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# Transfer of the Phosphatidyl Moiety of Phosphatidylglycerol to Phosphatidylethanolamine in *Escherichia coli*

KAZUSHIGE YOKOTA AND MAKOTO KITO*

*Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan*

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Phosphatidylglycerol was pulse-labeled with radioactive lipid precursors in a serine auxotroph of *Escherichia coli*. Most of the radioactivity of phosphatidylglycerol labeled in a serine-depleted medium was transferred to phosphatidylethanolamine during a chase in the presence of L-serine, but not in its absence. Metabolism of fatty acyl moieties labeled with [1-$^{14}$C]acetate, acylated glycerol moieties labeled with [2-$^{3}$H]glycerol, and phosphate moieties labeled with $^{32}P_i$, followed by a chase in the presence of cerulenin, showed that the intact phosphatidyl moiety of phosphatidylglycerol was transferred to phosphatidylethanolamine. The composition of phosphatidylethanolamine molecular species was unaltered and not perturbed by the transfer of the phosphatidyl moiety of phosphatidylglycerol. The increase of phosphatidylethanolamine with a concomitant decrease of phosphatidylglycerol was not coupled with the postulated turnover of phosphatidylglycerol to membrane-derived oligosaccharides and lipoprotein. It is suggested that phosphatidylglycerol is capable of providing its phosphatidyl moiety for the production of phosphatidylethanolamine in response to the relief of serine limitation by addition of L-serine.

*Escherichia coli* membranes contain phospholipids which are composed of phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin. Phosphatidylglycerol is an acidic phospholipid and comprises about 20 to 30% of the total phospholipids. Phosphatidylglycerol in *E. coli* membranes is known to be a cofactor of certain membrane-associated enzymes (20, 22). Rapid metabolism of phosphatidylglycerol has been shown by many workers (12, 13, 30, 40, 33). The turnover of phosphatidylglycerol occurs as follows: cardiolipin formation from 2 mol of phosphatidylglycerol (17), rapid exchange of nonacylated glycerol for free glycerol in cells (2, 29), and production of acyl phosphatidylglycerol (25). Furthermore, membrane-derived oligosaccharides in the periplasmic space containing *sn*-glycerol 1-phosphate residues are derived from the nonacylated glycerol of phosphatidylglycerol (37, 39). 1,2-Diglyceride is covalently attached to the sulfhydryl group of murein lipoprotein through a thioether linkage (6, 15). Wu and co-workers have shown that the glycerol moiety of this lipoprotein is donated by the nonacylated glycerol of phosphatidylglycerol, and acyl chains are derived by the deacylation of phospholipids (9, 10, 26, 27). Recently, a mutant defective in phosphatidylglycerol synthesis was used to show that the phosphatidylglycerol gene was linked to lipopolysaccharide metabolism (31). In this report we attempt to investigate the fate of phosphatidylglycerol, which is radioactively labeled in a serine auxotroph in the absence of L-serine. The transfer of the phosphatidyl moiety of phosphatidylglycerol to phosphatidylethanolamine in *E. coli* membranes is described.

## MATERIALS AND METHODS

**Chemicals and materials.** *Bacillus cereus* phospholipase C and cabbage phospholipase D were the products of Boehringer Mannheim GmbH, Mannheim, West Germany. Thin-layer plates precoated with Silica Gel G (no. 5721,5724,11845) were purchased from E. Merck AG, Darmstadt, West Germany. Funagel, FGI (150 Å [15.0 nm]) was obtained from Funakoshi, Tokyo. *E. coli* lipids were prepared as described previously (23). Standard phospholipids were purchased from Serdary Research Laboratories, London, Ontario, Canada. [1-$^{14}$C]acetate (56.0 mCi/mmol), [2-$^{3}$H]glycerol (10 Ci/mmol), and $^{32}P_i$ were purchased from New England Nuclear Corp., Boston, Mass. Cerulenin was purchased from Sigma Chemical Co., St. Louis, Mo. Chloramphenicol was obtained from Boehringer Mannheim.

**Culture, strain, and growth conditions.** A serine auxotroph, *E. coli* SerA$^-$ Kλt, of an *E. coli* K-12 derivative was a gift from L. I. Pizer (University of Colorado Health Science Center, Denver). Bacteria were grown in minimal M9 medium containing 0.2% glucose, 0.1% Casamino Acids, and 50 μg of L-serine per ml (24). Growth of cells was monitored with a Klett-Summerson photoelectric colorimeter (filter no. 66).

**Pulse-labeling and chase.** A serine-depleted medium, minimal M9 medium supplemented with 0.2% glucose,

was used for pulse-labeling with [1-$^{14}$C]acetate or [2-$^{3}$H]glycerol. A low-phosphate medium containing 1 mM phosphate and 0.2% glucose was used for the pulse-labeling with $^{32}P_i$ (8). Cells were grown to a density of approximately 50 Klett units at the early exponential growth phase and then harvested by centrifugation. Concentrated cells at 200 to 280 Klett units were used for pulse-labeling for 10 to 20 min in 50 ml of a serine-depleted medium. Pulse-labeling was terminated by addition of unlabeled acetate, glycerol, or $P_i$. Cells were washed with minimal M9 medium at 4°C and harvested by centrifugation. Portions of cells were subsequently chased at 37°C in serine-supplemented or depleted M9 medium, except for the $Ca^{2+}$ treatment. Treating cells with $Ca^{2+}$ was carried out in minimal Tris medium during the chase, by a modification of the procedure of Irvine et al. (18). The minimal Tris medium used for chase consisted of 0.05 M Tris-hydrochloride buffer, 0.08 M NaCl, 0.02 M KCl, 0.02 M $NH_4Cl$, and 0.003 M $Na_2SO_4$, pH 7.5. The chase was also carried out in a serine-depleted medium or in the serine-supplemented medium containing cerulenin. Effects of chemicals on lipid metabolism was investigated by additions during the chase in the serine-supplemented medium after pulse-labeling with [1-$^{14}$C]acetate. The final concentrations of chemicals in the cultures were as follows: hydroxylamine, 10 mM; $CaCl_2$, 0 to 50 mM; disodium EDTA·$2H_2O$, 0 to 43 mM; chloramphenicol, 100 μg/ml; sodium cyanide, 10 mM; sodium azide, 50 mM; 2,4-dinitrophenol, 2 mM.

