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Purification of Glucose-Inducible Outer Membrane Protein OprB of *Pseudomonas putida* and Reconstitution of Glucose-Specific Pores

EDWARD G. SARAVOLAC,[1]† NORMAN F. TAYLOR,[1] ROLAND BENZ,[2] AND ROBERT E. W. HANCOCK[3]*

Department of Chemistry and Biochemistry, University of Windsor, Windsor, Ontario, Canada N9B 3P4[1]; Lehrstuhl fur Biotechnologie der Universitat Wurzburg, Wurzburg, Germany[2]; and Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5[3]

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A 43,000 molecular-weight, glucose-inducible, organic acid-repressible protein (OprB) was identified in the outer membrane of *Pseudomonas putida*. OprB was surface expressed in whole cells, had a high β-sheet content, and was heat modifiable, as demonstrated by ^{125}I-labeling, circular dichroism spectroscopy, and mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. OprB was extracted from outer membrane preparations by using 2% Lubrol PX with 10 mM EDTA and purified by DEAE-Sephacel ion exchange chromatography following ammonium sulfate precipitation. Reconstitution experiments with black lipid membranes showed that OprB formed small, cation-selective pores which bound glucose (K_S = 110 mM) and other carbohydrates. However, the binding site of OprB appeared to be distinct from that of the maltodextrin-specific porin LamB from *Escherichia coli*.

Pseudomonas putida is a member of the *Pseudomonas fluorescens* branch (group I) of the family *Pseudomonadaceae*, a group of organisms that also includes *P. aeruginosa* (17). The outer membranes of *P. putida* and related pseudomonads contain a number of proteins which are homologous biochemically, immunologically and genetically to those described in *P. aeruginosa* (15). However, to date no functional studies of *P. putida* porins have been described.

One interesting class of porins are those outer membrane proteins which are involved in high-affinity substrate transport and contain channels with saturable binding sites for specific substrates. The best studied examples of this class are the maltodextrin-specific porin LamB (5, 6, 21) and nucleotide-specific porin Tsx (18) from *Escherichia coli* and the phosphate-specific porin OprP (12) and imipenem/basic amino acid-specific porin OprD (26) of *P. aeruginosa*.

LamB is part of a shock-sensitive maltose transport system which mediates the accumulation of maltose into the cell against 100,000-fold concentration gradients, and mutants of *E. coli* lacking LamB are impaired in maltose uptake (5, 7). LamB reconstituted into black lipid membranes has been shown to bind maltose and maltodextrins up to maltoheptose with K_d decreasing from 10 to 0.067 mM with increasing maltodextrin molecular weight (5, 6). On the basis of this data, a binding site that interacts with up to 5 glucose residues has been proposed (6). In contrast, the phosphate porin OprP appears to have a simple binding site comprising a ring of 3 lysine residues (12).

A less well-characterized system for carbohydrate uptake has been described in the pseudomonads. *P. aeruginosa* and *P. putida* have common pathways for the dissimilation of glucose (1, 16). Glucose uptake follows either an oxidative pathway in which it is oxidized extracellularly to gluconate or 2-ketogluconate on the cytoplasmic membrane and subsequently transported (1, 10) or a phosphorylative pathway in which it is transported directly by a high-affinity uptake system (K_m = 7 μM) and subsequently phosphorylated intracellularly (10, 16). High-affinity glucose uptake is induced by growth on glucose (10), and under these conditions a periplasmic glucose-binding protein (25) and the outer membrane porin protein OprB (previously called protein D1) are also induced in *P. aeruginosa* (14). A glucose-inducible outer membrane protein has also been observed in a *P. fluorescens* strain (9). Liposome-swelling assays have indicated that *P. aeruginosa* OprB may be selective for glucose (27), but there is as yet no evidence for a direct role of OprB in glucose transport nor an assessment of the glucose-binding kinetics of this porin.

