ACID PHOSPHOLIPASE A_1 AND A_2 IN THE CELLS, AND SUBCELLULAR REDISTRIBUTION OF THEIR ACTIVITIES IN THE CELLS INFECTED WITH MEASLES VIRUS

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SUMMARY

Presence of soluble acid phospholipase A_1 and A_2 was confirmed in the (lysosomes + mitochondria) fraction of cultured human amnion cell line, FL cells. Activity of these enzymes and acid phosphatase was detected in the cytosol fraction of FL cells harvested at 59 hr after infection with measles virus, indicating that these enzymes in the (lysosomes + mitochondria) fraction were released to the cytosol fraction during the maturation of measles virus in the cells. Further, it was confirmed that the release of acid phospholipase A_1 and A_2 almost paralleled the development of cytopathic effect.

Soluble phospholipase A_1 and A_2 (1) acting optimally in acid pH range have been reported to be present in the lysosomes (2, 3) of animal tissues. However, only a few studies on phospholipases in tissue culture cells have so far appeared (4).

The formation of multinucleated giant cells caused by infection with measles virus has been reported by many investigators (5-7). Recently, it has been revealed that lysophosphatidylcholine could induce the membrane fusion of chick erythrocytes or cultured cells (8-10), therefore, it seems that there is a close relationship between the change of phospholipase activities (11) and the cell fusion in tissue culture cells infected with measles virus.

In the present study, the activity of soluble acid phospholipase A_1 and A_2 was demonstrated in (Lyso + Mit) fraction of FL cells (12), and the change of their intracellular distribution (redistribution) by measles virus-infection was also investigated to obtain a clue to the formation of multinucleated giant cells described above.

MATERIALS AND METHODS

Abbreviations; GPC, sn-glycero-3-phosphorylcholine; PBS, phosphate buffered saline; YLE, yeast extract-lactalbumin hydrolysate-Earle's salt solution; CPE, cytopathic effect; Lyso, lysosomes; Mit, mitochondria; DPO, 2,5-diphenyloxazol; POPOP, 2,2'-p-phenylene-bis(5-phenyloxazol).

Cells and virus. FL cell monolayer was grown and harvested as described elsewhere (13). Measles virus (Sugiyama strain) (14) was inoculated at input multiplicity of 0.6-1.0 to fully grown cell sheets washed twice with PBS (15). After adsorption (1 hr at 37°C), YLE medium (13) supplemented with 2 % bovine serum was added and incubation was continued. CPE was assayed by the method of Collins and Roberts (16).

Substrates. 1-Acyl-2-(1-^{14}C)oleoyl-GPC (0.5-0.8 mCi/mmole) was prepared as reported previously (13), and 1-(1-^{14}C)palmitoyl-2-oleoyl-GPC (0.2 mCi/mmole) was prepared enzymatically by acylation of pure 1-(1-^{14}C)palmitoyl-GPC, which was obtained by deacylation with snake venom phospholipase A_2 (13) of dipalmitoyl-GPC-^{14}C of FL cells cultured in the growth medium containing palmitic acid-1-^{14}C. 2-(1-^{14}C)Oleoyl-GPC (0.53 mCi/mmole) was prepared from 1-alkenyl-2-(1-^{14}C)oleoyl-GPC (13) with iodine (17).

Subcellular fractionation. FL cells were homogenized with 0.25 M sucrose, and subcellular fractionation was carried out (18). The (Lyso + Mit) fraction was obtained by centrifugation of 450 x g supernatant at 17,500 x g for 15 min. The resulting pellet was then washed twice with 0.25 M sucrose and finally suspended in the sucrose solution. The supernatant from the 17,500 x g centrifugation was centrifuged at 105,000 x g for 90 min to give the cytosol fraction. Soluble and membrane sub-fractions of the (Lyso + Mit) fraction were obtained by the method of Beck et al (19).

Enzyme assays. Phospholipase A_1 and A_2 activities were determined as follows: 0.5 ml of subcellular fraction containing 0.5 mg of protein was added to the suspension of usually 20 nmoles of 1-acyl-2-(1-^{14}C)oleoyl-GPC in 0.5 ml of 0.2 M acetate buffer (pH 3.8 or 4.2, optimal for the activity of phospholipase A_1 or A_2 of the (Lyso + Mit) fraction, respectively). After incubation of the mixture at 37°C for 2 hr, lipids were extracted with 4.0 ml of chloroform-methanol (2:1, by vol.) (20), and separated chromatographically on Silica gel H thin layer plates, which were developed in chloroform-methanol-water (65:25:4, by vol.). Each radioactive spot was scraped and transferred to a scintilla-

Table 1 Hydrolysis of radioactive choline glycerophospholipids by phospholipid acylhydrolase of FL cell homogenate.