**Phospholipid analysis.** During the chase, portions of cultures were taken at given intervals and transferred into 5% trichloroacetic acid with carrier cells (5 to 10 mg). Lipids were extracted from the precipitates with chloroform-methanol (2:1, vol/vol) at 35°C (4). Samples of 50 μg of phospholipids of *E. coli* K-12, phosphatidylserine, and phosphatidic acid were added to the lipid extracts, which were washed with 0.2 M KCl. Phospholipids were separated by two-dimensional thin-layer chromatography, with chloroform–methanol–28% ammonia in water (200:120:15, vol/vol) as the first solvent system and chloroform-methanol-acetic acid (65:25:8, vol/vol) as the second solvent system. Preparation of phosphatidylethanolamine and phosphatidylglycerol was carried out with a one-dimensional solvent system of chloroform-methanol-acetic acid (65:25:8, vol/vol) on Funagel plates. For the measurement of radioactivity in the acylated glycerol moiety of phosphatidylglycerol, it was treated with phospholipase C (11, 23, 41), and radioactivity in the resultant 1,2-diglyceride was determined. Molecular

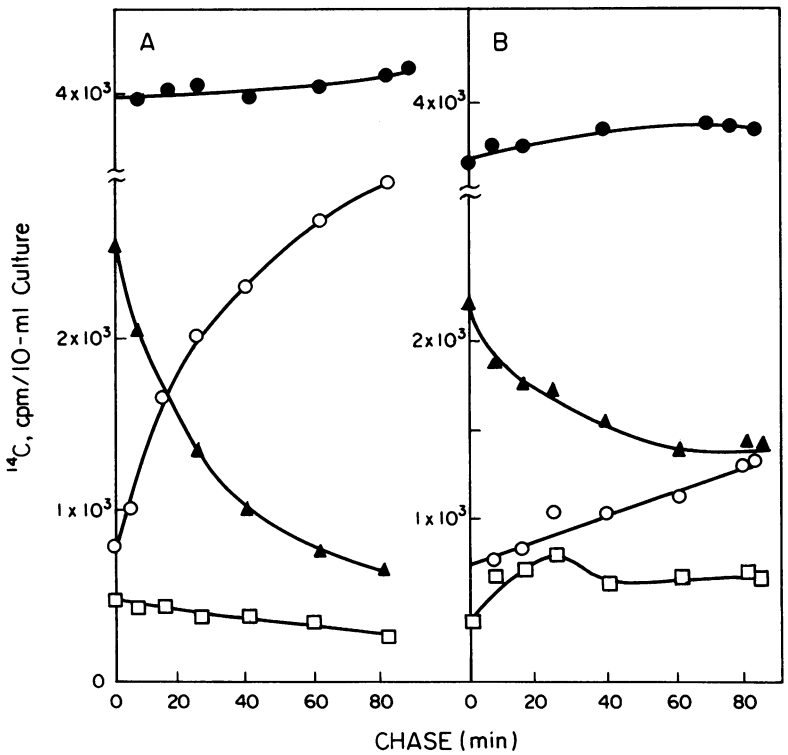


FIG. 1. Pulse-labeling and chase with [1-$^{14}$C]acetate in *E. coli* SerA$^-$ Kλt. Cells harvested at 50 Klett units were pulse-labeled with 20 μCi of [1-$^{14}$C]acetate (0.36 μmol) for 10 min at 37°C in a serine-depleted medium. Unlabeled acetate was added to dilute the specific activity 1,000-fold. One portion was chased in 100 ml of a serine-supplemented medium (A), and the other was chased in 100 ml of a serine-depleted medium (B). At various intervals, 10 ml of culture was removed, and the reaction was terminated at a final concentration of 5% trichloroacetic acid. Radioactivity of $^{14}$C-phospholipids was determined as described in the text. Symbols: ●, total phospholipids; ▲, phosphatidylglycerol; ○, phosphatidylethanolamine; □, cardiolipin.

species of phospholipids were determined by thin-layer chromatography with Silica Gel G impregnated with silver nitrate as described previously (23).

**Measurements of radioactivity.** Phospholipids separated on thin-layer plates were scraped from plates into scintillation vials. Measurements were carried out in a toluene-based scintillation fluid (23). Values were expressed as counts per minute. Phospholipids doubly labeled with [1-$^{14}$C]acetate and [2-$^{3}$H]glycerol were extracted from thin-layer plates, and disintegrations per minute were determined. The ratio of $^{3}$H to $^{14}$C was calculated by standardization. Radioactivity in water-soluble fractions was analyzed by using Aquasol-2 (New England Nuclear Corp.). Lipid-depleted pellets, which were applied for the determination of membrane-derived oligosaccharides and murein lipoprotein, were solubilized with NCS (Amersham International Limited, Amersham, England), and the radioactivity was determined in a toluene-based scintillation fluid (37, 39). Measurements were performed on a Packard Tri-Carb liquid scintillation spectrometer.

## RESULTS

**Pulse-labeling and chase with [1-$^{14}$C]acetate.** Pulse-labeling with [1-$^{14}$C]acetate of a serine auxotroph, *E. coli* SerA$^{-}$ Kλt, in a serine-depleted medium showed that radioactivity was preferentially incorporated into phosphatidylglycerol (Fig. 1). The transfer of the radioactivity of phosphatidylglycerol to phosphatidylethanolamine occurred during the chase in a serine-supplemented medium (Fig. 1A). The decrease in radioactivity in phosphatidylglycerol was concomitant with an increase in radioactivity of phosphatidylethanolamine, and the total radioactivity in the phospholipid fraction was not changed. The conversion of phosphatidylglycerol to phosphatidylethanolamine was depressed during the chase in a serine-depleted medium (Fig. 1B), and progressed in the presence of cerulenin in a serine-supplemented medium (data not shown).

**Pulse-labeling and chase with [2-$^{3}$H]glycerol.** Pulse-labeling with [2-$^{3}$H]glycerol in a serine-depleted medium resulted in preferential incorporation into phosphatidylglycerol which accounted for more than 90% of radioactivity in the lipid fraction. After dilution by addition of unlabeled glycerol, radioactivity was chased in the presence (Fig. 2A) or absence (Fig. 2B) of L-serine. In both cases, total radioactivity in phosphatidylglycerol was significantly reduced by the loss of the nonacylated glycerol moiety due to the exchange for free glycerol in cells (2, 29).

