JOURNAL OF BACTERIOLOGY, Feb. 1987, p. 605–611
0021-9193/87/020605-07$02.00/0

Vol. 169, No. 2

Fatty Acid Metabolism in *sn*-Glycerol-3-Phosphate Acyltransferase (*plsB*) Mutants

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Received 11 August 1986/Accepted 4 November 1986

Fatty acid metabolism was examined in *Escherichia coli plsB* mutants that were conditionally defective in *sn*-glycerol-3-phosphate acyltransferase activity. The fatty acids synthesized when acyl transfer to glycerol-3-phosphate was inhibited were preferentially transferred to phosphatidylglycerol. A comparison of the ratio of phospholipid species labeled with $^{32}P_i$ and [^{3}H]acetate in the presence and absence of glycerol-3-phosphate indicated that [^{3}H]acetate incorporation into phosphatidylglycerol was due to fatty acid turnover. A significant contraction of the acetyl coenzyme A pool after glycerol-3-phosphate starvation of the *plsB* mutant precluded the quantitative assessment of the rate of phosphatidylglycerol fatty acid labeling. Fatty acid chain length in membrane phospholipids increased as the concentration of the glycerol-3-phosphate growth supplement decreased, and after the abrupt cessation of phospholipid biosynthesis abnormally long chain fatty acids were excreted into the growth medium. These data suggest that the acyl moieties of phosphatidylglycerol are metabolically active, and that competition between fatty acid elongation and acyl transfer is an important determinant of the acyl chain length in membrane phospholipids.

The effect of cessation of phospholipid biosynthesis on cellular processes has been studied extensively in *Escherichia coli* with *plsB* mutants, which are conditionally defective in *sn*-glycerol-3-phosphate (glycerol-P) acyltransferase activity (for reviews, see references 32 and 34). The *plsB* mutation is a defect in the structural gene for the glycerol-P acyltransferase (24) and is located at min 92 of the *E. coli* chromosome (26). Membranes prepared from the *plsB* mutant have a glycerol-P acyltransferase activity with a K_m for glycerol-P that is 10-fold higher than the activity in membranes isolated from the isogenic wild-type strain (2). Physiological concentrations of glycerol-P are not sufficient to sustain phospholipid biosynthesis; however the enzymatic defect can be overcome by supplying exogenous glycerol or glycerol-P to the culture medium. The phospholipid biosynthetic rate decreases immediately after the removal of glycerol-P, whereas the production of protein, DNA, and RNA is unaffected for 30 min after glycerol-P starvation (28). The mass ratio of phosphatidylethanolamine (PtdEtn) to phosphatidylglycerol (PtdGro) is not altered in glycerol-deprived cultures, nor is the rate of PtdGro head group turnover (28). Thus, de novo phospholipid biosynthesis can be halted without immediately perturbing macromolecular synthesis or other metabolic processes.

The ability to block the incorporation of fatty acids into phospholipids via the de novo biosynthetic pathway in *plsB* strains suggests that they could be used to examine fatty acid turnover in membrane phospholipids. One fatty acid turnover process involves the transfer of 1-position fatty acids from PtdEtn to the major outer membrane lipoprotein followed by the resynthesis of PtdEtn (18a, 33). Turnover at the 1 position of PtdEtn accounts for the switch in the positional distribution of exogenous [1-^{14}C]oleate incorporated into PtdEtn after the starvation of *plsB* strains for glycerol-P (33). However, exogenous fatty acids are metabolically channeled through two independent specific acylation pathways (36), and our earlier experiments would not have revealed an acyltransferase process that utilizes acyl-acyl carrier protein (acyl-ACP) derived from fatty acid biosynthesis. This type of turnover process could be examined with a different labeling strategy, since acyl-ACP production does not appear to be coordinately regulated with glycerol-P acyltransferase activity (35). Cronan et al. (9) suggest that phospholipid and fatty acid synthesis are not tightly coupled, and they report that abnormally long chain fatty acids accumulate in the absence of phospholipid synthesis. On the other hand, Nunn et al. (31) conclude that these two processes are coordinately inhibited and point out that the continued increase in fatty acid labeling after glycerol-P starvation may be due to a change in the specific activity of the intracellular acetate pool. The accumulation of acyl-ACP immediately after the cessation of phospholipid synthesis (35) accounts for the production of abnormally long chain fatty acids, and the concomitant reduction in nonesterified ACP promotes the inhibition of fatty acid biosynthesis. The goal of the present study was to use the *plsB* genetic system to determine whether there is fatty acid turnover in membrane phospholipids other than PtdEtn.

