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Size of Diffusion Pore of *Alcaligenes faecalis*

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The diffusion pore of the outer membrane of *Alcaligenes faecalis* was shown to be substantially smaller than the *Escherichia coli* porin pore. In experiments with intact cells, pentoses and hexoses penetrated into the NaCl-expanded periplasm, whereas saccharides of M_r >342 did not. Cells treated with 0.5 M saccharides of M_r >342 weighed 33 to 38% less than cells treated with isotonic solution, suggesting that these saccharides do not permeate through the outer membrane. The diffusion rates of various solutes through the liposome membranes reconstituted from the M_r-43,000 outer membrane protein showed the following characteristics. (i) The relative diffusion rates of pentoses, hexoses, and methylhexoses appeared to be about 1.0, 0.6, and negligibly small, respectively. (ii) The diffusion rate of glucose appeared to be about 1/10th that with the *E. coli* B porin. (iii) The diffusion rate of gluconic acid was five to seven times higher than that of glucose. (iv) The diffusion rates of β-lactam antibiotics appeared to be 40 to <10% of those with the *E. coli* B porin.

The outer membrane of gram-negative bacteria constitutes a barrier against large-hydrophilic and hydrophobic molecules and guards the interior of the cell from hazardous agents (for reviews, see references 14 and 17). Yet the cell must incorporate nutrients and other essential substances to support its growth. To overcome this difficulty, cells have developed diffusion pores made of the pore-forming protein porin (12, 13). According to their sizes, the pores have been classified into roughly two groups: those with an exclusion limit of molecular weight (M_r) about 600 and those with an exclusion limit of M_r of several thousand. Earlier data indicated that the outer membrane pores of *Alcaligenes faecalis* and *Pseudomonas aeruginosa* belong to the latter group (4). However, the presence of such a porin in the outer membrane of *A. faecalis* has not been confirmed.

Recently, Caulcott et al. (3) suggested that the outer membrane pore of *P. aeruginosa* is smaller than that of *Escherichia coli*. Yoneyama and Nakae (20, 21) reexamined the outer membrane permeability of *P. aeruginosa* by weighing the centrifuged pellet and taking electron micrographs of hypertonic saccharide-treated cells. They obtained evidence that the outer membrane does not allow free diffusion of disaccharides (20, 21). These data contradict previous reports (e.g., see reference 7). Since *A. faecalis* is moderately resistant to a number of structurally unrelated antibiotics except some of the β-lactams and hence can cause opportunistic infections (8), it was worthwhile to reexamine the permeability of the outer membrane of *A. faecalis*.

This study reports that the outer membrane of *A. faecalis* allows the free diffusion of pentoses and probably hexoses and acts as a barrier against the access of uncharged disaccharides.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *A. faecalis* ATCC 19018 and a clinical strain (994) isolated from urine from a patient were used. *E. coli* ATCC 25922 and *E. coli* B were also used. MICs for *A. faecalis* and *E. coli* are shown in Table 2. Cells were grown overnight in L broth and were diluted with a 10-fold volume of prewarmed L broth. After 3.5 h at 37°C with shaking at 240 rpm, the cells were harvested by centrifugation, washed once with 25 mM sodium phosphate buffer–5 mM $MgCl_2$ (pH 7.0) (buffer A) containing 0.14 M NaCl, and resuspended in the same solution to about 50% (wt/wt).

Purification of porin. The partially purified protein fraction was obtained by washing the crude membrane preparation successively with a solution containing 2% sodium dodecyl sulfate and a solution of 2% sodium dodecyl sulfate and 0.4 M NaCl and then passing it through a Sephacryl S-300 column as described earlier (19). The dialyzed eluates were dissolved in 2 ml of 1.0% octaethylene glycol dodecylether and were subjected to isoelectric focusing (FBE 3000 isoelectric focuser; Pharmacia, Uppsala, Sweden) on a Sephadex isoelectric focusing slab gel containing 2% ampholytes of pH ranges 3.5 to 10 and 5 to 7 (equal volumes of both). The sample was focused for a total of 4,500 V · h (600 V for 1 h, 900 V for 3 h, and 1,200 V for 1 h) at 23°C. The sample eluted from the fractionated gel was adsorbed to a DEAE-Sephacel column (0.5 by 4 cm) equilibrated with 50 mM Tris hydrochloride (pH 8.0) and washed with an excess of 50 mM Tris hydrochloride (pH 8.0), and the column was eluted with a solution containing 0.4 M NaCl, 25 mM octyl glucoside, and 50 mM Tris hydrochloride (pH 8.0). Porin activity of the fractionated samples was assayed by reconstituting them into liposome membranes and by the liposome-swelling method.

