Isolation and Characterization of *Escherichia coli* K-12 Mutants Lacking Both 2-Acylglycerophosphoethanolamine Aciyltransferase and Acyl-Acyl Carrier Protein Synthetase Activity*

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2-Acyl-glycerophosphoethanolamine (2-acyl-GPE) acyltransferase and acyl-acyl carrier protein (acyl-ACP) synthetase are thought to be dual catalytic activities of a single inner membrane enzyme. A filter disc replica print method for the detection of acyl-ACP synthetase activity by colony fluorography was used to screen a mutagenized population of cells for acyl-ACP synthetase mutants (aas). All aas mutants lacked both acyl-ACP synthetase and 2-acyl-GPE acyltransferase activities in *vivo*. There was no detectable acyl-CoA-independent incorporation of exogenous fatty acids into phosphatidylethanolamine or the major outer membrane lipoprotein in aas mutants. Exogenous lysophospholipid uptake and acylation was also lacking in aas mutants. Lipoprotein acylation by phospholipids synthesized by the *de novo* biosynthetic pathway was not affected in aas mutants showing that this gene product was not directly involved in lipoprotein biogenesis. The aas mutants had an altered membrane phospholipid composition and accumulated both 2-acyl-GPE and acylphosphatidylglycerol. Acylphosphatidylglycerol accumulation was due to the transacylase activity of lysophospholipase L2 (the *pldB* gene product) since *aas pldB* double mutants accumulated 2-acyl-GPE, but not acylphosphatidylglycerol. The *aas* allele was mapped to 61 min of the *Escherichia coli* chromosome, and the deduced gene order in this region was *thyA-aas-lysA*. The biochemical, physiological, and genetic analyses of aas mutants support the conclusion that 2-acyl-GPE acyltransferase and acyl-ACP synthetase are two activities of the same protein and confirm that this enzyme system participates in membrane phospholipid turnover and governs the acyl-CoA independent incorporation of exogenous fatty acids and lysophospholipids into the membrane.

Membrane phospholipids are critically important in maintaining the structure of biological membranes, but in addition, portions of these lipids are used in the synthesis of other membrane-associated macromolecules. In *Escherichia coli*, the observed turnover of the phosphatidylglycerol headgroup is due to its transfer to either the cytosine of the major outer membrane lipoprotein to form a thioether linkage (1) or to the abundant periplasmic glucose polymers known as membrane-derived oligosaccharides (2). The diacylglycerol remaining in the membrane is phosphorylated by diacylglycerol kinase to yield phosphatidic acid which then re-enters the mainstream of the *E. coli* phospholipid biosynthetic pathway (3). Acyl moieties at the 1-position of PtdEtn¹ are also metabolically active, and this process is related to the transacylation reactions that occur during the maturation of the bacterial lipoproteins (4). The resulting 2-acyl-GPE is then recycled to PtdEtn by 2-acyl-GPE acyltransferase (4-6). This inner-membrane enzyme transfers fatty acids to the 1-position either from an acyl-ACP derivative or from nonesterified fatty acids in the presence of ATP and Mg²⁺ (5, 6). Aciyltransferase activity is blocked by nonspecific ACP antibodies indicating that ACP is required for the ATP-dependent acylation of 2-acyl-GPE (5). 2-Acyl-GPE acyltransferase/acyl-ACP synthetase was solubilized and purified from *E. coli* inner membranes (7). The biochemical data support the concept that both enzymatic reactions are catalyzed by a heterodimer composed of a membrane-bound acyltransferase subunit and ACP (7). The physiological function of 2-acyl-GPE acyltransferase is to regenerate PtdEtn from 2-acyl-GPE formed by transacylation reactions or phospholipase A₄ action. 2-Acyl-GPE acyltransferase is also thought to be required for the acyl-CoA-independent incorporation of exogenous fatty acids (8) and 2-acyl-lysophospholipids into the cell membrane (9). The goal of the present work was to isolate mutants lacking 2-acyl-GPE acyltransferase and acyl-ACP synthetase activity to corroborate the conclusion that 2-acyl-GPE acyltransferase/acyl-ACP synthetase are dual catalytic activities of the same protein complex, and to confirm the physiological role of the acyltransferase/synthetase in fatty acid and lysophospholipid uptake, and in maintaining membrane phospholipid composition.

