Suppression of the Lethal Effect of Acidic-Phospholipid Deficiency by Defective Formation of the Major Outer Membrane Lipoprotein in Escherichia coli

YOHKO ASAI, YUICHI KATAYOSE,† CHINAMI HIKITA,‡ AKINORI OHTA, AND ISAO SHIBUYA*
Department of Biochemistry, Saitama University, Urawa 338, Japan

Received 30 June 1989/Accepted 20 September 1989

Escherichia coli strains harboring the pgsA3 allele encode a defective phosphatidyglycerophosphate synthase is lethal for all but certain strains. Genetic analysis of such strains has revealed that the lethal effect is fully suppressed by the lack of the major outer membrane lipoprotein that consumes phosphatidylglycerol for its maturation.

Escherichia coli strains harboring the pgsA3 allele that encodes a defective phosphatidyglycerophosphate synthase have drastically reduced contents of acidic phospholipids but grow normally, except in certain media with very low osmotic pressures (20). However, we have noticed (21) that this mutation is not transferable by P1 transduction to strains other than those of the same genetic background as that of the original isolate (SD strains [20, 26]). Heacock and Dowhan also observed the lethal nature of this allele for all but SD strains and reported the absolute lethality of a null mutation in the pgsA gene (6). We therefore questioned whether the SD strains carry an unidentified mutation(s) that enables the cells to tolerate the pgsA3 allele, which is otherwise lethal. Here we report that the pgsA3 mutation is fully suppressed phenotypically by defects in the formation of the major outer membrane lipoprotein (3) present in the SD strains.

Bacterial strains used are listed in Table 1. Culture media used included LB (19), NBY (22, 26), and LBLS (same as LB except 0.5% instead of 1% NaCl). P1 vir transduction (19), transformation with plasmids (5), and recombinant DNA experiments (18) were carried out essentially as described elsewhere. The pgsA alleles were routinely examined by colony autoradiography of phosphatidylylycerophosphate synthase activity (24), and quantitative assay of the synthase was carried out for crude membrane fractions (23, 26). The amount of the free form of the major lipoprotein was estimated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8) of the 2% Triton X-100-insoluble envelope fractions (25), followed by staining with Coomassie brilliant blue and scanning in a densitometer (15).

Various E. coli strains were first examined for the ability to accept the pgsA3 allele by P1 transduction with a donor strain harboring both the pgsA3 allele and a nearby tetracycline (Tc) resistance marker (zed-508::Tn10). No pgsA3 mutants were obtained from the Tc transductants selected on LBLS plates with recipients of various genetic backgrounds other than SD strains, except for a single transductant with strain Hfr3000 (data not shown). The presence of the cls-1 allele in the recipient and glycerol in selective media, which were expected to cause a decrease in the consumption (26) and an increase in the synthesis of phosphatidylglycerol in certain strains (13), respectively, did not affect the results. Therefore, we assumed that SD strains and the pgsA3 transductant from Hfr3000 contained a mutation(s) that phenotypically suppressed the pgsA3 allele.

Although the putative suppression mutation in SD strains had no phenotype, the new mutant from Hfr3000 was auxotrophic for aromatic amino acids. Strain CH104 was an F' strain constructed by standard transduction and mating procedures to harbor this mutation in addition to various genetic markers (Table 1) and was used to characterize the suppression phenomenon by classical genetic methods. The mutation was recessive and mapped at around minute 37 on the chromosome, and we did not obtain its spontaneous revertants (less than 1/10⁶). Although it failed to complement the aroD6 mutation, the aroD6 allele did not suppress the pgsA3 phenotype. We therefore suspected that the suppressive mutation that originally emerged in the Hfr3000 transductant was a deletion that included aroD and/or nearby aroH and the gene responsible, upon mutation, for the suppression.