Determination of solute permeability of outer membrane of intact cells. (i) Penetration assay. The penetration of saccharides across the intact outer membrane was tested by the procedure described earlier (20, 21).

(ii) Weight assay. Determination of the weight of the cells treated with hypertonic saccharides was modified from the procedure described earlier (21). A cell suspension (100 μl) was centrifuged at 55,000 × *g* for 1 min, and the tube with the pellet was weighed with a precision balance (first precipitate). The pellet was suspended in 100 μl of solution containing 0.5 M saccharide (0.14 M NaCl for a control) and 0.5 U of glucose-6-phosphate dehydrogenase in buffer A. After 4 min at 23°C, the mixture was centrifuged at 103,000 × *g* for 1 min. The supernatant was saved (second supernatant). The remaining liquid was carefully removed, and the tubes were weighed as described above (second precipitate).

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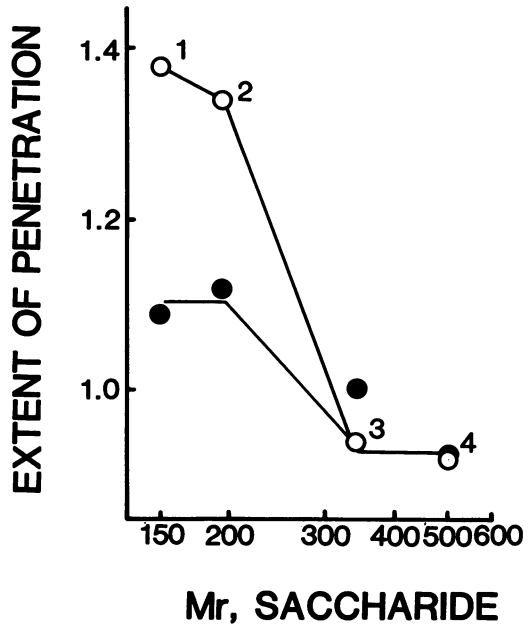

FIG. 1. Penetration of saccharide into the periplasm of plasmolyzed cells. *A. faecalis* ATCC 19018 cells were treated as described in Materials and Methods. The saccharides and enzyme recovered from the deplasmolyzed cells were quantified. Saccharides used were as follows: 1, ribose; 2, α-methylmannoside; 3, sucrose; and 4, raffinose. Open and closed symbols show the experiments using the plasmolyzed (450 mosM) and unplasmolyzed (300 mosM) cells, respectively.

The pellet was suspended in 100 μl of solution containing 0.14 M NaCl in buffer A. The suspension was kept at 23°C for 4 min and then centrifuged at 55,000 × *g* for 1 min, and the supernatant was saved as described above (third supernatant). It is assumed that the ratio of enzyme activity in the third supernatant to that in the second supernatant represents the intercellular water volume of the second precipitate. Therefore, the intercellular space of the second pellet was calculated as follows: second supernatant (100 μl) × (third supernatant/second supernatant)$_{\text{glucose-6-phosphate dehydrogenase}}$. The intercellular solute volume was converted to the intercellular solute weight as follows: intercellular space × density of 0.5 M test solute. Thus, the weight percent of the cell was computed as follows: [(net weight of second precipitate − weight of the intercellular test solute of second precipitate)/(net weight of first precipitate − weight of intercellular salt solution of first precipitate)] × 100.

Reconstitution of proteoliposome and determination of diffusion rate. Bacterial phospholipids and the purified porin (surfactant was removed by dialysis) were dried in the bottom of a tube as reported previously (15). The lipid film was suspended in 0.5 ml of one of the following solutions: (i) 40 mosM stachyose–1 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS)–NaOH (pH 7.2); (ii) 14 mosM stachyose–2 mM thiamine PP_i–4 mM Tris hydrochloride (pH 6.2); (iii) 12 mosM stachyose–4 mM sodium NAD–1 mM imidazole NAD (pH 6.0) (18, 22). The suspension was mixed on a Vortex mixer at the highest speed for 20 s. The liposome-swelling assay was done by the procedure reported previously (15), and the diffusion rate was calculated as described previously (14, 15). The porin-free liposome was impermeable to the test solutes used.

Preparation of test solutes. Test solutes were prepared as follows: (i) 40 mosM saccharide or 40 mosM amino acid in 1 mM MOPS buffer (pH 7.2); (ii) 18 mosM glucose or 18 mosM glucosamine hydrochloride in 1 mM thiamine PP_i–2 mM Tris base (pH 6.2); (iii) 18 mosM glucose, 18 mosM sodium gluconate, 18 mM dipolar ionic, 9 mM monoanionic, or 6 mM dianionic β-lactams in 1 mM sodium NAD–1 mM imidazole NAD (pH 6.0) (18, 22). The β-lactam solution was carefully adjusted to pH 5.8 to 6.2 with NaOH. The fine adjustment of the solute osmolarity was made by using liposomes without protein as reported earlier (15).

