Diffusion of β-Lactam Antibiotics through Liposome Membranes Reconstituted from Purified Porins of the Outer Membrane of Pseudomonas aeruginosa

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Pseudomonas aeruginosa has been recognized as one of the opportunistic pathogens that frequently cause infections in immunocompromised patients and patients with cystic fibrosis. A major problem in P. aeruginosa infection is the intrinsic resistance of the organism to a number of structurally unrelated antibiotics (3, 19). This resistance has been assumed to be due to low outer membrane permeability (1, 28). The rates of diffusion of β-lactam antibiotics through the intact outer membrane of P. aeruginosa have been shown to be 100 to 500 times lower than the corresponding rates for Escherichia coli (28). This low outer membrane permeability is largely attributable to the small size of the diffusion pores, as shown in both intact cells (6, 24, 25) and in vitro reconstitution experiments (26). Although sucrose seemed to diffuse very poorly through the P. aeruginosa outer membrane in most of our previous experiments (24-26), one of our earlier experiments showed an undetectable diffusion rate under the conditions of that study (8). Some investigators have reported that P. aeruginosa produces large outer membrane pores made of protein F (2, 9, 10, 30) but that the low permeability results from the closed state of these pores (1, 2, 30). Other investigators reported that the low rate of diffusion of β-lactam antibiotics is related to the structural alteration of protein F (7).

In recent years, imipenem has been introduced for the treatment of Pseudomonas infections and has been demonstrated to have powerful effects. However, some cases of unsuccessful imipenem therapy for P. aeruginosa infections resulted in the emergence of imipenem-resistant mutants. Such mutants often lacked the outer membrane protein D2 (5, 15, 20). Analyses of the outer membrane proteins of imipenem-resistant laboratory strains confirmed the results described above (4). These findings suggest that protein D2 may be a porin in P. aeruginosa. We recently purified the outer membrane proteins of P. aeruginosa and performed assays for the pore-forming activity in the reconstituted proteoliposomes. The results revealed that proteins C, D2, and E are capable of forming diffusion pores in the liposome membrane. The pores formed by these proteins are smaller than those formed by E. coli porins (27).

It is thus of considerable interest and urgency to study the diffusion of antibiotics through these newly identified porins. In the present study, we measured the rates of diffusion of β-lactam antibiotics through proteoliposomes that were reconstituted from purified protein C, D2, or E. Here we provide evidence that protein D2 allows efficient diffusion of carbapenems and that protein C allows the diffusion of antipseudomonal β-lactams.

MATERIALS AND METHODS

Bacterial strains and growth conditions. P. aeruginosa KG1079 is a protein F-defective mutant derived from strain PAO4089. E. coli B was also used. Cells were grown overnight in L broth containing 5 mM MgCl2 and were then diluted with a ninefold volume of the same prewarmed medium. After shaking at 240 rpm for 5 h at 37°C, the cells were harvested by centrifugation. The outer membranes were purified from P. aeruginosa KG1079 and E. coli B by the procedures described by Mizuno and Kageyama (16) and Smit et al. (21), respectively. Protein was quantified by the method of Lowry et al. (14).

Purification of porin. The OmpF porin of E. coli B was purified as described elsewhere (23). The surfactant in the porin solution was replaced with 34 mM octyl β-glucoside by gel filtration. Porins from the outer membrane of P. aeruginosa KG1079 were purified as follows. The purified outer membrane (20 mg of protein) was mixed with 10 ml of 44 mM octyl β-glucoside–10 mM Tris hydrochloride (pH 8.0). The mixture was subjected to sonic oscillation for 2 min and centrifuged at 100,000 × g for 30 min at 20°C. The soluble
fraction was applied to a high-performance liquid chromatography column (0.75 by 7.5 cm [inner diameter]; TSKgel-DEAE-5PW; Tosoh Corp., Tokyo, Japan) that was equilibrated with a solution of 34 mM octyl-β-glucoside-1 mM EDTA-10 mM Tris hydrochloride (pH 8.0). The column was washed with 20 ml of the same solution and eluted with a linear gradient of 100 to 180 mM NaCl in the same solution at a rate of 1.0 ml/min for 30 min.