MATERIALS AND METHODS

Chemicals and supplies. Sources for supplies were as follows: from New England Nuclear Corp., sodium [2-^{3}H]acetate (specific activity, 3.4 Ci/mmol) and β-[3-^{3}H]alanine (specific activity, 120 Ci/mmol), [2-^{14}C]malonyl coenzyme A (CoA; specific activity, 46.9 mCi/mmol); from Amersham Corp., carrier-free $^{32}P_i$ (specific activity, 46.9 mCi/mmol), [1-^{14}C]acetyl-CoA (specific activity, 44.1 mCi/mmol), and ACS scintillation solution; from Analtech, 250-μm Silica Gel H and G plates; from Separations Group, the Vydac HS201-C18 reverse-phase chromatography column; from Supelco Inc., the DEGS-PS gas chromatography column; from Millipore Corp., type HA 0.45-μm filters; from Sigma Chemical Co., β-alanine, tetracycline hydrochloride, Brij 58, and buffers; from Serdary Research Laboratories Inc., phospholipid standards. All chemicals and solvents were reagent grade or better.

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TABLE 1. List of strains

Strain	Genotype	Construction, source, or reference
Strain 8	*HfrC glpR glpD phoA8 relA1 fhuA22* T2^r *spoT1 pit-10*	CGSC[a]
BB26	*plsB26* of strain 8	1
BB26-36	*glpK*(Fr) of strain BB26	2
DF40	*pgi*	D. Clark
JA200 (pLC9-28)	Plasmid corrects *plsB* defect	26
JA200 (pLC26-5)	Plasmid corrects *fadL* defect	D. Clark
LS5283	*fadL*::Tn*10 fadR*	14
SJ5	*zjc*::Tn*10* of strain BB26-36	P1(SJ8) × BB26-36
SJ8	*plsB*$^+$ *zjc*::Tn*10* of strain BB26-36	P1(W1485::Tn*10*) × BB26-36
SJ10	*panD2 zad-220*::Tn*10*	16
SJ22	*panD2* of strain BB26-36	P1(SJ10) × BB26-36
SJ27	*pgi plsB*$^+$ of strain BB26	P1(UB1005) × BB26
SJ38	*plsB*$^+$ of strain BB26-36	P1(UB1005) × BB26-36
SJ48	*fadE* of strain BB26-36	33
SJ117	*plsB26 fadE*	Tet^s isolate of SJ48
SJ119	*plsB26 fadE fadL*	P1(LS5283) × SJ117
UB1005	*metBl relA1 spoT1* λ^- λ^r *gyrA216* F$^-$	5
W1485	*supE42*	6

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Bacterial strains and growth media. All strains used in this study were derivatives of *E. coli* K-12 and are listed in Table 1. The Tn*10* insertion close to the *plsB* locus was isolated by transducing strain BB26-36 with P1 phage grown on a random pool of tetracycline-resistant (Tet^r) isolates of strain W1485::Tn*10* (6, 8), and recombinants were selected for Tet^r and *plsB*$^+$. Selection for the loss of Tn*10* was performed by the method of Bochner et al. (4), as modified by Maloy and Nunn (27). The *plsB* mutation was scored on plates containing glucose-minimal medium with or without 0.04% glycerol-P (29), the *pgi* mutation was scored on glucose-tetrazolium indicator plates (13), and the Tn*10* marker was scored on plates containing 10 μg of tetracycline hydrochloride per ml. Glycerol-P acyltransferase activity was measured as described previously (38). Strains were grown in M9 minimal salts (29) plus thiamine (0.01%) containing the growth supplements indicated in each figure legend. Strain BB26-36 was grown in low-phosphate medium (28) when $^{32}P_i$ was used. 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 colorimeter units.

