PHOSPHOLIPID-DEPENDENT ACID PHOSPHOLIPASE A_1 FOUND IN CYTOSOL OF MAMMALIAN CELLS

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1. Introduction

Phospholipases A_1 in mammalian cells have generally been believed to be distributed in a membrane system, such as endoplasmic reticulum [1], lysosomal membrane [1] and plasma membrane [2], of cells except lysosomal phospholipase A_1, which is present in soluble form in the inner matrix [1,3,4]. We report here the presence of a new type of soluble acid phospholipase A_1 (EC 3.1.1.32) in the cytosol of FL cell line derived from human amniotic membrane [5] and also of rat tissues. This enzyme revealed its activity only in the presence of certain phospholipids or a nonionic detergent, Triton X-100, in acidic pH (optimum pH 3.6).

2. Materials and methods

FL cells (3×10^7) were grown as monolayers in yeast extract–lactalbumin hydrolysate–Earle's medium containing 10% bovine serum [6]. Cells at late logarithmic phase were harvested and homogenized with 0.25 M sucrose. Postnuclear fraction was centrifuged at 105 000 × *g* for 90 min to give the cytosol fraction which was used as the enzyme preparation. In this fraction, almost no lysosomal acid phospholipase A_1 and A_2 (EC 3.1.1.4) or acid phosphatase (EC 3.1.3.2) activities were detected [4].

1-Acyl-2-[1-^{14}C]oleoyl-GPC (0.5 mCi/mmol) was prepared enzymatically from 1-acyl-GPC using 1-[^{14}C]-oleic acid, CoA and ATP with FL cell homogenates [6]. 1-[1-^{14}C]Palmitoyl-2-oleoyl-GPC (0.2 mCi/mmol) was prepared by the same enzymatic acylation of 1-[1-^{14}C]palmitoyl-GPC obtained by the treatment of 1-[^{14}C]palmitic acid-labeled phosphatidylcholine of FL cells with snake (*Trimeresurus flavoviridis*) venom phospholipase A_2 [6]. 2-[1-^{14}C]Oleoyl-GPC (0.53 mCi/mmol) was obtained by hydrolysis with iodine [7] of 1-alkenyl-2-[1-^{14}C]oleoyl-GPC prepared by acylation of choline lysoplasmalogen [6]. [^{14}C]Trioleoylglycerol (0.4 mCi/mmol) was prepared from total lipid extracts of FL cells incubated for 6 h after the addition of 1-[^{14}C]oleic acid (5 μCi) to the growth medium.

Phosphatidylcholine and phosphatidylethanolamine obtained from egg yolk, cardiolipin from FL cells, phosphatidylinositol and phosphatidylserine from ox brain were purified by the column chromatography on silicic acid [8] and on DEAE-cellulose [9]. Phosphatidic acid was prepared from phosphatidylcholine by the action of cabbage phospholipase D [10]. Triton X-100 was the product of Roam and Haas (Philadelphia).

Acid phospholipase A_1 activity in the cytosol was determined as follows: a mixture of either 1-[1-^{14}C]-palmitoyl-2-oleoyl-GPC or 1-acyl-2-[1-^{14}C]oleoyl-GPC (each 18 000 dpm) and egg phosphatidylcholine (total 850 nmol) was used as substrate, to which an appropriate amount of phospholipid or Triton X-100 (final 0.1%, w/v), acetate buffer (pH 3.6, final 0.1 M) and the enzyme solution (0.2 mg as protein) were added, and the reaction mixture (total 1 ml) was incubated for 1 h at 37°C. Reaction products were extracted with chloroform–methanol (2:1, w/v) and

Abbreviation: GPC, *sn*-glycero-3-phosphorylcholine

separated by thin-layer chromatography [4]. Since 2-acyl-GPC acyl-hydrolase (EC 3.1.1.5) activity was not detectable when 2-[1-^{14}C]oleoyl-GPC (840 nmol) was incubated with the cytosol preparation (0.63 mg as protein) under the same condition as described above, phospholipase A_1 activity was determined by measuring the radioactivity of the formed ^{14}C-labeled fatty acid or [^{14}C]lysophosphatidylcholine (2-acyl-GPC) from the 1- or 2-labeled substrate, respectively. Protein was measured by Lowry's method [11].