Reconstitution of proteoliposomes and determination of diffusion rate. Proteoliposomes were reconstituted by a previously described procedure (17), with minor modifications. A mixture of phosphatidylcholine and dicetylphosphate in a molar ratio of 97 to 3 (total, 1 µmol) in chloroform was dried in the bottom of a tube under an N₂ gas stream. The dried lipid film was solubilized with 100 µl of 68 mM octyl-β-glucoside. The lipid was then mixed with an appropriate amount of porin or the purified outer membrane was dissolved in 34 mM octyl-β-glucoside, and the mixture was dialyzed for 3 days at 4°C against a large excess of distilled water containing Bio-Beads (Bio-Rad Laboratories, Richmond, Calif.). Spontaneously formed liposomes were dried again and suspended in 133 µl of one of the following solutions: (i) 18 mosM stachyose-1 mM 3-(N-morpholino)propanesulfonic acid (MOPS)-NaOH (pH 7.2); (ii) 14 mosM stachyose-2 mM thiamine PP₃-4 mM Tris hydrochloride (pH 6.2) (11); (iii) 12 mosM stachyose-4 mM sodium NAD-1 mM imidazole NAD (pH 6.0) (18, 29). For the permeability assay, 35 µl of proteoliposome suspension (0.26 µmol of lipid equivalent) was diluted with 665 µl of the isotonic test solute, and the change in optical density at 450 nm was recorded. The diffusion rate was calculated from the initial rate of change in optical density as described previously (27).

Preparation of test solutes. The following test solutes were prepared: (i) 18 mosM saccharides, dipolar ionic β-lactams, glycin, or peptides in 1 mM MOPS buffer (pH 7.2); (ii) 18 mosM glucose or glucosamine hydrochloride in 1 mM thiamine PP₃-2 mM Tris base (pH 6.2) (11); or (iii) 18 mosM saccharides, 18 mosM sodium gluconate, 9 mM mononoanionic β-lactams, 9 mM β-lactams with one positive and two negative charges, or 6 mM dianionic β-lactams in 1 mM sodium NAD-1 mM imidazole NAD (pH 6.0) (18, 29). Fine adjustment of the solute osmolarity was performed by using liposomes without protein.

Chemicals. The following chemicals were obtained from the indicated sources. Bio-Beads (SM-2), Bio-Rad Laboratories; n-octyl-β-D-glucoside, Dojindo Laboratories (Kumamoto, Japan); cephalaxin, cefaclor, and cephradine, Shionogi Pharmaceuticals (Osaka, Japan); ceftobiprole and piperacillin, Toyama Chemicals (Tokyo, Japan); cefazidime, Tanabe Pharmaceuticals (Osaka, Japan); cefoxolin, Takeda Chemicals (Osaka, Japan); aztreonam, Eai Co., Ltd. (Tokyo, Japan); imipenem, Banyu Pharmaceuticals (Tokyo, Japan); meropenem (SM-7338), Sumitomo Pharmaceuticals (Osaka, Japan).

RESULTS

Purification of porin. Proteins D₃ and E were eluted as homogeneous preparations by a single chromatographic procedure (see Materials and Methods; Fig. 1). Fractions enriched with protein C were pooled, diluted with a threefold volume of the buffer, and then applied onto the same column. Rechromatography under identical conditions yielded a homogeneous preparation of protein C (Fig. 1). The protein profile appeared essentially as shown in Fig. 1 when a gel containing 1/10 amount of sample was stained by the silver method. The reason for the slightly faster mobility of purified protein C (Fig. 1, lane B) than that of protein C in lanes F and G of Fig. 1 is not clear. We have seen a similar gel profile earlier (27). Two-dimensional gel electrophoresis by isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that protein C consisted of two protein spots with identical M₉s and a marginal pI difference (data not shown). These two proteins were included in protein C according to the nomenclature of Mizuno and Kageyama (16). It is possible that protein C in the crude membrane fraction forms a complex with other membrane component(s) and that this extra component might be eliminated during purification.