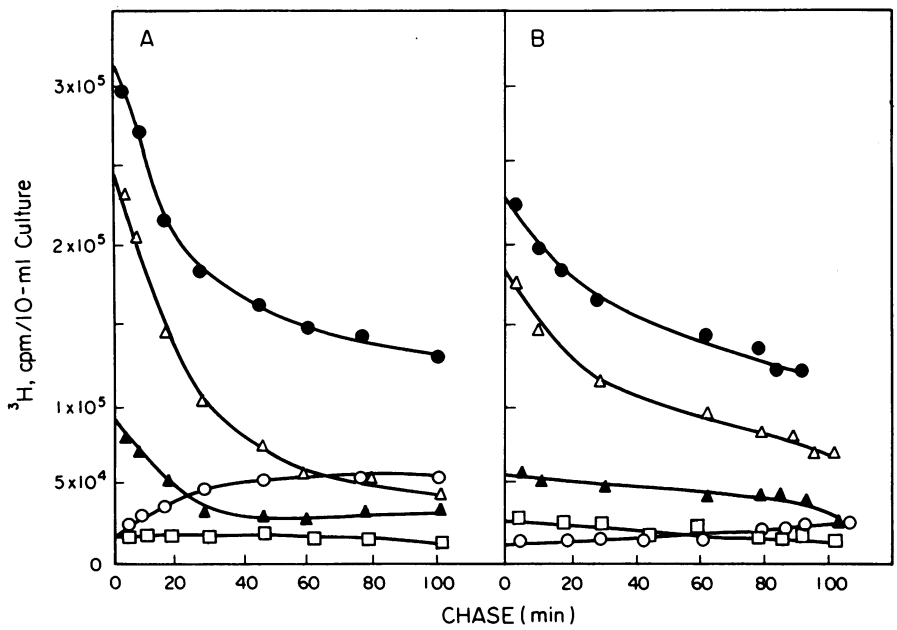


FIG. 2. Pulse-labeling and chase with [2-$^{3}$H]glycerol in *E. coli* SerA$^{-}$ Kλt. Cells harvested at 50 Klett units were pulse-labeled with 100 μCi of [2-$^{3}$H]glycerol (10 nmol) for 20 min at 37°C in a serine-depleted medium. Unlabeled glycerol was added to dilute the specific activity 1,000-fold. One portion was chased in 100 ml of a serine-supplemented medium (A), and the other portion was chased in 100 ml of a serine-depleted medium (B). At various intervals, 10 ml of culture was removed and the reaction was terminated at a final concentration of 5% trichloroacetic acid. Radioactivity of $^{3}$H-phospholipids was determined as described in the text. Symbols: ●, total phospholipids; △, intact phosphatidylglycerol; ▲, 1,2-diglyceride moiety of phosphatidylglycerol; ○, phosphatidylethanolamine; □, cardiolipin.

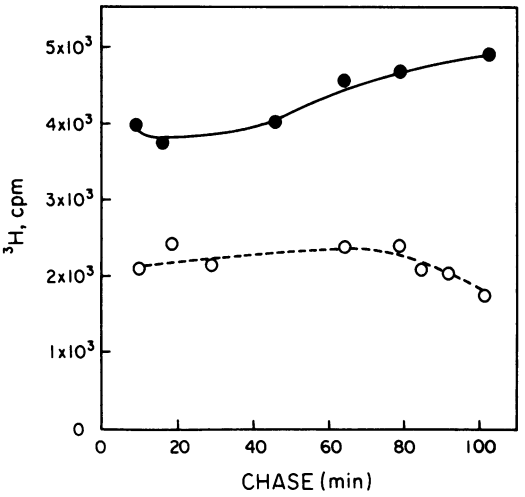


FIG. 3. Incorporation of [2-$^3$H]glycerol into lipid-depleted pellets during the chase. After phospholipid fractions were extracted from cells treated as described in the legend to Fig. 2, the radioactivity was determined for the lipid-depleted pellets as described in the text. Symbols: ●, serine supplemented; ○, serine depleted.

The increase in radioactivity of phosphatidylethanolamine was obtained only in a serine-supplemented medium. Phospholipase C was used to examine the turnover of the acylated glycerol moiety of phosphatidylglycerol. In the presence of L-serine, 60% of the radioactivity in the 1,2-diglyceride moiety of phosphatidylglycerol was metabolized within 100 min. About two-thirds of the acylated glycerol moiety was recovered in phosphatidylethanolamine, and the remaining radioactivity was lost from the lipid fraction. In the absence of L-serine, a small amount of the acylated glycerol moiety was lost. However, the fate of this radioactivity was not further examined.

The turnover of phosphatidylglycerol has been reported to correlate with the metabolism of membrane-derived oligosaccharides (37, 39). To determine whether the radioactivity in phosphatidylglycerol was transferred to membrane-derived oligosaccharides, we examined the radioactivity of the lipid-depleted pellets remaining after phospholipids were extracted from cells. The phosphatidyl moiety of phosphatidylglycerol rapidly decreased within the initial 30 min in the presence of L-serine (Fig. 2), whereas an increase in radioactivity of pellets including membrane-derived oligosaccharides was not seen during the initial 20 min (Fig. 3). It seems unlikely that the turnover of phosphatidylglycerol is closely related to the metabolism of membrane-derived oligosaccharides in this mutant cell.

**Pulse-labeling and chase of acylated glycerol moiety doubly labeled with [2-$^3$H]glycerol and [1-$^{14}$C]acetate.** Phosphatidylglycerol was pulse-labeled by the simultaneous addition of 100 μCi of [2-$^3$H]glycerol and 10 μCi of [1-$^{14}$C]acetate to a culture. The ratio of $^3$H to $^{14}$C disintegrations per minute in phosphatidylethanolamine and the 1,2-diglyceride moiety of phosphatidylglycerol was measured during the chase. At the initial chasing time, the ratio of $^3$H to $^{14}$C in phosphatidylethanolamine and the 1,2-diglyceride moiety of phosphatidylglycerol was about 23 and 11, respectively. Since phosphatidylglycerol and phosphatidylethanolamine are synthesized from CDP-diglyceride via a common intermediate, phosphatidic acid, in *E. coli*, the ratio of $^3$H to $^{14}$C in the 1,2-diglyceride moiety of phosphatidylglycerol should be similar to that in phosphatidylethanolamine (12, 23, 33). It is difficult to explain how the difference was caused.

