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Partial purification of PLA_2 inhibitor from the marine brown alga *Ishige okamurae*

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Introduction

Since past twenty years various efforts have been made to isolate biomedically active compounds from seaweed resource, however, there are few papers on the inhibitors of bacterial phospholipase A_2 (PLA_2) enzyme. The PLA_2 is an enzyme which cleaved fatty acids at *sn*-2 position of phosphatidylcholine. Inhibitor of this enzyme may be useful as therapeutic drug for the treatment of inflammatory disease. In the present study an active fraction has been partially purified from the seaweed *Ishige okamurae* by methanol-water (4:1) extraction of chloroform fraction, silica gel chromatography, and Sephadex LH-20 filtration. Taxonomically this brown alga falls under order Chordariales and family Ishigeaceae. The algae is widespread on rocks in upper and middle intertidial zone on rough open coast. Thallus is firm, dichotomously branched and is perennial, and luxuriant from winter to summer and mature in early summer.

Materials and methods

Algal collection and preparation of extracts

Collection of *I. okamurae* was carried out at intertidal zone from Namhe Island, Korea during May 2001 and May 2002 and Cheju Island, Korea during June 2001 and June 2003. Seaweed sample was washed off epiphytes and salts with tap water and distilled water, then air-dried at room temperature. It was ground into a powder using coffee grinder for 5 minutes and stored -20℃ until use. For each 200 g seaweed powder, 1L of methanol-water 4:1 was added and kept for 1 day at room temperature and then filtered. Same process repeated for three times and were combined. Solvents were evaporated *in vacuuo*. The extract was then successively fractionated into different classes according to polarity following the method of Harborne (1998). The crude extract was at first acidified to pH 2.0 by 2M H_2SO_4 and then successively extracted with $CHCl_3$ three times and then mixed. The chloroform extract (fraction III) then evaporated *in vacuuo* to get a gummy tar. After weight measurement it was soaked into methanol and stored in freeze showed main inhibitor against PLA_2.

Purification of the extract

5ml (1.0gm) of the moderately polar chloroform fraction (0.2 gm/ml) was then filtered on a silica gel (22g, 230-400mesh) column (1.8 x 50cm), and then eluted with each n-hexane, chloroform and methanol as eluants. The chloroform fraction showd best fraction for PLA_2 inhibition. Using 100% methanol as eluation Sephadex LH-20 (Amersham Biosciences AB, Sweden) column (2 cm x 85 cm) each 110 drops of fraction was collected at a flow rate of 0.5 mL min^{-1}.

Enzymatic Assay

Synthesis of substrate

4-nitro-3-(octanoyloxy) benzoic acid, is the substrate for PLA_2 enzymatic activity. This compound was synthesised following the method of Cho and Kezdy (1991).

Assay procedure

The silica gel and sephadex column fractions were assayed for PLA_2 activity. 15μL of extract (4mg/ml) was added in 3ml of 10mM Tris-HCl (pH 8.0) containing 100mM NaCl and 1mM $CoCl_2$. And then 15μL of PLA_2 (0.1-2.0 mg/ml) was mixed with 15μL of the substrate of 3.1mM. Then the absorbancy was measured at 410nm before incubation and after two hours incubation at 37℃ (Cho and Kezdy 1991).

Results and discussion

Substrate synthesis

120mg of 4-nitro-3-octanoloxy benzoic acid is synthesized from 183mg of 3-hydroxy-4-nitrobenzoic acid.

Silica gel and Sephadex LH-20 column chromatography

After eluting 550ml of n-hexane, chloroform fraction (dark green) showed the most active fraction and 15-60 fractions showed the most potential inhibitory fraction from Sephadex LH-20 column chromatography against bacterial PLA_2.

Reference

Harborne J B (1998) A guide to modern techniques of plant anlysis. Phytochemical Methods Chapman and Hall Pub. p 7.

Cho W and Kezdy FJ (1991) Chromogenic substrates and assay of Phospholipase A_2. Methods Enzymol. 197. pp 75-79.