Alteration of Fatty Acid Composition in a pgsA3 Mutant of Escherichia coli

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We examined the fatty acid composition in an Escherichia coli pgsA3 mutant lacking the potential to synthesize phosphatidylglycerolphosphate, a precursor of phosphatidylglycerol. The contents of C18:1cis–9 (oleic acid) and C18:1cis–11 (cis-vaccenic acid) in the total phospholipids extracted from the pgsA3 mutant growing at 37°C were higher and that of C14:0 was lower than the wild type cells, resulting in a higher level of unsaturation of fatty acids (ratio of unsaturated fatty acids to saturated ones) in the mutant. The higher level of the unsaturated fatty acids in the pgsA3 mutant was more obvious in cardiolipin than in phosphatidylethanolamine. On the other hand, at 28°C, at which the pgsA3 mutant shows limited cell growth, the content of unsaturated fatty acids in cardiolipin decreased in the pgsA3 mutant compared with the wild type. We consider that the pgsA3 mutant maintains cellular homeostasis by altering the level of unsaturated fatty acids in cardiolipin, and the mechanism is influenced by temperature.

Key words fatty acid composition; phosphatidylglycerol; Escherichia coli

Phosphatidylglycerol (PG) is an abundant phospholipid in Escherichia coli (E. coli) cell membranes.1) The pgsA gene is responsible for the synthesis of phosphatidylglycerolphosphate, a precursor required for PG synthesis.2) Since deletion of the pgsA gene causes a lethal phenotype, PG is essential for cell growth.3,4) The manner in which PG participates in cell growth, however, has remained unclear. Based on various biochemical findings, we proposed that the activity of DnaA protein, the initiation factor of chromosomal DNA replication in E. coli, is regulated by acidic phospholipids, such as PG and cardiolipin (CL).5–7) This speculation was supported by recent genetic studies.8) We also showed that the growth of temperature-sensitive dnaA mutants is more sensitive to various reagents interacting with phospholipids than that of wild type cells at permissive temperatures, suggesting the in vivo interaction of DnaA protein with membrane phospholipids.9,10) To understand the role of PG in cell proliferation, a study on the adaptation of cells to a lack of PG synthesis may provide useful information.

Shibuya and colleagues demonstrated that a strain lacking the lpp gene, which encodes a major outer membrane protein, grows normally at 37°C with a pgsA3 mutation, which causes a very small content of PG in cell membranes.11,12) It is well known that bacterial cells alter their fatty acid composition in order to maintain membrane homeostasis. For example, the content of unsaturated fatty acids in cell membranes becomes higher at lower temperatures.13) This phenomenon is thought to be an adaptation of cells in order to maintain membrane fluidity. Thus, we considered the possibility that the pgsA3 mutant adapts itself to the lack of PG synthesis through an alteration of its fatty acid composition. In this study, we investigated the influence of the pgsA3 mutation on the composition of fatty acids. The results suggest that the mutant cells adapt to insufficient PG synthesis by increasing the content of unsaturated fatty acids in CL.

MATERIALS AND METHODS

E. coli Strains JE5513 (Hfr man–l pps [pp–2], JE5513Tc (JE5513 uvrC279::Tn10), and YA5513Tc (JE5513Tc pgsA3) were kindly provided by Dr. Shibuya (Saitama University).11,12)

