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The Role of Specific Surface Loop Regions in Determining the Function of the Imipenem-Specific Pore Protein OprD of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa **OprD is a specific porin which facilitates the uptake of basic amino acids and imipenem across the outer membrane. In this study, we examined the effects of deletions in six of the proposed eight surface loops of OprD on the in vivo and in vitro functions of this protein. Native OprD formed very small channels in planar lipid bilayers, with an average single-channel conductance in 1.0 M KCl of 20 pS. When large numbers of OprD channels were incorporated into lipid bilayer membranes, addition of increasing concentrations of imipenem to the bathing solutions resulted in a progressive blocking of the membrane conductance of KCl, indicating the presence of a specific binding site(s) for imipenem in the OprD channel. From these experiments, the concentration of imipenem value of resulting in 50% inhibition of the initial conductance was calculated as approximately 0.6 μM. In contrast, no decrease in channel conductance was observed for the OprDΔL2 channel upon addition of up to 2.4 μM imipenem, confirming that external loop 2 was involved in imipenem binding. Deletion of four to eight amino acids from loops 1 and 6 had no effect on antibiotic susceptibility, whereas deletion of eight amino acids from loops 5, 7, and 8 resulted in supersusceptibility to β-lactams, quinolones, chloramphenicol, and tetracycline. Planar lipid bilayer analysis indicated that the OprDΔL5 channel had a 33-fold increase in single-channel conductance in 1 M KCl but had retained its imipenem binding site. The disposition of these loop regions in the interior of the OprD channel is discussed.**

The porins of gram-negative bacteria form water-filled channels that permit the diffusion of hydrophilic solutes across the outer membrane (7, 24). They are generally divided into two classes: nonspecific porins which permit the general diffusion of hydrophilic molecules below a certain size, and specific porins which facilitate the diffusion of specific substrates by virtue of containing stereospecific binding sites (7). *Pseudomonas aeruginosa* OprD is a specific porin for imipenem (22), a carbapenem which shows excellent activity against *P. aeruginosa*. However, the natural substrate for OprD is not imipenem but its structural analogs, basic amino acids and small peptides containing these amino acids (23). In vivo functional studies indicated that OprD could also selectively facilitate the diffusion of gluconate under growth-rate-limiting conditions (11). Although the protein has been characterized in vitro in liposome swelling assays and the presence of the binding site has been confirmed (22), very limited work has been done to study its physical properties in planar lipid bilayers. Ishii and Nakae (13) measured the single-channel conductance of OprD, which was 20 to 30 pS. They also observed larger channels (400 pS) that they suggested were capable of being induced under certain conditions. In their studies, the ion selectivity of the OprD channel was not measured, and no direct evidence for the presence of a specific binding site(s) for imipenem within the OprD channel was obtained from these studies. In this study, we purified OprD and reanalyzed its physical properties in the planar lipid bilayer system.

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To fully understand the molecular mechanism involved in the facilitated uptake of imipenem, a detailed knowledge of the molecular structure of OprD is required. In a previous paper (12), we proposed an OprD topology model based on multiple amino acid sequence alignments with the nonspecific porin superfamily and structural predictions. Site-directed mutagenesis was used to construct eight deletion mutants, one from each of the predicted external loops. Six of these deletion mutants were permissive for expression in the *P. aeruginosa* outer membrane. The loop 2 (L2) deletion mutant only partially reconstituted the imipenem supersusceptibility in a *P. aeruginosa oprD*::Ω background, suggesting that L2 might be involved in imipenem binding. However, the mechanism of imipenem transport and the interior architecture of the OprD channel were not characterized.

In this study, we examined these deletion mutants with regard to their in vivo functions in the transport of antibiotics, basic amino acids, and gluconate. In addition, two interesting mutant OprDs, OprDΔL2 and OprDΔL5, were purified, and their in vitro functions were analyzed in the black lipid bilayer system. The results allow us to propose a general architecture for the OprD channel and to explore the mechanism of imipenem transport through the OprD channel.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *P. aeruginosa* PAO1 strain H103 was used as the OprD-containing wild-type strain (26). *P. aeruginosa* H636 and H729 were *oprF*::Ω and *oprD*::Ω derivatives of strain H103 created by gene replacement with Ω interposon-mutated *oprF* and *oprD* genes, respectively (11, 25). *Escherichia coli* CE1248 (F$^-$ *recA56 phoE proA,B phoR69 ompB471 thr leu thi pyrF thy ilvA his lacY argG tonA rspL cod dra utr glpR*) (23) was a strain with mutations preventing the production of porins OmpF, OmpC, and PhoE.

