Characterization of Escherichia coli Cells Deficient in 1-Acyl-sn-glycerol-3-phosphate Acyltransferase Activity*

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A mutant of Escherichia coli K-12 defective in 1-acyl-sn-glycerol-3-phosphate acyltransferase has been isolated. At the permissive temperature for growth, 30 °C, 20% of the total cellular glycerophospholipids is 1-acyl-sn-glycerol-3-phosphate, as identified by mass spectral analysis and proton NMR. This percentage of 1-acyl-sn-glycerol-3-phosphate rises to about 30% when the temperature of the culture is shifted to 42 °C. This increase is primarily at the expense of phosphatidylethanolamine. Extracts from cells harboring the plsc mutation have no detectable 1-acyl-sn-glycerol-3-phosphate acyltransferase activity. The fatty acid composition of the accumulated 1-acyl-sn-glycerol-3-phosphate is about 60% cis-vaccenate and 40% palmitate, with no detectable amounts of palmitoleate or other fatty acids, consistent with the known fatty acid composition of the sn-1 position of glycerophospholipids.

The isolation of this gene, plsc, completes the list of genes known to be required for the synthesis of the major glycerophospholipids in E. coli.

Escherichia coli phospholipid biosynthesis mutations have been isolated and characterized in nearly every step in the pathways. These mutations are proving invaluable in the study of phospholipid biosynthesis, including the regulation of head group composition and fatty acid composition.

Escherichia coli carefully regulates the composition of its membrane glycerophospholipids. To be a biologically functional membrane, the membrane phospholipids must be at the boundary of the crystalline-fluid phase transition. The membrane is nonfunctional if all the lipids are in the ordered state or if they are all in the disordered state. There is a wide variation in fluidity that is tolerated; however, there does seem to be an optimal fluidity at which cell growth is more rapid (Cronan and Rock, 1987).

The tight mutations in phospholipid biosynthetic enzymes, in most cases, have resulted in cell death or arrest of cell growth (Cronan and Rock, 1987). Significant changes in phospholipid composition, in most cases, cause marked changes in the physiology of the cell.

In the present study, I characterize the effects of a mutation in the gene thought to encode the enzyme responsible for the second step in phospholipid biosynthesis, 1-acyl-sn-glycerol-3-phosphate acyltransferase (1-acyl-G-3-P acyltransferase). This enzyme can utilize either acyl-CoA or acyl-acyl carrier protein to donate a fatty acid to 1-acyl-G-3-P (Rock et al., 1981). This enzyme is thought to determine the temperature-dependent incorporation of saturated and unsaturated fatty acids at the sn-2 position of glycerophospholipids. Unlike the sn-1 fatty acid which is used as a fatty acid donor for lipoproteins (Jackowski and Rock, 1986) and lipopolysaccharide (Brozek et al., 1987), the sn-2 position fatty acid is stable, and there are no other known mechanisms for fatty acid addition to the sn-2 position, as there are for the sn-1 position (Hummel et al., 1981). Although much work has been done to characterize the sn-glycerol-3-phosphate acyltransferase and other phospholipid biosynthetic enzymes (Scheideler and Bell, 1989; Ray et al., 1970; Cronan and Rock, 1987), very little work has been done to characterize 1-acyl-G-3-P acyltransferase. The isolation of a mutation in the gene for 1-acyl-G-3-P acyltransferase, plsc, will enable us to better understand the functions of this enzyme and its involvement in the control of fatty acid composition.

EXPERIMENTAL PROCEDURES

Materials—[32P]Orthophosphate was obtained from Du Pont-New England Nuclear. Palmitoyl-CoA, fatty acid-free bovine serum albumin, and phospholipids were from Sigma. Silica Gel 60 plates (0.25 mm) were purchased from E. Merck, Darmstadt, Germany. Silica Gel was Bio-Sil A from Bio-Rad. Yeast extract and tryptone used in LB medium were obtained from Difco. Bicinchoninic acid was from Research Products International Corp. Bicinchoninic acid (BCA) was from Pierce Chemical Co.

Growth of Bacteria—Bacterial strains used are listed in Table I. Bacteria were grown in LB or M9 medium (Miller, 1972), supplemented with tetracycline (30 μg/ml) or streptomycin (100 μg/ml).