Other methods. Protein was determined by the method of Lowry et al. (10). Carbohydrates were quantitated by the phenol-sulfuric acid method (5). The glucose-6-phosphate dehydrogenase assay (21) and acrylamide gel electrophoresis (9) were as described earlier.

Reagents. Glucose-6-phosphate dehydrogenase was obtained from Boehringer Mannheim-Yamanouchi, Inc. (Tokyo, Japan). Octaethylene glycol dodecylether and octyl glucoside were purchased from Nikko Chemicals (Tokyo, Japan) and Dojindo Laboratories (Kumamoto, Japan), respectively. Antibiotics used were obtained from the following sources: tetracycline, Wakamoto Pharmaceuticals (Tokyo, Japan); chloramphenicol, P-L Biochemicals, Inc. (Milwaukee, Wis.); ofloxacin, gift from Daiichi Seiyaku Co. Ltd. (Tokyo, Japan); kanamycin and streptomycin, Meiji Pharmaceuticals (Tokyo, Japan); cefazolin, Fujisawa Pharmaceuticals (Tokyo, Japan); cefmetazole, Sankyo Co. Ltd. (Tokyo, Japan); cefoxitin, Merck Sharp & Dohme (Darmstadt, Federal Republic of Germany); cefoperazone and piperacillin, Toyama Chemicals (Tokyo, Japan); cefadroxil, Banyu Pharmaceuticals (Tokyo, Japan); sulbenicillin and cefsulodin, Takeda Chemicals (Osaka, Japan); cephalothin, cefamandole, moxalactam, cephalexin, cefaclor, cephaloridine, and gentamicin, Sionogi Pharmaceuticals (Osaka, Japan).

RESULTS

Penetration of saccharides. *A. faecalis* ATCC 19018 cells were plasmolyzed, and saccharide penetration was examined for 1, 3, 5, and 10 min. Arabinose and α-methylmannoside penetrated into the periplasm and equilibrated within about 1 min, but the sucrose- or raffinose-permeable space appeared to be negligibly small (data not shown). Similarly, cells were incubated with 28 mM saccharides of various M_rs for 3 min in the presence of hypertonic NaCl. The saccharides recovered from the deplasmolyzed cells showed that ribose and α-methylmannoside were highly permeable but that di- and trisaccharides were practically impermeable under these conditions (Fig. 1).

To validate the above result, we determined the exclusion limit by an assay technique based on a different principle. When cells are treated with outer membrane-impermeable hypertonic solutes, the osmotic pressure results in the dehydration and shrinkage of the cells (20). Cells treated with hypertonic saccharides of various sizes were weighed. The results (Fig. 2) indicated that the weights of the pellets of *A. faecalis* ATCC 19018 cells treated with 0.5 M sucrose, raffinose, and stachyose were about 36, 33, and 38% less, respectively, than that of the pellet of the isotonic NaCl-treated cells. Thus, it was evident that these saccharides do not permeate this outer membrane. On the other hand, the cells treated with 0.5 M arabinose, glucose, and α-methylmannoside weighed 2, 3, and 5% less, respectively, suggesting that these saccharides were able to permeate the membrane. A similar result was obtained with the clinical isolate (Fig. 2). Reproducibility of the result was examined by four independently repeated experiments. Weights (milligrams ±