Phospholipid and fatty acid analyses. Samples collected from a labeling experiment were placed in an ice slush, and the cells were harvested by centrifugation at 12,000 × *g* for 15 min. The cell pellet was washed with 5 ml of ice-cold unlabeled medium and then extracted by the method of Bligh and Dyer (3). Bacterial phospholipids were separated on Silica Gel G layers developed with chloroform-methanol-acetic acid (55:20:5, vol/vol), and neutral lipid classes were separated with hexane-diethyl ether-acetic acid (50:50:1, vol/vol). The distribution of radioactivity on the thin-layer plates was determined by using a Bioscan radioactivity imaging detector. Collection times varied between 5 and 20 min depending on the total amount of radioactivity applied to the plate. Fatty acid methyl esters were prepared by reacting the lipid sample with 5% HCl in anhydrous methanol. Methyl esters were extracted into hexane and dissolved in carbon disulfide, and the fatty acid compositions were determined by using a Varian 3700 gas chromatograph equipped with a column packed with Supelcoport (100/120 mesh) coated with 5% DEGS-PS and operated isothermally at 165°C. Integration accuracy was checked by using the National Heart Institute standard mixture D.

Quantitation of CoA thioesters. Cultures labeled with β-[3-^{3}H]alanine were harvested by centrifugation in a Beckman Microfuge-12 at 4°C, and the unwashed cell pellets were stored overnight at −70°C. The total cellular CoA content was determined in one of the duplicate cell pellets by thin-layer chromatography after 2-propanol lysis (16). For determining the thioester composition of the CoA pool, pellets were suspended in 24 μl of 1 N formic acid and Vortex mixed for 15 s every 5 min for a total of 30 min while in an ice slush. Just before analysis, 25 μl of 1 M dibasic potassium phosphate was added to bring the pH of the extract to 6.5. The sample was then filtered by using an Amicon micropartition system equipped with a YMT ultrafiltration membrane to remove high-molecular-weight material. Recovery of radioactive CoA was approximately 90%. CoA thioesters were fractionated by using a modification of the method of DeBuysere and Olson (10). A Perkin-Elmer Series 400 liquid chromatograph was equipped with a 4.6-mm by 25-cm 5-μm Vydac HS201-C18 reverse-phase column protected by a guard column packed with pellicular C18 reverse-phase material. The column effluent was monitored with a Perkin-Elmer LC-75 spectrophotometric detector operated at 254 nm and a Hewlett-Packard 3392A reporting integrator. Solvent A was 0.22 M potassium phosphate (pH 4.0), and solvent B was methanol-chloroform (98:2, vol/vol). The column was developed at a flow rate of 0.5 ml/min beginning with an 8.5-min linear gradient from 14 to 18% solvent B, a 0.1-min step to 24% solvent B, and then a 20-min linear gradient from 24 to 30% solvent B. The identity of the tritium-labeled CoA species was confirmed by comparisons with the retention times of known standards and by cochromatography with ^{14}C-labeled CoA thioesters. β-Alanine eluted in the void volume of the chromatographic system, and ACP did not elute from the column under these gradient conditions. Recovery of CoA derivatives was approximately 70%.

RESULTS

Genetic analysis of *plsB* mutants. Isogenic *plsB*$^+$ strains were used as controls. These strains were prepared from the corresponding *plsB* mutants by using P1 phage grown on strain UB1005 (Table 1). However, *plsB* strains also harbor the *plsX* defect, and both *plsB plsX*$^+$ and *plsB*$^+$ *plsX* strains

TABLE 2. Map position of the *zjc*::Tn*10* insertion

Cross (P1 donor × recipient)	Selection (*n*)	Recombination classes (*n*)
SJ8 (*zjc*::Tn*10*) × BB26-36 (*plsB*)	Tn*10* (100)	*plsB* (20) *plsB*$^+$ (80)
SJ5 (*zjc*::Tn*10 plsB*) × SJ38 (*plsB*$^+$)	Tn*10* (100)	*plsB* (79) *plsB*$^+$ (21)
SJ5 (*zjc*::Tn*10 plsB*) × SJ27 (*pgi*)	Tn*10* (191)	*pgi*$^+$ plsB (29) *pgi*$^+$ plsB$^+$ (2) *pgi plsB* (111) *pgi plsB*$^+$ (49)

have wild-type growth characteristics (25). To ensure that our strains were *plsB*$^+$ and not *plsX*$^+$, we isolated a Tn*10* insertion close to the *plsB* allele and used this genetic reagent to test for the presence of the *plsB* mutation in our control strains (Table 2). The location of *zjc*::Tn*10* at min 92 of the chromosome was confirmed by its 18% cotransduction frequency with the *pgi* marker in strain DF40 (data not shown), and a three-point cross established the gene order *zjc*::Tn*10*-*plsB*-*pgi* (Table 2). An interesting sidelight to this work was the finding that strain BB26 had a *pgi* defect, whereas strain BB26-36 did not. This point was not obvious, since *plsB pgi* double mutants did not yield the characteristic red color of *pgi* mutants (13) on tetrazolium indicator plates, but they exhibited the *pgi* growth phenotype (13) on glucose-minimal medium.

