SHORT COMMUNICATIONS

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Acylation of lysophosphoglycerides by *Escherichia coli*

An enzymatic acylation of lysolecithins into lecithin was first reported by LANDS[1] who found that this reaction was strongly catalyzed by rat liver microsomes. A great variety of animal tissues has since been demonstrated to convert lysophosphoglycerides having different polar head groups so as to give the corresponding diacyl phosphoglycerides (for reviews see refs. 2 and 3). Homogenates of green leaves were recently found to be also highly active in this respect[4]. MUNDER *et al.*[5] noticed the presence of lecithin in *Salmonella minnesota* after the exposure of these cells to lysolecithin; the normal cells were not found to contain any lecithin.

The present study gives direct evidence that *Escherichia coli* contains enzymes catalyzing a conversion of lysolecithin (lyso-PC) and lysophosphatidyl ethanolamine (lyso-PE) into the corresponding diacyl analogs by an acylation reaction. Using phospholipase A (EC 3.1.1.4) from *Crotalus adamanteus* venom, 1-acyl-glycero-3-phosphoryl choline and 1-acyl-glycero-3-phosphoryl ethanolamine were prepared from rat liver lecithin (PC) and phosphatidyl ethanolamine (PE) which had been labeled biosynthetically with [1-^{14}C]palmitic acid (New England Nuclear Corp.) and/or $^{32}P_i$ (Philips-Duphar Co., Amsterdam). The procedures involved, yielding compounds with a radioactive purity of over 97% as determined by thin-layer and paper chromatographic analyses, were essentially the same as those previously described[6,7]. *E. coli* NCTC 2276 was cultured in a medium containing, per l, bactopeptone, 15.0 g; glucose, 20 g; yeast extract 1.0 g and NaCl, 5 g. Culture with agitation at 37° was continued to the stationary phase. For each experiment, 200 ml of culture containing 40–50 mg of protein was used. The cells, isolated by centrifuging at 30000 × *g* for 15 min, were washed with 10 ml of 0.02 M Tris–HCl buffer (pH 7.4) containing 0.5% NaCl, and finally suspended in 10 ml of buffer and sonicated for 10 min in a salt–ice mixture.

Sonicated suspensions were used directly or separated into a supernatant and a particulate fraction by centrifuging at 22000 × *g* for 30 min. The particulate fraction, the supernatant fraction and the sonicated whole cells were diluted with buffer to maintain a protein concentration of 3–5 mg/ml. Protein was determined by a biuret method[8]. The labeled lyso derivatives and 4 µmoles of oleic acid (a generous gift from Unilever Co., The Netherlands) were sonicated in 5 ml of Tris buffer containing 0.04 M $MgCl_2$. Each tube was then allotted 1 ml of this suspension, 1 ml of *E. coli* preparation and 0.2 ml of Tris buffer containing 3 mg of disodium ATP and 0.14 mg of coenzyme A (Fluka-Buchs, Switzerland). Incubation was continued for various times with agitation at 37°. The reaction was stopped with 7 ml of methanol. After extraction of the lipids by the method of BLIGH AND DYER[9], each phospholipid was separated by thin-layer chromatography on activated silica gel G with the solvent chloroform–methanol–water (65:35:4, by vol.). Each labeled component was located with the aid of pure phosphoglycerides co-chromatographed with each lipid extract and visualized by exposure to iodine vapours. The distribution of radioactivity along

Abbreviations: PC, phosphatidyl choline (lecithin); PE, phosphatidyl ethanolamine.

the chromatoplates was measured by scanning, and provided an additional means for locating the labeled components. Each labeled substance was quantitatively recovered by transferring the silica gel into a small column and eluting with 100 ml of methanol. After removal of solvent, counting of monolabeled or doubly labeled components was performed according to VAN DEN BOSCH[10].