Gene Mapping—P1 transduction (Silhavy et al., 1984), Hfr and F' matings (Miller, 1972), and ColEl matings (Raetz et al., 1977) were done as previously described.

Quantitation of Lipid—Lipid analysis and phospholipid biosynthesis—Lipopoly saccharide and glycerophospholipid biosyntheses were quantitated as described (Galloway and Raetz, 1990).

Isolation of Unknown Lipid—To isolate sufficient quantities of the unknown lipid for NMR analysis and protein assays, strain 2-1 was grown at 30 °C in 1 liter of LB medium until late log phase of growth. This culture was added to an equal volume of LB at 30 °C. The culture was then transferred to a 42 °C water bath, and growth was continued for 2 h. Cells were harvested by centrifugation at 5000 × g, for 20 min. Total phospholipids were extracted under acidic Bligh-Dyer (Nishijima and Raetz, 1979) conditions as described earlier (Brozek et al., 1989). Pyridine was added at 1/5 volume, and the solvents were removed by rotary evaporation at 30 °C. The residue was dissolved in 10 ml of chloroform:methanol:water (26:10:1) and applied to a 20-ml silicic acid column (Bio-Sil A) equilibrated in the

1 The abbreviations used are: 1-acyl-G-3-P, 1-acyl-sn-glycerol-3-phosphate; PG, phosphatidylglycerol; PE, phosphatidylethanolamine.
same buffer. The column was chromatographed using the same solvant. Fractions (5 ml) containing the unknown lipid (detected by thin-layer chromatography and sulfuric acid charring) were pooled and dried by rotary evaporation. The residue was redissolved in chloroform:methanol:(28%) ammonia (13:5:1) and applied to a 10-ml silicic acid column (Bio Sil A) equilibrated in the same buffer. The column was washed with 75 ml of chloroform:methanol:(28%) ammonia and the unknown lipid eluted with chloroform:methanol:acetic acid:water (65:25:4:2). The position of the unknown lipid, which was separated on Silica Gel 60 TLC plates developed in chloroform:methanol:acetic acid:water (65:25:4:2). Fractions (5 ml) containing the unknown lipid were removed by centrifugation, and the glycerophospholipids were dried by rotary evaporation. The residue was redissolved in chloroform:methanol (25:15:4:2). The substrate, l-acyl-G-3-P, and the product, phosphatidic acid, were located by autoradiography, scraped from the plate, and counted in scintillation fluid.

**Phospholipid Analysis**—Cells were labeled with 32P, in LB medium as indicated. Incorporation of label was terminated by addition of 0.8 ml of culture to 3.0 ml of chloroform:methanol (1:2, v/v). The debris was removed by centrifugation, and the glycerophospholipids were extracted by the acidic two-phase partition of Bligh and Dyer (1959) as modified by Nishijima and Raetz (1979). A portion of the extract was analyzed by two-dimensional Silica Gel 60 thin layer chromatography (TLC) developed first in chloroform:methanol:water (65:25:4), dried, then developed in chloroform:methanol:acetic acid (65:25:10). The positions of the phospholipids were identified by autoradiography, the radioactive spots were scraped from the plate, and the radioactivity was determined by scintillation counting in Bioskim II scintillation mixture.

### RESULTS

**Isolation of Membrane Defect Mutants**—In order to obtain a mutant defective in membrane biosynthesis, E. coli strain SM105 was treated with nitrosoguanidine, and membrane synthesis mutants were selected by the method of Tsuruoka et al. (1988), by sedimentation through sucrose. Of the colonies isolated, 77% (18 of 25) were temperature-sensitive for growth, and 72% (13 of 18) of the temperature-sensitive colonies were sensitive to 0.2% deoxycholate in the growth medium. A disruption of the outer membrane of Gram-negative bacteria is often indicated by a sensitivity to deoxycholate (Raetz and Foulds, 1977; Misra and Benson, 1988; Hirota et al., 1977). One temperature-sensitive, deoxycholate-resistant strain, 2-1, was selected for further study.

**Initial Characterization of the Mutant Strain**—E. coli strain 2-1 carries a mutation, designated *pisc*, which causes the cells to accumulate on unidentified phospholipid, especially at 42 °C. Mutant (2-1) and wild-type (SM105) cells were pulse-labeled with [32P]orthophosphate to determine if this lipid is a normal precursor of the glycerophospholipids and not re-
Phage lysates prepared on the donor strains were used to transduce the recipient strain, SM2-1 (plc, metC::Tn10). Selection was for met+.