Diffusion of β-lactam antibiotics. To determine the rates of diffusion of β-lactam antibiotics under the optimum protein concentration, rates of liposome swelling were measured by using proteoliposomes containing different amounts of porin per fixed amount of lipid. The rates of diffusion of ribose, glucose, and imipenem increased linearly as the amount of protein D₂ was increased from 5 to 50 µg/µmol of lipid (Fig. 2). Other porins showed similar results (data not shown). In an earlier report (18), it was reported that the diffusion rates of saccharides are not proportionally related to the amount of E. coli porin. Therefore, to ensure that the results shown in Fig. 2 were not artifacts, a similar experiment was carried out with proteoliposomes containing 0.5 to 5 µg of the E. coli B OmpF porin. The results confirmed that plots of the rates of diffusion of glucose versus the amount of OmpF showed a hyperbolic curve, confirming the previous results (data not shown). The amounts of protein D₂ required for a permeability equivalent to that for 0.7 µg of E. coli B OmpF appeared to be roughly 14, 28, and more than 70 µg for the diffusion of ribose, glucose, and imipenem, respectively. The rates of diffusion of ribose, glucose, and sucrose through

FIG. 1. Electrophoretogram of purified porins from the outer membrane of P. aeruginosa. Porins were purified from P. aeruginosa KG1079 by the procedure described in the text. Protein was mixed with an equal volume of sample buffer (12) and was heated at 95°C for 5 min. Samples containing 4 µg protein (5 µl) were applied, and electrophoresis in a 10% acrylamide gel in the presence of sodium dodecyl sulfate was performed by the procedure described by Laemmli (12). Lane A, the M₉ markers (in thousands [K]) β-galactosidase (E. coli; Mr, 116,000), phosphorylase b (rabbit muscle; Mr, 97,400), albumin (bovine; Mr, 66,000), albumin (egg; Mr, 45,000), and carbonic anhydrase (M₉, 29,000); lane B, protein C; lane C, protein D₂; lane D, protein E; lane E, protein F; lane F, purified outer membrane from P. aeruginosa PAO1 (20 µg of protein); lane G, purified outer membrane from P. aeruginosa KG1079 (20 µg of protein). The nomenclature of the outer membrane proteins was that of Mizuno and Kageyama (16).
the protein D2 porin were roughly 6, 3, and 2% that of the E. coli B OmpF. The size of the P. aeruginosa porin pore seemed to be substantially smaller than that of E. coli. Since the P. aeruginosa porin was very inefficient, the rates of diffusion of β-lactams were measured with liposomes containing 50 μg of porin per μmol of lipid. Diffusion rates were expressed as values relative to the rate for glucose through the protein D2 pore (Tables 1 and 2). Note that one cannot compare the diffusion rates of saccharides between Tables 1 and 2 precisely because of the different buffer systems that were used. Determination of the diffusion rates for saccharides confirmed the previously reported permeability properties of proteins C, D2, and E (27).

In the first series of experiments, the rates of diffusion of dipolar ionic β-lactams were determined. Among them, the rates of diffusion of carbapenem, imipenem, and meropenem through the protein D2 pore appeared to be 32 and 56%, respectively, of the rate for glucose (Table 1). These values are markedly high compared with the rates for other dipolar ionic β-lactams. Although imipenem also diffused through the pores of proteins C and E relatively efficiently, the diffusion of meropenem through these porins was less efficient (Table 1). The diffusion rates of dipolar ionic cephalosporins through the pores of protein C, D2, or E were less than 5% of the rate for glucose, indicating that these drugs were hardly permeable through the P. aeruginosa outer membrane, thus confirming results of an earlier report (28). These results are inconsistent with those of a report that the rate of diffusion of cefalaridine is close to those of glucose and imipenem (22). The reason for this discrepancy is not clear. The rates of diffusion of ribose and glucose through the lipid membrane containing purified protein F, formally assigned to be porin, were about 2% that of glucose through protein D2 (Table 1). The rates of diffusion of imipenem and meropenem through protein F were less than 2% of the rate for glucose in protein D2 (Table 1). These results suggest that protein F does not form a pore for the permeation of saccharides or carbapenems.