Radioactive substrates mixed with egg yolk choline phospholipid (Total lipid-P nmoles/tube)	% of radioactivity (above) and the amounts* of reaction products (below)		
	Lysophosphatidylcholine-^{14}C	Phosphatidylcholine-^{14}C	Fatty acid-^{14}C
1-(1-^{14}C)Palmitoyl-2-oleoyl-GPC (100.0)	16.7 8.7	50.0 25.8	33.3 17.2
1-Acyl-2-(1-^{14}C)oleoyl-GPC (100.6)	30.8 16.0	49.2 25.5	20.0 10.3
2-(1-^{14}C)Oleoyl-GPC (81.0)	98.3 41.0	0.3 0.2	1.4 0.6

The mixture of substrate containing either 1-(1-^{14}C)palmitoyl-2-oleoyl-GPC (12,500 dpm) or 1-acyl-2-(1-^{14}C)oleoyl-GPC (24,000 dpm) and egg yolk phosphatidylcholine added to give the suitable amount for enzymatic reaction, 0.5 ml of cell homogenate (0.97 mg as protein), and 0.5 ml of 0.2 M acetate buffer (pH 4.0) was incubated for 2 hr at 37°C. Incubation of 2-(1-^{14}C)oleoyl-GPC (94,500 dpm) was also carried out under the same condition described above. Reaction products were extracted, separated, and the amount of each individual radioactive product was determined as described in the text. Figures represent the averages of duplicate determinations. * nmoles formed/mg protein/hr.

tion vial containing DPO-POPOP-toluene scintillation mixture and thixotropic gel (21). Since 2-acyl-GPC acylhydrolase activity was negligible in the assay system described above (see Table 1), phospholipase A_1 and A_2 activities were determined by measuring the amount of radioactive lysophosphatidylcholine and fatty acid, respectively. Acid phosphatase activity was determined using p-nitrophenylphosphate as substrate (22). Protein was determined by Lowry's method (23).

RESULTS AND DISCUSSION

Acid phospholipase A_1 and A_2 activities in FL cells. The extent of enzymatic hydrolysis of 1-acyl-2-(1-^{14}C)oleoyl-GPC with the homogenate of FL cells at various pH (3.6-9.1) was studied. Radioactive lysophosphatidylcholine and fatty acid were found to be formed optimally in acid range at pH 3.8 and 4.2, respectively. Radioactive fatty acid was formed to a certain extent at neutral pH also, although it was considerably lower than in acid pH.

Table 2 Activity distribution of acid phospholipase A_1, A_2 and acid phosphatase in the soluble and membrane sub-fractions of the (Lyso + Mit) fraction.

	Enzyme	Relative activity in sub-fraction		
		Soluble	Membrane	Whole
Specific activity	Phospholipase A_1*	60.5	1.8	4.7
	Phospholipase A_2**	21.2	0.8	2.1
	Acid phosphatase***	1,123.1	103.9	138.0
Total activity	Phospholipase A_1	17.4	7.7	26.3
	Phospholipase A_2	6.1	3.4	11.2
	Acid phosphatase	313.5	444.7	772.8

Preparation of the soluble and the membrane sub-fractions of the (Lyso + Mit) fraction, and the determination of the enzyme activities were carried out as described in the text. * nmoles of lysophosphatidylcholine-^{14}C formed/mg protein/hr, ** nmoles of fatty acid-^{14}C formed/mg protein/hr, *** nmoles of p-nitrophenol formed/mg protein/min, from the substrates.

