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Genetic manipulation of membrane phospholipid composition in *Escherichia coli: pgsA* mutants defective in phosphatidylglycerol synthesis

(phosphatidylglycerophosphate synthase/phosphatidylserine synthase/cardiolipin synthase/phosphatidylethanolamine)

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ABSTRACT **Unique mutants of *Escherichia coli* K-12, defective in phosphatidylglycerol synthesis, have been isolated from a temperature-sensitive strain incubated at its nonpermissive temperature. The parent strain had excess phosphatidylglycerol by harboring both the *pss-1* allele [coding for a temperature-sensitive phosphatidylserine synthase (EC 2.7.8.8)] and the *cls*$^-$ allele (responsible for a defective cardiolipin synthase). The newly acquired mutations caused better growth at higher temperatures. One of the mutations (*pgsA3*) has been identified in the structural gene for phosphatidylglycerophosphate synthase [glycerophosphate phosphatidyltransferase (EC 2.7.8.5)]. Phospholipid compositions of these mutants were remarkable; phosphatidylethanolamine was the sole major lipid. In media with low osmotic pressures, these cells grew more slowly than the wild-type cells. They grew normally without recovering from the phospholipid abnormality in media appropriately supplemented with sucrose and $MgCl_2$. Formation of cardiolipin and phosphoglycerol derivatives of membrane-derived oligosaccharides was reduced in a *pgsA3* mutant. *E. coli* strains having the *pgsA3*, *pss-1*, and *cls*$^-$ mutations, either individually or in combination, constitute an empirical system in which the molar ratio of three major membrane phospholipids can be variously altered.**

The specific functional roles of individual lipids in biological membranes are still not well understood at the molecular level. To understand the biological implications of molecular diversity of membrane lipids, manipulation of the composition of major membrane lipids followed by the analysis of resulting changes in membrane and cellular functions seems to be one promising approach. This will be most effectively and specifically achieved by genetic alterations that introduce defective mutations in the enzymes of the committed steps in individual lipid biogenesis (1).

Escherichia coli K-12 should serve as a useful model organism for this purpose since its membranes contain only three phospholipids with relatively simple fatty acid compositions. Phosphatidylethanolamine (PtdEtn, normally about 75% of the total extractable lipids), phosphatidylglycerol (PtdGro, about 20%), and cardiolipin (CL, about 5%) are the major extractable lipids. Defective mutations in all but one of the steps in the phospholipid biosynthetic pathway have been isolated and studied (2, 3). However, not all of the reported mutations are useful for the manipulation of phospholipid composition, as discussed previously (1). Potentially useful among these are temperature-sensitive mutations in phosphatidylserine (PtdSer) synthase (CDPdiacylglycerol:L-serine *O*-phosphatidyltransferase, EC 2.7.8.8), which catalyzes the committed step in PtdEtn synthesis (4, 5) and a defective mutation in CL synthesis (1, 6). Alteration of phospholipid composition by the combined introduction of these mutations (*pss-1* and *cls*$^-$) and the physiological consequences have been studied (1).

The genetic manipulation of phospholipid composition in *E. coli* had been limited by lack of mutations involving defects in PtdGro synthesis. Although defective mutations in *pgsA*, the structural gene for phosphatidylglycerophosphate (PtdGro-*P*) synthase (CDPdiacylglycerol:*sn*-glycerol-3-phosphate phosphatidyltransferase, EC 2.7.8.5), which catalyzes the committed step in acidic phospholipid synthesis, had been reported (5, 7), they caused an *in vitro* enzymatic defect and not an *in vivo* effect on PtdGro-*P* synthesis. Although in a temperature-sensitive mutant, defective both in *pgsA* and *pgsB*, acidic phospholipids decrease at its nonpermissive temperature, intermediates in lipopolysaccharide synthesis accumulate, and the cells lose viability (7–9); therefore, this setting is not suitable for our purpose.

