JOURNAL OF BACTERIOLOGY, Sept. 1981, p. 1121-1124
0021-9193/81/091121-04$02.00/0

Vol. 147, No. 3

Effects of Unsaturated Fatty Acids on the Morphogenesis of an Unsaturated Fatty Acid Auxotroph of *Escherichia coli*

YOSHITAKA KURIKI

Institute for Protein Research, Osaka University, Suita, Osaka, Japan

Received 27 January 1981/Accepted 6 June 1981

Supplementation of linoleate or linolenate in a culture medium caused abnormal morphogenesis in an unsaturated fatty acid auxotroph of *Escherichia coli* K-12.

Unsaturated fatty acid auxotrophs of *Escherichia coli* incorporate various unsaturated fatty acids into their membrane phospholipids. These mutants, therefore, provide a convenient tool for studies of the effects of the unsaturated fatty acid composition of membrane lipids on cell physiology (2, 11). This communication reports that the cell shape of an unsaturated fatty acid auxotroph of *E. coli* was markedly altered when grown in a medium supplemented with linoleate or linolenate. It is also reported that this morphological alteration was accompanied by an increase in a penicillin-binding protein and changes in the composition of membrane proteins.

E. coli strain O1e-28E$_1$ (5), an unsaturated fatty acid auxotroph, was grown in a medium supplemented with several unsaturated fatty acids, and the cell shape was observed by phase-contrast microscopy. Cells grown on oleate exhibited the normal rodlike shape at the logarithmic growth phase (Fig. 1B) and were elongated to form filaments upon entering the stationary phase (Fig. 1F). Since cells of the wild-type strain (W3110) were also elongated at the stationary phase, the elongation phenomenon was not unique for strain O1e-28E$_1$. With elaidate as the supplement, mutant cells also assumed rodlike and filamentous forms at the logarithmic and stationary phases, respectively (Fig. 1A and E). Anomalous morphogenesis was observed when the medium was supplemented with linoleate or linolenate. Linoleate-grown mutant cells were arc shaped and S shaped at the logarithmic phase (Fig. 1C). When elongated at the stationary phase, filamentous cells were hooked and sometimes formed loops (Fig, 1G). In linolenate-supplemented medium, arc-shaped cells also appeared at the logarithmic phase (Fig. 1D) and formed spirals upon elongation (Fig. 1H). About 80% of linoleate- and linolenate-grown cells showed the abnormal shapes. When rod-shaped cells growing on oleate were transferred to linolenate medium, the rod-to-arc morphological change was observed after a period corresponding to more than two generations, suggesting that morphogenesis induced by unsaturated fatty acids was a much slower process than induction of enzymes such as β-galactosidase by appropriate inducers (9).

Since the shape of *E. coli* cells is maintained by a rigid cage of peptidoglycan (16), and penicillin-binding proteins (PBPs) are involved in the synthesis of peptidoglycan (1), it was of interest to compare the contents of PBPs in cell envelopes prepared from cells grown under differing conditions. For this purpose, the binding of benzyl[^{14}C]penicillin to the envelope preparations was determined. Cell envelopes derived from oleate- and elaidate-grown cells bound benzyl[^{14}C]penicillin to almost the same extent as those from wild-type cells grown in the presence of any of the unsaturated fatty acids (Table 1). The binding capacities of the cell envelopes derived from linoleate- and linolenate-grown cells were 30 to 50% higher than those of wild-type cell envelopes. The PBPs that had bound benzyl[^{14}C]penicillin were then solubilized from the envelopes and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12) with the aid of fluorography (6). All the preparations gave seven PBP bands, a number in agreement with previous observations for the wild-type strain (Fig. 2) (13). A notable finding was that the band corresponding to PBP 6 was definitely denser in preparations derived from the linoleate- and linolenate-grown cells than in those derived from the oleate- and elaidate-grown cells. It was clear that the increased levels of PBP 6 were responsible for the higher penicillin-binding capacities of the envelopes from linoleate- and linolenate-grown cells. An attempt was then made to examine the structure of peptidoglycan by determining cross-linkage by the