In the present study, we have identified, purified, and characterized a glucose-inducible, organic acid-repressible protein from the outer membrane of *P. putida*.

MATERIALS AND METHODS

Bacterial strains and media. *P. putida* (ATCC 12633) and *P. aeruginosa* (ATCC 15692) were obtained from the American Type Culture Collection. Cells were maintained on nutrient agar and routinely cultured on the semidefined mineral-salts medium of Davis and Mingioli (8) containing 0.1% (vol/vol) trace solutions (2) with 0.2% of a carbon source. Cultures were grown up to 2 liters in flasks, or large-scale (12-liter) cultures were grown in a fermenter (model SF-116; New Brunswick Scientific, Melrose Park, Ill.). In either case, cells were grown at 30°C to an optical density (620 nm) of 0.9 to 1.1 and harvested at 4,000 × *g* for 20 min at 20°C.

Outer membrane preparation and OprB purification. The outer membrane preparation was prepared by the one-step procedure of Hancock and Carey (14) with the following modifications. First, the cell suspension was not pretreated with lysozyme. Second, before application to the sucrose density gradient, the outer membrane-enriched cell envelope fraction was harvested from the French-pressed cell suspension by centrifugation at 20,000 × *g* for 90 min at 4°C. To purify the glucose-inducible protein, the outer membrane preparation was suspended to a concentration of 2 mg of

* Corresponding author.
† Present address: Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

protein per ml in a mixture containing 2% Lubrol PX, 10 mM EDTA, and 20 mM Tris-HCl [pH 8.0] and incubated with stirring at room temperature for 30 min. Undissolved outer membrane was removed by centrifugation at 90,000 × *g* for 1 h. The supernatant containing extracted proteins was treated successively with 30, 40, and 90% saturated ammonium sulfate and incubated with stirring for 15 to 20 min at 23°C after the addition of each. The precipitates were harvested by centrifugation at 15,000 × *g* for 20 min (at 20°C), after which the floating pellet was collected by decanting the subnatant. Each precipitate was suspended in a minimum volume of distilled, deionized water, and ammonium sulfate was removed by passage through a column (1.5 by 26 cm) of Sephadex G-25 equilibrated with 0.2% Lubrol–10 mM EDTA–20 mM Tris-HCl (pH 8.0)–50 mM NaCl (column buffer). The 90% ammonium sulfate fraction was loaded onto a column (1.5 by 18 cm) of DEAE-Sephacel equilibrated with column buffer, and 3-ml fractions were collected. The column was washed with 60 to 80 ml of column buffer, and a 150-ml linear gradient of 50 mM to 400 mM NaCl was eluted in column buffer. The fractions containing OprB were pooled and stored at 4°C. OprB from *P. aeruginosa* was also purified by this method. Further purification of this protein for amino acid analysis was accomplished by electroelution of the protein band excised from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels by using the Bio-Rad Model 442 electroeluter (Bio-Rad, Richmond, Calif.) fitted with a 3,500-Da cut-off membrane cap. Bands of OprB from *P. aeruginosa* were visualized in the gel by staining in ice-cold 0.25 M KCl as previously described (11). SDS was removed by dialysis against distilled water.

Cell surface iodination with ^{125}I. Proteins expressed at the cell surface were determined by cell surface labeling with ^{125}I by using the method of Richardson and Parker (20).

SDS-PAGE. Outer membrane proteins were analyzed by SDS-PAGE with the 11% gel system of Lugtenberg et al. (17) with a 5% stacking gel. When reducing conditions were utilized, freshly prepared (or freshly frozen) dithiothreitol was added to the solubilization reduction mix to permit subsequent separation of OprB and OprF.

Circular dichroism spectroscopy. The circular dichroism (CD) spectrum of OprB from *P. putida* was determined in the Department of Biochemistry, University of Toronto. The spectrum was measured on a JASCO J-41A spectropolarimeter. The 0.1-cm path length quartz cuvette contained 0.75 μg of purified OprB per ml of 0.06% Lubrol PX–6.3 mM Tris-HCl (pH 8.0)–31 mM NaCl.