Selective incorporation of [2-^{3}H]acetate into PtdGro. To determine whether acyl moieties derived from fatty acid biosynthesis are transferred to phospholipid in the absence of de novo phosphatidic acid formation, strain SJ48 (*plsB fadE*) was labeled with [2-^{3}H]acetate in the presence and absence of glycerol-P. After 1-h of labeling in the presence of glycerol-P, the distribution of label among the phospholipid classes was typical for *E. coli* (Fig. 1A) and the same as that found with strain SJ38 (*plsB*$^+$) either in the presence or absence of glycerol-P (data not shown). Consistent with the observations of Nunn et al. (31), the extent of fatty acid labeling was variable and increased as the time between glycerol-P removal and the addition of [2-^{3}H]acetate increased. The pattern of phospholipid labeling after glycerol-P removal was significantly different than the wild-type pattern (Fig. 1). In the absence of glycerol-P, PtdGro predominated (Fig. 1B), and the PtdGro/PtdEtn ratio was 28-fold higher than in the supplemented control (Fig. 1a). The relative proportion of cardiolipin that was labeled also increased and a small amount of free fatty acid was observed. One explanation for the elevated PtdGro/PtdEtn ratio was that the PtdGro-phosphate synthase was a more effective competitor for the depleted CDP-diacylglycerol pool. To check this point, the ratio of phospholipids synthesized was quantitated in strain SJ48 by labeling with $^{32}P_i$ under similar conditions (Fig. 2). Phosphate incorporation into phospholipid was inhibited 80% after the removal of glycerol; in contrast to the acetate-labeling results, the PtdGro/PtdEtn ratio increased only threefold (Fig. 2B). Since PtdGro production from CDP-diacylglycerol is four times higher than that of PtdEtn (19–21), a bias toward PtdGro synthesis when CDP-diacylglycerol was limiting seemed plausible. These data show that de novo phospholipid synthesis was inhibited by glycerol-P starvation, and the selective synthesis of PtdGro from [2-^{3}H)]acetate indicated the presence of a fatty acid turnover process that draws on the acyl-ACP pool generated by fatty acid biosynthesis.

Effect of glycerol-P starvation on the CoA pool composition. Nunn et al. (31) proposed that the continued [1-^{14}C] acetate incorporation into fatty acids after glycerol-P starvation was due to a change in the specific activity of the intracellular acetyl-CoA pool. To evaluate the suitability of our labeling method for determining the magnitude of fatty acid incorporation into PtdGro, the effect of glycerol-P starvation on the CoA pool composition was examined with strain SJ22 (*plsB panD*). The *panD* defect allowed the CoA pool to be uniformly labeled with exogenous β-[3-^{3}H]alanine (17, 18). Strain SJ22 was grown in the presence of β-[3-^{3}H]alanine, and the β-[3-^{3}H]alanine-derived CoA metabolites were analyzed by reverse-phase high-pressure liquid chromatography (Fig. 3). Control cultures supplemented with glycerol-P (Fig. 3A) had a distribution of CoA species (malonyl-CoA, 4.8%; CoASH (nonesterfied CoA), 22.6%; succinyl-CoA, 37.8%; acetyl-CoA, 34.9%) that was comparable to the distribution