We describe here the isolation and some properties of unique *E. coli* mutants that are defective in PtdGro synthesis. They have extraordinary phospholipid compositions and should be useful in the experimental examination of functional roles of major phospholipids in *E. coli* membranes.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Culture Media. *E. coli* K-12 derivatives having a common genetic background (F$^-$ *glpD3 glpR2 glpKp phoA8 pyrD34 his-68 galK35 xyl-7 mtl-2 rpsL118*) were used: strain SD12 is wild type with regard to the phospholipid biosynthetic enzymes, strain SD9 (*pss-1 cls*) has a temperature-sensitive PtdSer synthase and a defective CL synthase, and strain SD90 is a *recA1* derivative of strain SD9. Construction and properties of these strains have been described (1, 10). A *thyA* derivative of strain SD103 (*pgsA3 pss-1 cls*) was mated with an Hfr PK191, and Thy$^+$ His$^+$ Str^r recombinants were selected from which strain SD312 (*pgsA3 cls*) was obtained. *recA1* derivatives of strains SD103 and SD136 were obtained by a standard method of trimethoprim selection (*thyA* introduction) and mating with Hfr KL16-99 (*recA1*) (11). Plasmid pPG2 is a 17.7-kilobase-pair derivative of pSC101 carrying an 8.1-kilobase-pair *E. coli* chromosomal fragment that contains the *pgsA* gene (12). pCLF1 is a 19.5-kilobase-pair mini-F derivative that contains a kanamycin-resistance determinant and the *cls* gene (13). Broth media

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Abbreviations: CL, cardiolipin; MDO, membrane-derived oligosaccharide(s); PtdOH, phosphatidic acid; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; PtdGro-*P*, phosphatidylglycerophosphate; PtdSer, phosphatidylserine; Mops, 4-morpholinepropanesulfonic acid.

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NBY (1) and LB, 4-morpholinepropanesulfonic acid (Mops) buffered synthetic medium (14) supplemented with 16 L amino acids (1), medium I (15), and medium K containing 1 mM KH_2PO_4, 1.5 mM $(NH_4)_2SO_4$, 80 μM $MgCl_2$, 0.5 μg of $FeSO_4$ per ml, and a total of 2.5 mg of 16 L amino acids (1) per ml were used. Synthetic media contained 25 μg of uracil, 5 μg of thiamine, and 100 μg of L-histidine per ml. Plates contained 1.5% agar.

Methods. Bacterial growth rates at different temperatures and at a single temperature were determined in a temperature-gradient biophotorecorder (model TN-112D, Toyokagakusangyo, Tokyo) and in a Bioscanner (model OT-BS-12, Ohtake-seisakusho, Tokyo), respectively. PtdGro-*P* synthase, PtdSer synthase, and CL synthase in cell extracts were assayed, and two-dimensional TLC of lipids uniformly labeled with $^{32}P_i$ were performed as described (1, 10). Incorporation of [^{3}H]glycerol by way of PtdGro into membrane-derived oligosaccharide (MDO) was measured according to the method of Kennedy (15).

RESULTS

Isolation of Mutants Defective in PtdGro Synthesis. When strain SD9 (*pss-1 cls*) was incubated on LB plates at 37°C, a temperature slightly higher than the upper growth limit for this strain (1), colonies formed at a frequency of 10^{-8} per plated cell. TLC of the lipid extracts and *in vitro* enzyme assay for the extracts from these putative revertants revealed that most of them were the *pss* revertants but some others had reduced levels of PtdGro content. Among the latter, strain SD103, having the lowest PtdGro content, and strain SD136, having an intermediate PtdGro content, were studied further. Table 1 shows the *in vitro* activities of three committed-step enzymes in the major phospholipid biosynthesis of the newly isolated mutants and related strains, and Table 2 shows their phospholipid polar headgroup compositions. PtdGro-*P* synthase activity was undetectable in extracts of strain SD103. It had an extremely low PtdGro content, giving rise to an unusual lipid composition; PtdEtn was the sole major phospholipid (Fig. 1). Other unique features of this phospholipid composition were the elevated levels of phosphatidic acid (PtdOH) and several other minor phosphorus-containing lipids. Strain SD136 had intermediate values of both *in vitro* PtdGro-*P* synthase activity and PtdGro content. However, total phospholipid contents of strains SD103 and SD136 were practically the same as those of the parent (SD9) and wild-type strain (SD12).

Identification of the Gene Altered by Mutations. In addition to the defects in *in vitro* activities of PtdGro-*P* synthase (Table 1), the following lines of evidence indicated that the newly acquired mutation in strain SD103, and most probably that in strain SD136 also, was in *pgsA*, the structural gene for PtdGro-*P* synthase. These alleles in strains SD103 and SD136 are designated as *pgsA3* and *pgsA36*, respectively.