**EXPERIMENTAL PROCEDURES**

_Materials—Sources of supplies were: Amersham, [9,10-²H]palmitic acid (specific activity 60 Ci/mmol) and ACS scintillation solution; Analtech Inc., thin-layer chromatography plates; Boehringer Mannheim, Rhizopus arrhizus lipase, Triton X-100, Tris, and ATP; Whatman, number 42 and 3MM filter paper circles; Du Pont-New England Nuclear, EN²HANCE spray scintillation mixture; Pierce Chemical Co., biocinchonic acid protein assay kit; Sigma, Brij-58 and N-methyl-N'-nitro-N-nitrosoguanidine; Serdy Research Laborato-

¹ The abbreviations used are: PtdEtn, phosphatidylethanolamine; ACP, acyl carrier protein; acyl-ACP, acyl-acyl carrier protein; 2-acyl-GPE, 2-acyl-sn-glycerol-3-phosphoethanolamine; PtdGro, phosphatidylglycerol; 2-acyl-GPC, 2-acyl-sn-glycerol-3-phosphocholine; GPE, sn-glycerol-3-phosphoethanolamine; IPTG, isopropyl-β-D-thiogalactoside; SDS, sodium dodecyl sulfate.

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ries, bacterial PtdEtn and phospholipid standards. ACP was purified by the method of Rock and Cronan (10). 2-Acyl-GPE was prepared by digestion of E. coli PtdEtn with R. arrhizus lipase as described by Homma and Nojima (11). 2-Acyl-GPE and 2-acyl-GPC concentrations were determined using the method of Stewart (12) and using E. coli PtdEtn as a standard. All other biochemicals and solvents were reagent grade or better.

**Bacterial Strains and Growth Conditions**—All strains used in this work were derivatives of E. coli K-12 and are listed in Table I. Minimal growth medium consisted of M9 minimal salts (17), 0.4% glycerol, 0.2% casamino acids, and 0.005% thiamine. Rich broth or agar was composed of 10 g/liter tryptone, 1 g/liter yeast extract, 5 g/liter NaCl, and 15 g/liter agar. The fadD allele in strains LCH22, LCH31, LCH40, and LCH41 by transposing the transposable element (18, 19) followed by transduction with Pl(BW6169)XLCH1 (see text) and selection of the progeny on lactose minimal plates. The final step in strain construction was transformation with recombinant plasmids. The pKEN126 plasmid contained a tandem duplication of lppR-lppD-lpp sequences and expressed the major outer membrane lipoprotein (20). The Pl3510 plasmid had a single copy of lppR-lppD-lpp- bla sequences and expressed lipoprotein-d-lactamase fusion protein (21). In both cases, lipoprotein gene expression was regulated by the lacUV5 promoter-operator region, and expression was induced by the addition of 1 mM IPTG to the growth medium (4, 20, 21). Strain LCH66 (aas-1 plbB12) was constructed by transduction of strain LCH2 with Pl(BW6169)XLCH1. Tetracycline-resistant recombinants were selected and screened for defective acyl-ACP synthetase activity by the colony autoradiography method. The cell density was measured using a Klett-Summerson colorimeter calibrated by determining the number of colony forming units per milliliter as a function of the colorimeter readings.