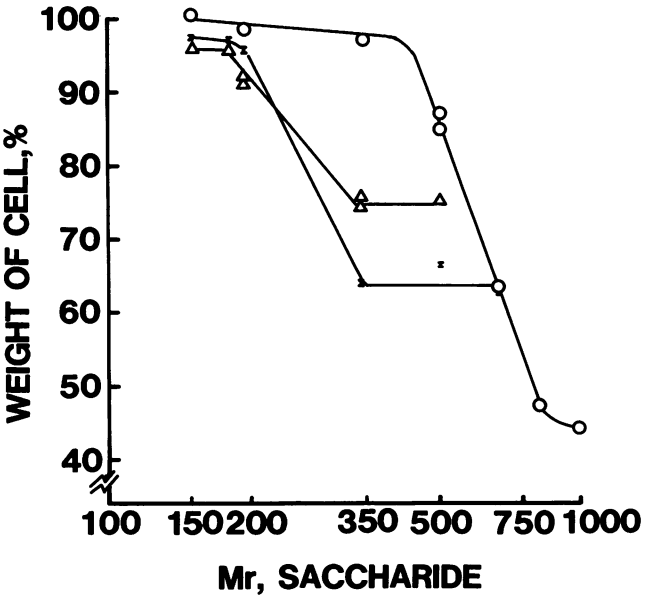

FIG. 2. Relative weights of cells treated with hypertonic saccharides. Experimental details and the calculation of the extent of solute permeability are given in Materials and Methods. Symbols: I, *A. faecalis* ATCC 19018, averages of four independent experiments ± standard deviations; △, clinically isolated *A. faecalis*; ○, *E. coli* B, average of two independent assays. Saccharides used were arabinose, glucose, α-methylmannoside, α-methylgalactoside, sucrose, cellobiose, maltotriose, raffinose, stachyose, maltopentaose, and maltohexaose.

standard deviation) of the cells treated with arabinose, glucose, α-methylmannoside, sucrose, raffinose, and stachyose appeared to be 97.85 ± 3.72, 97.14 ± 3.76, 95.72 ± 3.05, 63.96 ± 2.13, 66.35 ± 3.13, and 63.63 ± 1.61, respectively, indicating very high reproducibility (Fig. 2). In addition, the reliability of the assay method was examined by using *E. coli* B, whose outer membrane permeability is well established (12). The result confirmed the reported exclusion limit of the outer membrane, $M_r \approx 700$ (Fig. 2). This evidence indicates that the apparent exclusion limit of the outer membrane of *A. faecalis* must be close to the size of uncharged saccharides of M_r <340 and >194.

Properties of *A. faecalis* porin. The purified pore-forming outer membrane protein appeared to be nearly homogeneous on sodium dodecyl sulfate-acrylamide gel electrophoresis upon heating in the solubilizing buffer without salt (Fig. 3). The heated protein was located at the position corresponding to an apparent M_r of 43,000. The unheated protein moved much slower, suggesting that the protein forms oligomeric and higher aggregates. An unusual behavior of the purified porin was that the oligomeric aggregates became resistant to heating at 95°C for at least 10 min in sodium dodecyl sulfate if the solubilizing buffer contained 0.4 M NaCl. When the NaCl concentration was reduced, the aggregates dissociated into the monomeric form (Fig. 3). The isoelectric point of the purified porin appeared to be 3.8 to 4 (data not shown). The purified protein was tested for pore-forming activity by reconstituting proteoliposomes and assaying solute diffusion by the liposome-swelling technique (15). When the diffusion rate of glucose through the *A. faecalis* porin was compared with that through *E. coli* B OmpF, the former appeared to be roughly 1/15th and 1/7th of the latter at 0.2 and 0.5 μg of protein per μmol of phospholipid, respectively (Fig. 4). The reason for the nonlinear function of permeability versus the amount of porin is not clear at present. It was also observed with the *E. coli* porin (18).

Diffusion rates of saccharides. The relative diffusion rates of arabinose, ribose, mannose, galactose, and glucose appeared to be 1.02, 1.00, 0.73, 0.65, and 0.5, respectively (Fig. 5). The diffusion rates of methylhexoses, sucrose, and raffinose were undetectably low under the present experimental conditions (Fig. 5). Control experiments with the *E. coli* B porin confirmed the previous reports (11, 18; data not shown).

To gain more insight into the permeability properties of the *A. faecalis* porin, we tested the diffusion of glucose (uncharged, M_r 180), glucosamine (monocation, M_r 179), and gluconic acid (monoanion, M_r 196). To minimize the counter-ion effect, we tested the permeability of electrolytes by using liposomes made in the presence of impermeable

