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PHOSPHOLIPID METHYLATION IN MOPC-31C CELL MEMBRANES WITH MODIFIED PHOSPHOLIPID COMPOSITION

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SUMMARY --- The presence of the methylation pathway from phosphatidylethanolamine to phosphatidylcholine was first shown in MOPC-31C cells. Intermediate phospholipids of this pathway, phosphatidyl-N-monomethylethanolamine and phosphatidyl-N,N'-dimethylethanolamine, were accumulated in the cell membranes by adding choline analogues such as N-monomethylethanolamine and N,N'-dimethylethanolamine to the culture medium. These modified membranes had a striking character of enhanced phospholipid methylation. This enhancement could be explained by increases in the second and the third step of the methylation pathway from phosphatidylethanolamine.

INTRODUCTION --- Modification of the polar head group of membrane phospholipids has been reported in several mammalian cultured cells in relation to the structure and function of biological membranes (1-6). However, no significant alterations were found in the activities of membrane-bound enzymes in these modified cells (2-4,6), probably due to compensatory mechanisms against these modifications in the cells (2). We are interested in this kind of modification of membrane phospholipids from the view point of phospholipid methylation, since such modification could produce accumulation of PME and PDE within the membranes (1,2,4-6), both of which are intermediates in the synthesis of PC from PE by stepwise methylation (7). The importance of phospholipid methylation within the cells is suggested by the fact that the PC thus synthesized is richer in higher polyunsaturated fatty acids (8) than PC produced from CDP-choline and diglyceride (9). Moreover, membranes could be biologically transformed by conversion of phospholipid species. Indeed, recently phospholipid methylation was reported to affect various membrane properties, such as fluidity, phospholipid asymmetry, coupling of the β-adrenergic receptors with adenylate

Abbreviations: TME, trimethylethanolamine=choline; DME, N,N'-dimethylethanolamine; ME, N-monomethylethanolamine; E, ethanolamine; PE, phosphatidylethanolamine; PME, phosphatidyl-N-monomethylethanolamine; PDE, phosphatidyl-N,N'-dimethylethanolamine; PC, phosphatidylcholine; MOPC, mineral oil-induced plasmacytoma.

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cyclase, the numbers of β-adrenergic receptors and lactogenic binding sites, Ca^{++}-ATPase activity, leukocyte chemotaxis, mast cell histamine secretion and lymphocyte mitogenesis (10-18).

In this report, we modified membrane phospholipids of MOPC-31C cells by adding choline analogues, such as DME and ME, to the culture medium and examined the effect of this modification on phospholipid methylation. Enhancement of phospholipid methylation is a striking character of the modified membranes, in addition to the new finding of the presence of the methylation pathway from PE to PC in MOPC-31C cells.

MATERIALS AND METHODS

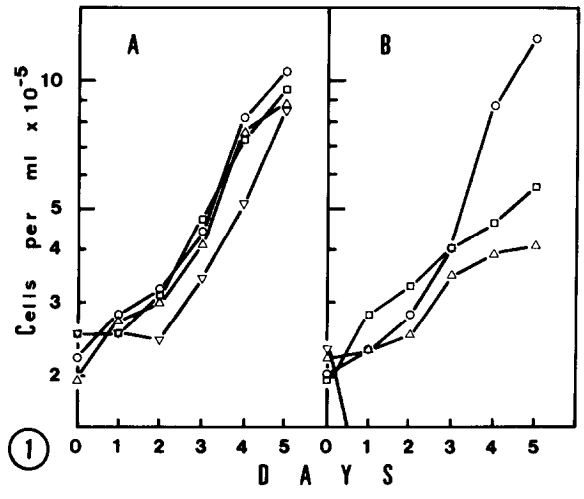
MOPC-31C cells (Flow Laboratories Inc., Rockville, Md., USA) were grown in Higuchi medium (19) with 10 μg/ml of choline (TME) or its analogues, such as DME, ME and E (Eastman Kodak Co., Rochester, N.Y., USA). Calf serum (Flow Laboratories), which had been dialyzed against 1,000 volumes of phosphate buffered-saline (20) for 24 hr, was also added to the medium at a concentration of 20% (v/v).