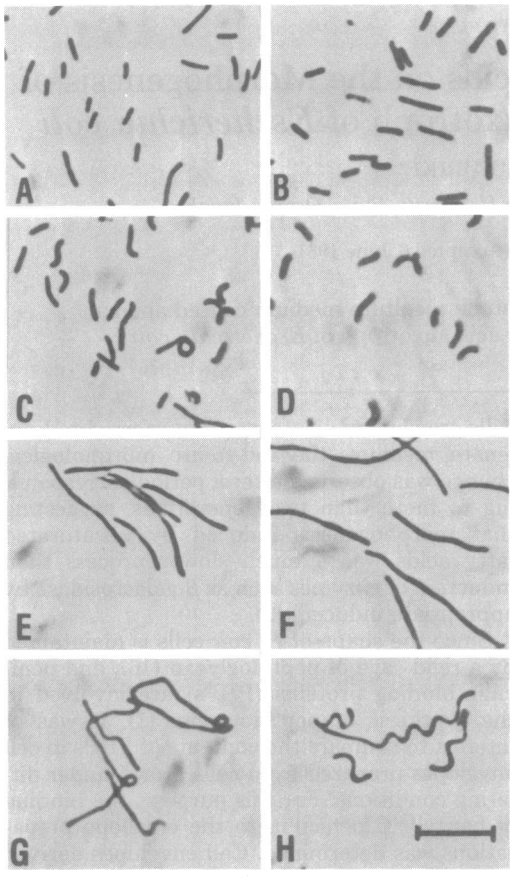

FIG. 1. *Cell shapes of E. coli strain O1e-28E$_1$ grown in the presence of various unsaturated fatty acids. The mutant strain, derived from E. coli K-12 strain W3110, was grown at 37°C with gentle shaking in medium E (15) supplemented with 0.5% glycerol, 0.1% Triton X-100, 0.2% Casamino Acids, and 0.01% unsaturated fatty acid (5). The doubling time under the growth conditions was 74 min in the oleate-containing medium, and the growth rate did not significantly differ depending on the unsaturated fatty acid supplemented. One drop of the culture was placed on a slide glass, covered with a cover slip, and photographed under phase-contrast conditions with a Nikon microscope. Cells were grown with elaidate (A and E), oleate (B and F), linoleate (C and G), and linolenate (D and H). A through D were taken at the middle logarithmic phase, and E through H were taken at the early stationary phase. The reference line in H represents 10 μm.*

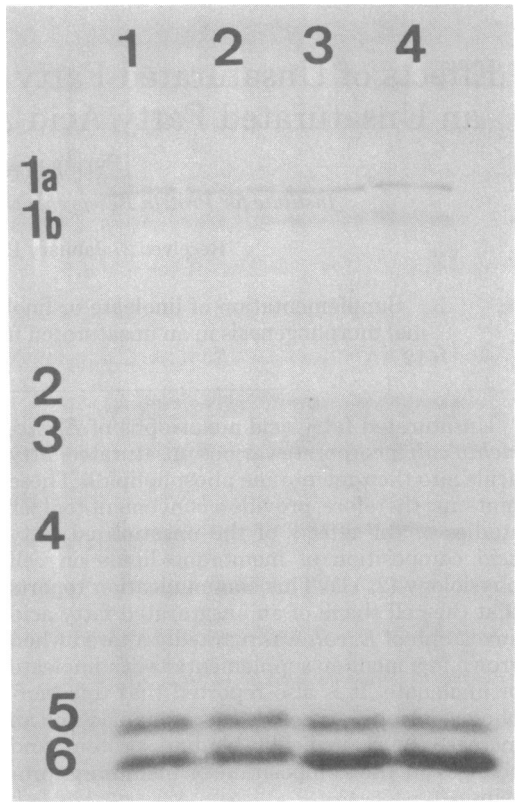

FIG. 2. *Analysis of PBPs in cell envelopes from E. coli strain O1e-28E$_1$ grown in the presence of various unsaturated fatty acids. Cells were grown, cell envelopes were prepared, and benzyl[^{14}C]penicillin-binding experiments were conducted as described in the legend of Table 1. PBPs labeled with benzyl[^{14}C]-penicillin were then isolated by the method of Spratt and Pardee (12), and a portion (7 μl) of the preparation was subjected to sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (14). Radioactive bands on the gel were detected by fluorography (6). PBPs are numbered in accordance with the method of Spratt and Pardee (12). PBPs were from cells grown with elaidate (lane 1; 420 cpm); oleate (lane 2; 460 cpm); linoleate (lane 3; 660 cpm); and linolenate (lane 4; 670 cpm).*

method of Kamiryo and Strominger (4). Peptidoglycan preparations prepared from cells grown on the four unsaturated fatty acids were found to be cross-linked to practically the same extent (data not shown). The possibility cannot be excluded that structural differences other than cross-linkage existed among the peptidoglycan preparations examined.