**Isolation of Acyl-ACP Synthetase Mutants**—A log-phase culture of strain UB1005 (6 × 10^6 cells/ml) grown in glycerol minimal medium was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine (6 μg per ml of cell suspension) for 1 h at 37 °C as outlined by Miller (17). Surviving single cell clones were grown on rich agar at 30 °C. About 94% of the cells were killed and 3% of the survivors had a Lac' phenotype. The replica printing method described by Raets (22) was used to isolate acyl-ACP synthetase mutants with a few modifications. After the mutagenized cells were transferred to a Whatman filter disc, they were permeabilized with a solution of 25% sucrose in 50 mM Tris-HCl, pH 7.4, 5 mg/ml lysozyme, and 10 mM EDTA. The replica print was then placed on a dry paper towel to remove excess liquid, transferred to another Petri dish, and rapidly frozen/thawed twice with liquid nitrogen. A 1-ml reaction mixture containing 0.1 M Tris-HCl, pH 8.0, 10 mM ATP, 100 mM MgCl₂, 2 mM dithiothreitol, 20 μM [3H]palmitic acid (specific activity 60 Ci/mmol) and 2% Triton X-100 was then applied to the replica print. After incubation at 42 °C for 1 h, the replica print was washed twice in chloroform/methanol/acetic acid (3:6:1, v/v) to remove unreacted [3H]palmitate and then precipitated with 94% ethanol. The washed replicate print was dried, sprayed with ENHANCE, and exposed to x-ray film at ~80 °C. Following fluorography, the replica print was stained with Coomassie Brilliant Blue to localize the colonies. Candidate mutants defective in acyl-ACP synthetase were identified by comparison of the blue-stained replica print with the corresponding fluorogram. Colonies were then purified and grown in rich medium at 30 °C and cell extracts prepared for the direct determination of acyl-ACP synthetase activity in vitro. Approximately 40,000 colonies were screened by autoradiography and two strains (LCH1 (aas-1) and LCH2 (aas-2)) that did not possess detectable acyl-ACP synthetase activity in cell lysates were found, and strain LCH1 was selected for further study. Although the screening was performed to potentially isolate temperature-sensitive mutants, strains with temperature-sensitive acyl-ACP synthetase activity were not found. Both LCH1 and

| Strain | Genotype | Source |
|--------|----------|--------|
| BW6169 | leuB6 tonA27 lacY or lacZ4 tsx-1 argA::TnlO thi-1 Hfr supE44 gal-6X | CGSC |
| CAG12135 | recD1901::TnlO | (13) |
| CAG18709 | zgc-3074::TnlO | (13) |
| DC67 | thy-1 fadL | David Clark |
| DC405 | zac-2::TnlO fadE | David Clark |
| DC451 | zac-2::TnlO fadD | David Clark |
| Hfr | Hfr::TnlO rapid mapping kit | CGSC (14) |
| LCH1 | aas-1 metB1 relA1 spoT1 gvb216 X' X' F' | See text |
| LCH2 | aas-2 metB1 relA1 spoT1 gvb216 X' X' F' | See text |
| LCH22 | aas-1 metB1 relA1 spoT1 gvb216 X' X' F' | See text |
| LCH29 | metB1 relA1 spoT1 gvb216 X' X' F' fadD | P1(DC451)×UB1005 |
| LCH30 | aas-1 metB1 relA1 spoT1 gvb216 X' X' F' fadD zac-2::TnlO | P1(DC451)×LCH1 |
| LCH31 | aas-1 metB1 relA1 spoT1 gvb216 X' X' F' fadD zac-2::TnlO | See text |
| LCH40 | aas-1 metB1 relA1 spoT1 gvb216 X' X' F' fadD zac-2::TnlO | See text |
| LCH41 | aas-1 metB1 relA1 spoT1 gvb216 X' X' F' fadD zac-2::TnlO | See text |
| LCH47 | thi-1 fadD argA81::TnlO | P1(BW6169)×DC67 |
| LCH61 | argA81::TnlO aas-1 metB1 relA1 spoT1 gvb216 X' X' F' | P1(BW6169)×LCH1 |
| LCH66 | aas-1 plbB12 thr-1 leuB6 lacY rfhD1 supE44 thi argA81::TnlO F' | P1(LCH6)×LKD29 |
| LKD29 | plbB12 thr-1 leuB6 lacY rfhD1 supE44 thi F' | (15) |
| SJ126 | fadD recA thi-1 leuB6 lacY1 tonA22 supE44 F' (lacF')| PKEN126 |
| SJ127 | fadD recA thi-1 leuB6 lacY1 tonA22 supE44 F' (lacF') | (4) |
| SJ169 | srl-1::TnlO recA metB1 relA1 spoT1 gvb216 X' X' F' | This study |
| UB1005 | metB1 relA1 spoT1 gvb216 X' X' F' | (16) |

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LCH2 lacked acyl-ACP synthetase activity at 30 and 42 °C.