In the second series of experiments, the diffusion rates of antipseudomonal monoanionic β-lactams were determined. The rates of diffusion of cefoperazone through the pores of proteins C, D2, and E were 26, 24, and 17%, respectively, of the rates for glucose (Table 2). Piperacillin was as diffusible as cefoperazone through the protein C pore, but its rates of diffusion through other porins were very low.

The third series of experiments was done to determine the rates of diffusion of antipseudomonal β-lactams bearing one positive and two negative charges. The rates of diffusion of cefazidime and ceftazolin through the protein C pore were 33 and 25% of the rates for glucose, and those through protein D2 and E pores were about 10% of the rates for glucose (Table 2).

The fourth series of experiments was performed to determine the diffusion rates of an antipseudomonal β-lactam antibiotic bearing two negative charges. The rates of diffusion of aztreonam through the pores of proteins C, D2, and E were 31, 4, and 14%, respectively of the rates for glucose, indicating that this β-lactam mainly diffuses through the P. aeruginosa outer membrane via protein C (Table 2). When the rates of diffusion of β-lactam antibiotics through proteoliposomes that were reconstituted from the purified

### Table 1. Relative rates of diffusion of dipolar ionic β-lactam antibiotics and saccharides

<table>
<thead>
<tr>
<th>Solute</th>
<th>(M_r)</th>
<th>Protein C</th>
<th>Protein D2</th>
<th>Protein E</th>
<th>Protein F</th>
<th>Outer membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>150</td>
<td>94.6 ± 24.9</td>
<td>193.5 ± 53.4</td>
<td>217.3 ± 18.1</td>
<td>2.1 ± 2.1</td>
<td>75.9 ± 17.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>180</td>
<td>56.0 ± 2.3</td>
<td>100.0 ± 17.9</td>
<td>161.2 ± 10.5</td>
<td>2.2 ± 0.9</td>
<td>47.5 ± 4.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>342</td>
<td>4.6 ± 3.6</td>
<td>1.5 ± 1.7</td>
<td>4.0 ± 3.9</td>
<td>0.8 ± 0.3</td>
<td>2.7 ± 3.0</td>
</tr>
<tr>
<td>Dipolar ionic β-lactams</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>299</td>
<td>9.9 ± 6.7</td>
<td>32.1 ± 3.2</td>
<td>14.7 ± 0.8</td>
<td>1.2 ± 0.9</td>
<td>7.0 ± 4.2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>383</td>
<td>0.8 ± 1.1</td>
<td>56.0 ± 12.3</td>
<td>2.0 ± 2.7</td>
<td>1.7 ± 0.5</td>
<td>11.3 ± 2.7</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>347</td>
<td>1.3 ± 2.1</td>
<td>0.9 ± 1.5</td>
<td>0.9 ± 0.8</td>
<td>1.0 ± 1.0</td>
<td>1.2 ± 1.2</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>368</td>
<td>0.1 ± 0.0</td>
<td>0.3 ± 0.5</td>
<td>0.6 ± 1.0</td>
<td>0.9 ± 0.5</td>
<td>1.8 ± 1.8</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>415</td>
<td>2.6 ± 2.2</td>
<td>3.6 ± 2.3</td>
<td>0.3 ± 0.4</td>
<td>0.9 ± 0.8</td>
<td>0.7 ± 0.3</td>
</tr>
</tbody>
</table>

* Liposomes were reconstituted from 50 μg of porin per μmol of lipid, as described in the text. The buffer system that was used was 18 mosM solute and 1 mM MOPS buffer (pH 7.2).

† Rates of diffusion are expressed as values relative to the swelling rate of the protein D2-containing liposomes for glucose. Data are means ± standard deviations of more than three independent assays.

‡ Protein F was purified from wild-type strain PA01 by the procedure described earlier (27).