During the analysis of PBPs, it was found that the electrophoresis patterns of the proteins from the envelopes were altered by the supplemented unsaturated fatty acids. As indicated with triangles in Fig. 3, the alterations were detected both in inner and outer membrane fractions, which were conveniently isolated by Sarkosyl treatment of the envelopes. It was notable that the intensities of the bands, represented by closed triangles, increased in preparations from linoleate- and linolenate-grown cells, but not in those derived from oleate- and elaidate-grown

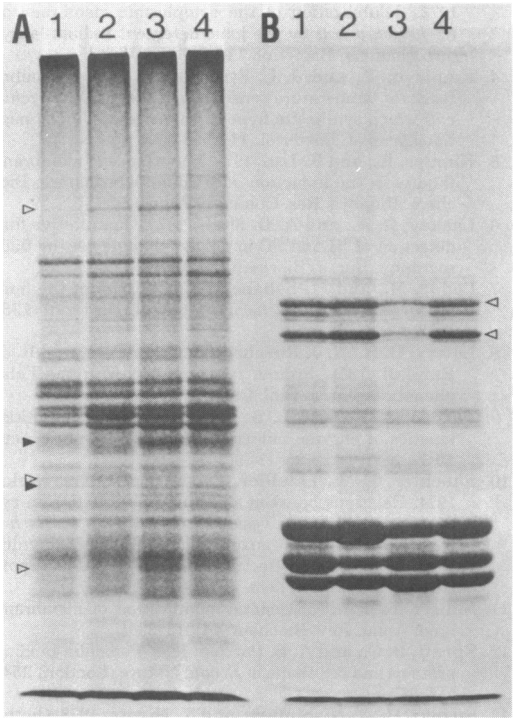

FIG. 3. *Electrophoresis patterns of membrane proteins from E. coli O1e-28E$_1$ grown in the presence of various unsaturated fatty acids. The cell culture and preparation of the cell envelopes were performed as described in the legend of Table 1. To solubilize the proteins constituting the inner membrane, a portion (0.3 ml) of the cell envelope preparation was incubated with 30 μl of 20% Sarkosyl at 23°C for 30 min, as described previously (3). After centrifugation at 100,000 × g for 60 min, 0.2 ml of the supernatant was mixed with 40 μl of 0.25 M Tris-hydrochloride (pH 6.8) containing 5% sodium dodecyl sulfate, and 25% glycerol, 0.7 M β-mercaptoethanol, and 0.05% phenol red. The mixture was boiled for 3 min and used as the preparation of inner membrane proteins. The precipitate after removal of the inner membrane proteins was suspended in 0.3 ml of 50 mM Tris-hydrochloride (pH 6.8) containing 1% sodium dodecyl sulfate, 0.4 M β-mercaptoethanol, 10% glycerol, and 0.01% phenol red and then incubated at 100°C for 3 min to solubilize the proteins present in the outer membrane. Insoluble material was removed by centrifugation at 100,000 × g for 60 min. The supernatant was used as the outer membrane protein preparation. These preparations were applied to the sodium dodecyl sulfate-polyacrylamide gel (14), and the proteins on the gel were stained with Coomassie brilliant blue R-250. In comparison with the electrophoresis patterns of the preparations derived from the oleate-grown cells, triangles indicated the protein bands which were altered by supplementation of different unsaturated fatty acids. Closed triangles show the protein bands altered in common by supplementation of linoleic acid and linolenic acid. (A) Proteins from the inner membranes (ca. 70 μg); (B) proteins from the outer membranes (ca. 35 μg). Lanes represent cells that were grown with elaidate (lane 1); oleate (lane 2); linoleate (lane 3); and linolenate (lane 4).*

cells. Although several other altered bands were observed (Fig. 3), reproducibility was poor in repeated experiments. The alterations may not have been due to the effects of supplementation of different unsaturated fatty acids.

The results presented above indicate clearly that the morphology of *E. coli* O1e-28E$_1$ cells was affected by unsaturated fatty acids supplemented in the medium. Abnormal morphogen-

TABLE 1. *Benzyl[^{14}C]penicillin binding to cell envelopes from E. coli strains W3110 (wild type) and O1e-28E$_1$ grown in the presence of various unsaturated fatty acids*[a]

Unsaturated fatty acid supplemented with:	Radioactivity bound (cpm/mg of protein)		
	Strain W3110	Strain O1e-28E$_1$	
		Expt 1	Expt 2
Elaidate	4,721	4,656	4,224
Oleate	4,460	4,700	4,653
Linoleate	4,518	6,200	6,648
Linolenate	4,790	5,934	6,745