- **TABLE II**

<table>
<thead>
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<th>Donor</th>
<th>Distribution of markers</th>
<th>Number</th>
<th>Percent</th>
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<tr>
<td>SO1023 (nupG)</td>
<td>met+ nupG plcC</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>met+ nupG plcC+</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>met+ nupG+ plcC</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>met+ nupG+ plcC*</td>
<td>113</td>
<td>70</td>
</tr>
<tr>
<td>CS1562 (tolC)</td>
<td>met+ tolC plcC</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>met+ tolC plcC+</td>
<td>14</td>
<td>33</td>
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<tr>
<td></td>
<td>met+ tolC plcC*</td>
<td>14</td>
<td>33</td>
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Fig. 1. Genetic map of *E. coli* K12 (64- to 66-min segment). Locations are calculated by the transduction frequencies shown in Table II and from Kato et al. (1988).

Fine mapping of **plcC** was accomplished using three-factor crosses with plcC, metC (65 min), and tolC (66 min) or nupG (64 min) using P1 transduction indicates that plcC maps very close to metC (71% co-transduction).

Three-factor cross of **plcC**, **metC**, and **tolC** or **plcC**, **metC**, and **nupG**. Phage lysates prepared on the donor strains were used to transduce the recipient strain, SM2-1 (plc, metC::Tn10). Selection was for met+.

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<td>met+ tolC plcC+</td>
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<tr>
<td></td>
<td>met+ tolC plcC*</td>
<td>14</td>
<td>33</td>
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**1-H NMR Spectroscopy of the Unknown Lipid**—As a first step in determining the structure of the unknown lipid, a 1-mg sample (prepared on a large scale as described under "Experimental Procedures") was subjected to 1-H NMR analysis at 500 MHz (Fig. 2). The lipid was prepared as a pyridinium salt, and it was dissolved in CDC13:CD30D (2:1, v/v). The spectra were run at 20 °C. As expected, several characteristic features of a glycerophospholipid spectrum were identified in the spectrum of the unknown (Fig. 2). These features include the methylene groups of the fatty acyl chain at 1.25 ppm, the protons of the terminal methyl groups resonating at 0.8 ppm, and the protons at the sn-1 position of a glycerol of a 1-acyl-glycerophospholipid at 4.1 ppm (if a fatty acid is attached to the sn-2 position, a peak would be expected at about 5.1 ppm (Kates, 1986)). The triplet at 2.3 ppm is characteristic of the protons on carbon 2 of fatty acid, and the resonance at 1.5 ppm is a feature of fatty acyl protons on the third carbon. Indeed, if the resonance at 1.5 ppm is selectively decoupled, the triplet of 2.3 ppm collapses to a singlet, as expected for a fatty acid (data not shown).

The triplet at 5.3 ppm is characteristic of protons attached to unsaturated carbons in a fatty acid. The methylene protons adjacent to the double-bonded carbons would be expected to resonate at approximately 1.9 ppm (Kates, 1986). As can be...
seen in Fig. 2, there is a resonance at this position. As expected, when the resonance at 1.9 ppm was selectively decoupled, the triplet at 5.3 collapsed to a singlet (data not shown).

To determine if the unknown compound resonates in the region of 4.3 ppm, which is obscured by the H$_2$O peak in Fig. 2, the spectra of the unknown was analyzed in CDCl$_3$:CD$_3$OD (4:1, v/v) at 37 °C, which shifted the water peak to 4.0 ppm. No additional resonances were observed in this spectrum (data not shown).

FAB Mass Spectrometry of the Unknown Lipid—To better identify the unknown lipid produced by strain 2-1, the purified lipid (see "Experimental Procedures") was subjected to fast atom bombardment mass spectrometry in the negative mode (Fig. 3). The two apparent mass peaks (M – H)$^-$, differing by 26 mass units, at m/z 409 ± 1 and 435 ± 1 (Fig. 3) indicate that the sample contains two species. These values of (M – H)$^-$, m/z of 435 ± 1 and 409 ± 1, are consistent with 1-acyl-G-3-P containing cis-vaccenate or palmitate, respectively. The expected atomic mass (M – H)$^-$ of 1-cis-vaccenoyl-sn-glycerol-3-phosphate is 435.52, and the expected atomic mass (M – H)$^-$ of 1-palmitoyl-sn-glycerol-3-phosphate is 409.48.