lpp, the structural gene for the major outer membrane lipoprotein, is located in this aroD region on the chromosome (1). In fact, the free form of this lipoprotein was not seen in the outer membrane fractions from all strains that tolerated the pgsA3 allele (Fig. 1), contrary to our previous idea based on a preliminary analysis (20). Globomycin sensitivity (11, 14) was consistent with this result: all strains lacking the free-form lipoprotein bands shown in Fig. 2 and pgsA3 mutants SD103 and SD312 were resistant to 50 μg of globomycin per ml, whereas all strains that were unable to fully tolerate the pgsA3 allele (Table 2) were sensitive to this inhibitor of signal peptidase II (9, 11). An lpp-2 mutant, JE5513T, tolerated the pgsA3 mutation, as did strains SD12T and CH104 (Table 2). Strain E610 harboring the lpp-3 allele also yielded pgsA3 transductants that grew equally normally. The lpp-3 (or lppD14, formerly mlpA) allele encodes a defective lipoprotein that is not modified with glycerol from phosphatidylglycerol because of a single amino acid replacement (Gly-14→Asp) (16). From strains CH105 (lpp") and JE5512T (lpp"), however, emerged pgsA3 transductants (Table 2) that grew weakly (Fig. 2). In this experiment, numbers of Thy" transductants were also scored to evaluate the frequencies of transduction to Tc'. Repeated transduction experiments with thymine prototrophs gave essentially the same results. The lpp" pgsA3 transductants grew only in NBY medium supplemented with high concentrations of

* Corresponding author.
† Present address: Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan.
‡ Present address: Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.
TABLE 1. *E. coli* K-12 strains used

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| SD12  | F− lpp-12(λ) galK35 gplD3 gplK glyK glyK pgr2his-68 phoA8 pyrD34 rpsL118 | 26; this work |
| SD103 | SD12 cis-l pgsA3 pss-l(Ts) | 20 |
| SD312 | SD103 his+ pps+ | 20 |
| Hfr3000 | Hfr thi-1 relA spoT1 supQ80 | CGSC\(^c\) |
| N3024 | uvrC279::Tn10 | CGSC |
| CH104 | F− Δ(lpp-aro) galK35 his-68 malA1 mgt-2 gyrA pyrD34 rpsL118 thyA trp-45 xyl-7 | This work |
| CH105 | CH104 aro+ lpp+ | P1(Hfr3000) × CH104 |
| YA104 | CH104 pgsA3 uvrC279::Tn10 | P1(SD103Tc) × CH104 |
| YA105 | CH105 pgsA3 uvrC279::Tn10 | P1(SD103Tc) × CH105 |
| JE5512 | Hfr man-l pps | 7; S. Mizushima |
| YA5512 | JE5512 pgsA3 uvrC279::Tn10 | P1(SD103Tc) × JE5512 |
| JE5513 | JE5512 lpp-2 (formerly lpo) | 7; S. Mizushima |
| YA5513 | JE5513 pgsA3 uvrC279::Tn10 | P1(SD103Tc) × JE5513 |
| E610 | Hfr lpp-3 (lppD14, formerly mlpA) pps | 27 |

* For simplicity, various thyA and uvrC279::Tn10 derivatives constructed in the present work are not listed in this table. They were obtained by trpE prophage selection and P1 transduction with a uvrC279::Tn10-harbouring strain, N3024 or SD103Tc as the donor, respectively, and are designated in the text and source or reference column of this table by adding suffixes T and Tc, respectively, to strain names.

\(^b\) The lpp-12 mutation was identified in this work.

\(^c\) CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

NaCl or in LB. Such culture conditions are known to suppress the formation of membrane-derived oligosaccharides (12) that also consume phosphatidylglycerol. These transductants lost viability quickly when stored in the cold. The reason for the emergence of weakly growing cells only from certain strains is not clear. Their genotypes (lpp\(^+\) pgsA3) were confirmed by in vitro assay of phosphatidylglycerophosphate synthase, by transduction to pgsA3\(^+\) followed by lipoprotein analysis, and by globomycin sensitivity.