[a] Each strain was grown in 500 ml of the medium (cf. the legend of Fig. 1) supplemented with the indicated unsaturated fatty acid until the optical density of the culture was about 0.92. The cells were harvested, washed with 10 mM sodium phosphate buffer (30 ml; pH 7.0), and stored at −70°C (wet weight, ca. 0.95 g). The frozen cells were suspended in the phosphate buffer (12.5 ml), sonicated in an ice bath for 4 min, and centrifuged at 1,000 × g for 10 min. The pellet was suspended in the phosphate buffer (5 ml), sonicated, and centrifuged as described above. The supernatants derived from the two centrifugations were combined and further centrifuged at 100,000 × g for 30 min to precipitate the cell envelope fraction. The resultant pellet was washed twice with the phosphate buffer (25 ml). Mild sonication was used to facilitate suspension of the pellet, and centrifugation was at 100,000 × g for 30 min. The cell envelope preparation thus obtained was suspended to a protein concentration of about 23 mg/ml in 50 mM sodium phosphate buffer (pH 7.0) containing 10 mM $MgCl_2$. A portion (0.3 ml) of the preparation was incubated with 90 μM benzyl[^{14}C]penicillin (58 Ci/mol; Radiochemical Centre, Amersham, England) at 30°C for 10 min, as described by Spratt and Pardee (12). The reaction mixture (10 μl) was diluted with 90 μl of water and applied to a paper disk. The disk was washed three times with 5% trichloroacetic acid, twice with ethanol-ether (2:1), and once with ether. The radioactivity retained on the dried paper disk was measured in a liquid scintillation spectrometer. Protein was determined by the method of Lowry et al., with bovine serum albumin as the standard (8).

esis of linoleate- and linolenate-grown cells was accompanied by an increased level of PBP 6 and detectable alterations in the composition of membrane proteins. Nothing is known at present of the mechanisms underlying these changes. Since most unsaturated fatty acids taken up by the cells exist as the acyl moieties of membrane phospholipids (7), it is conceivable that the state of the membrane is responsible for the observed anomalies. However, the fluidity of the membrane does not seem to be a major factor, because it has been reported that membrane lipids of *E. coli* cells are mostly in the fluid state under conditions similar to those employed in the present study (10). A possible clue to the problem may be the fact that oleate and elaidate, which do not cause abnormal morphogenesis, are both C_{18} acids containing only one double bond, whereas linoleate and linolenate, which induced anomalies, are C_{18} acids containing two and three double bonds, respectively. It is of interest that the unusual *trans* configuration of elaidate did not affect the morphogenesis of the mutant cells. The relationship between the observed changes in membrane proteins, including PBP 6, and cell shape is now under investigation.

We thank R. Sato for encouragement during this study and for critical review of the manuscript, and K. Izui for the strains of *E. coli* used in this study.

LITERATURE CITED

1. **Blumberg, P. M., and J. L. Strominger.** 1974. Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. Bacteriol. Rev. **38**:291-335.
2. **Cronan, J. E., Jr.** 1978. Molecular biology of bacterial membrane lipids. Annu. Rev. Biochem. **47**:163-189.
3. **Filip, G., G. Fletcher, J. L. Wulff, and C. F. Earhart.** 1973. Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium-lauryl sarcosinate. J. Bacteriol. **115**:717-722.
4. **Kamiryo, T., and J. L. Strominger.** 1974. Penicillin-resistant temperature-sensitive mutants of *Escherichia coli* which synthesize hypo- or hyper-cross-linked peptidoglycan. J. Bacteriol. **117**:568-577.
5. **Kimura, K., and K. Izui.** 1976. Importance of membrane fluidity in the induction of alkaline phosphatase. Biochem. Biophys. Res. Commun. **70**:900-906.
6. **Laskey, R. A., and A. D. Miels.** 1975. Quantitative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335-341.
7. **Leduc, M., and M. Schaechter.** 1978. Size of the lipid precursor pool in *Escherichia coli*. J. Bacteriol. **133**: 1038.
8. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
9. **Pardee, A. R., and L. S. Prestidge.** 1961. The initial kinetics of enzyme induction. Biochim. Biophys. Acta **49**:77-88.
10. **Shechter, E., L. Letellier, and T. Culik-Krzywicki.** 1974. Relations between structure and function in cytoplasmic membrane vesicles isolated from an *Escherichia coli* fatty-acid auxotroph: high-angle X-ray diffraction, freeze-etch electron microscopy and transport studies. Eur. J. Biochem. **49**:61-76.
11. **Silbert, D. F.** 1975. Genetic modification of membrane lipid. Annu. Rev. Biochem. **44**:315-339.
12. **Spratt, B. G., and A. B. Pardee.** 1975. Penicillin-binding proteins and cell shape in *E. coli*. Nature (London) **254**: 516-517.
13. **Suzuki, H., Y. Nishimura, and Y. Hirota.** 1978. On the process of cellular division in *Escherichia coli*: a series of mutants of *E. coli* altered in the penicillin-binding proteins. Proc. Natl. Acad. Sci. U.S.A. **75**:664-668.
14. **Tamaki, S., S. Nakajima, and M. Matsuhashi.** 1977. Thermosensitive mutation in *Escherichia coli* simultaneously causing defects in penicillin-binding protein-1Bs and in enzyme activity for peptidoglycan syntheses *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **74**:5472-5476.
15. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
16. **Weidel, W., and H. Pelzer.** 1964. Bagshaped macromolecules—a new outlook on bacterial cell walls. Advan. Enzymol. Regul. **26**:193-232.