Broad-host-range plasmid pUCP19 (21) was used for the overexpression of OprD in *P. aeruginosa*. Plasmid pXH2 contained the *oprD* gene, which was

TABLE 1. Effects of deletions on other antibiotic susceptibilities of *P. aeruginosa* strains

Strain[a]	MIC (μg/ml)[b]						
	Cefpirome	Cefotaxime	Aztreonam	Ciprofloxacin	Fleroxacin	Tetracycline	Chloramphenicol
H729	1.0	4.0	1.0	0.125	0.5	6.25	25
H729(pUCP19)	4.0	8.0	2.0	0.125	0.5	12.5	50
H729(pXH2)	4.0	8.0	2.0	0.125	0.5	6.25	25
H729(pHP1)	2.0	4.0	2.0	0.06	0.25	6.25	25
H729(pHP2)	4.0	8.0	1.0	0.125	0.5	6.25	25
H729(pHP5)	**0.5**	**2.0**	**0.5**	**0.0156**	**0.06**	**0.78**	**0.78**
H729(pHP6)	8.0	8.0	2.0	0.25	1.0	12.5	50
H729(pHP7)	**0.5**	**2.0**	**0.5**	**0.0312**	**0.125**	6.25	**3.12**
H729(pHP8)	**0.5**	**2.0**	**0.5**	**0.0312**	**0.125**	6.25	**3.12**

[a] Strains in boldface showed differences in antibiotic susceptibilities.
[b] Determined by the agar dilution method on Mueller-Hinton plates. Each MIC was determined three times independently. Values in boldface were different from the control values. The MIC of trimethoprim was 100 μg/ml for all strains.

cloned as the 2.1-kb *Bam*HI-*Kpn*I fragment into pUCP19 in the same orientation as the *lac* promoter (11). Plasmids pHE1 to pHE8 were pTZ19R containing the corresponding mutant *oprD* genes with deletions in the region encoding the predicted L1 to L8. Plasmids pHP1 to pHP8 were pUCP19 containing the corresponding mutant *oprD* gene with deletions in the region encoding the predicted L1 to L8 (12).

Strains were routinely grown on Luria broth (LB) medium (1.0% Bacto Tryptone, 0.5% yeast extract, 0.5% NaCl) or LB agar containing, in addition, 2% Bacto Agar. *P. aeruginosa* strains were also grown on Mueller-Hinton broth. The following antibiotics were used in selective media: ampicillin (75 μg/ml) for *E. coli* (replaced by carbenicillin [750 μg/ml] for *P. aeruginosa*) and kanamycin (300 μg/ml) for *P. aeruginosa*.

MC determinations. MICs were determined as previously described (11).

Purification of OprD. For the purification of OprD from *E. coli*, strain CE1248(pBK19R) was used, and cultures were grown at 37°C with 75 μg of ampicillin per ml, 0.4% glucose, and 1 mM isopropylthiogalactoside (IPTG) to an optical density at 600 nm of 0.8 to 1.0. For the purification of OprD from *P. aeruginosa*, strain H636(pXH2) lacking OprF and overexpressing OprD was used, and cultures were grown at 37°C with 750 μg of carbenicillin per ml and 0.4% glucose to an optical density at 600 nm of 0.8 to 1.0. Mutant proteins OprDΔL2 and OprDΔL5 were purified from CE1248(pHE2) and CE1248 (pHE5), respectively.