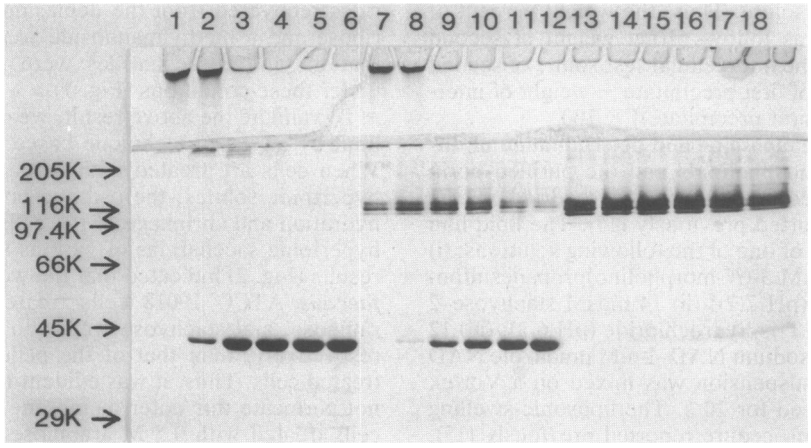

FIG. 3. Acrylamide gel electrophoresis of purified porin. The purified porin was dissolved in the sample buffer (0.7 mg/ml) without NaCl (lanes 1 to 6), with 0.1 M NaCl (lanes 7 to 12), and with 0.4 M NaCl (lanes 13 to 18). Mixtures were heated at 95°C for 0 (lanes 1, 7, 13), 1 (lanes 2, 8, 14), 2 (lanes 3, 9, 15), 3 (lanes 4, 10, 16), 5 (lanes 5, 11, 17), and 10 (lanes 6, 12, 18) min, and 3.5 μg of proteins per well was subjected to electrophoresis by the method of Laemmli (9). M_r markers used were carbonic anhydrase (29,000), egg albumin (45,000), bovine serum albumin (66,000), phosphorylase *b* (97,400), β-galactosidase (116,000), and myosin (205,000).

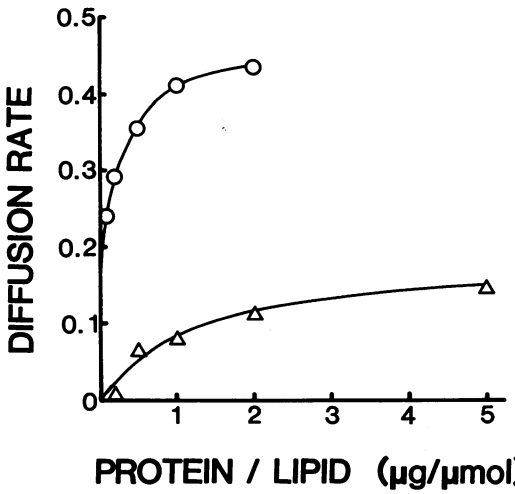

FIG. 4. Effect of porin concentration on the swelling rate of proteoliposomes. Proteoliposomes were reconstituted from the specified amount of porins in a solution containing 40 mosM stachyose and 1 mM MOPS (pH 7.2) as described in Materials and Methods. The test solute was 40 mosM glucose in 1 mM MOPS (pH 7.2). The liposome swelling assay was done as described in Materials and Methods. Symbols: ○, *E. coli* B; △, *A. faecalis*.

electrolytes having a countercharge to the test solute, as suggested by other investigators (18).

At first, the diffusion rates of glucose and glucosamine were compared by using liposomes containing thiamine PP_i. In this comparison, liposomes reconstituted from the *E. coli* B porin were used as a reference. The results in Fig. 6A show the following. (i) The diffusion rates of glucose and glucosamine through the *A. faecalis* porin were nearly comparable. (ii) The ratio of the diffusion rates of glucosamine and glucose through the *E. coli* B porin at 0.2 μg of porin per μmol of phospholipid were about 1.5, confirming previous reports (2). (iii) The diffusion rate of glucose through the *A. faecalis* porin at 0.2 and 0.5 μg of porin was roughly 10 and 5 times lower, respectively, than that through the *E. coli* B porin. This large difference in the diffusion rate of uncharged saccharide must be due to the different pore size.

In an analogy to the above experiment, the diffusion rates of glucose and gluconic acid through liposomes reconstituted from *A. faecalis* or *E. coli* B porins were compared. The results (Fig. 6B) demonstrated the following. (i) The diffusion rate of glucose through the *A. faecalis* porin appeared to be roughly eight times lower than that through the *E. coli* B porin at 0.2 μg of porin. (ii) The diffusion of gluconic acid through the *A. faecalis* porin was comparable with that through the *E. coli* B porin. (iii) Consequently, the diffusion rate of gluconic acid through the *A. faecalis* porin was eightfold higher than that of glucose at 0.2 μg of porin, indicating that the pore is anion selective. The anion selectivity of the *A. faecalis* porin was also demonstrated in the diffusion of glucose-6-phosphate (M_r 282), in that the diffusion rate of glucose-6-phosphate was 300% of that of glucose (data not shown).

Diffusion rate of amino acids. Since *A. faecalis* utilizes organic acids, we measured the diffusion of amino acids through the porin pore. Since preliminary experiments showed that the diffusion rate of methionine (M_r 149) through a liposome membrane containing the *A. faecalis* porin was about fourfold higher than that of ribose (M_r 150), we used 20 times less porin per phospholipid than in the assay for saccharide diffusion. The diffusion rates were normalized to that of glycine (M_r 75). The results (Fig. 7) indicated that the diffusion rates were inversely proportional to the M_r. The apparent exclusion limit of the porin pore to amino acids was calculated to be close to the size of a hypothetical amino acid with an M_r of approximately 300. This result suggested that the *A. faecalis* porin allows very efficient diffusion of charged compounds.