Cronan and Vagelos (1972) have shown that in normal E. coli, that palmitic acid is found almost exclusively at position 1 of glycerophospholipids, cis-vaccenic acid is found at positions 1 and 2, and palmitoleic acid is found almost exclusively at position 2. As expected for a strain of E. coli defective in the ability to add a fatty acid to position 2 of sn-glycerol-3-phosphate, only trace amounts of 1-palmitoleoyl-sn-glycerol-3-phosphate, atomic mass (M – H)$^-$ of 407.47, is visible by mass spectral analysis (Fig. 3). Taken together with the results of the proton NMR, the unknown lipid can be identified as being primarily 1-acyl-G-3-P containing palmitate or cis-vaccenate as the fatty acid. Both the mass spectral data and the NMR spectra indicate that about 60% of the 1-acyl-G-3-P molecules contain cis-vaccenate.

In the pathway of synthesis of the major phospholipids in E. coli (Cronan and Rock, 1987), 1-acyl-G-3-P is the substrate for the second reaction, the addition of a fatty acid to 1-acyl-G-3-P. This reaction is catalyzed by 1-acyl-G-3-P acyltransferase.

Determination of the Activity of 1-Acyl-G-3-P Acyltransferase—To demonstrate that the defect in strain 2-1 is due at least in part to 1-acyl-G-3-P acyltransferase, the activity of the acyltransferase was quantitated in vitro as described under "Experimental Procedures." Membrane extracts were isolated from strains grown at the permissive temperature (28 °C). For membrane extracts of wild-type strain SM105, the assay was linear with both time and protein concentration, in the concentrations and times used. Fig. 4 (lane 3) demonstrates that even if the concentration of protein in the extract is increased...
A 1-Acyl-sn-glycerol-3-phosphate Acyltransferase Mutant 17219

[Image 0x1 to 588x807]

FIG. 6. Analysis of phospholipids produced by SM105 and 2-1. The lipids were isolated as described under "Experimental Procedures" and separated using two-dimensional TLC. The first dimension (ascending) was developed using chloroform:methanol:water (65:25:4), and the second dimension (right to left) was developed using chloroform:methanol:acetic acid (65:25:10). The phospholipids were visualized by autoradiography, which was carried out for 12 h at -70 °C with Kodak XAR film. A, cells, growing in LB medium, were pulse-labeled with 32P (50 μCi/ml) for 5 min after a 1 h shift to 42 °C. Panel a, SM105 (plsc+). Panel b, 2-1 (plsc−). B, cells were continuously labeled with 32P. Cells were grown at 30 °C overnight with 32P in LB medium, then diluted in the same labeled medium and grown for several generations at 30 °C, followed by a 2-h shift to 42 °C. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; PA, phosphatidic acid; LPA, 1-acyl-G-3-P; ?, unknown hydrophobic lipid.

### TABLE III

<table>
<thead>
<tr>
<th>Strain</th>
<th>PE</th>
<th>PG</th>
<th>1-Acyl-G-3-P</th>
<th>Other Hydrophobic</th>
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</thead>
<tbody>
<tr>
<td>SM105 30 °C</td>
<td>62.0</td>
<td>19.5</td>
<td>0.2</td>
<td>18.3</td>
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<tr>
<td>SM105 42 °C</td>
<td>67.0</td>
<td>22.1</td>
<td>0.2</td>
<td>10.6</td>
</tr>
<tr>
<td>2-1 30 °C</td>
<td>36.3</td>
<td>9.9</td>
<td>37.5</td>
<td>16.3</td>
</tr>
<tr>
<td>2-1 42 °C</td>
<td>0.3</td>
<td>5.5</td>
<td>75.7</td>
<td>18.5</td>
</tr>
</tbody>
</table>