(*i*) A mixture of an equal amount of the extracts from strains SD103 and SD12 showed about one-half of the PtdGro-*P* synthase specific activity of that of strain SD12 (1.6 nmol of PtdGro-*P* per min/mg of protein at 30°C), eliminating the possibility of the production of an inhibitor by the new mutation.

(*ii*) The new mutation in strain SD103 was found to be closely linked to *uvrC*, a gene physically established to be proximal to *pgsA* (12, 16). This analysis was carried out by transducing strain SD103 by the $P1_{vir}$ lysate of strain AB1884 (*uvrC*), followed by the selection of *pgsA*$^+$ transductants on NBY medium supplemented with 50 mM sucrose and 20 mM $MgCl_2$ at 40°C, where strain SD103 was difficult to grow but strain SD9 grew well, as will be discussed later. Colonies that emerged on this plate were first examined for their capability of growth on the NBY plates at 42°C. *pss*$^+$ transductants and revertants grew under the latter conditions and were discarded. Thirty colonies were purified, and their UV sensitivity, PtdGro contents, and *in vitro* PtdGro-*P* synthase activity were examined. Nine of the 30 colonies were UV sensitive

Table 1. *In vitro* specific activities of the enzymes that catalyze the committed steps in major phospholipid biosynthesis

Strain (genotype)	PtdGro-*P* synthase*	CL synthase†	PtdSer synthase*
SD12 (wild type)	3.5	1.7	17.7
SD9 (*pss-1 cls*)	3.6	<0.1	<0.1
SD103 (*pss-1 pgsA3 cls*)	<0.1	<0.1	<0.1
SD136 (*pss-1 pgsA36 cls*)	0.16	<0.1	<0.1
SD312 (*pgsA3 cls*)	<0.1	<0.1	19.4

Cells grown to early stationary phase in NBY medium at 30°C were disrupted by sonication, and enzyme activities were assayed.
*nmol of product per min/mg of protein at 30°C.
†nmol of CL formed per mg of protein during 20-min incubation at 30°C.

Table 2. Phospholipid compositions of isolated *pgsA* mutants and related strains

Strain (genotype)	Composition*					Total lipid†
	PtdEtn	PtdGro	CL	PtdOH	Other	
SD12 (wild type)	76.2	18.0	5.2	0.1	0.5	170
SD9 (*pss-1 cls*)	67.6	31.8	0.1	0.1	0.4	150
SD103 (*pss-1 pgsA3 cls*)	88.6	1.3	0.4	2.6	7.1	160
SD136 (*pss-1 pgsA36 cls*)	87.5	5.1	0.6	1.2	5.6	160

Cells were grown at 30°C and labeled uniformly with ^{32}P (1 μCi/0.3 μmol per ml; 1 Ci = 37 GBq) in a synthetic medium Mops supplemented with uracil, thiamine, and amino acids to late exponential phase (5×10^8 cells per ml), and lipids were extracted and separated by two-dimensional TLC. Errors in duplicate analysis were <3% of each value.
*Molar percent of lipid phosphorus calculated from the radioactivity of each spot.
†Expressed as μmol of lipid phosphorus per g of dry weight of cells, calculated from the total radioactivity of all spots, the specific radioactivity of the culture medium, and the cell mass estimated from the protein content of the culture.

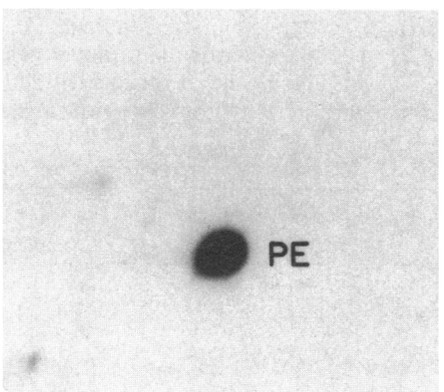

FIG. 1. Autoradiogram showing the unique phospholipid composition of strain SD103 (*pss-1 cls pgsA3*). Cells were labeled uniformly with ^{32}P in NBY medium at 30°C, and lipids were extracted from the cells of exponential phase and subjected to two-dimensional TLC, as described (1, 10). The chromatographic origin was the lower left corner. Under these conditions, other phospholipids are well separated from PtdEtn (PE) (1, 10).

and contained PtdGro at the level of strain SD9 and normal PtdGro-*P* synthase activities. All other 21 colonies, which were presumably unaltered SD103, showed wild-type UV sensitivity and lacked PtdGro-*P* synthase activity and PtdGro.

