2) (EC3.1.1.4) catalyzes the selective hydrolysis of the *sn*-2-acyl group in 1,2-diacyl-*sn*-glycero-3-phospholipids. PLA_2 is widely distributed in tissues of various organisms and is classified into extracellular and intracellular types. Extracellular PLA_2 is abundant in mammalian pancreas and snake venom, and these enzymatic and structural characteristics have been well studied (Arni & Ward, 1996; Dennis, 1983).

On the other hand, the PLA_2s from mammalian pancreas and snake venom have been used as diagnostic biochemical reagents. Stoll (1996) employed snake venom PLA_2 to analyze the positional distribution of fatty acids in glycerophospholipids from guinea pig and pig cardiac membranes. Mine (1997) examined structural and functional changes of hen egg-yolk low density lipoproteins (LDL) as a result of modifying its phospholipids using porcine pancreatic PLA_2. Additionally, commercial PLA_2, mainly produced from porcine pancreas, is used for industrial processes in the food industry. Soy lysophosphatidylcholine (lysoPC), which is an excellent emulsifier for food, is prepared by porcine pancreatic PLA_2-catalyzed hydrolysis of soy phosphatidylcholine (PC) (Aoi, 1990). The emulsion with soy lysoPC is stable under various conditions at high temperature, acidic solution and/or high salt concentration. Soy lysoPC is also a good solubilizer, and the interaction between soy lysoPC and protein is very strong. Dahlke, Buchold, Munch, and Paulitz (1995) reported

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that PLA_2 is suitable for the enzymatic degumming of edible oils. The enzymatic degumming of crude edible oils reduces the amounts of acids, bases and wastes during the refining processes. It also allows the extraction of PC and lysoPC as valuable by-products for the fortification of other foods. Recently, it was shown that LDL, modified by PLA_2, was removed from the circulation to the liver more rapidly than unmodified LDL (Labeque, Mullon, Ferreira, Lees, & Langer, 1993). Based on this finding, a novel therapy for hypercholesterolemia has recently been developed, that utilizes immobilized PLA_2 contained in an extracorporeal shunt.

In contrast, few studies exist on the enzymology and application of PLA_2 from marine invertebrates. In the previous study, we isolated PLA_2 from the pyloric ceca of starfish (*Asterina pectinifera*), and compared its enzymatic properties with those of porcine pancreatic PLA_2 (Kishimura & Hayashi, 1999b). The specific activity of the *A. pectinifera* PLA_2 for PC was about 30 times higher than that of the commercially available PLA_2 from porcine pancreas (Sigma). In addition, the *A. pectinifera* PLA_2 hydrolyzed PC more effectively than phosphatidylethanolamine (PE), like snake venom PLA_2 (Ibrahim, Sanders, & Thompson, 1964) but not porcine pancreatic PLA_2.

In this study, we partially purified PLA_2s from the pyloric ceca of the starfish (*Coscinasterias acutispina* and *Plazaster borealis*) and examined the characteristics of these enzymes.

2. Materials and methods

2.1. Materials

The starfish (*C. acutispina*) was caught off Uozu, Toyama Prefecture, Japan, and was stored at −20 °C until used. The starfish (*P. borealis*, *Solaster borealis*, and *Aphelasterias japonica*), sea urchin (*Strongylocentrotus franciscanus*), shellfish (*Neptunea arthritica* and *Patinopecten yessoensis*) and common squid (*Todarodes pacificus*) were caught off Hakodate, Hokkaido Prefecture, Japan and were stored at −20 °C until used. Porcine pancreatic PLA_2 was purchased from Sigma (St. Louis, MO, USA) and Amano Pharmaceutical Co. (Nagoya, Japan). Egg-yolk PC was purchased from Wako Pure Chemicals (Osaka, Japan). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA).

2.2. Preparation of crude enzyme solution

Crude enzyme solution was prepared from the pyloric ceca and viscera of the invertebrates by the same method as described by Kishimura and Hayashi (1999a). The pyloric ceca and viscera were homogenized in 4 volumes of chloroform–methanol (2:1, v/v) for 10 min, and the homogenates were filtered in vacuo on ADVANTEC No. 2 filter paper. Similarly, the residues were homogenized in 2 volumes of chloroform–methanol (2:1, v/v) and 1.3 volumes of acetone for 10 min, and then the residues were air-dried at room temperature. PLA was extracted by stirring the defatted powder for 3 h at 5 °C in 50 volumes of 50 mM Tris–HCl buffer at pH 8.0. The extracts were centrifuged at 10,000*g* for 10 min, and then the supernatants were concentrated by lyophilization into crude enzyme solution.

2.3. Purification of starfish PLA_2

The crude enzyme solutions were applied on a column (3.9×44 cm) of Sephacryl S-200 pre-equilibrated with 50 mM Tris–HCl buffer (pH 8.0), and PLA_2 was eluted with the same buffer. Main active fractions were concentrated by lyophilization and dialyzed against 10 mM Tris–HCl buffer (pH 8.0). The dialyzates were applied on a column (1.1×18 cm) of DEAE-cellulose, pre-equilibrated with 10 mM Tris–HCl buffer (pH 8.0). PLA_2 was eluted with a linear gradient of NaCl, from 0 to 0.5 M in 10 mM Tris–HCl buffer (pH 8.0), and main active fractions were obtained. The fractions were dialyzed against 10 mM Tris–HCl buffer (pH 8.0) and the dialyzates were concentrated by lyophilization. The concentrates were applied on a column (3.9×64 cm) of Sephadex G-50 pre-equilibrated with 10 mM Tris–HCl buffer (pH 8.0) and PLA_2 was eluted with the same buffer. Consequently, main active fractions were obtained. The final enzyme preparations were purified 63-fold (C-PLA_2) and 12-fold (P-PLA_2) from the crude enzyme solutions in yields of 14% and 5%, respectively. The C-PLA_2 and P-PLA_2 included small amounts of several proteins, as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

2.4. Lipid extraction and analysis

The extraction of tissue lipids and lipid analyses by thin-layer chromatography (TLC), preparative TLC, TLC-frame ionization detection method (TLC/FID) and gas–liquid chromatography (GLC) were performed as described by Hayashi (1989) and Hayashi and Kishimura (1996).

PC and PE were prepared from the total lipids of the squid mantle muscle using preparative TLC with chloroform–methanol–acetic acid–water (55:17:3:2, v/v/v/v) as a developing solvent.

2.5. PLA_2 activity assay

PLA_2 activity was measured as described by Kishimura and Hayashi (1999b). One unit of enzyme