We have further confirmed that the defect in the lpp gene is responsible for the suppression phenomenon by transforming lpp mutants with an lpp-bearing plasmid. The plasmid used here was a derivative of pSCL101 which carried a 1.5-kilobase-pair kanamycin resistance gene and the 2.8-kilobase-pair chromosomal lpp region of pKEN111 (15), but this lpp had a spontaneous promoterlike mutation to reduce

![FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the 2% Triton-insoluble fractions. Cells in the early stationary growth phase were analyzed for the free-form major outer membrane lipoprotein (bands indicated by an arrow) as described in the text. All strains analyzed had the wild-type pgsA allele. Lanes: 1, JE5512 (lpp\(^+\)); 2, JE5513 (lpp-2); 3, CH105 (lpp\(^+\)); 4, CH104 (Δlpp); 5, Hfr3000 (lpp\(^+\)); 6, SD12 (lpp-12).]

![FIG. 2. Growth characteristics of various lpp and pgsA mutants in NBY medium with different concentrations of NaCl. Cells grown to the early stationary phase in LB were transferred at time zero to NBY medium (147 mosmol/kg) (A and D) or to NBY supplemented with 80 mM NaCl (B and E) or with 320 mM NaCl (C and F). All media contained, in addition, 80 μg of thymine per ml. Growth curves were obtained by monitoring the optical density at 650 nm in a Bioscanner (model OT-BS-12; Ohtake-seisakusho, Tokyo, Japan). (A through C) Symbols: ○, JE5512Tc (lpp\(^+\) pgsA\(^+\)); ●, JE5513Tc (lpp-2 pgsA\(^+\)); Δ, YA5512 (lpp\(^+\) pgsA3); ▲, YA5513 (lpp-2 pgsA3). (D through F) Symbols: ○, CH105Tc (lpp\(^+\) pgsA\(^+\)); ●, CH104Tc (Δlpp pgsA\(^+\)); Δ, YA105 (lpp\(^+\) pgsA3); ▲, YA104 (Δlpp pgsA3).]

the overall expression to about 1.1-fold that of the wild-type allele on the chromosome (Y. Asai and I. Shibuya, unpublished data). Strain JE5513 (lpp-2 pgsA\(^+\)) gave kanamycin-resistant transfectants at a frequency of 4.2 × 10\(^{6}\)μg of DNA, but no transformant was obtained from strains YA5513 (Δlpp pgsA3) and SD312 (lpp-12 pgsA3). The plasmid vector without the lpp gene gave kanamycin-resistant transfectants at normal frequencies (10\(^{5}\) to 10\(^{7}\)μg of DNA) for all strains tested.

The present observations indicate that the pgsA3 mutation damages cells seriously, if not lethally; that its harmful effect is fully tolerated when the major outer membrane lipoprotein is deficient; and that a growth condition under which phosphatidylglycerol is expected to be saved by the reduction of

![TABLE 2. Full suppression of the pgsA3 mutation by defects in the major outer membrane lipoprotein\(^a\)].

| Recipient lpp allele | No. of transductants\(^a\) | pgsA3\(^+\)/Tc\(^c\) |
|---------------------|--------------------------|-----------------|
| SD12T | lpp-12 | 29 | ND | 25/26 |
| CH104 | Δlpp | 121 | 460 | 113/115 |
| JE5513Tc | lpp-2 | 197 | ND | 48/50 |
| E610 | lpp-3 | 184 | ND | 46/50 |
| Hfr3000Tc | Wild type | 8 | 97 | 0/8 |
| CH105 | Wild type | 13 | 230 | (2)/9 |
| JE5512Tc | Wild type | 154 | 356 | (13)/140 |

\(^a\) P1 transduction using strain SD103Tc (pgsA3 uvrC279::Tn10) as the donor. Selective plates were LB supplemented with tetracycline and 80 μg of thymine per ml and minimal A with required nutrients except for thymine. 