Amino acid analysis. Approximately 1.5-nmol samples of OprB from *P. putida* and *P. aeruginosa* were analyzed for amino acid content by Tak Leung in the laboratory of Lana Lee, Department of Chemistry and Biochemistry, University of Windsor, by using the Pico-Tag system (Waters Associates, Milford, Mass.). Detergent and lipopolysaccharide was removed from the proteins prior to amino acid analysis by phenol extraction as previously described (24).

Black lipid membrane experiments. Methodology for the reconstitution experiments with black lipid bilayer membranes have been previously described (3, 5). Briefly, the apparatus consisted of a Teflon chamber with a thin wall separating two aqueous compartments. The Teflon divider had small circular holes with an area of either 2 mm^2 (for macroscopic conductance measurements) or 0.1 mm^2 (for single-channel experiments). A 1% solution of diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Birmingham, Ala.) in *n*-decane was painted over the holes to form the membrane. Reconstitution experiments were initiated after the lipid bilayer membrane thinned out and turned optically black to incident light, indicating bilayer formation. The aqueous salt solutions (Merck, Darmstadt, Germany) were used unbuffered and had a pH of around 6. The temperature was kept at 25°C throughout.

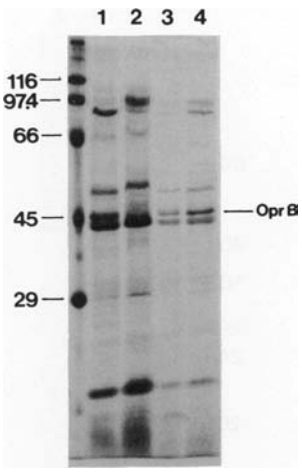

FIG. 1. Outer membranes prepared from *P. putida* grown on various carbon sources. Cells were grown on the following carbon sources. Lanes: 1, glucose; 2, gluconate; 3, maltose; 4, lactose. Molecular weight standards are given in thousands. The lower amounts of sample loaded in lanes 3 and 4 (10 μg of protein), as opposed to lanes 1 and 2 (30 μg of protein), reflects the poor growth of, and consequent poor yield of outer membranes from, *P. putida* on maltose and lactose.

The membrane current was measured with a pair of calomel electrodes switched in series with a voltage source and an electrometer (Keithley 602). In the case of the single-channel recordings, the electrometer was replaced by a current amplifier. The amplified signal was monitored with a storage oscilloscope and recorded with a tape or a strip chart recorder. Zero-current membrane potential measurements were performed by establishing a salt gradient across membranes containing 100 to 1,000 OprB channels as has been described earlier (7). Macroscopic conductance inhibition experiments were performed as described previously (5, 6, 12).

Assays. Protein concentrations were determined by the Lowry assay with the formulation of Scopes (22), with bovine serum albumin as the standard. D-[6-^{3}H]glucose (specific activity, 38 mCi/mmol; ICN Radiochemicals, Irvine, Calif.) binding to purified OprB from *P. putida* was performed by using the equilibrium dialysis method of Stinson et al. (25). Radioactivity was counted with the Beckman LS 7500 liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, Calif.) and Dupont Formula 963 liquid scintillation cocktail (NEN Research Products, Boston, Mass.).