Acyl-ACP Synthetase and 2-acyl-GPE Acyltransferase Assays—
Membranes were prepared by differential centrifugation of bacterial cell lysates as described previously (7). The standard acyl-ACP synthetase assay (23) contained 5 mM ATP, 10 mM MgCl2, 2 mM dithiothreitol, 50 μM [3H]palmitic acid, 100 μM 2-acyl-GPE, 10 μM ACP, 1% Triton X-100, 0.1 M Tris-HCl, pH 8.0, and the indicated amount of membrane protein in a final volume of 40 μl. At the end of 10 min at 37 °C, 30 μl of the assay mixture was withdrawn and deposited on a Whatman 3MM filter disc and washed with two changes of chloroform/methanol/acetic acid (85:15:10, v/v) to remove unreacted fatty acid. The filter papers were dried and counted in 3 ml of ACS scintillation solution to determine the amount of [3H]palmitoyl-ACP formed.

The standard 2-acyl-GPE acyltransferase assay contained 5 mM ATP, 5 mM MgCl2, 1 mM dithiothreitol, 50 μM [3H]palmitic acid, 100 μM 2-acyl-GPE, 10 μM ACP, 1% Triton X-100, 0.1 M Tris-HCl, pH 8.0, and the indicated amount of membrane protein in a final volume of 40 μl (5). Incubations were terminated after 10 min at 37 °C by adding 0.2 ml of ethanol. The mixture was evaporated to dryness under a stream of nitrogen, resuspended in chloroform/methanol (1:1, v/v), and the sample applied to the preadsorbent layer of a Silica Gel G plate. The plate was then developed with chloroform/methanol/acetic acid (85:15:10, v/v), the PtdEtn area was located with the Bioscan imaging detector, and the amount of [3H]PtdEtn formed was determined by scraping the Silica Gel from the plate and scintillation counting.

Isolation of Exogenous Fatty Acids and Lysoospholipids—
Strains were grown to the mid-logarithmic phase (approximately 5 × 10^8 cells/ml) in glycerol minimal medium containing 0.5% Brij-58 and labeled with 25 μCi/ml of [3H]palmitic acid. Culture samples (1 ml) were plated on agar media, and the cells were harvested by centrifugation at 12,000 × g for 15 min at 4 °C. The cell pellet was washed with ice-cold unlabeled medium. The lipids were extracted by the method of Bligh and Dyer (24) and were separated on Silica Gel G thin-layer chromatography plates developed with chloroform/methanol/acetic acid (55:20:5, v/v). The location of radioactivity on the thin-layer chromatography plates was determined by scraping the Silica Gel from the plate and scintillation counting. The same results were obtained with strain LCH2 (aas-2) (not presented). The acyltransferase/synthetase was postulated to be responsible for the uptake and incorporation of fatty acids into PtdEtn in strains (fadD) lacking acyl-CoA synthetase activity (8). In a control experiment, strain LCH29 (fadD) incorporated exogenous [3H]palmitate exclusively into PtdEtn as anticipated (8). In contrast, strain LCH30 (aas-1 fadD) did not incorporate exogenous [3H]palmitate into PtdEtn (Fig. 1) or any other phospholipid class (not shown) illustrating that aas mutants lacked the acyl-CoA-independent cotransduction frequencies were converted to map distance using the formula of Wu (27).

RESULTS

Isolation of Acyl-ACP Synthetase Mutants—A colony autoradiography method was developed to screen a mutagenized population of strain UB1005 for the presence of acyl-ACP synthetase activity. Acyl-ACP formation (a dark colony) was completely dependent on ATP and the addition of exogenous ACP to the assay mixture increased the intensity of colony labeling. However, there was sufficient endogenous ACP present to produce adequate colony labeling for routine screening. Two strains, LCH1 (aas-1) and LCH2 (aas-2), identified by this procedure had identical biochemical and physiological properties although strain LCH1 (aas-1) was used for most of the experiments in this report. Strain LCH1 did not have a growth phenotype under any of the standard laboratory culture conditions we employed. Filter disc screening of mutant colonies and assays of cell extracts were performed at 30 and 42 °C in order to recognize temperature-sensitive mutations in acyl-ACP synthetase; however, extracts from strains LCH1 and LCH2 lacked acyl-ACP synthetase activity at both 30 and 42 °C, and no temperature-sensitive mutants were found.

