

Influence of Growth Media on *Escherichia coli* Cell Composition and Ceftazidime Susceptibility

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Cell composition and surface properties of *Escherichia coli* were modified by using various growth media to investigate the role of yet uncharacterized components in ceftazidime susceptibility. An eightfold dilution of Luria broth was used as the basic growth medium and was supplemented with up to 4% phosphate, 5% glucose, or 12% L-glutamate. Decreases in cephaloridine and ceftazidime susceptibility, of two- and eightfold, respectively, were observed only in the glucose-enriched medium. The outer membrane permeability to ceftazidime and cephaloridine was evaluated by crypticity indices. Indices were unchanged under all growth conditions. Fluorometry of whole cells with 1-*N*-phenyl-naphthylamine showed that glucose does not affect the interaction of this hydrophobic probe with the membranes but showed that elevated concentrations of phosphate or glutamate cause a marked increase in cell hydrophobicity, which, in turn, correlates with an increase in the susceptibility of *E. coli* to nalidixic acid. Growth in phosphate- or glutamate-enriched media caused an augmentation in major phospholipid species and may explain the increased hydrophobicity and susceptibility of *E. coli* to nalidixic acid. These data showed that *E. coli* susceptibility to ceftazidime is not influenced by cell surface hydrophobicity and suggested that the contribution of a nonspecific lipophilic diffusion route for entry of ceftazidime into cells is not likely to occur or is distinct from that of more hydrophobic molecules such as nalidixic acid. Finally, the penicillin-binding proteins of the *E. coli* cells were also investigated. Penicillin-binding protein 8 was only markedly labeled with ¹²⁵I-penicillin V in inner membranes extracted from cells grown with glucose. Results of this study suggest that the unexpected change in penicillin-binding protein 8 observed in the presence of glucose may be responsible for the increase in MICs of cephaloridine and ceftazidime.

In general, the susceptibility of gram-negative bacteria to β -lactam antibiotics results from the relative effect of the outer membrane permeability and the periplasmic β -lactamase on the β -lactam periplasmic concentration. In turn, the periplasmic antibiotic concentration needed to affect the antibiotic inner membrane cell targets (the penicillin-binding proteins [PBPs]) depends on the affinities of these PBPs for the β -lactam (33).

Ceftazidime is a very potent broad-spectrum cephalosporin against members of the family *Enterobacteriaceae* and *Pseudomonas* spp. (9, 25), although it has been reported to have an unusually slow diffusion rate through the outer membrane of gram-negative bacteria (34). Recently, Nikaido and Normark (21) constructed a mathematical model to predict β -lactam MICs from the antibiotic penetration rate through the outer membrane and the β -lactamase hydrolysis rate in the periplasm. In the case of ceftazidime, the observed MIC was significantly different from the predicted value, and it was suggested that ceftazidime penetrates the outer membrane by an additional non-porin pathway (21). The great affinity of ceftazidime for PBP 3 is thought to be one of the reasons for its antibacterial activity (12, 19). Other factors such as the role of the murein hydrolases and the overall peptidoglycan metabolism have been involved in the mechanism that triggers the killing of bacteria by β -lactams (29), but it is still not clear how the bactericidal cascade of events occurs or how it is controlled.

In the present study, we attempted to modify the cell

composition of *Escherichia coli* by using various growth media to investigate the role of yet uncharacterized components in ceftazidime susceptibility. It is of primary interest to understand fully the mechanism of action of this very potent β -lactam which has a poor penetration rate through the outer membranes of gram-negative bacteria. Under our experimental conditions, changes in cell components were observed and efforts were made to understand how such changes affect ceftazidime susceptibility with regard to outer membrane permeability and to antibiotic interactions with PBPs.

MATERIALS AND METHODS

Bacterial strains and cultivation media. The basic growth medium used for *E. coli* JF568 (4) was Miller's Luria broth (GIBCO Canada, Inc.) diluted 1:8 with water (LB/8), resulting in a final mixture of 1.25 g of peptone, 0.63 g of yeast extract, and 1.25 g of sodium chloride per liter of broth. The supplementation of LB/8 with sodium phosphate, dibasic-potassium phosphate, monobasic (2:1), D-(+)-glucose, or L-glutamate was used to modify the cell composition of *E. coli* grown in these media. The pHs and osmolalities of these media are given in Table 1. The osmolality was measured in a vapor pressure osmometer (model 5500; Wescor, Inc., Logan, Utah).