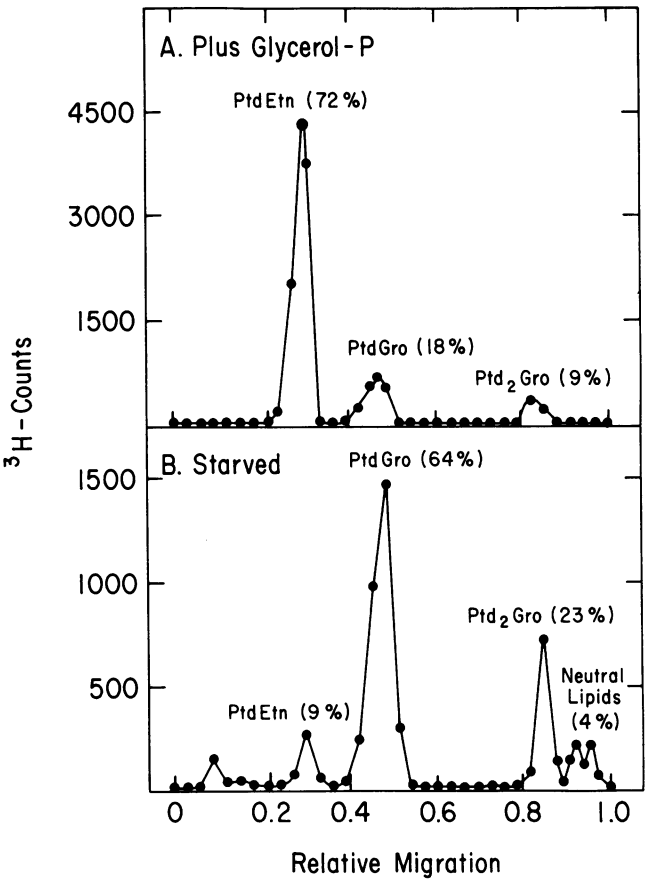

FIG. 1. Effect of glycerol-P starvation on the pattern of [2-^{3}H]acetate incorporation into the phospholipids of strain SJ48 (*plsB fadE*). Strain SJ48 was grown to a density of 1.5×10^8 cells per ml in M9 medium containing succinate (0.4%), acetate (0.2%), casein hydrolysate (0.1%), and glycerol-P (0.04%). Cells from duplicate cultures were harvested by filtration and washed three times with 5 ml of warm medium without acetate and either with (A) or without (B) glycerol-P. The cells were then suspended in the original culture volume of warm growth medium used in the washing step, and sodium [2-^{3}H]acetate (0.1 mCi/ml) was added to each culture. The cells in the starved culture assimilated 82% less [2-^{3}H]acetate than those supplemented with glycerol-P. The samples shown were removed 1 h after the initiation of acetate labeling, and the distribution of label among the phospholipid species was determined by thin-layer chromatography as described in Materials and Methods.