\(^b\) Number of tetracycline-resistant (Tc\(^+\)) and thymine-protoprotrophic (Thy\(^+\)) transductants formed from the same adsorption mixtures. ND, Not determined.

\(^c\) Number of pgsA3 transductants, as judged by colony autoradiography, per number of Tc\(^+\) transductants examined. Numbers in parentheses indicate the unusually weakly growing transductants described in the text.
membrane-derived oligosaccharides synthesis (12) also has a partial suppressive effect.

The major outer membrane lipoprotein consumes 1 mol of phosphatidylglycerol for its maturation (4) and is by far the most abundant protein of the cell (2, 10). Therefore, we have two alternative working hypotheses for the harmful effect of the pgsA3 mutation and the suppression by lipoprotein deficiency. (i) There are important membrane functions that specifically depend on phosphatidylglycerol and/or cardiolipin, which are not sufficiently formed in pgsA3 mutants but are saved for their essential purposes by the lipoprotein deficiency. (ii) A reduction of phosphatidylglycerol in pgsA3 mutants causes a harmful accumulation of unprocessed prolipoprotein in the cytoplasmic membrane. In both hypotheses, reduction of phosphatidylglycerol consumption to form membrane-derived oligosaccharides would lower the lethal effect of the pgsA3 mutation. Although the former hypothesis seems more likely, since a diglyceride-free mutant prolipoprotein in lpp-3 mutants neither accumulates much in the cytoplasmic membrane (16, 17) nor damages the cell growth (14, 27) and an lpp-3 pgsA3 double mutant grew normally, the molecular basis for the lethal effect of the pgsA3 allele remains to be experimentally determined.

We thank Shoji Mizushima for strains JES512 and JES513 and help in electrophoretic analysis of the lipoprotein, Barbara J. Bachmann for supplying us with many E. coli K-12 strains, Masayori Inouye for plasmid pKEN111, Henry C. Wu for strain E610, and Mamoru Arii for globomycin. We also thank Nobuyuki Uetake and Tomoko Kuroiwa for experimental help and Hiroshi Matsuaki for general help during the work.