The amount of radioactive fatty acid (17.2 nmoles) and lysophosphatidylcholine (8.7 nmoles) formed presumably by phospholipase A_1 and A_2, respectively, from mixed substrate (100 nmoles) of 1-(1-^{14}C)palmitoyl-2-oleoyl-GPC and egg phosphatidylcholine corresponded well to the amount of lysophosphatidylcholine (16.0 nmoles) and fatty acid (10.3 nmoles) formed from the mixture of 1-acyl-2-(1-^{14}C)oleoyl-GPC and egg phosphatidylcholine (100.6 nmoles) (Table 1). It was also confirmed that lysophospholipid acylhydrolase (lysophospholipase) activity under the same condition was nearly completely negligible. Furthermore, neither phospholipase C nor D activity could be demonstrated in FL cell homogenate.

These results indicate that the hydrolysis of acylesters of phosphatidylcholine was caused by the mixture of acid phospholipase A_1 and A_2 in FL cells.

Occurrence of acid phospholipase A_1 and A_2 in the soluble sub-fraction of the (Lyso + Mit) fraction of FL cells. Relative specific activity as well as total activity of acid phospholipase A_1 and A_2 in post nuclear fraction (450 x g supernatant fraction) of FL cell homogenate was found to be maximum in the

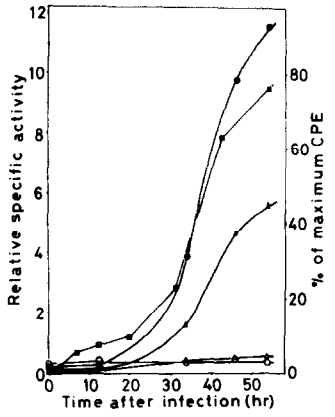

Fig. 1 Development of CPE and the activation of phospholipase A_1 and A_2 activities in the cytosol fraction of FL cells infected with measles virus. At appropriate times after infection with measles virus, cells were harvested, fractionated and the relative specific activities of phospholipase A_1 (nmoles of lysophosphatidylcholine-^{14}C formed/mg protein/hr: ●——●, infected: ○——○, control) and A_2 (nmoles of fatty acid-^{14}C formed/mg protein/hr: ▲—▲, infected △——△, control) in the cytosol fraction were determined as described in the text. CPE (■——■) was also assayed simultaneously (16).

(Lyso + Mit) fraction like that of acid phosphatase regarded as a lysosomal marker. It was also confirmed that the greater part of the lysosomal acid phospholipase A_1 and A_2 activities was recovered in the soluble sub-fraction (Table 2), indicating that these enzymes in the fraction were present in the matrix in soluble form or at most bound loosely to the membrane.

Release of acid phospholipase A_1, A_2 and acid phosphatase in the particulate fraction into the cytosol fraction of FL cells infected with measles virus. FL cells were fractionated into the particulate and the cytosol fraction at 59 hr after infection with measles virus, and acid phospholipase A_1, A_2 and acid phosphatase activities in the cell homogenate, particulate, and the cytosol fractions were determined. It should be noted (Table 3) that, although there was little or no difference between infected and control cell homogenates in the relative specific activity or the total activity of these enzymes, a marked decrease in these enzyme activities were found in the particulate fraction. Conversely, a marked increase occurred in their activities in the cytosol fraction of infected cells. The optimum pH of phospholipase A_1

Table 3 Redistribution of the activities of acid phospholipase A_1, A_2 and acid phosphatase in FL cells infected with measles virus.

Cells	Fraction	Relative activity					
		Specific			Total		
		Phospholipase A_1*	Phospholipase A_2**	Acid phosphatase***	Phospholipase A_1	Phospholipase A_2	Acid phosphatase
Normal FL cells (Control)	Homogenate	1.58	0.65	81.8	53.6 (100)	22.1 (100)	2,775 (100)
	Particulate	2.28	0.87	119.3	48.4 (90.3)	18.6 (84.2)	2,539 (91.5)
	Cytosol	0.21	0.10	25.1	1.5 (2.8)	0.7 (3.2)	178 (6.4)
Infected FL cells	Homogenate	1.48	0.64	85.4	45.3 (100)	19.6 (100)	2,613 (100)
	Particulate	0.47	0.21	64.9	10.2 (22.5)	4.7 (24.0)	1,413 (54.1)
	Cytosol	4.73	2.01	149.1	28.7 (64.4)	12.2 (62.2)	905 (34.6)