Outer membranes were isolated as described previously (9) and subjected to a three-step differential detergent solubilization to concentrate OprD and remove other membrane components. First, the pellet was extracted with 10 mM Tris-HCl (pH 8.0)–0.5% octyl-polyoxyethylene (octyl-POE) and then centrifuged at 45,000 rpm for 1 h. The supernatant was reserved, and the pellet was extracted with 10 mM Tris-HCl–3% octyl-POE–0.2 M NaCl and then centrifuged as described above. Finally, OprD was largely extracted from the pellet with 10 mM Tris-HCl–3% octyl-POE–0.1 M NaCl–5 mM EDTA and then centrifuged, and this supernatant was dialyzed against 10 mM Tris-HCl–5 mM EDTA–0.08% *N*,*N*-dimethyldodecylamine-*N*,*N*-oxide. The solubilized protein was loaded onto a fast protein liquid chromatography anion-exchange column (Mono Q; bed volume = 1.0 ml, flow rate = 0.5 to 1.0 ml/min) that had been equilibrated with 10 mM Tris-HCl–5 mM EDTA–0.08% *N*,*N*-dimethyl dodecylamine-*N*,*N*-oxide. The protein was eluted by applying a linear gradient of buffer which contained the ingredients specified above plus 0 to 1.0 M NaCl. After the first run, the fractions which contained the least contaminants were pooled and subjected to a second run with a much flatter salt gradient and a lower elution speed. OprD was eluted in a purified form during this step. The purified OprD was aliquoted and frozen at −70°C.

Black lipid bilayer techniques. All black lipid bilayer methods were performed exactly as described previously (2, 3, 8).

RESULTS

Effects of deletion on antibiotic susceptibilities. In a previous study (12), we examined the effects of deletions on imipenem susceptibility. Control experiments indicated that the plasmid copy numbers were similar in all strains tested and that the deletions did not grossly disrupt the integrity of the outer membrane in that MICs for polymyxin and gentamicin were unaffected by any deletion (12). Here we confirmed this result, since trimethoprim susceptibility was unaltered (note that trimethoprim is taken up via the hydrophobic pathway, whereas gentamicin and polymyxin utilize the self-promoted uptake). In addition, there were no significant differences in the growth rates for all strains, indicating that the deletions did not cause substantial metabolic disturbances. Therefore, the differences in MICs observed were due only to changes in OprD brought about by the deletions of the predicted loops.

MICs of several other antibiotics for strain H729 expressing the OprD deletion derivatives were assessed. The L1 and L6 deletions had no effect on antibiotic susceptibility (Table 1), whereas the L2 deletion affected only imipenem and meropenem susceptibility, as previously noted (12). In contrast, the deletion in L5 led to enhanced susceptibilities to several different kinds of antibiotics, including β-lactams (cefpirome, cefotaxime, and aztreonam), quinolones (ciprofloxacin and fleroxacin), chloramphenicol, and tetracycline [Table 1, H729(pXH2) expressing native OprD]. For chloramphenicol, the susceptibility increased 32-fold. Similar results were obtained for the deletions in L7 and L8 except that there were no differences in susceptibility to tetracycline (Table 1). None of these three deletions prevented reconstitution of imipenem susceptibility in an *oprD*::Ω background (12). These results indicated that the deletions in L5, L7, and L8 appeared to have resulted in a more open channel, leading to a significant increase in the permeability of the channel to antibiotics that were normally excluded. This could be explained by the deletions either changing OprD from a specific porin to a general porin or converting OprD to a specific channel with high general permeability as observed for the *E. coli* sucrose porin ScrY (20).

It has been previously demonstrated that lysine will compete with imipenem for uptake through the OprD channel, resulting in an increasing MIC as a function of lysine concentration (10). To determine if the deletions in L5, L7, and L8 also affected the passage of imipenem and lysine through OprD (i.e., from specific to nonspecific uptake), competition experiments were performed. The data (Fig. 1) were consistent with the conclusion that those deletions did not influence the binding of imipenem or lysine in the OprD channel, in contrast to the L2 deletion mutant (12).

Effects of deletion on sugar transport. To determine if the L2, L5, L7, or L8 deletion affected the function of OprD as a nonspecific channel for the transport of gluconate, strain H729 cells expressing those OprD derivatives were grown in BM2 minimal medium with gluconate as the carbon source at concentrations ranging from 0.5 to 10 mM. The growth rates of the strains expressing the L5, L7, or L8 deletion did not show significant differences from that of strain H729 expressing native OprD (11), suggesting that those deletions did not affect the passage of gluconate through the OprD channel. In contrast, H729(pHP2) did not grow in BM2 with gluconate as the