Biochemical Characterization of Strain LCH1—Membranes were isolated from strains UB1005 and LCH1 (aas-1) and the specific activities of 2-acyl-GPE acyltransferase and acyl-ACP synthetase were compared. Membranes from strain UB1005 had acyltransferase activity of 0.21 nmol/min/mg and synthetase activity of 20 pmol/min/mg. In contrast, strain LCH1 (aas-1) had <0.01 nmol/min/mg of acyltransferase activity and <0.1 pmol/min/mg of synthetase activity. The same results were obtained with strain LCH2 (aas-2) (not presented). The acyltransferase/synthetase was postulated to be responsible for the uptake and incorporation of fatty acids into PtdEtn in strains (fadD) lacking acyl-CoA synthetase activity (8). In a control experiment, strain LCH29 (fadD) incorporated exogenous [3H]palmitate exclusively into PtdEtn as anticipated (8). In contrast, strain LCH30 (aas-1 fadD) did not incorporate exogenous [3H]palmitate into PtdEtn (Fig. 1) or any other phospholipid class (not shown) illustrating that aas mutants lacked the acyl-CoA-independent cotransduction frequencies were converted to map distance using the formula of Wu (27).

FIG. 1. Strain LCH30 (aas-1 fadD) was unable to incorporate exogenous palmitic acid into PtdEtn. Strains LCH29 (fadD) and LCH30 (fadD aas-1) were grown in glycerol-minimal medium to a density of 3.2 × 10^8 cells/ml. [3H]Palmitic acid (25 μCi/ml) was added, and at the indicated times cells were harvested, extracted, and the amount of [3H]PtdEtn formed determined by thin-layer chromatography as described under "Experimental Procedures."
ent pathway for incorporation of fatty acids into phospholipid. A second physiological process ascribed to this enzyme system was the uptake and acylation of exogenous 2-acyl-lysophospholipids (9). This parameter was measured in aas mutants using exogenous 2-acyl-GPC as the lysophospholipid and [3H]palmitic acid (9). 2-Acyl-GPC uptake and acylation was easily detected in fadD mutants, but was absent in aas fadD double mutants (Table II). Phosphatidylethanolamine formation was also blocked in strain LCH1 (aas-1) which was capable of assimilating large amounts of exogenous fatty acid for phospholipid synthesis via the acyl-CoA-dependent (fadD) pathway (Table II), showing that the ACP-dependent acyltransferase/synthetase was the only mechanism for the uptake and acylation of 2-acyl-lysophospholipids in E. coli. These data illustrate that the aas mutation results in the concomitant loss of both 2-acyl-GPE acyltransferase and acyl-ACP synthetase activities in vitro and blocks the acyl-CoA-independent incorporation of exogenous fatty acids and lysophospholipids into the membrane in vivo.

Alterations in Phospholipid Composition Due to the aas Mutation—The presence of aas mutants did not cause a major change in the amounts of the major phospholipid species, but two minor phospholipid components accumulated in aas mutants. First, lysophosphatidylethanolamine content increased (Fig. 2) as was anticipated since this phospholipid is a substrate for the acyltransferase/synthetase. However, the largest difference between wild type and aas mutants was the accumulation of another phospholipid identified as acyl-PtdGro (Fig. 2). Acyl-PtdGro is formed by the action of lysophospholipase L2 which transfers the fatty acid from the 2-acyl-GPE to the headgroup glycerol of PtdGro (28, 29). Hydrolysis of 2-acyl-GPE to fatty acid and GPE is a second reaction catalyzed by lysophospholipase L2 (28). To confirm the identity of the phospholipid that accumulates in aas mutants, we examined the phospholipid composition of pldB mutants that lack lysophospholipase L2 activity (see Fig. 5). Strain LK29 (pldB12) did not contain detectable levels of acyl-PtdGro. Strain LCH66 (aas-1 pldB12) possessed elevated levels of lysophosphatidylethanolamine, but did not accumulate acyl-PtdGro (Fig. 3).