Steady state composition

SM105     70.5  22.1   0.3     6.9     0.1
2-1       32.1  16.2   28.8    20.0    1.9

10-fold and the specific activity of the 1-[32P]acyl-G-3-P is increased 6-fold, extracts from 2-1 do not catalyze the addition of a palmitate from palmitoyl-CoA to 1-acyl-G-3-P. This demonstrates a greater than 100-fold reduction in 1-acyl-G-3-P acyltransferase activity in strain 2-1 as compared to SM105. No activity could be detected from extracts of strain 2-1 under all conditions tested, including longer incubations and lower temperatures. The specific activity of 1-acyl-G-3-P acyltransferase in the wild-type cells was 5 nmol/min/mg of protein, in good agreement with previously published values (Ray et al., 1970). At the concentration of extract shown in Fig. 4, greater than 80% of the 1-acyl-G-3-P is converted to phosphatidic acid using the wild-type extract of SM106 (Fig. 4, lane 1). There is no conversion in the absence of acyl donor (Fig. 4, lane 2). Similar results were obtained with the isogenic pair JC200 (plsc+) and JC201 (plsc−). In addition, if JC201 harbors the plasmid pLC4-14 (plsc+), the specific activity of the 1-acyl-G-3-P acyltransferase increases to greater than 50-fold over wild-type activity (>26 nmol/min/mg of protein).

Growth and Viability of the plsc Mutant—To determine whether the plsc mutation caused cell death under nonpermissive conditions, or simply arrested growth of the cells, JC201 (plsc−) and its isogenic wild-type strain JC200, growing exponentially at 30 °C in LB medium, were shifted to 42 °C. The optical density of the culture, as measured by a Klett-Summerson colorimeter, plateaued 90 min after the shift and remained level for at least 7 additional h (Fig. 5A). However, cell viability immediately plateaued for about 5 h, at which point cell viability decreased dramatically, as judged by plating efficiency on LB agar at 30 °C (Fig. 5B). At 30 °C, the growth rate of the plsc mutant cells is approximately 60% the rate of the isogenic wild-type cells. It should be noted that even at 30 °C as the plsc strain starts to reach stationary phase, the cell viability decreases.

When cells are observed under the light microscope, there is no visible change in cell morphology of the plsc cells (JC201) for 2 h after shift to 42 °C. After 2 h at 42 °C, some filaments do start to form. This phenotype of formation of filaments under nonpermissive growth conditions is common to phospholipid biosynthetic mutants (Raetz, 1976; Hawrot and Kennedy, 1978). At no time is there evidence of cell lysis in the plsc mutant cells (data not shown).

Phospholipid Analysis of plsc Cells under Different Growth Conditions—The isolation of a severe mutation in 1-acyl-G-3-P acyltransferase raised several important questions. Are cells able to survive with significant amounts of 1-acyl-G-3-P in their membrane? As the concentration of 1-acyl-G-3-P in the membrane is increased, do cells compensate for this by a
change in the composition of other phospholipids synthesized? Is the synthesis of other nonstandard phospholipids stimulated by the accumulation of 1-acyl-G-3-P?

To answer these questions, the phospholipid composition of the cells was analyzed after separation of the lipids on two-dimensional thin layer chromatography (TLC). To determine the rate of synthesis of each of the phospholipids, cells were pulse-labeled with $^{32}$P for 5 min either at 30°C or after 1 h at 42°C. Phospholipids were extracted and separated as described under “Experimental Procedures.” Fig. 6A shows a typical autoradiogram of phospholipids labeled at 42°C and separated on two-dimensional TLC. As can be seen in panel b, pLS mutant cells (2-1) produce a large amount of 1-acyl-G-3-P, and the rate of incorporation of phosphorus into phosphatidyethanolamine (PE) is decreased dramatically when compared to phosphatidylglycerol (PG). Quantitation of the radioactive spots is shown in Table III. As can be seen in Table III, 37.5% of the phosphorus in the phospholipids synthesized at 30°C in strain 2-1 is 1-acyl-G-3-P. However, the ratio of PE to PG remains at near normal levels. This accumulation of 1-acyl-G-3-P at 30°C reflects the lack of in vitro activity of the 1-acyl-G-3-P acyltransferase even at low temperatures, as mentioned above. At 42°C, the amount of 1-acyl-G-3-P synthesized increases to 75.7% of the total phosphorus incorporated into phospholipid, with a dramatic decrease in PE biosynthesis. It should be noted that the rate of synthesis of PG also decreases, but not to the same extent.