(*iii*) The newly acquired mutations in strains SD103 and SD136 were complemented by a hybrid plasmid pPG2, a pSC101 derivative carrying the *pgsA* gene (12). Strains SD1030, SD1360, and SD90, *recA1* derivatives of strains SD103, SD136, and SD9, respectively, were transformed with pPG2 or with the vector pSC101. Transformants were selected on NBY plates supplemented with 20 mM $MgCl_2$ and 15 μg of tetracycline per ml. The addition of $MgCl_2$ was necessary to obtain transformants of strain SD1030 with pPG2, although $MgCl_2$ is generally known to antagonize the antibiotic (17). The specific activity of PtdGro-*P* synthase in the extract of strain SD1030 harboring pPG2 was 18.6 nmol of PtdGro-*P* per mg of protein per min [six times higher than that from strain SD90 with the vector pSC101, being due to an increase in gene dosage (12)], and the PtdGro content was restored (34% of the total phospholipid at 30°C). The PtdGro-*P* synthase activity and the PtdGro content of strain SD1360 were likewise restored by harboring pPG2.

(*iv*) Spontaneous revertants that grew on NBY medium supplemented with 50 mM sucrose and 20 mM $MgCl_2$ at 42°C and synthesized PtdGro normally were obtained at a frequency of about 10^{-7}.

Properties of *pgsA3* Mutants. Fig. 2 shows the temperature dependency of the growth rates of strains SD9 and SD103. Strain SD103 grew better than its parent at higher temperatures but worse at lower temperatures, suggesting the phospholipid composition unusually biased to PtdEtn was advantageous at higher temperatures.

Table 3 shows the phospholipid compositions of various *pgsA3* strains grown under several different culture conditions. Strain SD312 (SD103 *pss*$^+$) had essentially the same phospholipid composition and PtdGro-*P* synthase activity (Table 1) as those of strain SD103. PtdGro contents were lower in the cells grown in the NBY medium than those in the Mops medium. Introduction of the *cls*$^+$ gene into strain SD312 by transformation with a low-copy number hybrid plasmid, pCLF1, which contained the *cls* gene, did not cause an appreciable change in the phospholipid composition. Uniform labeling of the *pgsA3* mutants with [1-^{14}C]acetate gave radioactivity distributions of the extractable lipids similar to those with ^{32}P, indicating the absence of the accumulation of unusual phosphorus-free lipids in these mutants. Glycolipids X and Y, the intermediates in lipid A biosynthesis that accumulate in *pgsB* mutants (9), were not detected. Attempts to accelerate the turnover of PtdGro in strain SD312 by introducing the *cls* gene and by adding to the culture medium arbutin (*p*-hydroxyphenyl β-D-glucoside), a model substrate for phosphoglycerol transferase I of MDO biosynthesis (18), did not reduce the PtdGro content below 0.1%. The addition of sucrose and $MgCl_2$ to NBY medium that allows the active growth of strain SD103 at otherwise nonpermissive temperature, as described below, did not alter appreciably the lipid composition.

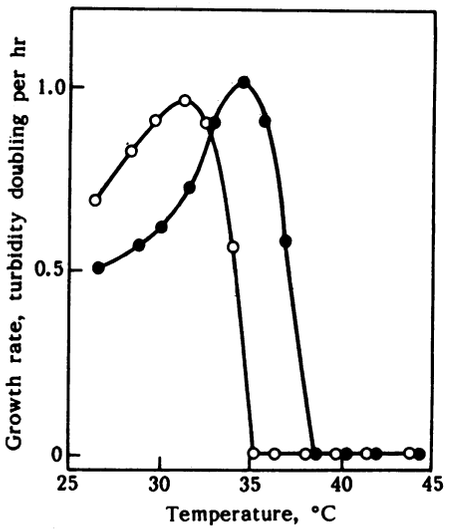

FIG. 2. Growth rates of strain SD103 (●) and its parent strain SD9 (○) at various temperatures. Growth curves in NBY medium were obtained in a temperature-gradient biophotorecorder, and growth rates were calculated from the exponential portions of the curves.