Diffusion of β-lactam antibiotics. To record the swelling rate in a proper range, we performed the assay using proteoliposomes containing 50 and 10 μg of porin per μmol of phospholipid for the *A. faecalis* and *E. coli* B porins, respectively. The diffusion rate of cefazolin through the *A. faecalis* and *E. coli* B porins was 0.2 and 0.18 arbitrary units, respectively. It was assumed, therefore, that the diffusion rate of cefazolin through the *E. coli* B porin is roughly five times higher than through the *A. faecalis* porin. Therefore, we calculated the diffusion rate of β-lactams relative to that of cefazolin. Table 1 compares a set of the results. The *A. faecalis* porin allowed the diffusion of the dianionic β-lactams sulbenicillin and moxalactam at about 104 and 56%, respectively, of the rate of cefazolin. Although the diffusion rates of monoanionic β-lactams varied from 100 to 20% of the rate of cefazolin, those of dipolar ionic β-lactams were approximately less than 20%. For example, the diffusion rates of cephalexin and cephaloridine were less than 5% of that of cefazolin. Cefsulodin having one positive and two negative charges diffused at about 50% of the rate of cefazolin.

As a control experiment, we assayed the diffusion rate of β-lactams through the *E. coli* B OmpF porin (Table 1). The results essentially confirmed the previously published data (18, 22). Thus, the small pore size and anion selectivity of the *A. faecalis* porin was confirmed.

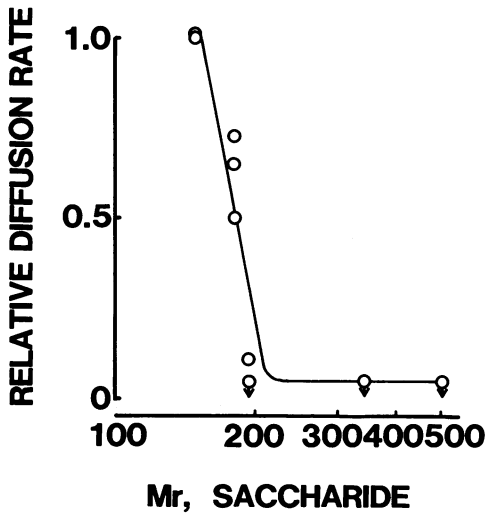

FIG. 5. Relative diffusion rates of uncharged saccharides. Proteoliposomes were prepared from 3.75 μmol of phospholipids and 25 μg of the *A. faecalis* porin in 0.5 ml of 40 mosM stachyose–1 mM MOPS (pH 7.2) by the procedure described in Materials and Methods. Test solutes were 40 mosM saccharides in 1 mM MOPS (pH 7.2). The diffusion rates are presented as relative to the diffusion rate of ribose. Arrows indicate diffusion rates of ≦0.05. Saccharides used were arabinose, ribose, mannose, galactose, glucose, α-methylglucoside, α-methylgalactoside, sucrose, and raffinose.