FL cells, harvested at 59 hr after infection with measles virus, were homogenized in ice-cold 0.25 M sucrose and centrifuged at 105,000 x g for 90 min. Pellet designated as the particulate fraction was resuspended in 0.25 M sucrose. As control experiment, normal FL cells were fractionated, and enzyme activities were determined in the same condition as the infected-cells. Percent of total activity in each fraction to total activity in the homogenate was also shown in the parenthesis. * nmoles of lysophosphatidylcholine-^{14}C formed/mg protein/hr, ** nmoles of fatty acid-^{14}C formed/mg protein/hr, *** nmoles of p-nitrophenol formed/mg protein/min.

and A_2 in the cytosol fraction of the infected cells was pH 3.8 and 4.2, respectively, and was similar to that of soluble phospholipase A_1 and A_2 in the (Lyso + Mit) fraction. These results indicate that acid phospholipase A_1, A_2 and acid phosphatase in the (Lyso + Mit) fraction were released to the cytosol fraction during the maturation of measles virus in FL cells.

It was also found that the release of the lysosomal acid phospholipase A_1 and A_2 to the cytosol fraction of FL cells infected with the virus almost paralleled the development of cytopathic effect, which had been characterized by the formation of the multinucleated giant cells (5) (Fig. 1).

On the other hand, lysophosphatidylcholine-induced membrane fusion (8-10) and the formation of lysophosphatidylcholine in erythrocyte membrane fused by Ca^{2+} (24) have recently been reported. Considering the relation between membrane-fusion and lysophosphatidylcholine, the parallelism described above suggests as one possibility that phospholipase A_1 and A_2 released from the lysosomal particulates into the cytosol fraction may hydrolyze phosphatidylcholine present in the plasma membrane to yield lysophosphatidylcholine, which may immediately be reacylated (13) to its original form, while the membrane-fusion is being accomplished.

REFERENCES

1. van Deenen, L.L.M. and de Haas, G.H., Ann. Rev. Biochem., Vol. 35, 157 (1966)
2. Franson, R., Waite, M. and LaVia, M., Biochemistry, 10, 1942 (1971)
3. Nachbaur, J., Colbeau, A. and Vignais, P.M., Biochim. Biophys. Acta, 274, 426 (1972)
4. Elsbach, P., Holmes, K.V. and Choppin, P.W., Proc. Soc. Exptl. Biol. Med., 130, 903 (1969)
5. Aoyama, Y., Japan J. Exptl. Med., 29, 535 (1959)
6. Toyoshima, K., Hata, S., Takahashi, M., Miki, T. and Okuno, Y., Biken's J., 3, 241 (1960)
7. Cascardo, M.R. and Karzon, D.T., Virol., 26, 311 (1965)
8. Poole, A.R., Howell, J.I. and Lucy, J.A., Nature, 227, 810 (1970)
9. Croce, C.M., Sawicki, W., Kritchevsky, D. and Koprowski, H., Exptl. Cell Res., 67, 427 (1971)
10. Keay, L., Weiss, S.A., Cirulis, N. and Wildi, B.S., In Vitro, 8, 19 (1972)
11. Matsumoto, M. and Suzuki, Y., 9th International Congress of Biochemistry (Stockholm, 1-7, July, 1973), Abstract Book p. 415

12. Fogh, L. and Lund, R.D., Proc. Soc. Exptl. Biol. Med., 94, 532 (1957)
13. Matsumoto, M. and Suzuki, Y., J. Biochem., 73, 793 (1973)
14. Matumoto, M., Arita, M. and Oda, M., Japan J. Exptl. Med., 35, 319 (1965)
15. Dulbecco, R. and Vogt, M., J. Exptl. Med., 99, 167 (1954)
16. Collins, F.D. and Roberts, W.K., J. Virol., 10, 969 (1972)
17. Eibl, H. and Lands, W.E.M., Biochemistry, 9, 423 (1970)
18. de Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. and Appelmans, F., Biochem. J., 60, 604 (1955)
19. Beck, C. and Tappel, A.L., Biochim. Biophys. Acta, 151, 159 (1968)
20. Folch, J., Lees, M. and Sloane-Stanley, G.H., J. Biol. Chem., 226, 497 (1957)
21. Snyder, F. and Stephens, N., Analytical Biochem., 4, 128 (1962)
22. Igarashi, M. and Hollander, V.P., J. Biol. Chem., 243, 6084 (1968)
23. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J. Biol. Chem., 193, 265 (1951)
24. Toister, Z. and Loyter, A., J. Biol. Chem., 248, 422 (1973)