As demonstrated in Fig. 1A, incubation of lyso-PE with sonicated cells of *E. coli* produced an increasing amount of water-soluble products with a corresponding decrease of lipid-soluble material. Similar observations were made with lyso-PC as substrate. In both cases glycerophosphate (as identified by chromatography in propanol–ammonia–water (6:3:1, by vol.): R_F 0.12, and phenol–water (5:2, w/w): R_F 0.27, was a major breakdown product. With an increase of time an increment of labeled PE production was observed at the expense of lyso-PE (Fig. 1B). Besides these 2 com-

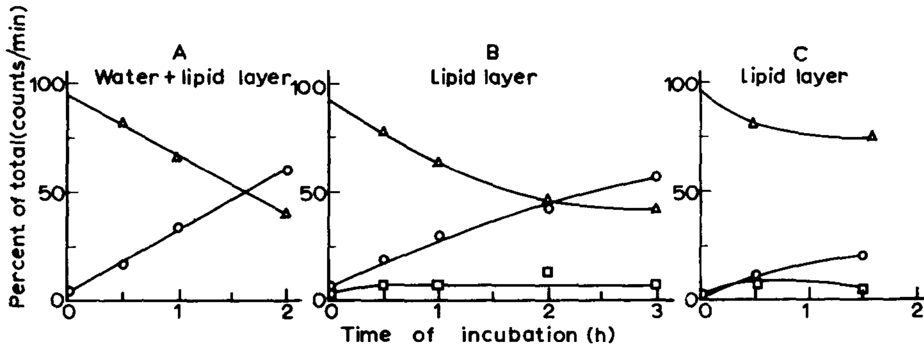

Fig. 1. The incubation mixture, maintained at pH 7.4, consisted of 1 ml of sonicated cells, 1 ml of substrate prepared by sonicating labeled lyso derivative and 0.8 μmole of oleic acid with 0.02 M Tris buffer containing 0.004 $MgCl_2$ and 0.2 ml of Tris buffer containing 3 mg of disodium ATP and 0.14 mg coenzyme A. After incubation at 37°, the reaction was stopped with 7 ml of methanol. (A) The water and lipid phases obtained by the method of BLIGH AND DYER[9] were counted separately: △, lipid phase; ○, water phase. The lipid phase components were separated by thin-layer chromatography. (B) △, lyso-PE; ○, PE; □, remainder of activity recovered on chromatoplate. (C) △, lyso-PC; ○, PC; □, remainder of activity. The total radioactivity recovered was about of 80% of the amount initially present in the substrate added.

ponents, some radioactivity was detected in other regions of the chromatograms. In a number of experiments the major part of the remainder was located in an area defined by R_F 0.05 to 0.15, the nature of this component being unidentified. Experiments with lyso-PC (Fig. 1C) similarly showed a conversion into PC, although this phospholipid, in contrast to PE, is not a constituent of *E. coli*. Further experiments with fractionated cells showed that the conversion of monoacyl into diacyl phosphoglycerides is brought about mainly by the particulate fraction, whereas production of water-soluble compounds was dominant in the supernatant fraction.

In order to reveal the nature of the former conversion, experiments were performed with lyso-PE labeled with both ^{14}C-labeled fatty acid and $^{32}P_i$. The isotopic ratio of the PE formed was found to be very close to that of the lyso-PE used as substrate (Table I). On the basis of these results it can be concluded that the major part of [1-^{14}C]acyl-glycero-3-[^{32}P]phosphoryl ethanolamine subjected to the particulate fraction of *E. coli* was acylated as an entity to give PE. In similar experiments with lyso-PC as substrate, the PC formed was subjected to the action of phospholipase A:

TABLE I

RATIO OF ^{14}C AND ^{32}P ACTIVITIES OF PHOSPHATIDYL ETHANOLAMINE FROM DOUBLY LABELLED LYSOPHOSPHATIDYL ETHANOLAMINE BY PARTICULATE FRACTIONS OF *E. coli*

The incubation conditions were essentially the same as described for Fig. 1A–C except that the particulate fraction of sonicated cells was used. After 1 h incubation at 37°, the lipids were separated and counted for ^{14}C and ^{32}P.

$^{14}C/^{32}P$ *ratio of lyso-PE substrate** *A*	$^{14}C/^{32}P$ *ratio of PE formed* *B*	*B/A***
1.04*	0.85	0.82
2.14	2.53	1.17
1.41	1.27	0.90
0.57*	0.74	1.30

* The ratios are for the substrate prior to incubation. In the 2 designated cases, the substrate remaining after incubation was counted and showed no significant change in its ratio.
** Average B/A = 1.05; S.D. 0.24.

87% of the labeled fatty acid initially present in the 1-acyl-glycero-3-phosphoryl choline utilized was demonstrated to be located at the C-1 position of PC. The significance of the acylation of lyso phosphoglycerides in lipid metabolism of *E. coli* needs further investigation. In any case the ability of particulate and membrane fractions of cells to acylate lysophosphoglycerides is an ubiquitous phenomena.

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