Protein Acylation in aas Mutants—In strains (fadD) lacking acyl-CoA synthetase activity, exogenous fatty acids were transported into the cell, esterified to PtdEtn, and then transferred to the amino terminus of lipoprotein (4, 5, 8). To clarify the role of aas in these steps, protein acylation by exogenous [3H]palmitate was examined in aas mutants carrying either the pKEN126 or pJG310 expression vectors (Fig. 4). In the control strains, SJ126 (fadD pKEN126) and SJ127 (fadD pJG310), fatty acids were incorporated into PtdEtn and efficiently transferred to both the lipoprotein (Lpp) and the lipoprotein-β-lactamase (Lpp-bla) fusion protein. In contrast, strains LCH31 (aas fadD pKEN126) and LCH22 (aas fadD pJG310) failed to incorporate exogenous [3H]palmitate into either phospholipids or lipoproteins. Strains LCH40 (aas fadD pKEN126) and LCH41 (aas fadD pJG310) were capable of uptake and esterification of exogenous fatty acids by the acyl-CoA-dependent pathway, and in these two strains both phospholipids and lipoproteins were labeled by exogenous [3H]palmitate. These data show that the acyltransferase/synthetase was required for incorporation of exogenous fatty acids into PtdEtn, but this enzyme system was not involved in the transacylation of fatty acids from PtdEtn to the lipoproteins.

Genetic Location of aas on the Bacterial Chromosome—the replica print method was used to localize the aas gene on the E. coli chromosome. Strain LCH1 was mated with a series of Hfr:Tn10 strains (14), and Tet recombinants were selected and assayed for acyl-ACP synthetase activity. These experiments localized the aas gene between 60 and 65 min of the chromosome. Next, a series of P1-mediated transduction experiments was employed using selectable markers in this region of the chromosome, and the gene was localized to 61 min (Fig. 5). To confirm the gene order in this region, a three-point transduction experiment was performed by infecting strain LCH1 (aas-1) with P1, phage grown on strain LCH47 (argA:Tn10 thyA). Tet recombinants (242) were selected and scored for thyA on plates and aas using the colony fluorography method. The results were: thyA− aas−, 19; thyA− aas−, 25; thyA− aas−, 8; and thyA− aas−, 190; confirming the gene order as argA-thyA-aas. We did not find a recombinant that was defective in acyl-ACP synthetase but not 2-acyl-GPE acyltransferase or vice versa supporting the concept that a single gene encodes both activities.

**DISCUSSION**

Our results provide additional support for the conclusion that 2-acyl-GPE acyltransferase and acyl-ACP synthetase are
2-Acyl-GPE Acyltransferase/Acyl-ACP Synthetase Mutants

FIG. 3. Phospholipid composition of aas and aas pldB mutants. Strains LK29 (pldB12) and strain LCH66 (aas-1 pldB12) were continuously labeled with [32P]orthophosphate during logarithmic growth in rich medium. Cells were harvested at a density of 1 × 10⁹ cells/ml, and the phospholipids were extracted and analyzed by two-dimensional thin-layer chromatography as described under "Experimental Procedures." A total of 7.2 × 10⁶ cpm was spotted onto each thin-layer plate, and both autoradiographs were exposed for 72 h.

FIG. 4. Lipoprotein acylation in aas mutants. Strains (LCH31, LCH40, LCH41, SJ126, and SJ127) were constructed with the indicated relevant genotypes harboring either the pKEn126 plasmid expressing lipoprotein (lpp) or the pLG310 plasmid expressing a fusion protein composed of the signal sequence and first nine amino acids of the lipoprotein gene fused to the β-lactamase gene (lpp-bla). In both cases, the expression of the acyl proteins was induced by IPTG. Strains were labeled with [3H]palmitic acid (25 μCi/ml) for 1 h either in the presence or absence of IPTG. Samples of total cell protein were fractionated using a 15% acrylamide SDS/urea gel and the radiolabeled components visualized by fluorography as described under "Experimental Procedures." Dual catalytic activities of the same protein. The first step in the acyltransferase catalytic cycle is the ATP-dependent ligation of a fatty acid to an enzyme-bound ACP subunit (Fig. 6). ACP is not covalently attached to the acyltransferase but is dissociated by high ionic strength conditions accounting for the acyl-ACP synthetase activity (7). The acyl-ACP intermediate is then transferred to 7-acyl-GPE to form lppEtn (Fig. 6). Physiological analysis of aas mutants confirms the role of 2-acyl-GPE acyltransferase/acyl-ACP synthetase in membrane phospholipid metabolism. First, this enzyme system is responsible for the uptake and incorporation of exogenous fatty acids into the 1-position of lppEtn (Fig. 6). There is no detectable phospholipid synthesis from exogenous fatty acids in aas fadD double mutants (Fig. 1), illustrating that the acyl-CoA synthetase (fadD) and acyl-ACP synthetase (aas) are components of the only two pathways for the uptake and incorporation of extracellular fatty acids in E. coli. Second, the inability of aas fadD and aas mutants to acylate exogenous fatty acids is associated with a single protein species (7). The aas mutants were selected solely on the basis of defective acyl-ACP synthetase activity; however, the mutants obtained lacked both acyltransferase and synthetase activities in vitro. The aas allele behaves as a single genetic locus in P1-mediated transductions and is located at min 61 of the chromosome (Fig. 5). Cloning and DNA sequencing of the aas locus will be the next step in verifying that a single structural gene encodes both activities.