Analysis of Fatty Acid Composition Exponentially growing E. coli cells in LB medium were harvested by centrifugation when the optical density at 600 nm reached 0.5. The total lipids were extracted by the Bligh-Dyer Method14 and applied to an SPE cartridge (NH2-phase) to separate fraction I containing phosphatidylethanolamine (PE) and fraction II containing CL and PG.15) Fraction II was applied to an SPE cartridge (SI phase), and CL and PG were eluted in this order with a solvent consisting of a mixture of chloroform and methanol (9:1) and then acetic acid of different concentrations (0–25%). The phospholipids were esterified with 14% trifluorooboron in anhydrous methanol, and their fatty acid contents were analyzed by gas chromatograph. The apparatus used was a Hewlett Packard 5890A gas chromatograph with a capillary column (0.25 mm i.d.×15 m, 0.25 μm, Supelcowax 10, Supelco Inc., Bellefonte, U.S.A.) and a hydrogen flame ionization detector. The column was operated at 160°C for 20 min, and the temperature was raised by 4°C/min to 190°C for 30 min. Helium was used as the carrier gas at a flow rate of 27 cm/s. The injection volume was 2 μl (split ratio 1:50).

RESULTS

Phospholipid Composition in the pgsA3 Mutant As shown in Table 1, the PE: CL: PG ratios of the mutant and the wild type strains growing at 37°C were 91:6:3 and 72:8:20, respectively. These results are consistent with those reported in the literature.16) Similar results were also obtained for the composition of phospholipids in both strains growing at 28°C (Table I).

Fatty Acid Composition in the pgsA3 Mutant We de-
determined the composition of fatty acids in the total phospholipids and in each phospholipid (PE, CL) in the pgsA3 mutant and wild type cells growing at 37 °C, at which the mutant shows the normal growth phenotype.12) The contents of C18:1cis-9 (oleic acid) and C18:1cis-11 (cis-vaccenic acid) were higher, and that of C14:0 was lower in the pgsA3 mutant than in the wild type cells (Fig. 1A). We determined the level of unsaturation of fatty acids (the ratio of unsaturated fatty acids to saturated ones) in the mutant and wild type cells. As shown in Table 2, the level of unsaturation of fatty acids in the total phospholipids extracted from the mutant growing at 37 °C was 30% higher than that extracted from the wild type cells.

Table 1. Composition of Phospholipids in pgsA3 Mutant and Wild Type Cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth temperature (°C)</th>
<th>Phospholipid (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PE</td>
</tr>
<tr>
<td>JE5513Tc (Wild type)</td>
<td>37</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>YA5513Tc (pgsA3)</td>
<td>37</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
</tr>
</tbody>
</table>

Exponentially growing cells were centrifuged when the optical density at 600 nm reached 0.5. After extraction of the total phospholipids, individual phospholipids were separated by solid phase extraction. Their contents were then calculated from the fatty acid content measured by the capillary GC method as described under Materials and Methods.

The analysis of the fatty acid composition of each phospholipid revealed that the influence of the pgsA3 mutation on the fatty acid composition is different between CL and PE. For example, the C18:1cis-11 content of PE in the mutant growing at 37 °C was higher, whereas that in CL was lower, than its content in the wild type cells (Figs. 1B and 1C). The content of C18:1cis-9 in CL was markedly higher in the pgsA3 mutant growing at 37 °C, but not in PE in comparison with the wild type cells. As a result, the increase in the level of unsaturation of fatty acids caused by the pgsA3 mutation is more apparent in

Table 2. Level of Unsaturation of Fatty Acids in pgsA3 Mutant and Wild Type Cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phospholipid</th>
<th>Growth temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>37 °C</td>
</tr>
<tr>
<td>JE5513Tc (Wild type)</td>
<td>Total</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>PE</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>CL</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>PG</td>
<td>0.80</td>
</tr>
<tr>
<td>YA5513Tc (pgsA3)</td>
<td>Total</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>PE</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>CL</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>(PG)</td>
<td>(1.03)</td>
</tr>
</tbody>
</table>

After separation of each phospholipid, the content of fatty acids was determined and the level of unsaturation of fatty acids (ratio of unsaturated fatty acids to saturated ones) (mol/mol) was calculated. The values for PG in the pgsA3 mutant are given as reference values because of low content.