As the chase proceeded in the presence of L-serine, the ratio of $^3$H to $^{14}$C declined in

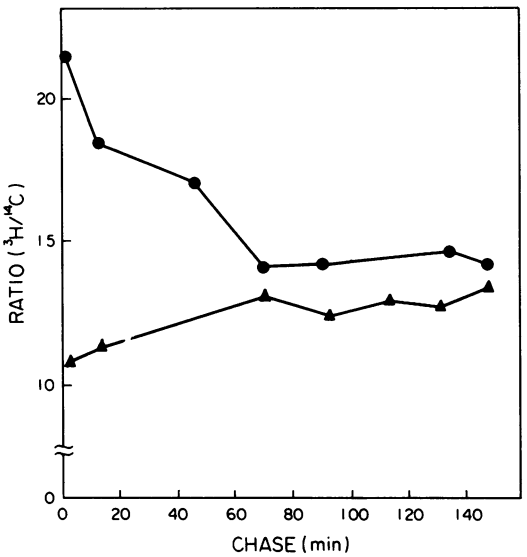


FIG. 4. Pulse-labeling and chase by double labeling method with [1-$^{14}$C]acetate and [2-$^3$H]glycerol in *E. coli* SerA$^-$ Kλt. Cells harvested at 50 Klett units were pulse-labeled with 10 μCi of [1-$^{14}$C]acetate and 100 μCi of [2-$^3$H]glycerol for 10 min at 37°C in a serine-depleted medium. Unlabeled acetate and glycerol were added to dilute the specific activity 1,000-fold. The chase was initiated in 100 ml of a serine-supplemented medium. At various intervals, 10 ml of culture was removed, and the reaction was terminated at a final concentration of 5% trichloroacetic acid. Radioactivity of phospholipids was determined as described in the text. Values are shown as the ratio of $^3$H to $^{14}$C in disintegrations per minute. Symbols: ●, phosphatidylethanolamine; ▲, 1,2-diglyceride moiety of phosphatidylglycerol.

phosphatidylethanolamine from 21 to 14. The decrease in the ratio of $^{3}H$ to $^{14}C$ in phosphatidylethanolamine presumably results from the dilution of a slight amount of preexisting phosphatidylethanolamine by the phosphatidylethanolamine newly synthesized from phosphatidylglycerol (Fig. 4). The result suggests that the conversion of phosphatidylglycerol to phosphatidylethanolamine proceeded by direct utilization of the 1,2-diglyceride moiety of phosphatidylglycerol.

**Transfer of the [$^{32}P$]phosphatidyl moiety of phosphatidylglycerol to phosphatidylethanolamine.** The transfer of the phosphatidyl moiety of phosphatidylglycerol to phosphatidylethanolamine was also examined by $^{32}P_i$ pulse-labeling. After pulse-labeling with $^{32}P_i$ in a serine-depleted and low-phosphate medium, unlabeled inorganic phosphate was added. The chase was initiated in the absence of L-serine for an initial period of 15 min, after which L-serine was added. The increase of radioactivity in phosphatidylethanolamine was greater than the decrease of radioactivity in phosphatidylglycerol in a serine-supplemented medium (Fig. 5A). This may be due to the de novo synthesis of phosphatidylethanolamine from $^{32}P$-labeled nucleotides. When the chase was carried out in the presence of cerulenin and L-serine, no increase in radioactivity in the total phospholipid fractions was seen (Fig. 5B). However, the radioactivity in phosphatidylglycerol decreased, and that in phosphatidylethanolamine increased. The result indicates a supply of the phosphatidyl moiety from phosphatidylglycerol to phosphatidylethanolamine.

**Analysis of molecular species of phosphatidylglycerol and phosphatidylethanolamine during the chase.** As shown in Fig. 2, the acylated glycerol labeled with [2-$^{3}H$]glycerol from phosphatidylglycerol was recovered in phosphatidylethanolamine. A portion of each sample used in this experiment was used for molecular species analysis. The molecular species of phosphatidylglycerol that showed the major decrease in radioactivity was the 1-palmitoyl-2-palmitoleoyl species (Fig. 6A). Phosphatidylethanolamine increased during the initial 40 min (Fig. 6B). When the conversion of phosphatidylglycerol to phosphatidylethanolamine

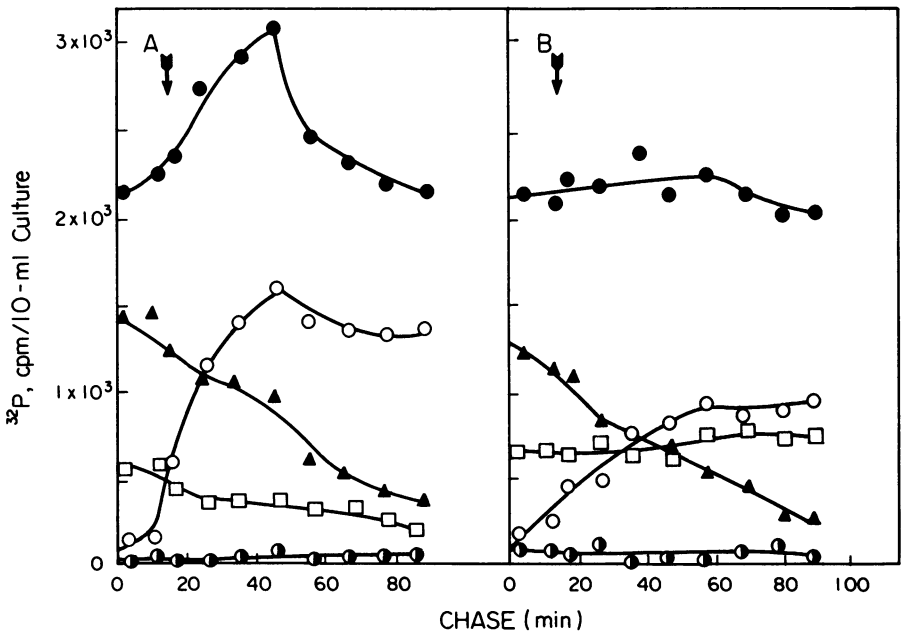


FIG. 5. Pulse-labeling and chase with $^{32}P_i$ in *E. coli* SerA$^-$ Kλt. Cells harvested at 50 Klett units were pulse-labeled with 200 μCi of carrier-free $^{32}P_i$ for 10 min at 37°C in a low-phosphate medium at 1 mM phosphate. Unlabeled $P_i$ (50 mM) was added to dilute the specific activity 50-fold. The chase was initiated in the absence of L-serine for 15 min, followed by the addition of L-serine at the time indicated by the arrows. One portion was chased in 100 ml of a serine-supplemented medium (A), and the other portion was chased in 100 ml of a serine-supplemented medium containing 200 μg of cerulenin per ml (B). At various intervals, 10 ml of culture was removed, and the reaction was terminated at a final concentration of 5% trichloroacetic acid. Radioactivity of $^{32}P$-phospholipids was determined as described in the text. Symbols: ●, total phospholipids; ▲, phosphatidylglycerol; ○, phosphatidylethanolamine; □, cardiolipin; ◑, phosphatidic acid.