This work was supported by grants-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED
1. Bachmann, B. J. 1983. Linkage map of Escherichia coli K-12, edition 7. Microbiol. Rev. 47:180–230.
2. Braun, V. 1975. Covalent lipoprotein from the outer membrane of Escherichia coli. Biochim. Biophys. Acta 415:335–377.
3. Braun, V., and K. Behn. 1969. Chemical characterization, spatial distribution and function of a lipoprotein (murein-lipoprotein) of the E. coli cell wall. The specific effect of trypsin on the membrane structure. Eur. J. Biochem. 10:426–438.
4. Chattopadhyay, P. K., and H. C. Wu. 1977. Biosynthesis of the covalently linked diglyceride in murein lipoprotein of Escherichia coli. Proc. Natl. Acad. Sci. USA 74:5318–5322.
5. Chung, C. T., S. L. Niemela, and R. H. Miller. 1989. One-step preparation of competent Escherichia coli: transformation and storage of bacterial cells in the same solution. Proc. Natl. Acad. Sci. USA 86:2172–2175.
6. Heacock, P. N., and W. Dowhan. 1987. Construction of a lethal mutation in the synthesis of the major acidic phospholipids of Escherichia coli. J. Biol. Chem. 262:13044–13049.
7. Hirota, Y., H. Suzuki, Y. Nishimura, and S. Yasuda. 1977. On the process of cellular division in Escherichia coli: a mutant of E. coli lacking a murein lipoprotein. Proc. Natl. Acad. Sci. USA 74:1417–1420.
8. Hussain, M., S. Ichihara, and S. Mizushima. 1980. Accumulation of glyceride-containing precursor of the outer membrane lipoprotein in the cytoplasmic membrane of Escherichia coli treated with globomycin. J. Biol. Chem. 255:3707–3712.
9. Hussain, M., S. Ichihara, and S. Mizushima. 1982. Mechanism of signal peptide cleavage in the biosynthesis of the major lipoprotein of the Escherichia coli outer membrane. J. Biol. Chem. 257:5177–5182.
10. Inouye, M., J. Shaw, and C. Shen. 1972. The assembly of a structural lipoprotein in the envelope of Escherichia coli. J. Biol. Chem. 247:8154–8159.
11. Inukai, M., M. Takeuchi, K. Shimizu, and M. Araki. Mechanism of action of globomycin. J. Antibiot. 31:1203–1205.
12. Kennedy, E. P. 1982. Osmotic regulation and the biosynthesis of membrane-derived oligosaccharides in Escherichia coli. Proc. Natl. Acad. Sci. USA 79:1092–1095.
13. Kobayashi, T., A. Ohta, and I. Shibuya. 1986. Membrane phospholipid synthesis in Escherichia coli: alteration by glycerol and physiological consequences in a pss mutant. J. Biochem. 99:1393–1400.
14. Lai, J.-S., W. M. Philbrick, S. Hayashi, M. Inukai, M. Araki, Y. HirotA, and H. C. Wu. 1981. Globomycin sensitivity of Escherichia coli and Salmonella typhimurium: effects of mutations affecting structures of murein lipoprotein. J. Bacteriol. 145:657–660.
15. Liu, N., K. Nakamura, and M. Inouye. 1981. Expression of the Serratia marcescens lipoprotein gene in Escherichia coli. J. Bacteriol. 146:861–866.
16. Lin, J. J. C., H. Kanazawa, J. Ozols, and H. C. Wu. 1978. An Escherichia coli mutant with an amino acid alteration within the signal sequence of outer membrane lipoprotein. Proc. Natl. Acad. Sci. USA 75:4891–4895.
17. Lin, J. J. C., H. Kanazawa, and H. C. Wu. 1980. Assembly of outer membrane lipoprotein in an Escherichia coli mutant with a single amino acid replacement within the signal sequence of prolipoprotein. J. Bacteriol. 141:550–557.
18. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. Miyazaki, C., M. Kuroda, A. Ohta, and I. Shibuya. 1985. Genetic manipulation of membrane phospholipid composition in Escherichia coli: pgsA mutants defective in phosphatidylglycerol synthesis. Proc. Natl. Acad. Sci. USA 82:7530–7534.
21. Nishijima, S., Y. Asami, N. Uetake, S. Yamagoe, A. Ohta, and I. Shibuya. 1988. Disruption of the Escherichia coli cld gene responsible for cardiolipin synthesis. J. Bacteriol. 170:775–780.
22. Ohta, A., and I. Shibuya. 1977. Membrane phospholipid synthesis and phenotypic correlation of an Escherichia coli pss mutant. J. Bacteriol. 133:443–447.
23. Ohta, A., K. Waggner, A. Radominska-Pyrek, and W. Dowhan. 1981. Cloning of genes involved in membrane lipid synthesis: effects of amplification of phosphatidylglycerophosphate synthase in Escherichia coli. J. Bacteriol. 147:552–562.
24. Raetz, C. R. H. 1975. Isolation of Escherichia coli mutants defective in enzymes of membrane lipid synthesis. Proc. Natl. Acad. Sci. USA 72:2274–2278.
25. Schmidt, C. A. 1971. Effect of ethylenediaminetetraacetic acid, Triton X-100, and lysozyme on the morphology and chemical composition of isolated cell walls of Escherichia coli. J. Bacteriol. 108:553–563.
26. Shibuya, I., C. Miyazaki, and A. Ohta. 1985. Alteration of phospholipid composition by combined defects in phosphatidylserine and cardiolipin synthases and physiological consequences in Escherichia coli. J. Bacteriol. 161:1086–1092.
27. Yen, D. W., and H. C. Wu. 1978. Physiological characterization of an Escherichia coli mutant altered in the structure of murein lipoprotein. J. Bacteriol. 133:1419–1426.