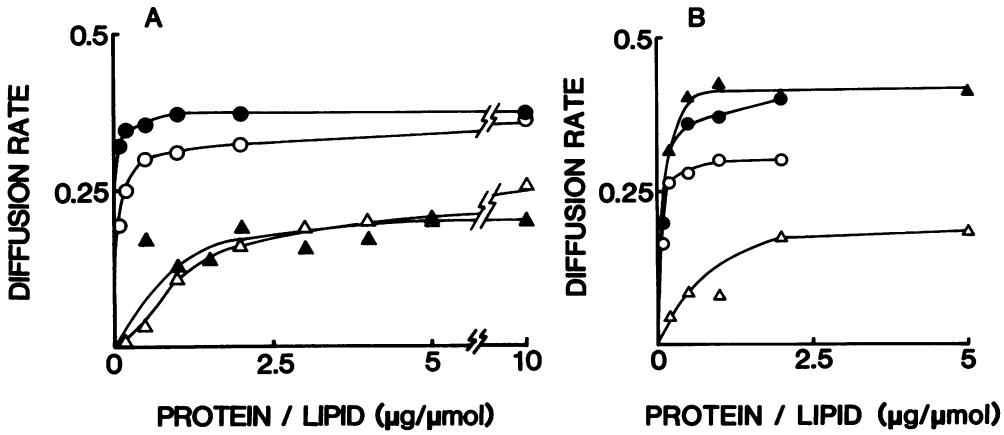

FIG. 6. Comparison of diffusion rates between glucose and its derivatives. Proteoliposomes were prepared from specified amounts of the *A. faecalis* or *E. coli* B porin in 0.5 ml of a solution containing either 14 mosM stachyose, 2 mM thiamine PP_i, and 4 mM Tris hydrochloride (pH 6.2) (A) or 12 mosM stachyose, 4 mM sodium NAD, and 1 mM imidazole NAD (pH 6.0) (B). The rate of liposome swelling was obtained by the method described in Materials and Methods. Test solutes were as follows: (A) ○, △, 18 mosM glucose in 1 mM thiamine PP_i–2 mM Tris hydrochloride; ●, ▲, 18 mosM glucosamine hydrochloride in 1 mM thiamine PP_i–2 mM Tris hydrochloride; (B) ○, △, 18 mosM glucose in 1 mM sodium NAD–1 mM imidazole NAD; ●, ▲, 18 mosM sodium gluconate in 1 mM sodium NAD–1 mM imidazole NAD. Triangles and circles indicate rates obtained with liposomes containing *A. faecalis* and *E. coli* B porins, respectively.

DISCUSSION

Our results indicate that the outer membrane of the intact *A. faecalis* cell allows the diffusion of pentoses and hexoses but acts as a barrier against saccharides with M_rs larger than about 342. The diffusion rate of hexoses appeared to be 50 to 75% of that of pentoses as determined by the liposome swelling assay. The diffusion rates of sucrose and raffinose were undetectably low under the conditions of this study. These results conflict with those of a previous report (4). In favor of the present results over the earlier one would be the demonstration of porin function corresponding to the diffusion properties of the intact outer membrane.

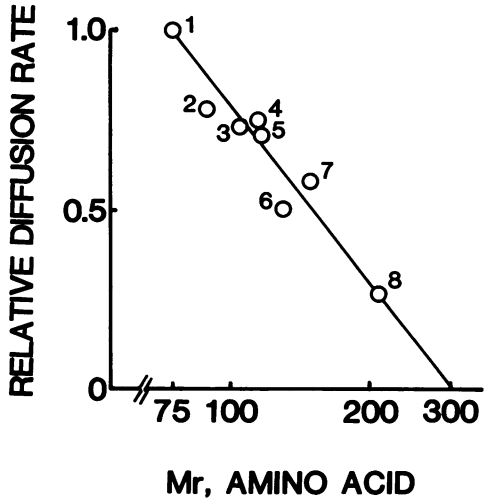

FIG. 7. Relative diffusion rates of amino acids. Proteoliposomes were reconstituted from 3.75 μmol of phospholipids and 1.25 μg of the *A. faecalis* porin in the presence of 40 mosM stachyose and 1 mM MOPS (pH 7.2). Test solutes were 40 mosM of an amino acid in 1 mM MOPS (pH 7.2). Amino acids used were as follows: 1, glycine; 2, alanine ; 3, serine; 4, proline; 5, valine; 6, leucine; 7, methionine, and 8, tryptophan.

On the basis of the data obtained from the liposome swelling assay, the apparent exclusion limit of the *A. faecalis* porin was calculated to be about the size of uncharged saccharides of M_r 200 to 250 or the size of a hypothetical amino acid of M_r approximately 300. To compare the diffusion of nonelectrolytes with that of negatively or positively

TABLE 1. Relative diffusion rates of β-lactam antibiotics[a]

β-Lactam	Relative diffusion rate		Ratio of diffusion rates (A/B)[b]
	A. faecalis (50 μg of porin/μmol of phospholipid) (A)	*E. coli* B (10 μg of porin/μmol of phospholipid) (B)	
Monoanionic			
Cefazolin	1.0 (0.20)[c]	1.0 (0.18)[c]	0.22
Cephalothin	1.04	0.32	0.65
Cefoxitin	0.40	0.56	0.14
Cefamandole	0.73	0.64	0.22
Cefmetazole	0.86	0.73	0.23
Piperacillin	0.22	0.13	0.33
Cefoperazone	0.32	0.16	0.40
Dianionic			
Sulbenicillin	1.04	0.59	0.35
Moxalactam	0.56	0.43	0.26
Dipolarionic			
Cephalexin	<0.05	0.70	<0.01
Cefaclor	0.22	0.51	0.08
Cefadroxil	0.12	0.43	0.05
Cephaloridine	<0.05	0.23	<0.04
Having one positive and two negative charges (cefsulodin)	0.50	0.40	0.25

[a] The diffusion rates were obtained by the liposome-swelling method as described in the text. The swelling rates were normalized to the diffusion rates of cefazolin in the respective liposomes.

[b] Ratio of the diffusion rates was the proportion of the swelling rates of liposomes containing *A. faecalis* porin to those of liposomes containing *E. coli* B porin. Since different amounts of porins were used in the rate assay, the ratio of A/B was multiplied by a factor 0.2.