For use in a whole-cell β -lactam permeability assay, *E. coli* cells were transformed with plasmid DNA encoding a ceftazidime-hydrolyzing TEM-10 β -lactamase by using a calcium chloride procedure (18). The plasmid DNA was

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purified by a rapid method (27) from a clinical isolate of *Klebsiella pneumoniae* described by Quinn et al. (24).

Measurement of growth rate. The growth rate of *E. coli* was measured in all supplemented media. Each measure was derived from three separate growth curves obtained from three distinct experiments plotted by the MS-2 Abbott Research System (Dallas, Tex.). The calculated growth rate of *E. coli* in the supplemented media was compared with the rate measured for the control culture by using Student's *t* test. A *P* value of less than 0.01 was considered to be significant.

Antibiotic susceptibility testing. Antibiotic MICs were determined by a broth dilution technique (10). Antibiotic inhibitory activities were also evaluated by monitoring growth of *E. coli* cells in the presence of drugs at 35°C by using the MS-2 Abbott Research System.

Cell component analyses. (i) Inner and outer membranes. Inner and outer membranes of *E. coli* were isolated as described previously (16). Cells from 1-liter cultures were harvested by centrifugation at 4°C. The cells were suspended in 15 ml of 20% sucrose–50 mM Tris hydrochloride (pH 7.9)–0.2 mM dithiothreitol and were disrupted by three passages through a French pressure cell (18,000 lb/in²). Debris was removed by slow-speed centrifugation. The supernatant was loaded onto a discontinuous sucrose gradient (52 to 70%) and was centrifuged for 16 h at 100,000 × *g* in a Beckman Ti70.1 rotor at 4°C. Inner and outer membranes were collected separately, suspended in 50 mM Tris hydrochloride (pH 7.9), and stored frozen (–20°C). Protein concentration was determined by the method of Lowry et al. (15). Inner or outer membranes (25 µg) were suspended in electrophoresis sample buffer containing sodium dodecyl sulfate (SDS) and 2-mercaptoethanol. The samples were heated to 100°C for 4 min and were loaded into 10% discontinuous SDS-polyacrylamide gels for electrophoresis. Gels were stained with 0.1% Coomassie brilliant blue.

(ii) LPS. Whole-cell lysis for lipopolysaccharide (LPS) was performed by the procedure of Hitchcock and Brown (14). LPS preparations were applied onto SDS-polyacrylamide gels (15%) containing 4 M urea, and visualization was achieved by silver staining (28).

(iii) RELs. As described previously for the lipid extraction from whole cells (5), bacteria were grown in broth to the early stationary phase, harvested by centrifugation, washed twice with saline, suspended in water, and freeze-dried. The readily extractable lipid (REL) fraction was obtained by chloroform-methanol (2:1) extraction of freeze-dried whole cells (11). The phosphate content of the REL fraction was determined by the method of Ames and Dubin (1). Phospholipids were resolved by thin-layer chromatography on silica gel G plates. Chromatograms were developed to a distance of 10 cm in chloroform-methanol-acetic acid-water (85:15:10:3.5), dried, and developed to a distance of 16 cm in hexane-diethyl ether (4:1). Resolved lipids were visualized with iodine vapor and identified by comigration with standard phospholipids.

(iv) PBPs. Radiolabeling of PBPs from *E. coli* inner membranes was performed by a modification of the method of Spratt (26), as described by Preston et al. (23), using ¹²⁵I-penicillin V as the labeled β-lactam (3). Membrane fractions of 20 µl containing 10 to 20 µg of protein in phosphate-buffered saline were incubated with 6 to 20 µg of ¹²⁵I-penicillin V (37.3 Ci/mmol) per ml for 15 min at room temperature. The samples were thereafter loaded for SDS-polyacrylamide gel electrophoresis as described above. Dried gels were subjected to Kodak X-Omat film for auto-

radiography (6 to 48 h) at –70°C. In PBP competition experiments, the membranes were pre-labeled with various concentrations of a competing β-lactam for 10 min prior to the addition of ¹²⁵I-penicillin V. The concentration of the competing β-lactam needed to block 50% of the subsequent binding of the radiolabeled penicillin to a particular PBP (*I*₅₀) was determined by scanning the PBP profiles obtained on the X-ray film with an LKB UltraScan XL apparatus as described previously (17).

Cell surface properties. (i) Cell surface hydrophobicity. The relative bacterial surface hydrophobicity was evaluated by fluorometry of whole cells by using the hydrophobic probe 1-*N*-phenyl-naphthylamine (NPN) by the procedure described before (6). Exponentially grown cells were washed and suspended in 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.2) to an *A*₆₀₀ of 0.3 before the addition of NPN (50 µM) at room temperature. Fluorescence emissions were recorded with an LS-5B luminescence spectrometer (Perkin-Elmer, Norwalk, Conn.). Excitation and emission wavelengths for NPN were set at 350 and 420 nm, respectively, with slit widths of 5 nm.