§ Liposomes were reconstituted from the outer membrane (50 μg of protein per μmol of lipid).
TABLE 2. Relative rates of diffusion of negatively charged β-lactam antibiotics and saccharides

<table>
<thead>
<tr>
<th>Saccharide</th>
<th>M_r</th>
<th>Protein C</th>
<th>Protein D₂</th>
<th>Protein E</th>
<th>Outer membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribose</td>
<td>150</td>
<td>109.7 ± 17.1</td>
<td>186.5 ± 22.4</td>
<td>235.3 ± 38.2</td>
<td>67.8 ± 3.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>180</td>
<td>59.3 ± 15.3</td>
<td>100.0 ± 10.4</td>
<td>217.6 ± 28.5</td>
<td>35.9 ± 3.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>342</td>
<td>1.3 ± 1.1</td>
<td>2.2 ± 3.6</td>
<td>10.2 ± 7.3</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>Monoanionic β-lactams</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>644</td>
<td>26.1 ± 8.9</td>
<td>24.1 ± 5.6</td>
<td>17.0 ± 5.1</td>
<td>7.2 ± 5.7</td>
</tr>
<tr>
<td>Pipercillin</td>
<td>516</td>
<td>26.4 ± 10.6</td>
<td>8.2 ± 7.1</td>
<td>5.1 ± 5.1</td>
<td>6.8 ± 2.9</td>
</tr>
<tr>
<td>Dianionic β-lactam, aztreonam</td>
<td>433</td>
<td>31.6 ± 6.8</td>
<td>4.4 ± 3.2</td>
<td>14.7 ± 0.6</td>
<td>8.1 ± 2.4</td>
</tr>
</tbody>
</table>

α Liposomes were reconstituted from 50 μg of porin per μmol of lipid in the presence of 12 mosM stachyose-4 mM sodium NAD-1 mM imidazole NAD (pH 6.0), as described in the text.
β Rates of diffusion are expressed as values relative to the swelling rate of the protein D₂-containing liposomes for glucose. Data are means ± standard deviations of more than three independent assays.
γ Liposomes were reconstituted from the outer membrane (50 μg of protein per μmol of lipid).
δ Soluble solutions were prepared to 18 mosM in a solution of 1 mM sodium NAD-1 mM imidazole NAD (pH 6.0).
ε Test solutes were prepared to 9 mM in the same buffer system described in footnote d.

Solute selectivity of porin pores. Since carbapenems and anionic antipseudomonal antibiotics diffused mainly through the protein D₂ and C pores, respectively, the solute selectivities of three species of porin pores were tested by using solutes with similar M_r values but different charges, i.e., glucose (uncharged), glucosamine (monocation), and glucuronic acid (monoanion). The rates of diffusion of glucosamine (M_r 179) through the pores of proteins C, D₂, and E were about 3, 14, and 1 time higher, respectively, than the respective rates for glucose (Fig. 3). These results indicate that the rates of diffusion of glucosamine relative to those of glucose were highest for protein D₂ among the three porin species tested. The rates of diffusion of glucuronic acid (M_r 195) through the pores of proteins C, D₂, and E were about 1.5 to 2 times higher than the respective rates for glucose (Fig. 3), suggesting that there is no significant anion selectivity.

To assess the solute selectivity of the protein D₂ pore, the rates of diffusion of glycin, diglycin, and triglycin were determined; and the results were compared with those for saccharides. Table 3 shows the following features. (i) The diffusion rates of (glycin)₀, saccharides were inversely proportional to the solute M_r. (ii) The diffusion rates of (glycin)₂ (M_r 189) appeared to be 0.94, 3.47, and 0.62 through the pore of proteins C, D₂, and E, respectively, compared with the rates of diffusion of glucose through the respective porins. These results suggest that (glycin)₃ diffuses well through the pore of protein D₂.

DISCUSSION

The intrinsic antibiotic resistance of P. aeruginosa is determined mainly by two factors. One is the instability of antibiotics against antibiotic-modifying enzyme(s), e.g., chromosomally encoded cephalosporinase (13). Another factor is the permeability barrier at the outer membrane (1, 28). This marked barrier function of the outer membrane is
mostly due to the presence of small diffusion pores that allow the diffusion of uncharged saccharides that are about the same size or smaller than a disaccharide (6, 24–26). Recently, we have identified three outer membrane proteins, C, D2, and E, that function as porins to form small diffusion pores (27). The copy number of these porins seems to be relatively low, as examined by two-dimensional gel electrophoresis (J. Ishii and T. Nakae, unpublished data). In the present study, we studied the diffusion of β-lactam antibiotics through liposome membranes that were reconstituted from homogeneously purified porin C, D2, or E.