Growth rates of the *pgsA3* mutants were found to depend on the composition of the culture medium. The temperature sensitivity of strain SD103 was ameliorated by combined addition of 400 mM sucrose and 20 mM $MgCl_2$ to the NBY medium (Fig. 3*A*); these compounds also increased the growth rate of strain SD312 at 42°C (Fig. 3*B*). NBY medium supplemented with 50 mM sucrose and 20 mM $MgCl_2$ did not support the growth of strain SD103 at 40°C or 42°C but did allow growth of its parent strain SD9 (1). These conditions were employed to distinguish between the two strains as described above. As summarized in Table 4, strain SD312 did not grow in a low ionic strength medium, medium K, at 37°C but the addition of 0.1% glucose to this medium fully remedied the defect caused by mutation. Supplementation of medium K with NaCl, sucrose, and an amino acid mixture of appropriate concentrations allowed the growth of strain SD312, although the rates were lower than those of strain SD12 grown under the same conditions. Addition of 20 mM Mg^{2+} or Fe^{2+} did not allow the growth of strain SD312 in medium K.

The possible effect of the extremely low level of PtdGro on

Table 3. Phospholipid compositions of various strains harboring the *pgsA3* allele under different culture conditions

Strain (genotype)	Supplement	Label	Composition				
			PtdEtn	PtdGro	CL	PtdOH	Other
SD103 (*pss-1 pgsA3 cls*)	None	^{32}P	95.7	0.3	<0.1	1.8	2.2
	None	^{14}C	84.7	1.0	0.1	6.7	4.7
	Sucrose + Mg	^{32}P	84.7	1.8	0.7	4.2	8.6
SD312 (*pgsA3 cls*)	None	^{32}P	91.0	0.4	0.3	2.9	5.4
	None	^{14}C	95.8	0.2	0.1	1.1	2.8
SD312/pCLF1 (*pgsA3 cls*/*cls*$^+$)	Km	^{32}P	92.9	0.2	0.3	3.1	3.5
	Arbutin + Km	^{32}P	92.5	0.1	0.3	3.2	3.9

Cells were grown at 30°C in NBY medium with supplements as indicated, and lipids were analyzed as described in the footnotes to Table 2, except that some cultures were labeled with [1-^{14}C]acetate: ^{32}P was replaced with 1 μCi of [1-^{14}C]acetate per ml where indicated, and the values represent the percent ^{14}C radioactivity in each lipid species. Under these conditions, ^{14}C was incorporated exclusively into fatty acid residues (data not shown). Sucrose + Mg, 400 mM sucrose and 20 mM $MgCl_2$; arbutin, 32 mM arbutin; Km, 25 μg of kanamycin sulfate per ml.

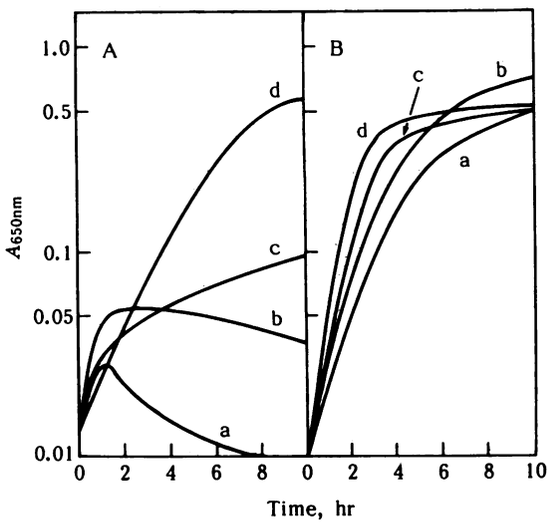

FIG. 3. Remedial effects of sucrose and $MgCl_2$ added in NBY medium on the growth of SD103 (*pss-1 cls pgsA3*) (*A*) and SD312 (*cls pgsA3*) (*B*). Samples (0.2 ml) of early stationary cultures (10^9 cells per ml) in NBY medium at 30°C were inoculated at time zero into 10 ml of prewarmed (42°C) NBY medium with various supplements as shown, and the A_{650} was recorded continuously in a Bioscanner maintained at 42°C. Curve a, NBY medium without supplement; curve b, NBY medium with 20 mM $MgCl_2$; curve c, NBY medium with 400 mM sucrose; curve d, NBY medium with 20 mM $MgCl_2$ and 400 mM sucrose.