The steady state phospholipid compositional analysis of the pLS strain 2-1 was accomplished with a steady state labeling of cells with $^{32}$P. Cells were grown for several hours at 30°C, then shifted to 42°C for 2 h. Phospholipid analysis was done as described under “Experimental Procedures.” Wild-type cells (SM105) accumulated nearly undetectable amounts of 1-acyl-G-3-P; however, the mutant cells produced a large amount (29.8% of the total cellular phospholipid). The ratio of PE to PG also decreases when the steady state lipids are measured. As can be seen in Fig. 6B in addition to 1-acyl-G-3-P, a new very hydrophobic lipid is detected in the pLS strain that is only found in very small amounts in the wild-type strain. This new lipid was not detected in pulse-labeled cells, either at 30°C or 42°C. Similar results were obtained using the isogenic pair JC200 (pLS) and JC201 (pLS+).

**DISCUSSION**

Previously, mutations had been obtained in each step of the synthesis of the major phospholipids except for the second step, the acylation of 1-acyl-G-3-P to form phosphatic acid. Studies using these mutations have greatly increased our understanding of how cells regulate the composition of their membrane lipids. These mutant strains have also aided in our understanding of the effects of certain membrane perturbations. The identification of the gene for 1-acyl-G-3-P acyltransferase in this work completes the list of genes known to be required for the synthesis of the major phospholipids of *E. coli.*

The mutation in the pLS gene slows down the synthesis of PG, PE, and cardiolipin, particularly at the nonpermissive temperature for growth (42°C). As synthesis of the more common phospholipids ceases, there is an increase in concentration, up to 30% of the total lipids, of a new lipid identified by mass spectral and NMR analysis to be 1-acyl-G-3-P. This high concentration of a precursor lipid at the time of cell death is similar to the accumulation of phosphatic acid in the CDP-diglyceride synthetase mutant (cds-8; Ganong and Raetz, 1982). Also like the cds mutation, the pLS mutation causes an increase in the apparent rate of synthesis of PG relative to PE, although this effect is much more pronounced in the pLS mutation. Unlike the cds mutant, this apparent increase in the concentration of PG relative to PE is observed in the final lipid composition at the time of cell death. This apparent increase in the rate of synthesis of PG may reflect the greater turnover of the glycerol head group, which is used in the synthesis of membrane-derived oligosaccharides (vanGolde et al., 1973) and lipopolysaccharides (Wu et al., 1983). The cause of the chemical accumulation of PG is unknown, but may reflect a control by the cell, triggered by the large accumulation of the detergent-like molecule 1-acyl-G-3-P.

Ballesta and Schaechter (1971) in their studies on the synthesis of phospholipids in *E. coli* after the removal of nutrients from the growth medium concluded that synthesis of PE is decreased under conditions of increased membrane synthesis, and the apparent increased rate of synthesis of PG is not related to growth and cell division, but presumably to other processes that are carried out by nongrowing metabolically active cells. Because the pLS cells are no longer growing at 42°C, this may explain the increase in the ratio of PG to PE. However, Ballesta and Schaechter (1971) only examined the rate of incorporation of a precursor into the phospholipids, which does not necessarily reflect the actual lipid concentration.

The loss in activity of 1-acyl-G-3-P acyltransferase appears to be immediate after the shift to 42°C, as evidenced by the lack of increase in optical density within 1 h of the shift. Although the cells no longer continue to grow, they are still viable for 3 h after the shift to 42°C. The fact that the cells are still viable 2 h after the shift to the nonpermissive temperature is unexpected because at this point 30% of the total phospholipids present is the detergent 1-acyl-G-3-P.

The isolation of a conditional lethal mutation in 1-acyl-G-3-P acyltransferase confirms the assumption that this enzyme is distinct from the sn-glycerol-3-phosphate acyltransferase responsible for the addition of a fatty acid to the sn-1 position of a phospholipid. Cessation of growth in this mutant could result from the inability of the cell to make lipids, preventing membrane growth. Alternatively, a change in the composition of the membrane may render an essential membrane protein inactive. On the other hand, the accumulation of 1-acyl-G-3-P, a detergent-like molecule, may itself adversely affect membrane function and prove lethal to the cell. The resolution of such questions requires a detailed study of membrane function under conditions in which the lipid composition is carefully regulated. This mutation should prove useful in the study of the regulation of head group composition and fatty acid composition.

**REFERENCES**


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