(ii) Cell permeability assay. Cell permeability to β-lactams was evaluated by determining crypticity indices (CIs), which were calculated as the ratio of the rate of hydrolysis by the freed periplasmic β-lactamase from broken cells to the rate of hydrolysis by the periplasmic β-lactamase of intact cells (35). *E. coli* cells containing plasmid-encoded β-lactamase were grown in various media, collected by centrifugation, and suspended to a final *A*₆₀₀ of 0.2 in the presence of 100 µM ceftazidime or cephaloridine. The rates of β-lactam hydrolysis were determined at 37°C by monitoring the decrease of UV *A*₂₅₆ over time in a Beckman DU-70 spectrophotometer with a Peltier temperature controller. The hydrolysis rates by equivalent portions of sonicated cells were also determined to provide the CI. Rates for whole cells were corrected for β-lactamase leakage by using a supernatant control.

RESULTS

Growth rate. The growth rate of *E. coli* in the control culture was 0.15 ± 0.01 Δ*A*₆₇₀/h and was not significantly modified in any of the media supplemented with glucose. The growth rate of *E. coli* was significantly reduced in media with 4% phosphate (0.11 ± 0.01 Δ*A*₆₇₀/h; *P* < 0.005) or 10 and 12% glutamate (0.06 ± 0.01 and 0.04 ± 0.01 Δ*A*₆₇₀/h, respectively; *P* < 0.001), indicating that broths with very high osmolalities (>500 mmol/kg of solvent; Table 1) impair growth.

Antibiotic susceptibility testing. We evaluated the effects of supplemental glucose, phosphate, or glutamate on antibiotic MICs determined by a tube dilution technique for *E. coli* JF568. In the unsupplemented control broth, the MICs of cephaloridine, ceftazidime, and nalidixic acid were 1.25, 0.15, and 5 µg/ml, respectively. Two- and eightfold decreases in cephaloridine and ceftazidime susceptibilities were observed in glucose-enriched medium, while a fourfold increase in nalidixic acid susceptibility was observed in glutamate-enriched medium. There were no changes in cephaloridine or ceftazidime MICs in broths supplemented with phosphate or glutamate. The significance of these MIC variations was supported by growth curves obtained from the MS-2 Abbott Research System (Fig. 1). The MICs determined in tubes and with the MS-2 Abbott Research System were slightly different but were reproducible for both conditions.

TABLE 1. Osmolality and pH of the supplemented LB/8 media used in this study^a

| Supplement (%) | pH | Osmolality (mmol/kg of solvent) |
|----------------|-------------|---------------------------------|
| None | 6.95 ± 0.11 | 36 ± 2 |
| Glucose | | |
| 0.1 | 6.95 ± 0.09 | 40 ± 2 |
| 0.5 | 6.94 ± 0.08 | 52 ± 3 |
| 1.0 | 6.93 ± 0.08 | 69 ± 1 |
| 5.0 | 6.89 ± 0.06 | 289 ± 1 |
| Phosphate | | |
| 0.1 | 7.24 ± 0.03 | 44 ± 2 |
| 0.4 | 7.23 ± 0.02 | 72 ± 2 |
| 1.0 | 7.19 ± 0.07 | 150 ± 4 |
| 4.0 | 7.11 ± 0.14 | 519 ± 7 |
| L-Glutamate | | |
| 1.0 | 6.83 ± 0.13 | 104 ± 4 |
| 5.0 | 6.92 ± 0.01 | 510 ± 3 |
| 10.0 | 6.97 ± 0.03 | 1125 ± 8 |
| 12.0 | 6.99 ± 0.06 | 1270 ± 7 |

^a Values are means ± standard deviations.

Cell component analyses. The outer and inner membrane protein profiles of *E. coli* cells grown in different media are shown in Fig. 2. The major outer membrane proteins of *E. coli* were identified by their respective molecular weights (4). The LamB outer membrane protein was absent in cells from the glucose-enriched medium, and the proportion of OmpF and OmpC was different only in the basic growth medium (LB/8). No significant changes in the inner membrane protein electrophoretic profiles (Fig. 2) or in the LPS banding patterns of cells grown in the different media were observed (data not shown). The RELs separated by thin-layer chromatography showed that cells grown in the basic medium or in glucose had a minor difference in lysophosphatidylethanolamine content, while cells from phosphate- or glutamate-enriched media showed an augmentation in all identified phospholipid species (Fig. 3).