[c] Swelling rates determined by liposomes containing appropriate amounts of porin per micromole of phospholipid.

TABLE 2. MICs of antibiotics against the strains used[a]

Strain	MIC (μg/ml)																		
	CEZ	CET	CFX	CMD	CMZ	CPZ	PIPC	SBPC	LMOX	CFS	CEX	CCL	CDX	CER	SM	KM	GM	TC	CP	OFLX
A. faecalis																				
ATCC 19018	12.5	1.56	0.78	0.39	0.78	1.56	0.39	12.5	<0.19	6.25	6.25	0.78	6.25	12.5	100	100	0.78	3.12	25	0.78
994	12.5	1.56	1.56	0.39	1.56	1.56	0.78	50	<0.19	6.25	6.25	0.78	12.5	6.25	>100	>100	6.25	100	25	12.5
E. coli																				
ATCC 25922	1.56	12.5	6.25	0.78	1.56	<0.19	1.56	12.5	<0.19	50	6.25	1.56	12.5	3.12	6.25	3.12	0.39	1.56	6.25	<0.19
B	3.12	3.12	3.12	0.78	0.78	<0.19	1.56	3.12	<0.19	50	12.5	0.78	12.5	3.12	12.5	6.25	0.78	0.78	3.12	<0.19

[a] MIC was determined by the agar dilution method, using a plate containing 20 ml of Mueller-Hinton agar medium. Cells (5×10^3) were spotted with a loop, and the growth was observed after 18 h of incubation at 37°C. Abbreviations: CEZ, cefazolin; CET, cephalothin; CFX, cefoxitin; CMD, cefamandole; CMZ, cefmetazole; CPZ, cefoperazone; PIPC, piperacillin; SBPC, sulbenicillin; LMOX, moxalactam; CFS, cefsulodin; CEX, cefalexin; CCL, cefaclor; CDX, cefadroxil; CER, cephaloridine; SM, streptomycin; KM, kanamycin; GM, gentamicin; TC, tetracycline; CP, chloramphenicol; and OFLX, ofloxacin.

charged solutes, we measured the diffusion of D-glucose (a nonelectrolyte), D-gluconic acid (1-carboxyl-D-glucose), and D-glucosamine (2-amino-D-glucose). The results showed that the ratio of the diffusion rate of gluconic acid over that of glucose was 5 to 7. We therefore conclude that the porin pore of *A. faecalis* selects anionic molecules.

It is generally observed that *A. faecalis* is moderately resistant to antibiotics, and therefore it is possible that a barrier function of the outer membrane is involved in drug resistance. We determined the diffusion of β-lactam antibiotics through the porin pore in vitro and found that the diffusion rates of mono- and dianionic β-lactams were generally 20 to 40% of that through the *E. coli* B porin (Table 1). The diffusion rates of these anionic β-lactam antibiotics seem exceptionally high compared with the diffusion rates of uncharged saccharides considering their relatively high M_rs. In fact, the MICs of structurally unrelated antibiotics such as tetracycline, chloramphenicol, ofloxacin, and aminoglucosides (streptomycin, kanamycin, and gentamicin) were 2 to 32 times higher than those for *E. coli* (Table 2). On the other hand, MICs of the negatively charged β-lactam antibiotics such as cephalothin, piperacillin, cefoxitin, cefamandole, cefmetazole, sulbenicillin, moxalactam, and cefsulodin in *A. faecalis* were roughly comparable with or slightly lower than those for *E. coli* (Table 2). The diffusion rates of the dipolar ionic β-lactams in *A. faecalis* were lower than those in *E. coli* despite the fact that the MICs of these antibiotics for those bacteria are comparable. Because the reason for this discrepancy cannot be explained only by outer membrane diffusion, it may be due to other factors (6, 16).

The PhoE porin of *E. coli* K-12 and protein P of *P. aeruginosa* form anion-selective pores (1, 2). A previous study reported that the PhoE pore showed a lower permeability toward large, uncharged, and dipolar ionic solutes compared with that of the OmpF pore in vitro, whereas monoanionic and dianionic compounds diffused at much higher rates (18). The anion-selective property of the PhoE porin is basically the same as that of the *A. faecalis* porin. The fact that the porin forms a small and anion-selective pore is immediately relevant to the chemotherapy of *A. faecalis*, except for β-lactam antibiotics.

(After finishing this study, we learned from H. Nikaido that Leora Zalman [Ph.D. thesis, University of California, Berkeley, 1982] has shown by liposome-swelling assays that the size of *A. faecalis* porin channel is significantly smaller than that of *E. coli* porins.)

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