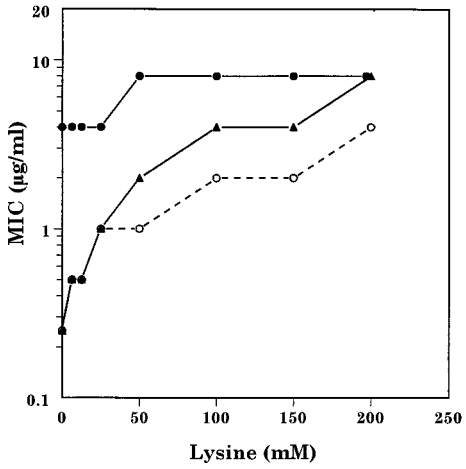

FIG. 1. Effect of L-lysine concentration in BM2 glucose medium on the susceptibility of OprD-defective strain H729 (filled circles), H729 expressing OprD with a deletion in predicted L5, H729(pHP5) (open circles), and H729 expressing native OprD, H729(pXH2) (triangles). The same results as observed for H729(pHP5) were obtained for strains H729(pHP7) and H729(pHP8).

carbon source, possibly because of alterations in the channel interior in this mutant.

Effects of deletion on single-channel conductance of porins. The physical properties of porins were further investigated in black lipid bilayer experiments. Wild-type OprD, OprDΔL2, and OprDΔL5 were purified. At nanomolar concentrations, all porins were able to increase the specific conductance of the lipid bilayer by several orders of magnitude. The time courses of the increase were similar. After a rapid increase during 10 to 40 min, the membrane conductance increased at a much slower rate. The addition of the porins at much lower concentrations to the aqueous phases bathing lipid bilayer membranes allowed the resolution of stepwise increases in conductance (Fig. 2), presumably as a result of the incorporation of individual porin units into the membrane, as suggested for other porins (1). Regarding wild-type OprD, for 170 measured single-channel events, the average single-channel conductance in 1 M KCl was 20 pS, which was at least 10 times smaller than those of most other porins studied to date. The only exception was the *E. coli* nucleotide-specific porin Tsx, with an average single-channel conductance of 10 pS. The purified OprD from the *E. coli* clone and from *P. aeruginosa* did not show any differences in single-channel conductance, indicating that OprD expressed from the cloned gene was properly folded in the *E. coli* outer membrane. Ishii and Nakae (13) demonstrated occasional open channels of OprD with a much higher conductivity (400 pS), especially in the presence of lipopolysaccharide. However, we did not observe any such events in hundreds of measurements and more than dozen independent experiments. One possibility for such large channels might have been the contamination by other porins in the preparation.

For OprDΔL2, the average single-channel conductance (26 pS in 1.0 M KCl) was slightly bigger than that of OprD. However, mutant OprD with a deletion in L5 showed more than a 30-fold increase in the average single-channel conductance (675 pS in 1.0 M KCl) compared with that of the wild-type OprD (Fig. 2B). The much bigger single-channel conductance confirmed the suggestion presented above that the deletion increased the general permeability of the channel, which, in turn, increased the susceptibilities of H729(pHP5) to different antibiotics (Table 1). For all channels studied, increasing the

