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The Positional Distribution of Fatty Acids in *Escherichia coli* Phospholipids Is Not Regulated by *sn*-Glycerol 3-Phosphate Levels

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Large changes in the intracellular concentration of *sn*-glycerol 3-phosphate had no effect on the acyl chain distribution of the phospholipids of *Escherichia coli*. This result directly contradicts the prediction by other workers based on in vitro experiments.

The phospholipids of *Escherichia coli*, like virtually all naturally occurring phospholipids, have a saturated fatty acid (palmitate) at position 1 and an unsaturated fatty acid (palmitoleate or *cis*-vaccenate) at position 2 (3). *sn*-Glycerol 3-phosphate (G3P) acyltransferase is the first committed step in the phospholipid biosynthetic pathway and catalyzes the transfer of a fatty acid from a thioester of either coenzyme A (CoA) or acyl carrier protein (ACP) to G3P. The product of this reaction, lysophosphatidic acid (1-acylglycerol 3-phosphate), is then acylated to phosphatidic acid by a second acyltransferase (2, 3).

In their in vitro studies on acyltransferase specificity, Okuyama and co-workers (8, 9) reported that the specificity of the acyltransferase system is dependent on the G3P concentration, with higher concentrations giving less specificity. From these data, these workers have suggested that abnormally high intracellular concentrations of G3P would result in the synthesis of phospholipids with unusual acyl chain distributions (9). For reasons given previously (2), this hypothesis seemed unlikely; however, direct data on this point were lacking.

We have examined the distribution of fatty acyl groups in phospholipids from cells grown with different intracellular concentrations of G3P. The levels of G3P were manipulated either by the carbon source provided or by use of mutants deficient in the regulation of G3P synthesis.

In the first approach, we have taken advantage of the data of Lowry et al. (6) on the G3P pools of cultures of the *E. coli* K-12 strain Hfr3000 YA139 (erroneously called Hfr139) grown on different carbon sources. By using the extremely sensitive methods developed in their laboratory, these workers demonstrated that the G3P pools of glycerol-grown cells are 16-fold higher (2.8 mM) than those of glucose-grown cells (0.18 mM). Due to the sensitivity of the

TABLE 1. *Fatty acid composition of strain Hfr3000 YA139*[a]

Carbon source	Wt of methyl ester (%)					UFA/SFA ratio[b]
	C_{14}	C_{16}	$C_{16:1}$	C_{17v}	$C_{18:1}$	
Total acids						
Glucose	1	26	42	8	24	2.70
Glycerol	2	24	49	7	19	2.85
G3P	2	26	46	8	24	2.57
Position 1 acids						
Glucose	1	70	tr	tr	29	0.41
Glycerol		68			32	0.47
G3P		68			32	0.47

[a] Strain Hfr3000 YA139 (also known as Hfr139) is a *thi panB* auxotroph of *E. coli* K-12. Cultures were grown at 37°C on the minimal medium of Lowry et al. (6) with glucose at 22 mM or glycerol (or G3P) at 44 mM. The cultures were harvested during exponental growth at a cell density of 0.15 mg/ml of culture. The growth conditions were precisely those of Lowry et al. (6). The phospholipids were extracted (10), and a portion was digested with the phospholipase A_2 activity of king cobra venom as described by Nutter and Privett (7). The products of the phospholipase digestion were separated by thin-layer chromatography on silica gel G plates developed first in petroleum ether-ether-CH_3COOH (70:30:2) and then in $CHCl_3$-CH_3OH-CH_3COOH (50:40:10). The fatty acyl groups of the untreated phospholipids and of the lysophospholipids were converted to the methyl esters with sodium methoxide in methanol (10), and the esters were dissolved in carbon disulfide and analyzed by gas chromatography on a column of 12% diethylene glycol succinate on 100/120 Chromosorb W. The fatty acids were myristic acid (C_{14}), palmitic acid (C_{16}), palmitoleic acid ($C_{16:1}$), *cis*-9,10-methylene hexadecanoic acid (C_{17v}), and *cis*-vaccenic acid ($C_{18:1}$).

[b] UFA, Unsaturated fatty acid; SFA, saturated fatty acid.

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methods used, the measurements were done on extremely small cell samples, thus minimizing the possibility of pool changes during harvesting and extraction of the cells (6).

We grew strain Hfr3000 YA139 on either glycerol or glucose under precisely the conditions given by Lowry et al. (6) and examined the distribution of the acyl chains in the phospholipid molecules extracted from the cells (Table 1). The unsaturated-to-saturated ratios in the phospholipids (of either the total fatty acids or those in position 1) of glucose- and glycerol-grown cultures were virtually identical. We also examined cells grown on glycerol 3-phosphate as the sole carbon source. Such cells are also expected to have an elevated intracellular G3P pool (5), but again, no unusual fatty acid distribution was found.

In the second approach, we examined strains having a G3P synthase (biosynthetic G3P dehydrogenase) deficient in feedback inhibition by G3P (1, 4). These strains overproduce G3P and had intracellular G3P pools of 2.4 to 2.8 mM, which are 10- to 12-fold more than the normal level of 0.24 mM (1). We found that these strains have phospholipid acyl group distributions indistinguishable from their isogenic wild-type strains (Table 2).