PBPs from inner membranes prepared from *E. coli* cells were identified by radioactive labeling with ¹²⁵I-penicillin V (Fig. 4). The PBP 8 (30 kDa) from cells grown in the glucose-enriched medium was markedly labeled compared with PBP 8 of inner membranes isolated from cells grown in the other media. This difference was consistently observed by using three different membrane preparations. In a PBP competition experiment (Fig. 5), PBP 8 was not targeted preferentially by ceftazidime, which bound with greater affinity to PBP 3. The I₅₀s of ceftazidime were 2 and <0.3 μg/ml for PBPs 8 and 3, respectively. PBP 8 bound unlabeled penicillin V and imipenem with high affinity (I₅₀, <0.3 μg/ml), while mecillinam showed no significant binding to that protein (I₅₀, >10 μg/ml) (data not shown).

Cell surface properties. Fluorometry of whole *E. coli* cells with NPN showed that supplemental glucose did not affect the interaction of this hydrophobic probe with the membranes (Table 2). A marked increase in cell hydrophobicity was seen only in 4% phosphate- and glutamate-enriched media.

The *E. coli* outer membrane permeability barrier to cephaloridine and ceftazidime was evaluated by determining CIs. No significant variations in CIs (*P* > 0.02) were observed for cells grown in the different media (Table 3).

DISCUSSION

One of our interests was to understand how ceftazidime penetrates the outer membrane of gram-negative bacteria. Such information may help in designing new molecules with resistances to β-lactamases similar to that of ceftazidime and a high affinity to PBPs but which cross the bacterial outer membrane barrier with a higher velocity. We planned the present study to create modifications in the cell surface of *E. coli* by using various growth media to investigate the role of yet uncharacterized components in ceftazidime susceptibility. This work was based on a previous study that showed that bacterial growth in various media results in changes in lipid composition (7) and that studies with bacteria with mutations that affect the structure of the LPS-phospholipid bilayer showed changes in bacterial susceptibility to hydrophobic or membrane-interacting antibiotics (20). The hope was to change *E. coli* surface properties (hydrophobicity and phospholipid content) to investigate a possible nonspecific lipophilic diffusion pathway for ceftazidime. Cephaloridine, a dipolar ionic β-lactam that penetrates readily through the water-filled hydrophilic pores of the outer membrane of gram-negative bacteria (34), and the more hydrophobic antibiotic nalidixic acid, which may diffuse through the LPS-phospholipid bilayer in a nonspecific manner (a non-porin pathway) (13), served as controls in this study.

As expected, supplementation of the basic growth medium with L-glutamate or phosphate provoked significant changes in *E. coli* cell surface hydrophobicity, as measured by the interaction of NPN with the bacterial membrane (Table 2). The high hydrophobicity noted under these conditions correlated well with the increased amounts of phospholipid species seen in the readily extractable lipids of bacteria (Fig. 3). Correspondingly, the susceptibility of *E. coli* to nalidixic acid was increased in glutamate-enriched broth (Fig. 1C). The increased susceptibility of *E. coli* to nalidixic acid in 4% phosphate was not as obvious (a twofold reduction of the MIC), but could be seen by using the MS-2 Abbott Research System (data not shown). These data indicated that the hydrophobic antibiotic, nalidixic acid, may be able to penetrate bacteria by passing through the hydrophobic domain of the outer membrane, as was suggested in a study with an LPS-deficient mutant (13).

The increase in cell surface hydrophobicity and the phospholipid content of bacterial cells grown in L-glutamate or phosphate had no effect on the susceptibility of *E. coli* to ceftazidime or cephaloridine. This may indicate that a β-lactam such as ceftazidime is not likely to use a nonspecific lipophilic diffusion pathway, as nalidixic acid seems to do for penetration through the outer membrane (13; this study). Accordingly, there was no change in the CIs measured for ceftazidime or cephaloridine with *E. coli* cells grown under all conditions (Table 3). Unless the rate of diffusion of ceftazidime through this hypothetical non-porin pathway is so low that the calculated CIs cannot establish a difference, diffusion of ceftazidime through water-filled porins may be the only major route of entry for this β-lactam. The discrepancy of the observed ceftazidime MIC and the predicted MIC calculated with the mathematical model of Nikaido and Normark (21), and which led them to suggest a non-porin pathway for the entry of ceftazidime into *E. coli* cells, may have resulted from the consideration of using in their model the ceftazidime affinity measurement for only one of the PBPs, 1a, 1b, 2, or 3, with the exclusion of any other PBP.