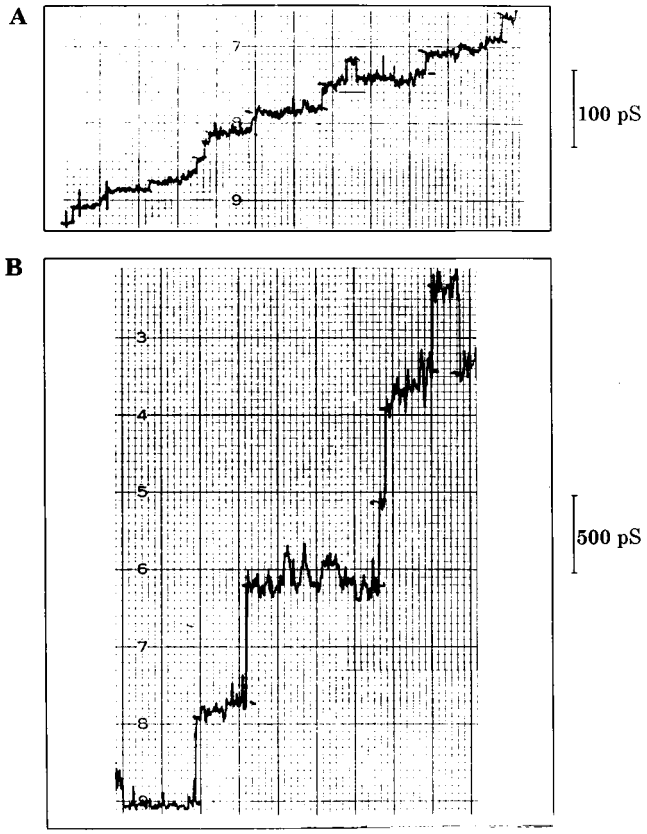

FIG. 2. Chart recording of the stepwise increase in current upon addition of purified porins to the aqueous phase (1 M KCl, pH 7) bathing a lipid bilayer membrane made from 1% oxidized cholestrol in *n*-decane. (A) Native OprD; (B) OprDΔL5. The applied voltage was 20 mV. Note that the resolution of the chart recorder was higher in panel A than in panel B.

size of the cation in the solution bathing the membranes caused a steady decrease in the average single-channel conductance, which was little affected by changing the size of anion (Table 2), indicating these channels were cation selective. For the L5 deletion variant, a linear relationship was demonstrated between the salt (KCl) concentration and single-channel conductance (Table 2). In contrast, increasing the salt concentration from 1 to 3 M KCl resulted in a relatively small increase in conductance for the native OprD and OprDΔL2 channels (Table 2). A similar result for *E. coli* hemolysin (4) has been

TABLE 2. Average single-channel conductances of the native and mutant OprD pores in different salt solutions

Aqueous salt solution	Conductance (pS)		
	OprD	OprDΔL2	OprDΔL5
0.3 M KCl	—[a]	—	229
1.0 M KCl	20	26	675
3.0 M KCl	26	31	1,711
1.0 M CsCl	15	<10	557
1.0 M LiCl	<10	—	272
1.0 M K-MOPS	19	25[b]	640

[a] —, the single-channel conductance was too small for the resolution of the equipment to detect.

[b] 1.0 M K-MOPS (K-morpholine-propanesulfonic acid) was replaced by 1.0 M KNO_3, since the membrane was too noisy in 1.0 M K-MOPS in the presence of OprDΔL2.

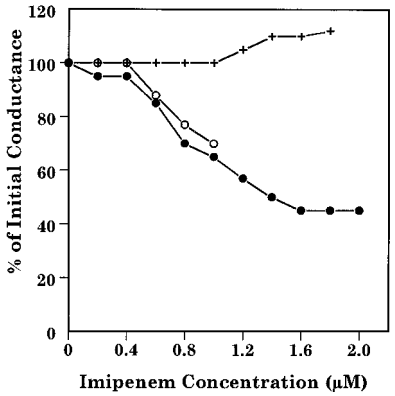

FIG. 3. Macroscopic conductance inhibition experiments to assess the binding of imipenem to native OprD (filled circles), OprDΔL2 (pluses), and OprDΔL5 (open circles). Purified porin was added to the salt solution (1 M KCl) bathing a lipid bilayer membrane. The increase in conductance due to insertion of porin pores was monitored until the rate of increase had slowed (1 to 2 h) and the membrane contained more than 100 channels. At this time, aliquots of imipenem, to increase the concentration in 0.2 μM steps, were added to the bathing solutions at both sides of the membrane, and the decrease of conductance due to binding of imipenem to the interior of the channel, blocking the movement of KCl through this channel, was monitored until the conductance stabilized (about 2 min). Additional aliquots were then added as described above. A representative experiment is shown.

interpreted as being due to the point charges at the pore mouth which caused a substantial surface potential. For OprD, these charges would be assumed to be due to anionic (acidic) amino acids that would tend to attract cations and repel anions.