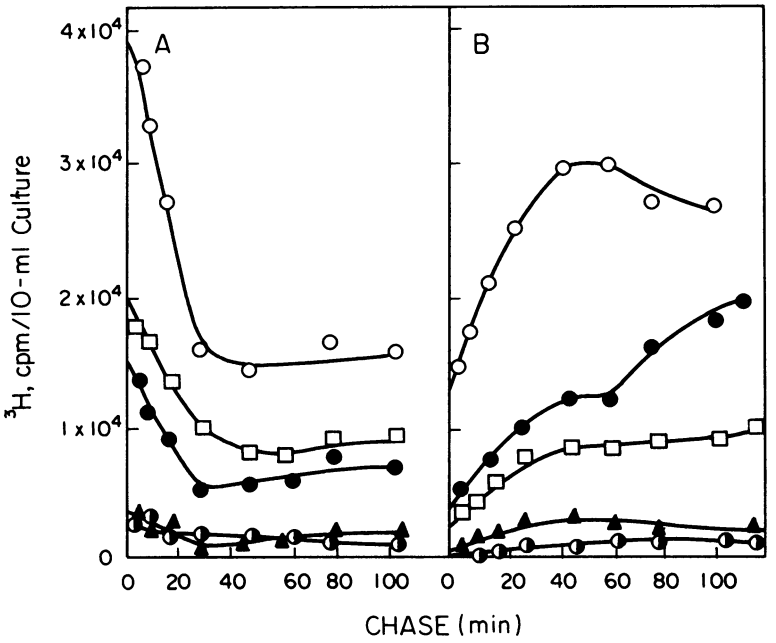


FIG. 6. Turnover of the molecular species in phosphatidylglycerol and phosphatidylethanolamine. Radioactivity of $^3$H-phospholipids was analyzed as described in the text. (A) Phosphatidylglycerol; (B) phosphatidylethanolamine. Symbols: ○, 1-palmitoyl-2-palmitoleoyl; ●, 1-palmitoyl-2-cyclopropanized; □, 1-palmitoyl-2-*cis*-vaccenoyl; ▲, 1-*cis*-vaccenoyl-2-palmitoleoyl; ◑, 1-*cis*-vaccenoyl-2-*cis*-vaccenoyl.

reached a plateau at 60 min, the change of the 1-palmitoyl-2-palmitoleoyl species to the 1-palmitoyl-2-cyclopropanized species was prominent. The results indicate that the 1-palmitoyl-2-*cis*-9,10-methylene hexadecanoyl species was the major component of the 1-palmitoyl-2-cylopropanized species.

Molecular species compositions of phosphatidylethanolamine and phosphatidylglycerol (Fig. 7) were similar to those in *E. coli* K-12 (19). From the results in Fig. 6B, the 1-palmitoyl-2-*cis*-vaccenoyl species was not modified to the corresponding species containing cyclopropane fatty acids. Hence, it was possible to compare the composition of the molecular species of phosphatidylethanolamine with that of phosphatidylglycerol. The proportion of the 1-palmitoyl-2-*cis*-vaccenoyl species in phosphatidylglycerol was about 25% (Fig. 7A), whereas that in phosphatidylethanolamine was about 15% (Fig. 7B). With regard to the proportion of the 1-palmitoyl-palmitoleoyl plus the 1-palmitoyl-2-cyclopropanized species, which was derived from the former compound during the chase, average values of about 65 and 78% were obtained in phosphatidylglycerol (Fig. 7A) and phosphatidylethanolamine (Fig. 7B), respectively.

**Effects of various substances on the conversion of phosphatidylglycerol to phosphatidylethanolamine.** It has been reported that hydroxylamine inhibits the reaction of phosphatidylserine decarboxylase in vivo (14, 36). To determine the pathway from phosphatidylglycerol to phosphatidylethanolamine in this mutant strain, hydroxylamine was added during the chase to a final concentration of 10 mM. A proportion of 42.3% of the total radioactivity in phospholipids was found in phosphatidylserine. However, the radioactivity did not increase in phosphatidylethanolamine (Fig. 8). The effects of $Ca^{2+}$ on the *E. coli* membrane have been well studied. $Ca^{2+}$-treated cells have the ability to take up DNA (3, 35). Nonspecific increased permeability of the outer membrane of *E. coli* (5) and fusion of lipid vesicle to the membrane of *Salmonella typhimurium* (21) have been caused by $Ca^{2+}$ treatment. Transfer of the radioactivity from phosphatidylglycerol to phosphatidylethanolamine was inhibited by 50 mM $CaCl_2$ (Fig. 9).

Treatment of cells with 0.5 mM EDTA is known to increase sensitivity toward antibiotics and lysozyme (16, 28). No effect was shown on the transfer of the phosphatidyl moiety of phosphatidylglycerol to phosphatidylethanolamine at 0.43 and 43 mM (data not shown). If phospholipase were involved in the above-mentioned conversion, EDTA could be expected to affect the turnover of phosphatidylglycerol (7, 12, 33). Hence, participation of phopholipase in this reaction was unlikely.

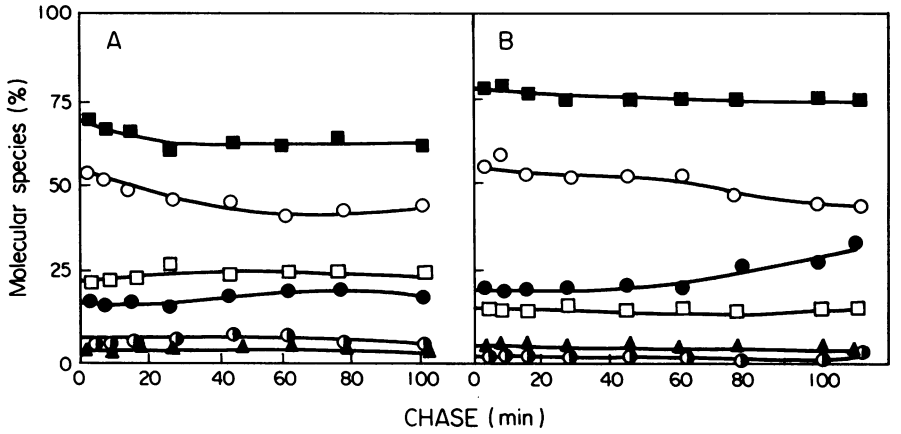


FIG. 7. Compositions of the molecular species in phosphatidylglycerol and phosphatidylethanolamine during the chase. Determination was carried out as described in the legend to Fig. 6 and in the text. (A) Phosphatidylglycerol; (B) phosphatidylethanolamine. Symbols: ■, 1-palmitoyl-2-palmitoleoyl plus 1-palmitoyl-2-cyclopropanized. Other symbols are the same as in Fig. 6.

Other chemicals were also examined in vivo. In the presence of L-serine, sodium azide, sodium cyanide, and 2,4-dinitrophenol inhibited both the turnover phosphatidylglycerol and the increase in phosphatidylethanolamine (data not shown). Chloramphenicol and cerulenin, however, showed no effect on this reaction (data not shown).