In this study, we observed an unexpected decrease in the susceptibility of *E. coli* to ceftazidime and cephaloridine

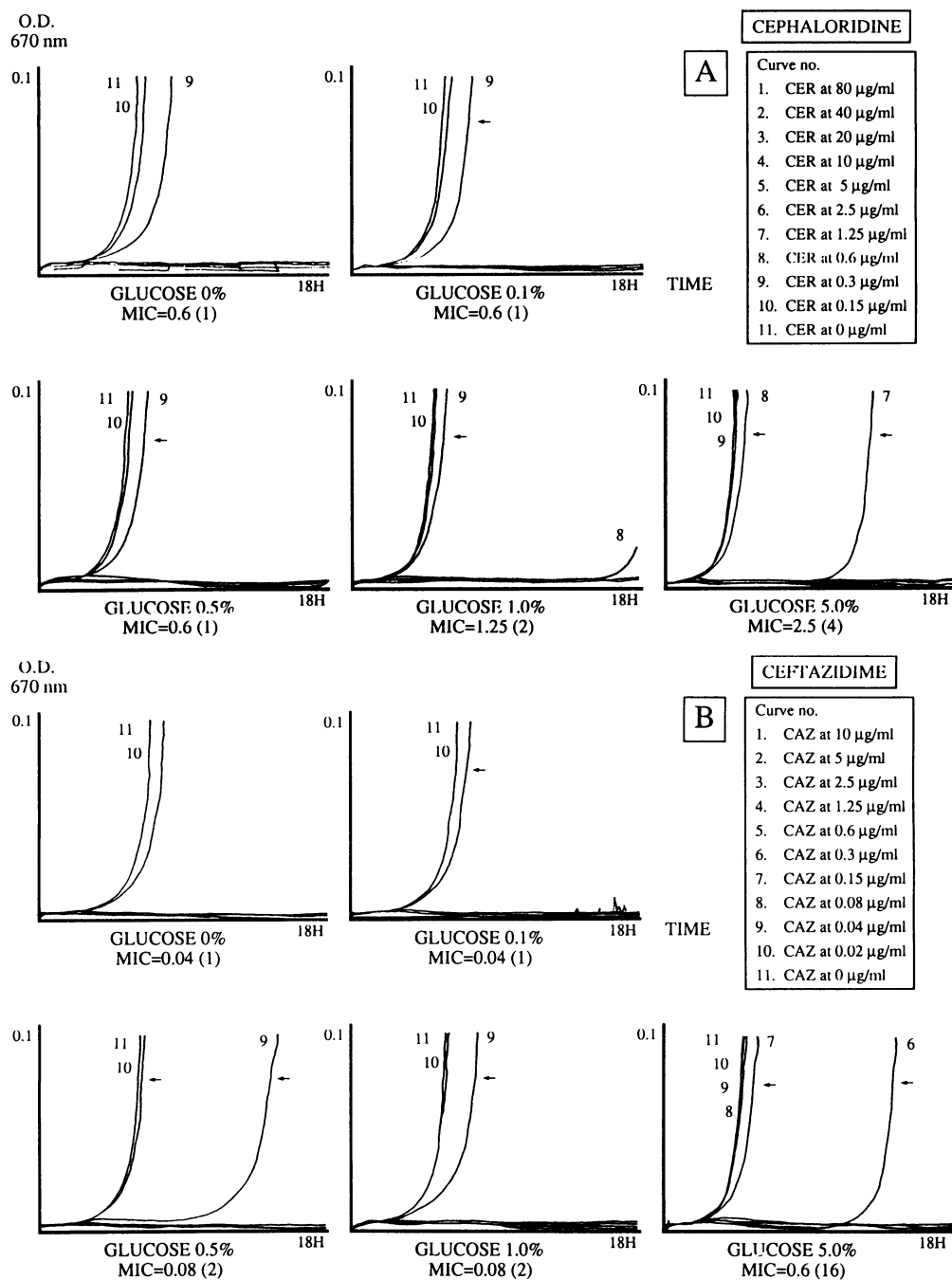


FIG. 1. *E. coli* cell growth measured as the optical density (O.D.) at 670 nm as a function of time and monitored by the MS-2 Abbott Research System. Cells were grown in an eightfold dilution of LB supplemented with glucose (A and B) or L-glutamate (C). Curves 1 to 11 represent growth obtained in decreasing concentrations of cephaloridine (A), ceftazidime (B), and nalidixic acid (C). The arrows indicate the tendency of the curves. The antibiotic MIC in each set of curves is also indicated with the ratio to the control MIC obtained in broth without supplement given in parentheses.