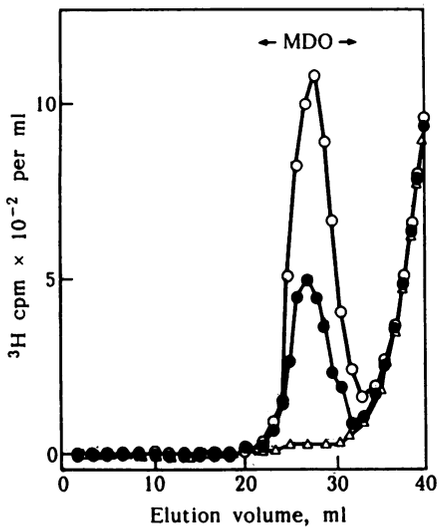

FIG. 4. Incorporation of [2-^{3}H]glycerol into MDO fractions of strains SD312 (*pgsA3 cls*) (●), SD12 (wild type) (○), and R6 (*galU*) (△). Cells were grown in medium I containing [2-^{3}H]glycerol (specific activity: 1 mCi/2.5 mmol) at 37°C to late exponential phase, and MDO was extracted and fractionated by gel filtration as described by Kennedy (15). The peak position corresponded to molecular weight 2500. Strain R6 (4) was used as a negative control that cannot form the oligosaccharide backbone of MDO; its *in vitro* specific activity of the *galU* gene product, UDP-glucose pyrophosphorylase, was 2.4% (0.79 nmol·min^{-1} per mg of protein) of the other two strains.

the other cellular component syntheses that supposedly utilize PtdGro as a substrate was examined in strain SD312. The most abundant product from PtdGro, CL, was reduced by a factor of about 20 in comparison with strain SD12 (Table 3). Fig. 4 demonstrates that the formation of another major product, MDO, that utilizes phosphoglycerol from PtdGro (18) was reduced by a factor of 2. Strains SD312 and SD12 had essentially the same growth rates in minimal medium either with fructose or maltose as carbon source (doubling time: 1.60 ± 0.17 hr), implying that the enzyme II in the phosphotransferase system, which supposedly requires PtdGro as an integral component and is responsible for the transport of fructose (19), was functional in strain SD312.

DISCUSSION

Previous efforts by Raetz and co-workers to isolate *E. coli pgsA* mutants defective in PtdGro synthesis (5, 7) had been unsuccessful. After examination of some 290,000 mutagenized cells by colony autoradiography, they obtained *pgsA* mutants with enzymatic defects in PtdGro-*P* synthase only *in vitro*, not *in vivo*. Likewise, our attempt to isolate *pgsA* mutants having PtdGro-*P* synthases with elevated K_m values for glycerophosphate, by selecting glycerol auxotrophs from a strain harboring mutations in glycerophosphate metabolism (*plsB*$^+$ BB26-36, ref. 20), only yielded *plsB* and *gpsA* mutants (unpublished data). The strategy in the present work to isolate *pgsA* mutants defective in *in vivo* PtdGro synthesis was based on an assumption that having PtdEtn as the sole membrane phospholipid is more advantageous than having a large amount of PtdGro (strain SD9) at higher temperatures since PtdGro has a phase-transition temperature some 30°C lower than those of PtdEtn and CL with the same fatty acid compositions (21). In fact, spontaneous *pgsA* mutants were obtained when strain SD9 (*pss-1 cls*) was incubated at its nonpermissive temperature. The difference in growth rate between the isolated *pgsA3* mutant (SD103) and its parent, SD9, (Fig. 2) implies a bypass nature of the mutation.