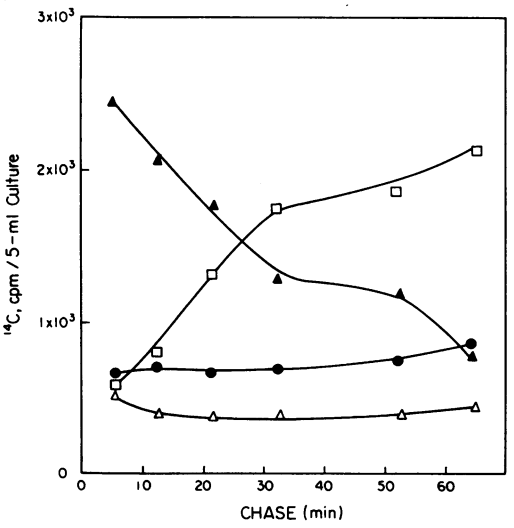


FIG. 8. Effect of hydroxylamine on the turnover of phosphatidylglycerol and the production of phosphatidylethanolamine. Cells harvested at 50 Klett units were pulse-labeled with 30 μCi of [1-$^{14}$C]acetate for 10 min at 37°C in a serine-depleted medium. Unlabeled acetate was added to dilute the specific activity 1,000-fold. One portion was removed and chased in 30 ml of a serine-supplemented medium in which 10 mM hydroxylamine was included at the starting point. At various intervals, 5 ml of culture was removed, and the reaction was terminated at a final concentration of 5% trichloroacetic acid. Radioactivity of phospholipids was determined as described in the text. Symbols: ▲, phosphatidylglycerol; □, phosphatidylserine; ●, phosphatidylethanolamine; △, cardiolipin.

## DISCUSSION

When phosphatidylglycerol was synthesized in a serine-deficient *E. coli* strain, SerA$^-$ Kλt, in the presence of [1-$^{14}$C]acetate and [2-$^3$H]glycerol, the acyl chains and glycerols of phosphatidylglycerol became labeled. When transferred to media containing L-serine, rapid turnover of phosphatidylglycerol occurred, and the diglyceride moiety was recovered in phosphatidylethanolamine (Fig. 1A and 2A). The ratio of $^3$H to $^{14}$C in phosphatidylethanolamine became approximately equal to that in phosphatidylglycerol during the chase (Fig. 4). Whereas total radioactivity in the $^{32}$P-phospholipid fractions remained essentially constant during the chase, the [$^{32}$P]phosphatidyl moiety of phosphatidylglycerol was recovered in that of phosphatidylethanolamine in the presence of cerulenin (Fig. 5B). Thus, the pathway from phosphatidylglycerol to phosphatidylethanolamine probably proceeds via phosphatidic acid in which two acyl moieties and the acylated glycerol were bound together. Hydroxylamine has been shown to inhibit phosphatidylserine decarboxylase in vivo (14, 36). More than 40% of the phospholipid radioactivity was in phosphatidylserine upon addition of L-serine. This indicates that the reactions from phosphatidylglycerol to phosphatidylethanolamine proceeded via phosphatidylserine as an intermediate. Thus, it is likely that the reactions from phosphatidylglycerol to phosphatidylethanolamine progressed sequentially as follows: phosphatidylglycerol → phosphatidic acid → CDP-diglyceride →

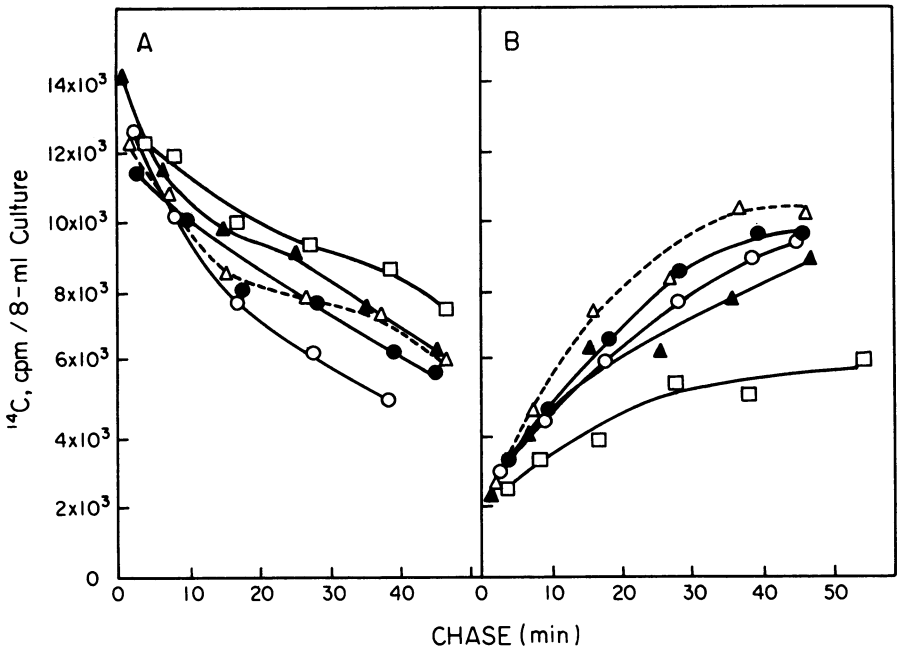


FIG. 9. Effect of $Ca^{2+}$ on the turnover of phosphatidylglycerol and the recovery of radioactivity in phosphatidylethanolamine. Cells harvested at 50 Klett units were pulse-labeled with 20 μCi of [1-$^{14}$C]acetate for 10 min at 37°C in a serine-depleted medium. Unlabeled acetate was added to dilute the specific activity 1,000-fold. The pulsed culture was divided into several portions. In 50 ml of a serine-supplemented medium, $CaCl_2$ was added to the culture at the starting point. At various intervals, 8 ml of culture was removed, and the reaction was terminated at a final concentration of 5% trichloroacetic acid. Radioactivity was determined as described in the text. (A) Phosphatidylglycerol; (B) phosphatidylethanolamine. Symbols: (○) 0 mM, (△), 0.2 mM, (●) 2 mM, (▲) 10 mM, and (□) 50 mM $Ca^{2+}$.

phosphatidylserine → phosphatidylethanolamine (12, 33).

The increase in phosphatidylethanolamine did not proceed independently of the turnover of phosphatidylglycerol. This was supported by the following evidence. The turnover of phosphatidylglycerol and production of phosphatidylethanolamine occurred inefficiently during the chase in the absence of L-serine (Fig. 1B and 2B). Treatment with 50 mM $Ca^{2+}$ or energy inhibitors, which inhibited the production of phosphatidylethanolamine in the presence of L-serine, depressed the turnover of phosphatidylglycerol. Under these conditions, neither phosphatidic acid nor diglyceride was detected during the chase, regardless of the presence or absence of L-serine (data not shown).