when the basic medium was supplemented with glucose (Fig. 1A and B). This observation was interesting, since the effect of glucose seemed specific for β -lactams and was more pronounced on ceftazidime. Supplementation with glucose did not affect significantly the growth rate of *E. coli* and did not result in observable changes in phospholipid composition or cell surface hydrophobicity (Fig. 3 and Table 2). Moreover, supplementation with glucose did not affect the

ability of ceftazidime to permeate the outer membrane of *E. coli* (Table 3). The expected repression of the LamB outer membrane protein in a glucose-enriched medium (Fig. 2) cannot account for the decrease in ceftazidime susceptibility, since LamB forms only very small pore channels (2). Also, the relative expression of OmpF and OmpC, which is controlled by the osmotic pressure of the growth medium, the carbon source, or the temperature (8), was not different

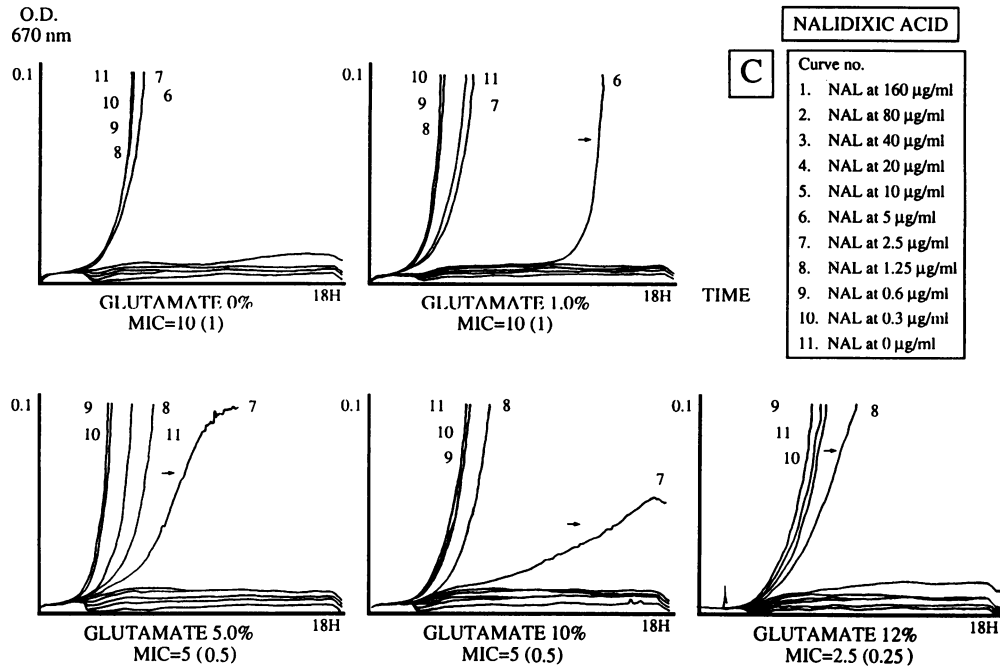


FIG. 1—Continued.

in membranes extracted from cells grown in glucose-, L-glutamate-, or phosphate-enriched media (Fig. 2) and did not result in a change in CIs (Table 3). Furthermore, we did not observe any decrease in susceptibility to ceftazidime by using media supplemented with sodium chloride (0.1, 0.5, 1, 2, and 4%), although these media were of higher osmolality (up to $1,321 \pm 13$ mmol/kg of solvent) (data not shown). In addition, *E. coli* susceptibility to ceftazidime was still decreased in media supplemented with glucose at 1 and 5%, even though we maintained the osmolality at 298 ± 9 mmol/kg by the addition of sodium chloride in all glucose-

enriched media and in the control culture (data not shown). These data show that changes in osmolality alone do not correlate with observed changes in *E. coli* susceptibility to ceftazidime in glucose-enriched media. We then investigated other specific components that would be involved more specifically in the bactericidal action of β -lactams, the PBPs.

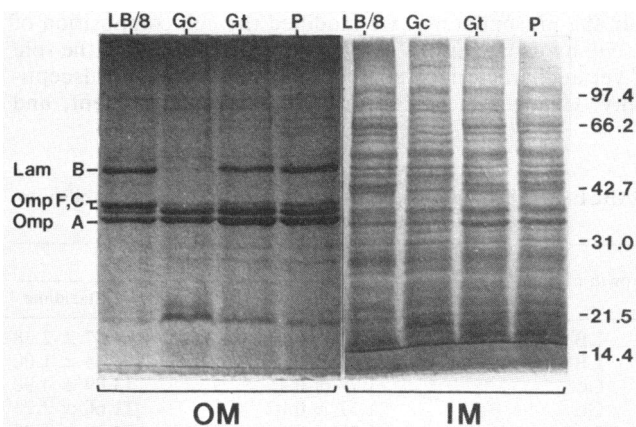


FIG. 2. Outer membrane (OM) and inner membrane (IM) proteins of *E. coli* JF568 electrophoresed on a SDS-polyacrylamide (10%) slab gel. Outer and inner membranes were extracted from cells grown in LB/8, LB/8 supplemented with 5% glucose (Gc), LB/8 supplemented with 10% L-glutamate (Gt), and LB/8 supplemented with 4% phosphate (P). Major outer membrane proteins are indicated on the left, and molecular weight markers (in thousands) are indicated on the right.