![Fig. 1. Fatty Acid Composition in pgsA3 Mutant and Wild Type Cells](image-url)

Extraction of the total phospholipids and purification of each phospholipid were performed as described under Materials and Methods. The fatty acids content in the total phospholipid (A), PE (B), and CL (C) was determined (See Materials and Methods). The composition of each fatty acids is shown as a relative value to the total content of fatty acids.
CL than in PE (Table 2).

Effect of Temperature on Fatty Acid Composition in the pgsA3 Mutant

It was reported that the pgsA3 mutation caused cold-sensitive growth when it was introduced into the SD9 strain (pgs-1, cls). In this study, we examined the growth of YA5513Tc (pgsA3, JE5513Tc) at both 37°C and 28°C and found that the mutant showed a slow-growth phenotype at 28°C but not at 37°C, compared with the wild type cells (data not shown). It is well known that bacterial cells adapt themselves to changes in incubation temperature by altering their fatty acid composition. Thus, we posed the question of whether or not the pgsA3 mutant is able to alter its fatty acid composition at 28°C. The level of unsaturation of fatty acids in the total phospholipids from the wild type cells was higher at 28°C than at 37°C, whereas the levels were the same at 28°C and 37°C in the pgsA3 mutant (Table 2). This is caused by a decrease in the content of C16:1 and a smaller increase in the content of C18:1cis-11 at the lower incubation temperature in the mutant (Fig. 1A).

Analysis of the fatty acid composition of each phospholipid at 28°C revealed that the effect of the pgsA3 mutation on the fatty acid composition of CL is different from that on PE. The most distinct difference is that the contents of C18:1cis-9 and C18:1cis-11 in PE were increased by the mutation, whereas those in CL were decreased or unchanged (Figs. 1B and 1C). As a result, at 28°C the level of unsaturation of fatty acids in CL was drastically decreased by the mutation, while the level in PE slightly increased (Table 2).

DISCUSSION

In this report, we examined the influence of the pgsA3 mutation on the composition of fatty acids in phospholipids. At 37°C, at which the mutant shows a normal growth phenotype, the mutation increases the level of unsaturation of fatty acids, especially in CL, suggesting that the mutant has adapted itself to the lack of PG synthesis by increasing the content of unsaturated fatty acids in CL. Compared with 37°C, the content of unsaturated fatty acids in CL decreased at 28°C in the pgsA3 mutant, whereas it increased in the wild type cells. The pgsA3 mutant shows a slow-growth phenotype at 28°C. Thus, the pgsA3 mutant seems to be unable to adapt itself to low temperatures, resulting in a restricted growth phenotype. Recently, it was reported that the lethal phenotype resulting from deletion of the pgsA gene is suppressed by the rhnA mutation, which induces stable DNA replication. Since stable DNA replication does not require the function of DnA protein, this result suggests that PG plays an essential role in the initiation of DNA replication in vivo through regulation of the activity of DNA protein. A high level of unsaturation of fatty acids is essential for activation of DNA protein by acidic phospholipids in vitro. Therefore, we consider that acidic phospholipids containing unsaturated fatty acids are necessary for the activity of DNA protein in vivo.

It was reported that the pgsA3 mutant shows an immotile phenotype, which is caused by a decrease in the promoter activity of the flhD gene, the master operon for flagella synthesis. The immotile phenotype and decreased expression of the flhD gene in the pgsA3 mutant are obvious at 28°C, but not at 37°C. This is consistent with our findings that the pgsA3 mutation caused an increase in the content of CL containing unsaturated fatty acids at 37°C but not at 28°C (Table 2). Therefore, we consider the possibility that the immotile phenotype of the mutant is caused by its low content of acidic phospholipids containing unsaturated fatty acids. Regarding the mechanism of how acidic phospholipids containing unsaturated fatty acids are involved in flagellation, we consider the involvement of DNA binding proteins, such as DnaA protein and topoisomerase I. These proteins bind to acidic phospholipids, and this interaction requires unsaturated fatty acids in the acidic phospholipids.

Acknowledgments

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REFERENCES