Physiological role of 2-acyl-GPE acyltransferase/acyl-ACP synthetase in membrane phospholipid turnover and uptake. Exogenous fatty acids are first converted to acyl-CoA derivatives and then incorporated into PtdEtn by the de novo biosynthetic pathway. In fadD mutants, acyl-CoA formation is blocked revealing a mechanism for the incorporation of exogenous fatty acids into PtdEtn via the 2-acyl-GPE acyltransferase/acyl-ACP synthetase pathway. This pathway allows the incorporation of exogenous 2-acyl-lysophospholipids into the membrane. Lyso phospholipid acylation is blocked in aas mutants showing that there is no comparable acyl-CoA-dependent pathway for lyso phospholipid incorporation into the membrane. Double mutants (aas fadD) do not incorporate fatty acids into any phospholipid species.

FIG. 5. Genetic map location of the aas gene. Arrangement of chromosomal markers and the aas locus in the 61-min region of the genetic map deduced from P1-mediated transductions (arrows) and three-point crosses (see text).
lysophospholipids (Table II) shows that the acyltransferase/synthetase pathway is the only route for the esterification of extracellular lysophospholipids. Third, the fact that acyl-GPE and acyl-PtdGro accumulate in aas mutants (Figs. 2 and 3) indicates that the resynthesis of PtdEtn is the primary metabolic fate of 2-acyl-GPE. The possibility that the acyltransferase may also be directly involved in transferring fatty acids to the amino terminus of the lipoprotein is ruled out by our experiments (Fig. 4). Although lipoprotein acylation by exogenous fatty acids is blocked in aas fadD double mutants, lipoprotein acylation does occur in aas mutants via the acyl-CoA synthetase (fadD)-dependent incorporation of exogenous fatty acids into PtdEtn which are then transferred to the lipoprotein (Fig. 4).

The aas mutation does not have a major effect on membrane phospholipid composition, but the minor alterations observed point to the function of the acyltransferase/synthetase system and lysophospholipase L₀ in membrane phospholipid metabolism (Fig. 7). 2-Acyl-GPE acyltransferase appears to be the most economical mechanism for scavenging 2-acyl-GPE generated from phospholipid turnover, and the accumulation of acyl-GPE in aas mutants indicates that the acyltransferase/synthetase is the most active pathway under normal growth conditions. However, E. coli inner membranes also contain a specific phospholipase that either degrades 2-acyl-lysophospholipids to fatty acid and GPE or catalyzes the transfer of fatty acids to PtdGro to form acyl-PtdGro (15, 28, 29). This route for the catabolism of 2-acyl-GPE provides an alternate mechanism to prevent 2-acyl-GPE from accumulating.

The observation that acyl-PtdGro accumulates in aas mutants demonstrates that the lysophospholipase L₀ pathway is significant in the absence of acyltransferase/synthetase activity. Blocking the lysophospholipase L₀ route in pldB mutants does not lead to a large increase in acyl-GPE accumulation (Fig. 3) indicating that there are other enzymes that catalyze 2-acyl-GPE. A membrane-associated transacylase activity has been characterized that converts two 2-acyl-GPEs to PtdEtn (11) and GPE (10), and there is a second lysophospholipase located in the soluble fraction (30). Both of these activities are distinct from the pldB gene product (14), and the role of these enzymes in controlling 2-acyl-GPE levels is not clear. Although lysophospholipids are potent detergents and would be expected to disrupt membrane structure if present in high concentrations, the lack of a growth phenotype in aas mutants illustrates that there are multiple mechanisms to prevent the accumulation of lysophospholipids and that the elevated levels of acyl-GPE observed in these strains are not deleterious.

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