The reactions from phosphatidylglycerol to phosphatidylethanolamine are indicative of two pools of phosphatidylglycerol with regard to its turnover: (i) phosphatidylglycerol that can be recovered in phosphatidylethanolamine, and (ii) phosphatidylglycerol that is not recovered in phosphatidylethanolamine, but is lost from the lipid fraction (Fig. 2).

The turnover of phosphatidylglycerol with respect to the phosphatidyl moiety and nonacylated glycerol moiety has been examined elsewhere (2, 6, 9, 10, 12, 17, 25–27, 29, 31, 33, 37, 39). The reaction reported here may be related to the previous reports on the turnover of phosphatidylglycerol, as follows.

(i) The nonacylated glycerol of phosphatidylglycerol is exchanged with free glycerol in the cells (2, 10, 29). It was shown in Fig. 2 that the radioactivity of nonacylated glycerol of phosphatidylglycerol was lost regardless of the presence of L-serine. The exchange of the polar head of phosphatidylglycerol with L-serine could not be involved for the production of phosphatidylethanolamine, since the composition of molecular species of the newly formed phosphatidylethanolamine was distinguished from that of phosphatidylglycerol (Fig. 7). The proportion of molecular species of phosphatidylethanolamine was maintained at a constant level and was not perturbed by the transfer of certain molecular species from phosphatidylglycerol to phosphatidylethanolamine during the chase. The characteristic compositions of molecular species of phosphatidylglycerol and phosphatidylethanolamine suggested that the nonspecific exchange

of the polar head group between both phospholipids did not take place. However, we cannot rule out the possibility of an enzymatic reaction which is selective for molecular species.

(ii) The reaction for the production of membrane-derived oligosaccharides involves the transfer of the nonacylated *sn*-glycerol 1-phosphate of phosphatidylglycerol. The nonpolar byproduct, 1,2-diglyceride, is recovered in phosphatidic acid (34, 37, 39). The radioactivity of lipid-depleted pellets was analyzed after the removal of phospholipid fractions. The nonacylated glycerol of phosphatidylglycerol labeled with [2-$^3$H]glycerol was not efficiently incorporated into the lipid-depleted pellets in connection with the turnover of phosphatidylglycerol (Fig. 3). This incorporation was not adequate to correlate the production of phosphatidylethanolamine with the metabolism of membrane-derived oligosaccharides.

(iii) It has been reported that phosphatidic acid is produced from phosphatidylglycerol after the transfer of nonacylated glycerol to the N-terminal residue in murein lipoprotein (10, 26, 27). Lipoprotein also does not contribute greatly to the decrease in phosphatidylglycerol radioactivity. As mentioned above, [2-$^3$H]glycerol was not efficiently incorporated into the lipid-depleted pellets under the conditions of our experiment.

(iv) It is possible that the action of hypothetical phospholipases is responsible for the production of 1,2-diglyceride or phosphatidic acid (1, 12, 32, 33, 38). However, the results indicate that most of the phosphatidylglycerol labeled during serine limitation was metabolized and recovered in phosphatidylethanolamine in the presence of L-serine. In the absence of L-serine, no increase in 1,2-diglyceride or phosphatidic acid was detected. Generally, $Ca^{2+}$ is required for the activity of phospholipases (7). The production of phosphatidylethanolamine during the chase was not activated even if $Ca^{2+}$ was added to the medium and was not depressed by treating cells with EDTA. Treatment with 50 mM $Ca^{2+}$ was inhibitory to the conversion. The evidence for the existence of phospholipase C or phospholipase D in *E. coli* membranes is circumstantial and has not been confirmed (12, 33).

The recovery of the nonpolar moiety with the concomitant turnover of phosphatidylglycerol is specific for phosphatidylethanolamine. The evidence that the composition of molecular species of phosphatidylethanolamine was different from that of phosphatidylglycerol, and was kept in constant proportion, may be attributed to the cellular response for the production of phosphatidylethanolamine. Phosphatidylglycerol was selectively synthesized in a serine-depleted medium, whereas phosphatidylethanolamine was not synthesized. Therefore, the ratio of phosphatidylglycerol to phosphatidylethanolamine in *E. coli* membrane is different from that under normal conditions. It appears that the rapid conversion of phosphatidylglycerol to phosphatidylethanolamine is initiated to normalize the phospholipid composition. It would be interesting to know whether the production of phosphatidylethanolamine with the concomitant turnover of phosphatidylglycerol can take place normally in a wild-type *E. coli* strain.

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## LITERATURE CITED

1. **Albright, F. R., D. A. White, and W. J. Lennarz.** 1973. Studies on enzymes involved in the catabolism of phospholipids in *Escherichia coli*. J. Biol. Chem. **248**:3968–3977.
2. **Ballesta, J. P. G., C. L. de Garcia, and M. Schaechter.** 1973. Turnover of phosphatidylglycerol in *Escherichia coli*. J. Bacteriol. **116**:210–214.
3. **Bergmans, H. E. N., I. M. van Die, and W. P. M. Hoekstra.** 1981. Transformation in *Escherichia coli*: stage in the process. J. Bacteriol. **146**:564–570.
4. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
5. **Brass, J. M., W. Boos, and R. Hengge.** 1981. Reconstitution of maltose transport in *malB* mutant of *Escherichia coli* through calcium-induced disruptions of the outer membrane. J. Bacteriol. **146**:10–17.
6. **Braun, V.** 1975. Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **415**:335–377.
7. **Brockerhoff, H., and R. G. Jensen.** 1974. Lipolytic enzymes, p. 266–288. Academic Press, Inc., New York.
8. **Carty, C. E., and L. O. Ingram.** 1981. Lipid synthesis during the *Escherichia coli* cell cycle. J. Bacteriol. **145**:472–478.
9. **Chattopadhyay, P. K., J. S. Lai, and H. C. Wu.** 1979. Incorporation of phosphatidylglycerol into murein lipoprotein in intact cells of *Salmonella typhimurium* by phospholipid vesicle fusion. J. Bacteriol. **137**:309–312.
10. **Chattopadhyay, P. K., and H. C. Wu.** 1977. Biosynthesis of the covalently linked diglyceride in murein lipoprotein of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5318–5322.
11. **Christie, W. W.** 1973. Lipid analysis, p. 277–279. Pergamon Press Inc., Elmsford, N.Y.
12. **Cronan, J. E., Jr.** 1978. Molecular biology of bacterial membrane lipids. Annu. Rev. Biochem. **47**:163–189.
13. **Cronan, J. E., Jr., and P. R. Vagelos.** 1972. Metabolism and function of the membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta **265**:25–60.
14. **Dowhan, W., W. T. Wickner, and E. P. Kennedy.** 1974. Purification and properties of phosphatidylserine decarboxylase from *Escherichia coli*. J. Biol. Chem. **249**:3079–3084.
15. **Hantke, K., and V. Braun.** 1973. Covalent binding of lipid to protein: diglyceride and amide-linked fatty acid at the N-terminal end of the murein-lipoprotein of the *Escherichia coli* outer membrane. Eur. J. Biochem. **34**:284–296.
16. **Hardway, K. L., and C. S. Buller.** 1979. Effect of ethylenediaminetetraacetate on phospholipids and outer membrane function in *Escherichia coli*. J. Bacteriol.