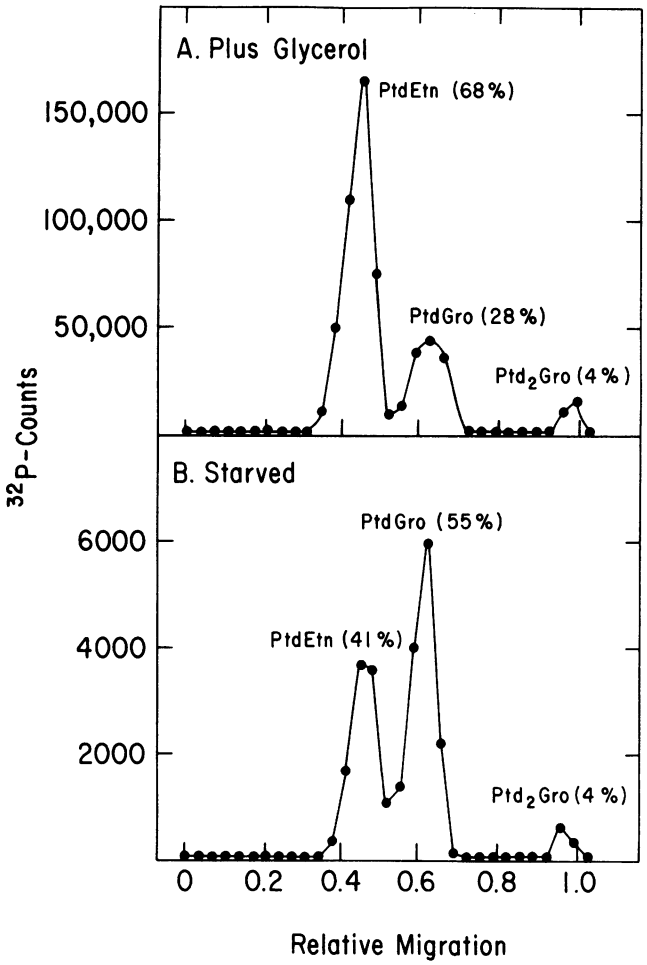

FIG. 2. Effect of glycerol deprivation on the pattern of $^{32}P_i$ incorporation into the phospholipids of strain BB26-36 (*plsB*). Strain BB26-36 was grown to a density of 1.5×10^8 cells per ml in the low-phosphate medium supplemented with glycerol (0.4%) as described by McIntyre et al. (28). Cells from duplicate cultures were harvested by filtration and were washed three times with 5 ml of warm medium either with (A) or without (B) glycerol. The cells were then suspended in the original volume of warm growth medium used in the washing step, and carrier-free $^{32}P_i$ (50 μCi/ml) was added. The samples shown were removed 1 h after the addition of $^{32}P_i$, and the distribution of label among the phospholipid species was determined by thin-layer chromatography as described in Materials and Methods.

of label observed in other strains of *E. coli* grown on succinate as the carbon source (data not shown). There was a significant change in the CoA pool composition as early as 5 min after the removal of glycerol-P (Fig. 3B). A reduction in the acetyl-CoA component to 9.5% of the total and the disappearance of malonyl-CoA was reflected in a corresponding increase in the proportion of succinyl-CoA (62.6%) and CoASH (27.9%). The acetyl-CoA pool continued to contract with time after glycerol-P starvation until it was no longer detectable at 30 min (data not shown). A shrinking acetyl-CoA pool is consistent with the induction of the glyoxylate shunt operon by the inactivation of the *fadR* repressor protein due to the accumulation of fatty acids (for a review, see reference 30). The decreased concentration of acetyl-CoA after glycerol-P starvation was consistent with a significant increase in the specific activity of the acetyl-CoA pool. Therefore, it is difficult to estimate the actual rate of fatty acid incorporation into PtdGro.

Fatty acid chain length and glycerol-P acyltransferase activity. To address the role of the glycerol-P acyltransferase in controlling the product distribution of fatty acid biosynthesis, strain BB26-36 (*plsB*) was grown in the presence of different concentrations of glycerol-P to gradually impair the acyltransferase system. The average carbon number of the membrane fatty acids in strain BB26-36 was higher than in the isogenic control strain SJ38 at the highest concentration of glycerol-P tested, and the average chain length increased substantially as the glycerol-P supplement was lowered (Fig. 4). The most notable increases were in stearate and *cis*-vaccenate, although at growth-limiting concentrations of glycerol-P (<0.2 mg/ml) both eicosanoic and *cis*-eicosenoic acids were also detected. In a complementary study, the fatty acid composition of strain JA200, harboring a hybrid plasmid containing the *plsB* gene (pLC9-28), was compared with that of the same strain containing the ColE1 plasmid with an irrelevant insert (pLC26-5). The glycerol-P acyltransferase specific activity was elevated 7.8-fold in the overproducing strain, and the fatty acids of this bacterium had an average carbon number of 16.0 compared with 16.5 for the isogenic control. This decreased average carbon