Effects of deletion on the substrate specificity of porins. To demonstrate that the OprD channel possessed a specific binding site(s) for imipenem, a macroscopic conductance inhibition experiment was performed. Large bilayer membranes (2 mm^2) were formed in 1 M KCl. A small amount of purified OprD was added to one side of the membrane, and the conductance started to rise rapidly for 10 to 40 min and thereafter continued to rise at a decreasing rate. At this time, membrane conductance had increased 2 to 3 orders of magnitude, and more than 1,000 channels were present in the membrane. Aliquots (60 μl) of imipenem solution (20 μM) were added to the aqueous solution at both sides of the membrane, and the conductance decreased to a new level over a period of about 2 min (Fig. 3). The ability of imipenem to block KCl movement provided direct evidence that the OprD channel contained an imipenem binding site(s). In addition, by plotting the data as the reciprocal of percent inhibition of conductance as a function of the reciprocal of imipenem concentration, it was possible to derive an I_{50} value (i.e., a concentration of imipenem resulting in 50% inhibition of the original conductance) of 0.58 ± 0.20 μM in four separate determinations. In these macroscopic conductance measurements, we were unable to decrease, by imipenem addition, the conductance of KCl by more than 66% for the OprD porin. There are at least two potential explanations for this finding. First, it is possible that even with imipenem associated with its binding site in the OprD channel, the channel might not be completely blocked, resulting in significant residual conductance, as found for the sucrose porin (20). Second, the conductance of individual OprD channels is so low that even a 0.3% contamination by the CE1248 porin previously described (10) would be sufficient to explain this residual non-imipenem-inhibitable conductance.

For OprDΔL2, no decrease in conductance was observed with the addition of up to 2.4 μM imipenem in eight separate experiments (Fig. 3). These results indicated that OprDΔL2 had a much lower affinity to imipenem, which further suggested that the deleted stretch was involved in the specific binding of imipenem. This in vitro result was in complete agreement with the in vivo functional data for H729(HP2) (12). In case of OprDΔL5, the progressive decrease in conductance upon the addition of imipenem solution was still observed (Fig. 3) and an I_{50} value similar to that measured for native OprD was obtained, suggesting that the deletion did not prevent the specific binding of imipenem. Therefore, OprDΔL5 was still a specific porin but with much higher general permeability like that of the *E. coli* sucrose porin ScrY (20).

DISCUSSION

Functional studies of the deletion mutants permitted us to suggest how the external loops are situated with respect to the interior of the OprD channel. The evidence supported the involvement of L2 in imipenem binding. Compared with the cloned wild-type OprD, the L2 deletion mutant only partially reconstituted supersusceptibility to imipenem and meropenem in the OprD-defective background of strain H729 (12), indicating that the uptake of imipenem and meropenem was seriously affected by this deletion. Consistent with this observation, imipenem had a reduced ability to bind to this mutant, as judged by its inability to inhibit the macroscopic conductance of KCl through channels formed by OprDΔL2.

Recently the maltodextrin-specific LamB porin of *E. coli* has been crystallized (19). While LamB appears to belong to a separate family of 18-β-stranded porins, it has many conceptual similarities to the 16-β-stranded porin superfamily (of which OprD is proposed to be a member [12]), since both types of porins form trimers of monomers constructed as β-barrels in which the β-strands are interconnected by short periplasmic turns and longer surface loops (5, 14, 19, 25). In the crystal structure of specific porin LamB (19), more than one loop was folded inside the channel, including L3, which was entirely folded into the barrel to form the eyelet (most constricted region of the channel), whereas L1 and L6 from the same monomer and L2 from the adjacent monomer were folded inward to different extents. The general porins also have constriction zones that are restricted by L3 residues. Our own data for OprD did not preclude the coinvolvement of L3, which, in all of the structurally defined porins, folds into the center of the channel to form the eyelet region determining channel diameter and selectivity. In addition, we cannot disprove the alternative possibility that L2 is involved in stabilizing L3, which contains the actual binding-site residues. A third possibility is that L2 represents an additional binding site that is not located in the constriction zone. If this were correct, we would predict that the substrates of OprD would bind to two sites progressively or in tandem, as has been proposed for LamB (19). This third possibility is favored by the architecture of the structurally defined porins in which L2 extends towards and interacts with neighboring subunits.