The cells were homogenized essentially as described by Harrison et al. (21); namely they were washed with 10 mM Tris-HCl (pH 7.6) containing 10 mM KCl and 1 mM $MgCl_2$, suspended in the same buffer (1-2 x 10^7 cells/ml), and disrupted by 40 strokes of a tightly fitting Teflon pestle in a glass homogenizer. Then one volume of hypertonic buffer (0.5 M sucrose, 90 mM Tris-HCl, pH 7.6, 40 mM KCl, 1 mM $MgCl_2$) was added and nuclei and undisrupted cells were precipitated by centrifugation at 1,000 x g for 5 min. The supernatant was centrifuged at 100,000 x g for 1 hr and the resulting pellet was used as the membrane fraction for phospholipid methylation.

Reaction mixture (50 μl), consisting of 0.1 M Tris-HCl (pH 8.6), 10 mM $MgCl_2$, 82.5 μM [methyl-^{14}C]-S-adenosyl-L-methionine (60.6 mCi/mmol, the Radiochemical Centre, Amersham, UK) and membranes (approximately 150 μg protein), was incubated at 37°C for 30 min or 1 hr. Lipids were extracted (12) and separated by two dimensional silica gel-thin layer chromatography (22). The spots corresponding to methylated phospholipids were scraped off and their radioactivities were measured in a liquid scintillation spectrometer. Phospholipid compositions (22) and protein contents (23) were analyzed by published methods.

RESULTS

Effect on cell growth --- First we examined the most suitable conditions for modification of membrane phospholipids of MOPC-31C cells with choline analogues. For this purpose we examined the effects of choline analogues on cell growth. As shown in Fig.1, at concentration of 10 μg/ml none of the analogues affected growth appreciably, though there was a slight growth lag in the presence of E. However, all the analogues were more or less inhibitory at a concentration of 40 μg/ml, which was used to modify membrane phospholipids of LM cells (2). Lower concentrations of the analogues (1 μg/ml) also had no effect on cell growth (data not shown), and phospholipids were not effectively substituted at this low concentration, as



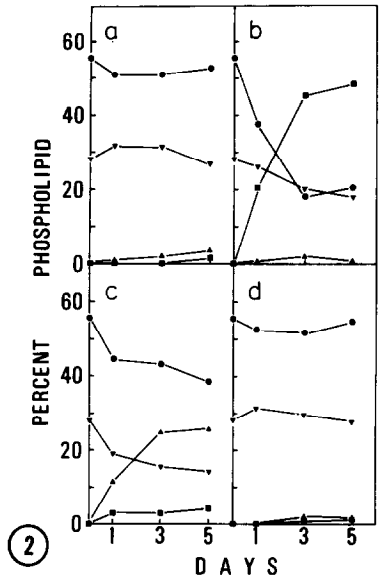


Fig.1. Growth of MOPC-31C cells in the presence of choline or its analogues. Stock cells, cultured in the presence of 1 μg/ml of TME, were washed once with Higuchi medium (60 x g, 5 min), and then suspended in the same medium with 20% dialyzed calf serum and either 10 μg/ml (A) or 40 μg/ml (B) of choline or its analogues (○;TME, □;DME, △;ME, ▽;E.). Growth of the cells at 37°C was followed by counting cells in a hemocytometer.

Fig.2. Phospholipid composition of MOPC-31C cells grown in the presence of choline or its analogues. MOPC-31C cells were grown in Higuchi medium with 10 μg/ml of choline or its analogues and 20% dialyzed calf serum for the indicated times (a;TME, b;DME, c;ME, d;E). Cells were harvested and washed with phosphate-buffered saline (60 x g, 5 min). Total cellular lipids were extracted and phospholipid compositions were analyzed as described previously (22). ●;PC, ■;PDE, ▲;PME, ▼;PE.

described in the following section. Thus we employed choline analogues at final concentrations of 10 μg/ml for modification of membrane phospholipids of MOPC-31C cells.

Phospholipid compositions of modified cells --- The alterations in phospholipid composition of the membranes of MOPC-31C cells on addition of choline analogues to the culture medium are shown in Fig.2. TME caused essentially no change in the phospholipid composition (Fig.2 a), whereas DME and ME increased the corresponding phospholipids, PDE and PME, respectively, with concomitant decreases of PE and PC (Fig.2 b,c). Decrease of PC was especially marked on DME-treatment. Incubation with choline analogues for 3 days was sufficient for modification of the membrane phospholipids with PDE and PME: the former reached 45%, and the latter 25% of the

TABLE I Phospholipid methylation in membrane fractions of MOPC-31C cells modified with choline analogues