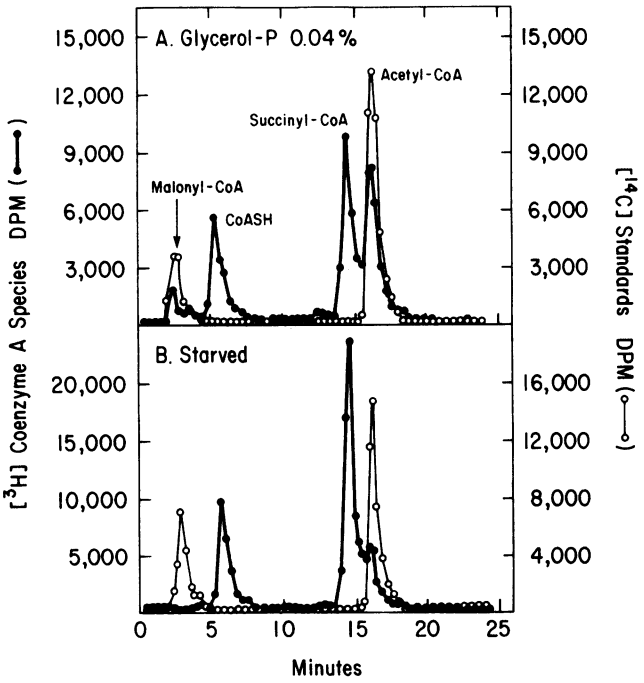

FIG. 3. Contraction of the acetyl-CoA pool after the removal of glycerol-P from strain SJ22 (*plsB panD*). Strain SJ22 was grown to a density of 1.5×10^8 cells per ml in M9 medium containing succinate (0.4%), casein hydrolysate (0.1%), glycerol-P (0.04%), and β-[3-^{3}H]alanine (4 μM; 6 Ci/mmol). Duplicate cultures were harvested by filtration and were washed three times with 5 ml of warm medium either with or without glycerol-P. The cells were suspended in the original volume (10 ml) of medium containing 4 μM β-alanine with (A) or without (B) glycerol-P. The sample from the starved culture (B) was taken 5 min after the removal of glycerol-P. The distribution of tritium among the components of the CoA pool was determined by reverse-phase high-pressure liquid chromatography as described in Materials and Methods. Peaks were identified by the comparison of their retention times with known standards, and the identifications were confirmed by spiking the samples with [2-^{14}C]malonyl-CoA and [1-^{14}C]acetyl-CoA.

number was due to an increase in the palmitoleate/*cis*-vaccenate ratio, and the strain harboring the *plsB* clone had 2% myristic acid compared with 0.4% for the control.

We observed that free fatty acids were generated after glycerol-P starvation and were excreted from the cell. Strain SJ48 (*plsB fadE*) released a low level of fatty acids into the medium during growth in the presence of glycerol-P, and removal of the glycerol-P supplement resulted in a 3.5-fold increase in the apparent rate of labeled fatty acid export (data not shown). Interestingly, the kinetics of acetate-labeled fatty acid export in the absence of phospholipid synthesis was the same in strain SJ119 (*plsB fadE fadL*), suggesting that the *fadL* gene product was not required for this fatty acid efflux process. The composition of the extracellular fatty acids is shown in Table 3. In both cases the fatty acids were deficient in palmitoleic acid, but after the removal of the glycerol-P supplement there was an increase in the average carbon number and the appearance of eicosanoic and *cis*-eicosenoic acids. Cyclopropane fatty acids were not detected. The cyclopropane ring is only introduced into unsaturated fatty acids attached to phospholipid (37), and the absence of these modified fatty acids supports our hypothesis that the extracellular fatty acids are derived from de novo biosynthesis rather than from phospholipid turnover.

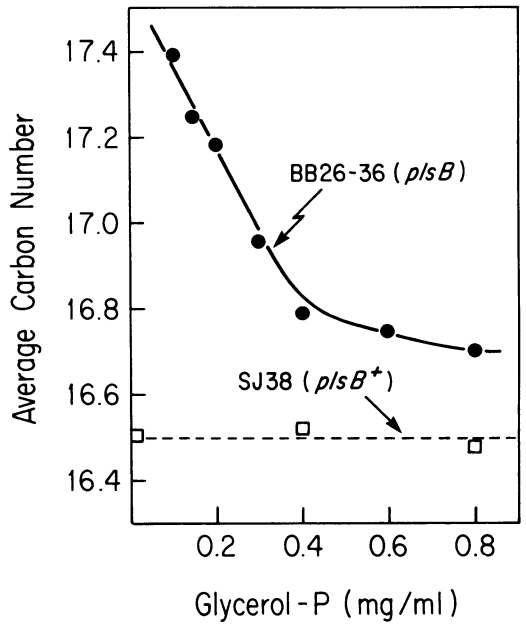

FIG. 4. Average carbon number of fatty acids in the phospholipids of strains BB26-36 (*plsB*) and SJ38 (*plsB*$^+$) grown in the presence of different concentrations of glycerol-P. Strains were grown to the late log phase in M9 medium containing glucose (0.4%), casein hydrolysate (0.1%), and glycerol-P (0.4 mg/ml). The cells were washed twice in medium devoid of glycerol-P, and flasks containing the glucose medium plus the indicated amount of glycerol-P were inoculated with 10^7 cells per ml and maintained at 37°C for 16 h. The strains were harvested, the lipids were extracted, and the fatty acid methyl esters were analyzed by gas chromatography as described in Materials and Methods. Strain BB26-36 (*plsB*) did not reach stationary phase (2×10^9 cells per ml) on the three lowest concentrations of the glycerol-P supplement. These cell densities and corresponding glycerol-P supplements were: 9×10^8, 0.2 mg/ml; 6×10^8, 0.15 mg/ml; and 4×10^8, 0.1 mg/ml. Growth was not detected in cultures that did not contain glycerol-P.