Among the dipolar ionic β-lactams, imipenem and meropenem diffused mainly through protein D2 pores (Table 1). These results are consistent with the observation that strains isolated from P. aeruginosa-infected patients in whom imipenem therapy was unsuccessful lacked protein D2 (5, 15, 20). A question then arises as to why imipenem-resistant mutants primarily lack protein D2. We interpret the available data as follows. Since the relative amounts of proteins C, D2, and E (pl, 6.6) in the outer membrane of the wild-type strain are roughly 34, 100, and 23, respectively (unpublished data; calculated from the densitometric trace of the electrophorogram), the relative diffusion rates of imipenem through these pores in the intact outer membrane must be roughly 3, 32, and 3, respectively. Therefore, the role of protein D2 in imipenem diffusion is predominant and the roles of proteins C and E are almost negligible. The reasons why protein D2 allows efficient diffusion of carbapenems may be explained as follows. (i) The molecular weights of imipenem and meropenem are smaller than those of other β-lactams. (ii) Protein D2 allows the efficient diffusion of compounds bearing a positive charge (Fig. 3). The rates of diffusion of dipolar ionic cephalems that are less effective against P. aeruginosa, e.g., cephalaxin, cefaclor, and cephaloridine, were very low, suggesting that a low rate of diffusion across the outer membrane and high β-lactamase susceptibility may be the reasons for the ineffectiveness of these antibiotics. It is still not known why the rates of diffusion of dipolar ionic cephalems with a relatively low Mr are low compared with those of carbapenems.

The relative rates of diffusion of cefoperazone, piperacillin, ceftazidime, cefsoludin, and aztreonam through protein C pores of intact cells were calculated to be about 9 to 11% of the rate of glucose through the protein D2 pore, when the diffusion rates were normalized according to the relative amount of protein C in the intact outer membrane. The rates of diffusion of these β-lactams through the protein D2 pore varied from 4 to 24%. The rates of diffusion of these antibiotics through the protein E pore were 1 to 4% of the rate of glucose diffusion through the protein D2 pore when the rates were normalized according to the relative amount of protein E in the intact outer membrane. Therefore, the level of participation of protein E in the diffusion of these β-lactams seems to be very small, and we assume that the β-lactams listed in Table 2 permeate the P. aeruginosa outer membrane mainly via the pores of proteins C and D2. It is not clear whether protein C or protein D2 plays the major role in the diffusion of antipseudomonal β-lactams listed in Table 2. However, observations that cefsulodin-ofloxacin-resistant mutants primarily produce decreased amounts of protein C and that the rates of diffusion of β-lactams through protein C-defective outer membranes are substantially low (Ishii and Nakae, unpublished data) suggest that these antipseudomonal β-lactams primarily permeate through the protein C pore. It is noteworthy that most of such protein C mutants produced a slightly reduced amount of protein D2, suggesting that protein D2 plays only a secondary role in the diffusion of these antibiotics (Ishii and Nakae, unpublished data).

Another question to be answered is how these antipseudomonal β-lactams with Mrs of over 400 can pass through the protein C pore, for which the apparent saccharide molecular weight exclusion limit is only 250 to 260 (27). Since the apparent exclusion limit is the Mr of the test solute, for which the rate of diffusion is about 1% of the rate for pentoses, β-lactam antibiotics with similar sizes may diffuse through the pore at a low rate. A similar case has been reported. Negatively charged β-lactam antibiotics with Mrs of over 400 diffuse efficiently through the pore of the Alcaligenes faecalis porin, for which the apparent molecular weight exclusion limit for uncharged saccharide is about 220 (11). We are unable to explain the high rates of diffusion of cefoperazone through all three porin pores. It is possible that β-lactams are contaminated with an impurity, as mentioned previously (29), or that the effects of the counterion was measured.

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LITERATURE CITED