We propose that L5, L7, and L8 fold partly into the OprD channel to restrict the channel diameter at its entrance. Deletion of these loops did not affect the susceptibility to imipenem but led to enhanced susceptibilities to those antibiotics, including β-lactams, quinolones, chloramphenicol, and, for ΔL5, tetracycline, which have been proposed to cross the outer membrane through the hydrophilic pathway. In good agreement with this finding, the single-channel conductance of OprDΔL5 (675 pS) was more than 30 times higher than that of native OprD (20 pS). Interestingly, the deletions studied did not

affect the specific binding site(s), as confirmed by the antagonistic effects of lysine on imipenem MICs for the mutants and the retention of the ability of imipenem to inhibit macroscopic conductance for the OprDΔL5 channels. Therefore, these three loops were not involved in the binding site. The MICs of chloramphenicol and tetracycline for H729(pHP7) and H729 (pHP8) were four- and eightfold higher than the MICs for H729(pHP5), respectively. From their chemical structures, these two antibiotics are quite bulky, and the four-ring-structured tetracycline is even bulkier than chloramphenicol. We propose that in order to allow the maximum passage of these two antibiotics, the channel has to be more open. Therefore, the differences in MICs could be explained if the deletions in L7 and L8 did not open the channel as widely as did the deletion of L5. Deletions in L1 and L6 did not significantly affect susceptibilities to imipenem or the other antibiotics tested, suggesting that these two loops were not as important in determining the channel size or selectivity.

Previous studies of *P. aeruginosa* have indicated an important role for low outer membrane permeability in the known high intrinsic resistance of this organism to antibiotics (18). On the other hand, recent studies have placed more emphasis on efflux (15, 16). The studies reported here supported the assumption that outer membrane permeability is an important determinant of antibiotic susceptibility. The single-channel conductance of the native porin OprD (20 pS) was far smaller than that of OprDΔL5 (675 pS). The small size of the native OprD channel presumably served to maintain the low intrinsic permeability of the *P. aeruginosa* outer membrane (17). In contrast, the L5 deletion mutant channel had a dramatic effect on antibiotic susceptibility. The only difference between H729(pXH2) and H729(pHP5) was that the latter contained a deletion of eight amino acids from OprD, resulting in the presence of a large channel in the *P. aeruginosa* outer membrane. The supersusceptibility of H729(pHP5) thus supported the critical role of the outer membrane of *P. aeruginosa* as a barrier to antibiotics (18). Presumably a saturable antibiotic efflux system would be more easily overwhelmed in the OprDΔL5 mutant because of substantially enhanced antibiotic diffusion across the outer membrane through this mutant channel.

The journey of imipenem and basic amino acids through the OprD channel may be depicted as follows. The initial prescreening regarding size and charge might be done by the loops exposed at the cell surface. These could function as a primary filter, concentrating the substrates such as imipenem and basic amino acids, especially when they were in low concentrations in the medium. The substrate molecule would then enter the mouth of the channel, which would be constricted by L5, L7, and L8. As in the maltose transport system, small peptides and imipenem are elliptical molecules, which would exceed, in their long axes, the exclusion limit of the OprD channel. Therefore, residues located at the mouth would be expected to orient imipenem and basic peptides so that they would be aligned to the pore axis. As suggested above, it is possible that L2 residues serve this function. It is unknown whether the concept of a ""greasy slide"" observed for LamB (18) could be applied to the OprD channel, but for the efficient transport of such extended molecules, we feel that there must be residues located along the channel to guide their diffusion. The substrate molecules would then encounter the constriction zone about halfway through the channel, at which position side chains, comprising charged residues of L3 and/or L2, and the barrel wall would bind to substrate molecules in a highly stereospecific manner influenced by the size, geometry, and charge of the substrate. This would presumably account for the specificity of the channel. After binding at the narrow constriction zone, the imipenem or basic amino acid molecules would be effectively released into the periplasm by a classical ""knock-on"" mechanism as long as the diffusion gradient was oriented from outside to inside. In spite of the structural similarity in side chains between imipenem and basic amino acid-containing dipeptides, it is obvious that imipenem has a carbapenem nucleus which is different from the peptide backbone, and this might explain the different affinities between imipenem and basic amino acids as described previously (23). The higher affinity of the channel for imipenem indicates that imipenem could fit in the specific binding site(s) better than basic amino acids. On the other hand, it is possible that the specific binding sites are located in such a way that they could have good but not very high affinity for various nutrients of similar structures, such as basic amino acids and small peptides containing these amino acids. Those carbapenems that do not utilize OprD and contain one additional positive group at position 1 or 6 (6) might be excluded from the mouth because of their bulkiness or because they might not be able to fit into the specific binding sites.

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