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Uptake and Acylation of 2-Acyl-Lysophospholipids by *Escherichia coli*

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The efficiency of extracellular 2-acyl-lysophospholipid incorporation into *Escherichia coli* membranes and the acyl donor utilized to acylate the 2-acyl-lysophospholipid was determined. Exogenous 2-acyl-lysophospholipids were acylated via the acyl-acyl carrier protein synthetase/2-acylglycerophosphoethanolamine acyltransferase pathway. The maximum extent of 2-acyl-lysophospholipid incorporation into the membrane was approximately 2.5% of the normal phospholipid biosynthetic rate.

Escherichia coli is an important experimental organism for examining the role of phospholipids in the structure and function of biological membranes (for reviews, see references 4 and 11). The genetic manipulation of membrane phospholipid composition has been the main experimental tool for the modification of membrane lipid composition as a result of the inability of *E. coli* to incorporate intact phospholipids into its membrane (11). The deep rough derivative of a *plsB* mutant is capable of incorporating intact 1-acyl-*sn*-glycerol-3-phosphate into phospholipid (9). Although this approach circumvents the glycerol phosphate acyltransferase, normal membrane phospholipid species are synthesized. Homma et al. (5) report that extracellular 2-acyl-lysophospholipids can be incorporated into the membranes of *E. coli*, suggesting the possibility that membrane phospholipid composition could be significantly altered by 2-acyl-lysophospholipid medium supplements. The goal of this work was to determine the biochemical pathway for the incorporation of 2-acyl-lysophospholipids and to assess the utility of this approach to alter membrane phospholipid composition.

The strains used were derivatives of *E. coli* K-12: BB26-36 [*plsB26 plsX glpD3 glpR2 glpK*(Fr) *phoA8 relA1 fluA22* T2^r *spoT1 pit-10* HfrC λ^+] (1) and strain SJ55 [*fadD plsB26 plsX zea-2*::Tn*10 glpD3 glpR2 glpK*(Fr) *phoA8 relA1 fluA22* T2^r *spoT1 pit-10* HfrC λ^+) (Fr stands for feedback resistant). Strain BB26-36 was phenotypically wild type for fatty acid transport and β-oxidation. Strain SJ55 was prepared by transduction of strain BB26-36 with P1 bacteriophage grown on strain DC451 (*fadD zea-2*::Tn*10*) followed by selection on tetracycline-containing plates. A single-colony isolate from this transduction that did not grow on oleate as a carbon source was labeled strain SJ55. Strains were grown in M9 minimal salts (10) containing 0.4% succinate, 0.2% acetate, 0.2% casein hydrolysate, 0.04% glycerol, and 0.01% thiamine. Cultures were grown at 37°C, and the cell number was measured with a Klett-Summerson colorimeter calibrated by determining the number of CFU per milliliter as a function of the colorimeter readings. Protein was determined by the microbiuret method (14) with bovine serum albumin as the standard.

Two metabolic labeling approaches were used to evaluate the uptake and incorporation of 2-acyl-lysophospholipids. 2-Acyl-*sn*-glycero-3-phosphocholine (2-acyl-GPC) was used as a prototypic lysophospholipid so that we would be able to easily distinguish the acylation of exogenous lysophospholipids from de novo biosynthesis. Lysophospholipids were prepared from the corresponding diacyl-phospholipids by digestion with either *Rhizopus* lipase (for the 2-acyl-lysophospholipid) or phospholipase A_2 (for the 1-acyl-lysophospholipid) as described previously (5, 12, 13). Phospholipid biosynthesis in strain BB26-36 (*plsB*) or strain SJ55 (*fadD plsB*) was suppressed by harvesting the culture at 5×10^8 cells per ml and resuspending the cells for 15 min in media without glycerol phosphate. The cells were then supplemented with the compounds listed in Table 1 and labeled with either [1-^{14}C]palmitic acid (10 μCi/ml; specific activity, 58 Ci/mol) or [2-^{3}H]acetic acid (0.1 mCi/ml; specific activity, 3.4 Ci/mmol) for 1 h. The cells were then washed and extracted by the method of Bligh and Dyer (2), and the phospholipids were fractionated by thin-layer chromatography on Silica Gel H layers developed with chloroform-methanol-acetic acid (55:20:5). The locations of radioactive phospholipid species were determined by using the Bioscan Imaging detector. The absolute amount of radioactivity in each phospholipid species was determined by scraping the Silica Gel into a vial, adding 0.1 ml of water to inactivate the gel, and then adding 3 ml of scintillation cocktail. The positional distribution of the radiolabel was determined by digesting isolated phospholipid classes with phospholipase A_2 (12), and the amounts of radioactivity associated with the 1- and 2-positions were determined by Bioscan imaging and liquid scintillation counting.