137:62–68.
17. **Hirschberg, C. B., and E. P. Kennedy.** 1972. Metabolism of the enzymatic synthesis of cardiolipin in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **69**:648–651.
18. **Irvine, R. T., T. J. Macalister, R. Chan, and J. W. Costerton.** 1981. Citrate-tris(hydroxymethyl)aminomethane-mediated release of outer membrane sections from the cell envelope of a deep-rough (heptose-deficient lipopolysaccharide) strain of *Escherichia coli* O8. J. Bacteriol. **145**:1386–1396.
19. **Ishinaga, M., R. Kanamoto, and M. Kito.** 1979. Distribution of phospholipid molecular species in outer and cytoplasmic membranes of *Escherichia coli*. J. Biochem. (Tokyo) **86**:161–165.
20. **Ishinaga, M., M. Nishihara, M. Kato, and M. Kito.** 1976. Function of phosphatidylglycerol molecular species in membranes: activation of membrane-bound *sn*-glycerol 3-phosphate acyltransferase in *Escherichia coli*. Biochim. Biophys. Acta **431**:426–432.
21. **Jones, N. C., and M. J. Osborn.** 1979. Interaction of *Salmonella typhimurium* with phospholipid vesicles: incorporation of exogenous lipids into intact cells. J. Biol. Chem. **252**:7398–7404.
22. **Kito, M., M. Ishinaga, and M. Nishihara.** 1978. Function of phospholipids on the regulatory properties of solubilized and membrane-bound *sn*-glycerol-3-phosphate acyltransferase of *Escherichia coli*. Biochim. Biophys. Acta **529**:237–249.
23. **Kito, M., M. Ishinaga, M. Nishihara, M. Kato, S. Sawada, and T. Hata.** 1975. Metabolism of the phosphatidylglycerol molecular species in *Escherichia coli*. Eur. J. Biochem. **54**:55–63.
24. **Kito, M., and L. I. Pizer.** 1969. Phosphatidic acid synthesis in *Escherichia coli*. J. Bacteriol. **97**:1321–1327.
25. **Kobayashi, T., M. Nishijima, Y. Tamori, S. Nojima, Y. Seyama, and T. Yamakawa.** 1980. Acyl phosphatidylglycerol of *Escherichia coli*. Biochim. Biophys. Acta **620**:356–368.
26. **Lai, J. S., W. M. Philbrick, and H. C. Wu.** 1980. Acyl moieties in phospholipids are the precursors for the fatty acids in murein lipoprotein of *Escherichia coli*. J. Biol. Chem. **255**:5384–5387.
27. **Lai, J. S., and H. C. Wu.** 1980. Incorporation of acyl moieties of phospholipids into murein lipoprotein in intact cells of *Escherichia coli* by phospholipid vesicle fusion. J. Bacteriol. **144**:451–453.
28. **Leive, L.** 1965. A nonspecific increase in permeability in *Escherichia coli* produced by EDTA. Proc. Natl. Acad. Sci. U.S.A. **53**:745–750.
29. **Lugon, C., and J. P. G. Ballesta.** 1976. Metabolism of phosphatidylglycerol in cell-free extracts of *Escherichia coli*. Eur. J. Biochem. **65**:207–212.
30. **Nishihara, M., M. Ishinaga, M. Kato, and M. Kito.** 1976. Temperature-sensitive formation of the phospholipid molecular species in *Escherichia coli* membranes. Biochim. Biophys. Acta **431**:54–61.
31. **Nishijima, M., C. E. Bulawa, and C. R. H. Raetz.** 1981. Two interacting mutations causing temperature-sensitive phosphatidylglycerol synthesis in *Escherichia coli* membranes. J. Bacteriol. **145**:113–121.
32. **Proulx, P. R., and L. L. M. van Deenen.** 1967. Phospholipase activity of *Escherichia coli*. Biochim. Biophys. Acta **144**:171–174.
33. **Raetz, C. R. H.** 1978. Enzymology, genetics, and regulation of membrane phospholipid synthesis in *Escherichia coli*. Microbiol. Rev. **42**:614–659.
34. **Raetz, C. R. H., and K. F. Newman.** 1979. Diglyceride kinase mutants of *Escherichia coli*: inner membrane association of 1,2-diglyceride and its relation to synthesis of membrane-derived oligosaccharides. J. Bacteriol. **137**:860–868.
35. **Sabelnikov, A. G., and I. V. Domaradsky.** 1981. Effect of metabolic inhibitors on entry of exogenous deoxyribonucleic acid into $Ca^{2+}$-treated *Escherichia coli* cells. J. Bacteriol. **146**:435–443.
36. **Satre, M., and K. P. Kennedy.** 1978. Identification of bound pyruvate essential for the activity of phosphatidylserine decarboxylase of *Escherichia coli*. J. Biol. Chem. **253**:479–483.
37. **Schulman, H., and E. P. Kennedy.** 1977. Relation of turnover of membrane phospholipids to synthesis of membrane-derived oligosaccharides of *Escherichia coli*. J. Biol. Chem. **252**:4250–4255.
38. **Van Den Bosch, H., and P. R. Vagelos.** 1970. Fatty acyl-CoA and fatty acyl carrier protein as acyl donors in the synthesis of lysophosphatide and phosphatide in *Escherichia coli*. Biochim. Biophys. Acta **218**:233–248.
39. **van Golde, L. M. G., H. Schulman, and E. P. Kennedy.** 1973. Metabolism of membrane phospholipids and relation to a novel class of oligosaccharides in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:1368–1372.
40. **Wicken, A., and K. W. Knox.** 1980. Bacterial cell surface amphiphiles. Biochim. Biophys. Acta **604**:1–26.
41. **Yokota, K., R. Kanamoto, and M. Kito.** 1980. Composition of cardiolipin molecular species in *Escherichia coli*. J. Bacteriol. **141**:1047–1051.