From these data, we conclude that the observations of Okuyama and co-workers (8, 9) are not physiologically relevant and are thus an artifact of their in vitro system. We believe that the artifact is due to the use of acyl-CoA rather than ACP as the acyl donor. We have recently presented kinetic evidence (C. O. Rock, S. Goelz, and J. E. Cronan, Jr., submitted for publication) that acyl-CoA and ACP substrates have different acyl donor sites in the acyltransferase system, although the G3P sites are kinetically identical. From these and other data (Rock et al., submitted for publication) we have concluded that ACP rather than acyl-CoA is the physiological acyl donor.

In an experiment relevant to the work of Okuyama et al. (8, 9), we examined the effect of G3P concentration of the acyltransferase system as-

TABLE 2. *Fatty acid composition of mutants with elevated G3P pools*[a]

Strains	Wt of methyl esters (%)					UFA/SFA ratio[b]
	C_{14}	C_{16}	$C_{16:1}$	C_{17v}	$C_{18:1}$	
Total acids						
Strain 8	2	34	39	4	22	1.81
SEG40	3	32	46	4	15	1.86
Position 1 acids						
Strain 8	1	80			19	0.23
SEG40	4	81			15	0.18

[a] Strain SEG40 is a G3P independent transductant of strain BB20 (*gpsA*) from a phage stock grown on BB26-36-R2 (1, 4), which carries a feedback-resistant biosynthetic G3P dehydrogenase (the *gpsA* gene product). Strain 8 is the parent of strain BB20 and thus strains SEG40 and 8 should be nearly isogenic except for the altered G3P dehydrogenase carried by the former strain. The strains were grown as described in Table 1, footnote *a*, with glucose as the carbon source. The fatty acids were analyzed as described in Table 1, footnote *a*. A similar experiment with a set of strains isogenic except for one strain carrying the feedback-resistant dehydrogenase lesion in strain BB26-36-R1 (1) gave a very similar result.

[b] UFA, Unsaturated fatty acid; SFA, saturated fatty acid.

TABLE 3. *Effect of G3P concentration on acyltransferase products*[a]

Substrate	Product formed[b]			LPA/PA ratio
	G3P (μM)	LPA (pmol)	PA (pmol)	
palmitoyl-CoA	125	74	120	0.6
	500	137	103	1.3
palmitoyl-ACP[c]	125	71	8.6	8.3
	500	142	16	8.9
cis-vaccenoyl-ACP	125	38	71	0.5
	500	36	74	0.5

[a] The acyltransferase assay contained Tris-hydrochloride (pH 8.5), 0.1 M; bovine serum albumin (fatty acid-free), 1 mg/ml; palmitoyl-CoA, 12.5 μM or ACP, 25 μM; $MgCl_2$, 5 mM, either 125 or 500 μM *sn*-[*U*-^{14}C]glycerol 3-phosphate (10,000 cpm/nmol), and purified inner membranes from *E. coli* K-12 strain Lin 8 (4 μg of membrane protein per reaction) in a final volume of 40 μl. After incubation at 21°C for 5 min, 0.2 ml of $CHCl_3$–CH_3OH (1:2 [vol/vol]) was added to stop the reaction. This solution was evaporated to dryness under N_2, redissolved in 30 μl of $CHCl_3$–CH_3OH (1:1 [vol/vol]), and spotted on Whatman SG-81 silicic acid-impregnated paper previously dipped in 0.4 M Na_2CO_3 and dried at 100°C for 3 h just before use. The solvent system used was $CHCl_3$–CH_3OH–CH_3COOH–H_2O (70:20:12:4 [vol/vol]) followed by petroleum ether–ether–CH_3COOH (46:42:1 [vol/vol]). The paper was developed twice in the same direction. The second solvent system (run for 20 cm) moved neutral lipids to the front, whereas the first solvent system (run for 14.5 cm) resolved lysophosphatidic acid and phosphatidic acid. The paper was cut into 0.5-cm strips and counted in Aquasol (New England Nuclear Corp.) scintillation solution. The methods used were a modification of those of Ray and Cronan (11). Very similiar results were obtained in three other experiments.

[b] LPA, Lysophosphatidic acid (monoacyl G3P); PA, phosphatidic acid (diacyl G3P).

[c] Diglyceride was also formed in the reaction. At 125 and 500 μm concentrations of G3P, the amounts formed were 16 and 42 pmol, respectively.

sayed with either an acyl-CoA or an ACP as the acyl donor (Table 3). We observed that at 500 μM, a G3P concentration fivefold above the Michaelis constant (90 to 100 μM) for G3P (11), the ratio of lysophosphatidic acid to phosphatidic acid formed from palmitoyl-CoA differed markedly from that found at a 125 μM concentration of G3P. However, if palmitoyl-ACP or *cis*-vaccenoyl-ACP was the acyl donor, the same ratio of products was observed at both G3P concentrations. It should be noted that we used G3P concentrations identical to those of experiments reported by Okuyama and Yamada (8).

In conclusion, the modulation of the intracellular G3P pool played no role in the regulation of phospholipid acyl group specificity in *E. coli*. The specificity of acyl transfer from ACP in vitro was not affected by the G3P concentration, whereas transfer from CoA was affected. Taken together, these results support our conclusion (Rock et al., submitted for publication) that ACP rather than acyl-CoA is the acyl donor in vivo.

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