3. Results and discussion

3.1. *Positional specificity of phosphatidylcholine hydrolysis by phospholipase in FL cell cytosol and the effect of phosphatidylinositol or Triton X-100 on the enzyme*

Phospholipase activity in FL cell cytosol was determined using either 1-[1-^{14}C]palmitoyl-2-oleoyl-GPC or 1-acyl-2-[1-^{14}C]oleoyl-GPC as substrate. When these substrates were incubated with cytosol preparation in the absence of phosphatidylinositol or Triton X-100, no detectable phospholipid splitting-enzyme activity was recognized. However, when phosphatidylinositol or Triton X-100 was added to the reaction mixture, either significant amount of radioactive fatty acid from 1-[1-^{14}C]palmitoyl-2-oleoyl-GPC or radioactive lysophosphatidylcholine from 1-acyl-2-[1-^{14}C]oleoyl-GPC was released. As shown in table 1, the amount of radioactive fatty acid released from 1-[1-^{14}C]-palmitoyl-2-oleoyl-GPC coincided fairly well with the amount of radioactive lysophosphatidylcholine from 1-acyl-2-[1-^{14}C]oleoyl-GPC. The released amount of either the radioactive lysoderivative or fatty acid from the respective 1- or 2-labeled substrate was very small, indicating that the activity of the acid phospholipase A_2 and/or 2-acyl-GPC acylhydrolase might be very low in the cytosol under the present reaction condition. Neither radioactive diglyceride nor phos-

Table 1
Hydrolysis of 1- or 2-labeled phosphatidylcholine by FL cell cytosol in the presence or absence of phosphatidylinositol or Triton X-100

Substrate (850 nmol)	Additions	Reaction products [nmol (left) or % of radioactivity in parentheses (right)]				
		Lysophosphatidylcholine		Phosphatidyl-choline	Fatty acid	
		1-[1-^{14}C]Palmitoyl-GPC fraction	2-[1-^{14}C]Oleoyl-GPC fraction		[^{14}C]Palmitic acid fraction	[^{14}C]Oleic acid fraction
1-[^{14}C]Palmitoyl-2-oleoyl-GPC	–	5.9 (0.7)		837.3 (98.5)	6.8 (0.8)	
	Phosphatidyl-inositol (final, 0.7 mM)	12.7 (1.5)		433.5 (51.0)	403.8 (47.5)	
	Triton X-100 (final, 0.1%)	10.2 (1.2)		255.0 (30.0)	584.8 (68.8)	
1-acyl-2-[1^{14}C]-oleoyl-GPC	–		7.6 (0.9)	835.6 (98.3)		6.8 (0.8)
	Phosphatidyl-inositol (final, 0.7 mM)		414.8 (48.8)	425.9 (50.1)		9.3 (1.1)
	Triton X-100 (final, 0.1%)		559.3 (65.8)	278.0 (32.7)		12.7 (1.5)

Reaction mixture (1 ml) containing 850 nmol of either 1-[1-^{14}C]palmitoyl-2-oleoyl-GPC or 1-acyl-2-[1-^{14}C]oleoyl-GPC, additions (phosphatidylinositol or Triton X-100), 0.1 M acetate buffer (pH 3.6), enzyme preparation (protein 0.33 mg) of FL cell cytosol was incubated for 2 h at 37°C

phatidic acid was detectable in the reaction products, indicating that the possibility of the presence of acid phospholipase C (EC 3.1.4.3) or phospholipase D (EC 3.1.4.4) in het cytosol of FL cells was excluded.

From these results, the presence of phospholipid- or Triton X-100-dependent phospholipase A_1 in the cytosol of FL cells was evident.

3.2. *Effect of the concentration of added certain phospholipids or Triton X-100 on the activity of cytosol acid phospholipase A_1 in FL cells*

The hydrolysis of phosphatidylcholine (0.25 mM) by the cytosol acid phospholipase A_1 was markedly stimulated by the addition of several kinds of phospholipids (fig.1). Most potent phospholipid stimulant was phosphatidylinositol, which showed the maximum stimulation of the formation of 2-acyl-GPC at 0.69 mM. Cardiolipin, phosphatidylserine, phosphatidic acid and phosphatidylethanolamine also showed the same stimulation maximally at 0.20 mM, 0.31 mM, 0.25 mM and 1.05 mM, respectively. It was also confirmed that Triton X-100 stimulated the cytosol acid phospholipase A_1 activity maximally at 0.08–0.1% (w/v).