Table 4. Dependence of the growth rate of strain SD312 on supplements to medium K

Supplement	SD312	SD12
None (medium K)	0.00	0.48
Glucose, 0.1%	0.63	0.63
NaCl, 30 mM	0.33	0.59
NaCl, 100 mM	0.55	0.71
Sucrose, 400 mM	0.50	ND
Amino acids, 2.5 mg/ml*	0.14	0.56

Growth rates (turbidity doubling per hour) were calculated from the exponential regions of the growth curves obtained in a Bioscanner maintained at 37°C. ND, not determined.
*Amino acids: a mixture of 16 amino acids of the composition described (1), totaling 5.0 mg/ml.

The phospholipid polar headgroup compositions of *pgsA3* mutants were remarkable; up to 95% of the extractable lipids was PtdEtn, and the levels of acidic phospholipids were extremely low, even though the compositions varied to some extent depending upon the background genotypes and culture conditions (Table 3). Cells with these abnormal lipid compositions were still able to grow as actively as wild-type cells in media of appropriate compositions (Table 4, Fig. 3). Phospholipid polar headgroup compositions of biological membranes are widely variable from species to species, but, in a given type of membrane, the composition changes within a rather narrow range depending on the cell age and environmental conditions. Such a specific phospholipid composition has been considered necessary for optimal function of a membrane. Living organisms have been assumed to possess regulatory mechanisms that maintain such membrane compositions. Some lower eukaryotic cells have been shown to possess precise mechanisms to maintain certain critical parameters of lipid composition, such as the ratio of zwitterionic to acidic phospholipid species, even in mutant cells with altered phospholipid biosynthesis (22, 23). In the present *E. coli pgsA3* mutant, the membrane must differ from the wild type at least in two respects, electrostatic charge and phase-transition temperature, while supporting the essential membrane functions, as implied by the capability of cell

growth. The results of the present work together with our previous observations with other phospholipid manipulations (1, 4, 10) indicate that the phospholipid composition required for the survival of *E. coli* cells is surprisingly flexible. Nonetheless, it appears that the phospholipid composition of wild-type cells is the one best suited for this organism. Manipulations of the membrane composition did not result in better growth and certain alterations in phospholipid composition seem to be lethal in more severe environments.

Strains harboring the *pgsA3* allele produced very small amounts of PtdGro and CL but showed compensatory rises in the amounts of PtdOH and other minor phosphorus-containing lipids (Tables 2 and 3). Although the *in vitro* PtdGro-*P* synthase activity in the extract was below the detection limit (Table 1), the small amounts of PtdGro in *pgsA3* strains (Tables 2 and 3) were most probably formed by the low residual activity of the mutated enzyme, as were the cases in other phospholipid mutants (2). Suboptimal activities of PtdGro synthesis in *pgsA3* mutants resulted in decreased syntheses of some cellular components that supposedly utilize PtdGro as substrate. CL was less than that in wild-type cells by a factor of about 17 even in the presence of a plasmid containing the *cls* gene (bottom two lines of Table 3). This provides further *in vivo* evidence indicating that CL is synthesized from PtdGro; Pluschke *et al.* observed a decreased rate of PtdGro turnover in a *cls* mutant, implying that PtdGro is consumed during the production of CL in wild-type cells (6). The incorporation of labeled glycerol into MDO was decreased by a factor of 2 (Fig. 4). This observation supports the notion that PtdGro serves *in vivo* as the phosphoglycerol donor in MDO synthesis (18). However, we have been unable to observe deficiencies in less abundant PtdGro-requiring materials in *pgsA3* mutants such as the enzyme II in phosphotransferase system or outer membrane lipoprotein (data not shown). It is uncertain at present whether acidic phospholipids are altogether nonessential in *E. coli* cells or whether the small residual levels maintain membrane functions that are dependent on PtdGro and CL. To resolve this, an attempt to isolate deletion mutants missing the *pgsA* gene entirely should be useful. The roles of PtdGro and CL in the mutants could be compensated in part by the elevated level of PtdOH. The anionic requirement could be satisfied even by membrane phosphoproteins or other anionic peptides.

We now have deficient mutations as well as cloned genes (12, 13, 24) for all three enzymes that catalyze the committed steps in major phospholipid syntheses, and when introduced in *E. coli* cells, individually as well as in combination, they give rise to a variety of modified membrane lipid compositions. This empirical approach should facilitate studies of specific functional roles of individual phospholipids and of membrane biogenesis and functions.

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