The ability of phosphatidylcholine (PtdCho) and its degradation products to alter the membrane phospholipid composition and support the growth of strain BB26-36 was examined (Table 1). When deprived of *sn*-glycerol-3-phosphate (glycerol-P), the cells stopped growing and the levels of [1-^{14}C]palmitate and [2-^{3}H]acetate incorporation under these conditions define the extent of phospholipid labeling in the absence of significant net synthesis. The addition of glycerol-P resulted in continued cell growth, a concomitant increase in phospholipid synthesis measured with [2-^{3}H[acetate, and a twofold increase in [1-^{14}C]palmitate incorporation. The difference in the phosphatidylethanolamine/phosphalidylglycerol (PtdEtn/PtdGro) ratio between the glycerol-P-starved and supplemented cells labeled with [2-^{3}H]acetate was the same as reported previously (3). Supplementation of the cultures with *sn*-glycero-3-phosphocholine (GPC) mimicked glycerol-P in supporting growth and normal rates of phospholipid biosynthesis (Table 1). These observations were attributed to the action of GPC phosphodiesterase (the *glpQ*

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TABLE 1. Phospholipid synthesis in strain BB26-36 supplemented with choline phosphoglycerides

Growth supplement	Amt of phospholipid (cpm per mg of protein)					
	PtdCho		PtdEtn		PtdGro	
	[^{14}C]16:0[a]	[^{3}H]acetate[b]	[^{14}C]16:0	[^{3}H]acetate	[^{14}C]16:0	[^{3}H]acetate
None	–	–	3,100	4,961	2,029	7,834
Glycerol-P (3.7 mM)	–	–	5,042	61,432	4,811	9,127
GPC (3.7 mM)	–	–	5,314	71,119	4,546	10,546
2-Acyl-GPC (0.2 mM)	1,029	954	3,541	32,131	4,148	9,362
PtdCho (0.2 mM)	–	–	3,753	34,481	4,397	10,037

[a] Cells were labeled with 10 μCi of [1-^{14}C]palmitic acid (16:0) per ml for 1 h.
[b] Cells were labeled with 0.1 mCi of [2-^{3}H]acetic acid per ml for 1 h.
[c] –, Not detected.

gene product) that is located in the periplasmic space and that degrades GPC to glycerol-P (8). Strain BB26-36 was able to grow, albeit at a lower rate, when supplemented with 2-acyl-GPC. 2-Acyl-GPC resulted in a large stimulation of PtdEtn synthesis measured with [2-^{3}H]acetate, indicating that 2-acyl-GPC was degraded to glycerol-P. However, PtdCho synthesis was also detected in cell exposed to 2-acyl-GPC. The efficiency of PtdCho labeling was much greater with exogenous [1-^{14}C]palmitate (11.8% of the total) than with [2-^{3}H]acetate (2.2% of the total). In both cases, radioactivity was preferentially localized (84%) in the 1-position of PtdCho (not shown). Supplementation of the cells with PtdCho yielded results similar to those obtained with 2-acyl-GPC, except that PtdCho synthesis was not detected.

To determine whether lysophospholipids were being acylated via an acyl carrier protein (ACP)- or coenzyme A (CoA)-dependent pathway, we measured PtdCho formation in strain SJ55 (*fadD plsB*), in which the CoA-dependent pathway was blocked owing to the inactivation of acyl-CoA synthetase (*fadD*). Synthesis of [^{14}C]PtdCho from exogenous [1-^{14}C]palmitate was not inhibited in strain SJ55 (1,156 cpm/mg per h) compared with strain BB26-36 (Table 1). Blockage of the CoA-dependent acylation routes eliminated the synthesis of PtdGro from exogenous [1-^{14}C]palmitate and increased the proportion of [^{14}C]PtdCho formed to 27% of the total labeled phospholipid. These data point to the acyl-ACP synthetase/2-acylglycesophosphoethanolamine (2-acyl-GPE) acyltransferase pathway for the acylation of 2-acyl-GPC. This conclusion was corroborated by determining the positional distribution of [^{14}C]palmitate in the PtdCho and PtdEtn products (Table 2). In all cases, >80% of the carbon-14 was found in the 1-position of both PtdCho and PtdEtn. The rate of 1-acyl-GPC labeling was 50% of the 2-acyl-GPC uptake rate, and, importantly, the carbon-14 was also selectively localized in the 1-position. This result was surprising, but acyl migration occurs in lysophospholipids and a small percentage of the 2-acyl form is always present in 1-acyl-lysophospholipid preparations, or, alternatively, there may be an isomerase activity in the *E. coli* envelope that stimulates this interconversion. Nonetheless, it is clear that the 2-acyl isomer is selectively acylated and incorporated into the membrane.