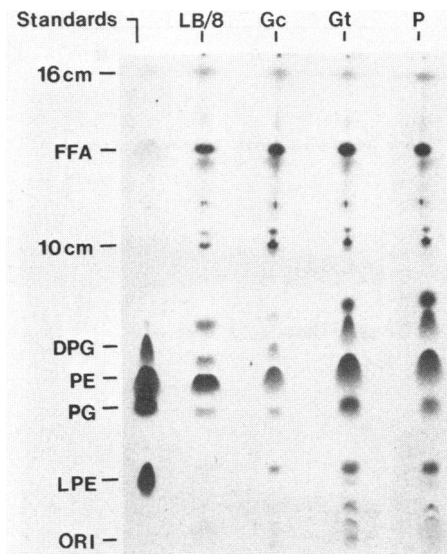


FIG. 3. Thin-layer chromatography of the RELs from whole *E. coli* cells grown in LB/8, LB/8 supplemented with 5% glucose (Gc), LB/8 supplemented with 10% L-glutamate (Gt), or LB/8 supplemented with 4% phosphate (P). ORI indicates where the REL samples were applied. Identification of visualized lipids was done by comigration with the following standards: free fatty acids (FFA), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and lysophosphatidylethanolamine (LPE). ORI, Origin.

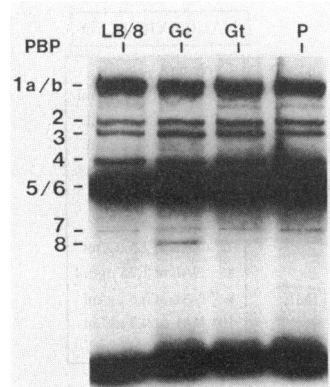


FIG. 4. Autoradiograph of *E. coli* JF568 grown in LB/8, LB/8 supplemented with 5% glucose (Gc), LB/8 supplemented with 10% glutamate (Gt), or LB/8 supplemented with 4% phosphate (P). Inner membrane proteins (20 μ g) were labeled for 15 min with 125 I-penicillin V (20 μ g/ml) and electrophoresed on an SDS-polyacrylamide (10%) slab gel. The PBPs of *E. coli* are indicated on the left.

Our results showed a correlation between the decreased susceptibility of *E. coli* to ceftazidime (and to a lesser degree to cephaloridine) and the expression of PBP 8 in glucose-enriched media (Fig. 4). Although we showed that ceftazidime had little affinity for PBP 8 (Fig. 5), more work is needed to demonstrate a role of that PBP in the mechanism that results in the reduced susceptibility of *E. coli* to ceftazidime. The type and rate of peptidoglycan synthesis varies with the physiological state of bacterial cells, which, in turn, varies with the growth conditions. For example, it is known that slow-growing *E. coli* cells are more resistant to the

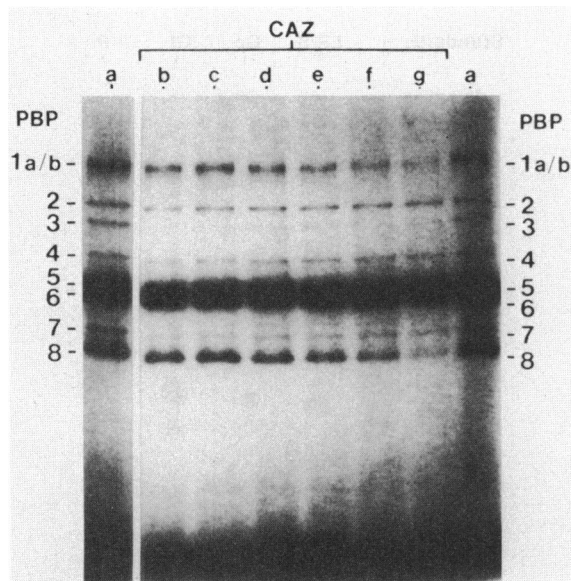


FIG. 5. Autoradiograph of *E. coli* JF568 grown in an LB/8 supplemented with 5% glucose in a PBP competition experiment with ceftazidime. Inner membrane proteins (10 μ g) were preincubated for 10 min with the competing β -lactam at 0 (lane a), 0.3 (lane b), 0.6 (lane c), 1.25 (lane d), 2.5 (lane e), 5 (lane f), and 10 (lane g) μ g/ml. Inner membrane proteins were then labeled with 6 μ g of 125 I-penicillin V per ml for 15 min and electrophoresed on an SDS-polyacrylamide (10%) slab gel. The PBPs of *E. coli* are indicated on both sides.