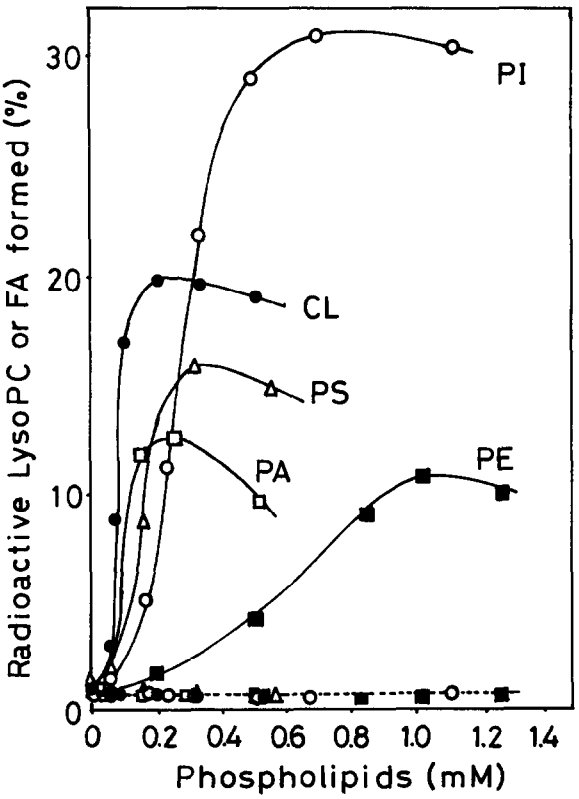

Fig.1. Effect of the addition of phospholipids on the activity of the cytosol acid phospholipase A_1 of FL cells. The reaction mixture (1 ml) containing 0.25 mM mixture of 1-acyl-2-[1-^{14}C]-oleoyl-GPC and egg phosphatidylcholine, an appropriate amount of phospholipid, 0.1 M acetate buffer (pH 3.6) and the enzyme preparation (protein 0.2 mg) of FL cell cytosol was incubated for 1 h at 37°C. (———) Radioactive lysoPC; (- - - -) radioactive FA

Abbreviations: PI, phosphatidylinositol; CL, cardiolipin; PS, phosphatidylserine; PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; FA, fatty acid.

Purified lipase preparation [12,13] has been reported to hydrolyze the ester bond at the 1-position of phosphoglycerides. However, [^{14}C]trioleoylglycerol (850 nmol) was not hydrolyzed by the enzyme preparation under the same condition as acid phospholipase A_1 assay.

Similar acid phospholipase A_1 activity could also be detected in the cytosol of rat tissues such as thymus, kidney, lung, intestinal mucosa, liver, muscle and testis (table 2), indicating that this enzyme might be a common component in the cytosol of mammalian cells.

The stimulation of the cytosol acid phospholipase A_1 by phospholipids or Triton X-100 may be explained by alteration of the physical state of the substrate, i.e.,

Table 2
Acid phospholipase A_1 activity in the cytosol of rat tissues

Rat tissues	Acid phospholipase A_1 activity in the cytosol (nmol of 2-acyl-GPC formed/mg protein/h)
Thymus	702.5
Kidney	679.2
Lung	410.5
Intestinal mucosa	395.2
Liver	101.5
Skeletal muscle	65.2
Testis	50.4

Wistar rats (70–150 g) were fasted for 18 h and killed by decapitation. Tissues were excised, washed with phosphate buffered saline. The cytosol fraction of each tissue was prepared by centrifugation of its postnuclear fraction in 0.25 M sucrose at 105 000 × *g* for 90 min. Enzyme activity was determined as described in the text, in the presence of Triton X-100, using the mixture of 1-acyl-2-[1-^{14}C]oleoyl-GPC and egg phosphatidylcholine (total 850 nmol)as substrate

formation of the mixed substrate-stimulator micelles [14] and/or by the direct interaction of the stimulator to enzyme protein resulting in consequent conformation change and activation of the enzyme. The final conclusion on the stimulation mechanism of cytosol phospholipase A_1 should be understood from the experiments using purified enzyme. This enzyme is, however, rather unstable, although its purification is now in progress.

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