Supplement	^{14}C-Methyl Incorporation* (pmol/mg/30 min)			
	PME	PDE	PC	Total***
TME	-**	1.41± 0.12	2.89± 0.81	4.30± 0.77
DME	-	-	62.34±10.25	62.34±10.25
ME	-	76.44±14.19	23.07± 7.55	99.51± 8.58
E	0.14± 0.01	1.74± 0.27	2.73± 0.66	4.61± 0.87

MOPC-31C cells were grown for three days in medium with 10 μg/ml of choline or its analogues and 20% dialyzed calf serum. Membrane fractions were prepared from the modified cells and incorporation of methyl groups from [methyl-^{14}C]-S-adenosyl-L-methionine was measured as described in the MATERIALS AND METHODS. Phospholipid methylation in the membrane fraction from cells grown in the absence of choline analogues was essentially the same to that of TME- or E-treated cell membranes. -; Not detected. Less than 0.035 pmol/mg/30 min. *; Average and standard deviation for three separate experiments. **; With larger amounts of TME-treated cell membranes (0.84 mg protein) radioactive PME was detected (0.12 pmol/mg/30 min). ***; Methyl incorporation in mouse liver microsomes was 6.29 nmol/mg/30 min (24).

total phospholipids under the present conditions. E-treatment, on the other hand, caused no increase of PE in membrane phospholipids (Fig.2 d). This is in contrast to the case in LM cells, in which PE become predominant on E-treatment (2,4,22). A higher concentration of E (40 μg/ml) could not be used for MOPC-31C cells because it killed the cells within one day. Moreover lower concentrations of ME or DME (1 μg/ml) resulted in replacement of only less ten percent of the total phospholipids by PME or PDE.

Phospholipid methylation in modified membranes --- The increases of PME and PDE in the membranes prompted us to examine whether these intermediate phospholipids could affect phospholipid methylation. As shown in TABLE I, membranes obtained from TME- or E-treated cells showed a very low level of phospholipid methylation, just simply compared with microsomes prepared from mouse liver (legend to TABLE I). The radioactive products in TME-treated cell membranes were mainly PDE and PC (TABLE I), but using large amounts of membranes, radioactive PME was also detectable (legend to TABLE I), indicating the presence of all three methylation steps of

PE in the membrane preparation. In contrast, ME- and DME-treated cell membranes both showed greatly increased phospholipid methylation: i.e., 15-20 times that in TME- or E-treated cell membranes (TABLE I). The main radioactive product in DME-treated cell membranes was PC, whereas both PDE and PC were found as products in ME-treated cell membranes. No radioactivity was found in PDE and PME in DME- and ME-treated membranes, respectively, under the present experimental condition.

DISCUSSION

In this study we observed that the membrane phospholipids of MOPC-31C cells were modified by addition of choline analogues at final concentrations of 10 μg/ml to the culture medium. The present results and others (1-6) suggest that this technique is widely applicable for modification of the base moiety of membrane phospholipids in various mammalian cultured cells. However, in MOPC-31C cells, addition of E to the culture medium did not result in increase in the PE content of the membranes, which we could not explain at present.

Our most interesting finding in this work is that treatments with ME and DME greatly increased phospholipid methylation in the cell membranes, since these modifications only produced no or a little effect on the activities of the several membrane-bound enzymes so far reported (2,4-6). The low levels of phospholipid methylation in TME- and E-treated cell membranes could be explained by the fact that the first step of methylation is rate-limiting (7,25,26). In ME- and DME-treated cell membranes, however, increased phospholipid methylation was observed though the enzyme activities for the first methylation seemed to be the same among TME-, DME- and ME-treated cells, judging from the metabolic behavior of the radioactive PE to PC within these cells.(These results will be reported elsewhere.). The possibility that the enzyme molecules catalyzing the second and the third methylation were induced by the modification is excluded by the finding that the exogeneous addition of PME and PDE to TME-treated cell membranes also increased phospholipid methylation (data not shown). All these results led us to conclude that the observed stimulation could be due to increase in available substrate and/or activation of the enzyme(s) for utilization of these phospholipids.

Recently, Hirata et al. reported that phospholipid methylation has important roles in the receptor-mediated information transfer across the membranes (12-14, 16-18). Thus the present technique leading to enhancement of the second and the third step of the methylation pathway from PE to PC is useful for elucidation of the biological roles of these partial reactions, especially in relation to immunoglobulin G secretion in MOPC-31C cells. Further studies in this line are now in progress.

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