TABLE 3. Composition of the fatty acids excreted from strain SJ48 (*plsB fadE*) in the presence and absence of a glycerol-P growth supplement

Fatty acid[a]	% of fatty acid[b]	
	Without glycerol-P	With glycerol-P
14:0	ND	Trace
16:0	35.3	39.0
16:1	3.0	3.1
18:0	23.2	31.9
18:1	24.4	26.0
20:0	8.1	ND
20:1	5.9	ND

[a] Number of carbons: number of double bonds. Cyclopropane derivatives were not detected.

[b] ND, Not detected; trace, <0.5%. The unsaturated fatty acid/saturated fatty acid ratios were 0.50 without glycerol-P and 0.41 with glycerol-P.

DISCUSSION

The selective incorporation of fatty acids into PtdGro in the absence of de novo phosphatidic acid formation suggests that the PtdGro acyl moieties are metabolically active. The complexities in determining the specific activity of the acetate pool in our experiments probably result in an overestimate of fatty acid incorporation into PtdGro and preclude a quantitative estimate of the rate of fatty acid turnover. Support for this view comes from the observations that fatty acid turnover in PtdGro is not apparent in classical pulse-chase experiments on growing cells and that turnover at the 1-position of PtdEtn is slow, amounting to only 3 to 5% of this phospholipid per generation (33). Even a small rate of turnover could be of major significance to other cellular processess, since the phospholipids are one of the more abundant components of the cell. One possibility is that the preexisting fatty acids are degraded or exported, and the turnover process provides a mechanism for the remodeling of PtdGro fatty acid composition. However, PtdGro turnover may be related to the biosynthesis of other molecules. The most promising candidates appear to be the lipoproteins in light of the experiments of Wu and collaborators (22, 23), who have shown that lipoprotein acyl moieties are derived from membrane phospholipids. PtdGro can serve as a source for these acyl groups, as indicated by the transfer of label from this phospholipid to lipoprotein in vesicle fusion experiments (7). The 1-position turnover cycle in PtdEtn has been tied to the acylation of the amino terminus of the lipoprotein (18a); however the phospholipid that is the source for the two ester-linked lipoprotein fatty acids remains to be firmly established. Further work will be required to determine whether PtdGro is also specifically involved in lipoprotein acylation.

Our compositional data are consistent with the hypothesis that competition between acyl transfer and chain elongation is a primary determinant of the observed fatty acid structures in membrane phospholipids. The initial proposal that the substrate specificity of the β-ketoacyl-ACP synthase reactions is the key factor (15) was questioned by Cronan et al. (9), who report that abnormally long chain fatty acids are synthesized in the absence of phospholipid biosynthesis. The rapid change in the precursor pool composition (Fig. 3), the increase in the specific activity of the acetate pool (31), and the persistence of elevated acyl-ACP concentrations after glycerol-P starvation (35) raise the possibility that the very long chain fatty acids only appear to be a significant

fraction of the total pool in the acetate-labeling studies. However, our analytical data (Fig. 4, Table 3) show that there is an overall increase in the average carbon number of the free fatty acids synthesized after glycerol-P starvation and in the membrane acyl group composition under growth conditions that impair glycerol-P acyltransferase activity. On the other hand, overproduction of the acyltransferase slightly reduces the average carbon number in membrane phospholipids. The product distribution of fatty acid biosynthesis is also modified by altering the activity of the condensing enzymes. Strains that overproduce β-ketoacyl-ACP synthase I have a significant increase in the average fatty acid chain length in their membrane phospholipids (12). The activity of β-ketoacyl-ACP synthase II is naturally temperature sensitive, and alteration in the activity of this condensing enzyme regulates the palmitoleate/*cis*-vaccenate ratio (for a review, see reference 11). Thus, chain length composition in *E. coli* appears to be regulated by the kinetic competition between the glycerol-P acyltransferase and the β-ketoacyl-ACP synthases.

ACKNOWLEDGMENTS

We thank S. Pickren and K. Lee for their technical assistance and J. E. Cronan, Jr., W. D. Nunn, and D. Clark for bacterial strains.

This work was supported by Public Health Service grant GM 28035 from the National Institutes of Health, Cancer Center (CORE) Support Grant CA 21765, and the American Lebanese Syrian Associated Charities. C.L.C. was supported by National Research Service Award T32 CA09346 from the National Cancer Institute.

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