TABLE 2. Relative hydrophobicity of *E. coli* cells grown in supplemented LB/8 measured by NPN fluorescence

| Supplement (%) | Relative hydrophobicity (NPN fluorescence) ^a |
|----------------|---|
| None | 4.6 \pm 0.5 |
| Glucose | |
| 0.1 | 5.2 \pm 0.2 |
| 0.5 | 5.1 \pm 0.2 |
| 1.0 | 5.0 \pm 0.3 |
| 5.0 | 4.5 \pm 0.2 |
| Phosphate | |
| 0.1 | 4.8 \pm 0.6 |
| 0.4 | 4.9 \pm 0.4 |
| 1.0 | 5.2 \pm 0.4 |
| 4.0 | 7.3 \pm 0.8 ^b |
| L-Glutamate | |
| 1.0 | 4.4 \pm 0.8 |
| 5.0 | 5.8 \pm 1.2 |
| 10.0 | 7.6 \pm 0.0 ^c |
| 12.0 | 8.0 \pm 0.2 ^c |

^a Values are means \pm standard deviations of four independent measurements.

^b $P < 0.01$ (4% phosphate versus LB/8; Student's *t* test).

^c $P < 0.001$ (10 and 12% L-glutamate versus LB/8; Student's *t* test).

bactericidal action of β -lactams (29), a phenomenon that correlates with changes in the rate of synthesis of peptidoglycan and its composition (22, 30) as well as with a decreased sensitivity of murein to autolysins (31). Recently, β -lactams with high affinities to the low-molecular-mass PBP 7, a protein resembling our PBP 8 (a 30-kDa imipenem binding protein), were found to be more potent for lysing nongrowing bacteria (32). It is possible that, in our study, bacterial cells exposed to the glucose-enriched medium synthesized a similar autolysin-resistant peptidoglycan through the activity of PBPs with unknown physiological significance, such as PBP 8. The synthesis of such a peptidoglycan would have resulted in a lower susceptibility to β -lactam-induced cell death.

In the present study, we modified the cell composition of *E. coli* by using various growth media to investigate the role of yet uncharacterized components in ceftazidime susceptibility. Changes in cell surface, phospholipid content, and

TABLE 3. Effect of growth media on *E. coli* cell permeability to cephaloridine and ceftazidime evaluated by CIs

| Growth medium ^a | CI ^b | |
|----------------------------|-----------------|------------------|
| | Cephaloridine | Ceftazidime |
| LB | 1.03 \pm 0.01 | 14.87 \pm 2.38 |
| LB/8 | 1.09 \pm 0.06 | 11.34 \pm 1.00 |
| Gc | 1.72 \pm 0.15 | 13.89 \pm 4.98 |
| Gt | 1.51 \pm 0.02 | 11.66 \pm 2.73 |
| P | 1.01 \pm 0.01 | 14.18 \pm 7.45 |

^a Abbreviations: LB, Luria broth; LB/8, eightfold dilution of LB; Gc, LB/8 supplemented with 5% glucose; Gt, LB/8 supplemented with 10% L-glutamate; P, LB/8 supplemented with 4% phosphate.

^b CIs were determined by using *E. coli* JF568 transformed with a plasmid encoding a ceftazidime-hydrolyzing β -lactamase. The CI represented the ratio of the rate of hydrolysis of substrate at 100 μ M by the β -lactamase of broken cells to the rate of hydrolysis by whole cells. Data shown are means \pm standard deviations.

PBPs were observed under our experimental conditions. Analysis of these changes with consideration of cell susceptibility to ceftazidime indicated that this β -lactam is not likely to use a nonspecific lipophilic diffusion route to penetrate the outer membrane of *E. coli*. Also, our results suggested that, under our experimental conditions, the addition of glucose in the medium may reduce ceftazidime-induced cell killing, perhaps through an unknown physiological activity associated with PBP 8. More data are needed to understand why ceftazidime is particularly influenced by this effect, whereas cephaloridine is affected to a lesser extent.

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