TABLE 2. Positional distribution of exogenous [1-^{14}C]palmitate incorporated into phospholipids of strain SJ55 (*plsB fadD*)

Growth supplement	% Distribution of radioactivity			
	PtdEtn		PtdCho	
	1-Position	2-Position	1-Position	2-Position
None	84	16	–[a]	–
Glycerol-P (3.7 mM)	82	18	–	–
1-Acyl-GPC (0.2 mM)	83	17	81	19
2-Acyl-GPC (0.2 mM)	85	15	86	14

[a] –, Not detected.

Our experiments indicate that 2-acyl-lysophospholipids are incorporated into *E. coli* membranes by the acyl-ACP synthetase/2-acyl-GPE acyltransferase pathway (Fig. 1). The selective incorporation of acyl moieties into the 1-position and the higher efficiency of labeling with exogenous fatty acids compared with acyl-ACP formed from [2-^{3}H]acetate support this view (Tables 1 and 2). Furthermore, elimination of the acyl-CoA-dependent pathway enhanced the labeling of the 1-position of PtdCho rather than blocking incorporation (Table 2; Fig. 1), ruling out the involvement of acyl-CoA intermediates (13). A third possible incorporation route could be the transacylation of 2-acyl-lysophospholipids by diacylphospholipids (6). However, this mechanism does not appear to contribute significantly to PtdCho synthesis from 2-acyl-GPC, because of the small amounts of [^{3}H]PtdCho formed in cells labeled with [2-^{3}H]acetate (Table 1).

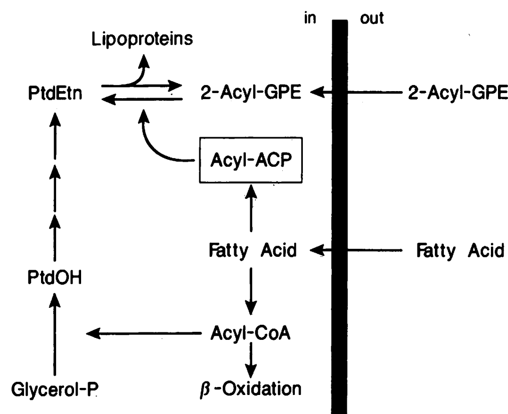

FIG. 1. Pathways for the incorporation of exogenous lipids into the *E. coli* membrane. One route is the transport of fatty acids into the cell and their conversion to acyl-CoA derivatives that are either incorporated into phospholipid via the de novo biosynthetic pathway or, alternatively, degraded by the β-oxidation system. The second pathway involves both fatty acids and 2-acyl-lysophospholipids. Phospholipid formation by this route is due to the sequential action of acyl-ACP synthetase and 2-acyl-GPE acyltransferase. The box around the acyl-ACP pool indicates that these acyl-ACPs are segregated from the acyl-ACP pool derived from fatty acid biosynthesis and cannot be elongated or utilized by the glycerol-P acyltransferase. PtdOH, phosphatidic acid.

Supplementation of *E. coli* with various 2-acyl-lysophospholipids does not appear to be a useful method for the drastic alteration of membrane phospholipid composition. Different genetic backgrounds and labeling protocols yielded variable percentages of 2-acyl-GPC incorporation into PtdCho owing to differences in metabolic channeling of the label (Table 1; Fig. 1). Our best estimate of the extent of 2-acyl-GPC synthesis comes from the information in Fig. 1 and our previous experience with this labeling protocol that indicates that the amount of PtdEtn synthesized under these conditions is about 5% of the total phospholipid-biosynthetic rate (7, 12). since PtdCho synthesis was half of the PtdEtn labeling in this experiment, an upper limit on PtdCho production is estimated to be 2.5% of the normal rate. This number is corroborated by the [^{3}H]acetate labeling experiments in which the rate of PtdCho synthesis appeared to be 2.2% of the PtdEtn labeling (Table 1). Although this level of incorporation does not allow the extensive replacement of PtdEtn by other phospholipids, this approach may prove valuable for introducing molecular probes attached to phospholipid polar head groups or 2-acyl moieties into normally growing *E. coli* cells.

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