RESULTS

Induction and repression of OprB. When outer membrane prepared from *P. putida* grown on glucose as the sole carbon source was subjected to SDS-PAGE, a 43-kDa glucose-inducible protein (OprB) was observed (Fig. 1). The glucose-inducible OprB was repressed by growth on organic acids such as gluconate and the citric acid cycle intermediates

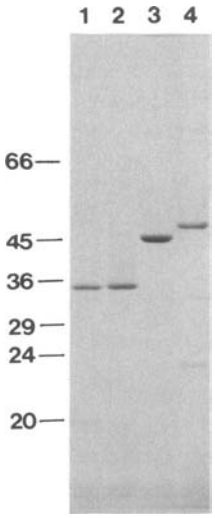

FIG. 2. Heat-modifiable mobility of glucose-inducible proteins from *P. putida* and *P. aeruginosa*. Lanes 1 and 4, *P. aeruginosa* OprB incubated in the solubilization reduction mix at 30 and 95°C, respectively, prior to electrophoresis; Lanes 2 and 3, *P. putida* OprB incubated at 30 and 95°C, respectively. Molecular weight markers are given in thousands.

TABLE 1. Amino acid composition of purified OprB from *P. putida* and *P. aeruginosa* and comparison with LamB

Residue	No. of residues/100 amino acid residues (%)		
	P. putida OprB[a]	*P. aeruginosa* OprB[a]	*E. coli* LamB[b]
Asparagine + aspartic acid	11.6 (0.48)	12.0 (0.30)	15.9
Glutamine + glutamic acid	12.9 (0.32)	12.2 (0.15)	10.6
Serine	6.0 (0.07)	5.7 (0.10)	6.7
Glycine	12.8 (0.04)	11.3 (0.16)	11.9
Histidine	2.1 (0.23)	1.9 (0.00)	1.5
Alanine	10.0 (0.21)	7.4 (0.05)	8.5
Proline	3.8 (0.04)	4.2 (0.05)	2.0
Tyrosine	3.1 (0.03)	4.2 (0.10)	5.2
Valine	7.5 (0.31)	8.6 (0.24)	5.2
Methionine	1.3 (0.09)	1.0 (0.10)	3.0
Isoleucine	2.9 (0.07)	3.6 (0.22)	4.7
Leucine	7.4 (0.05)	8.6 (0.24)	4.7
Phenylalanine	4.0 (0.61)	3.7 (0.10)	5.0
Lysine	4.1 (0.23)	5.9 (0.13)	4.7
Arginine	4.7 (0.07)	5.3 (0.95)	4.0
Threonine	4.3 (0.09)	4.5 (0.15)	6.2

[a] Amino acid analysis performed by T. Leung in the laboratory of L. Lee (University of Windsor) by using the Waters Pico Tag system, with average of four determinations. Standard deviations are in brackets.
[b] Calculated from the sequence data of Clement and Hofnung (7).

citrate, malate, and succinate. OprB was also induced by growth of cells in the presence of lactose or maltose. However, the minimal growth on the latter two carbon sources was probably sustained by yeast extract present in the media. This pattern of induction and repression was virtually identical to that observed for the glucose-inducible pore-forming protein OprB (D1) of *P. aeruginosa* (14).

Purification and properties of OprB. The purification procedure previously utilized by Hancock and Carey (14) for *P. aeruginosa* OprB was modified by the substitution of 2% Lubrol PX for Triton X-100, since this permitted monitoring of purification by UV adsorbance. By using the procedure described in Materials and Methods, the glucose-inducible OprB band was obtained in greater than 95% purity from *P. putida* (Fig. 2, lanes 2 and 3) and in only slightly lower purity from *P. aeruginosa* (Fig. 2, lanes 1 and 4). The DEAE-Sephacel elution profiles for OprB from *P. putida* and *P. aeruginosa* were virtually identical (not shown), and this suggested that there may be other similarities between the two glucose-inducible proteins. OprB from *P. aeruginosa* had been previously described as heat modifiable on SDS-PAGE. After SDS-PAGE following solubilization at 100°C for 10 min, *P. aeruginosa* OprB ran at an apparent molecular mass of 47.5 kDa (Fig. 2), similar to that previously described (13, 14). This was approximately 4,500 Da larger than heat-modified *P. putida* OprB, which ran at 43 kDa. However, when the proteins were solubilized at room temperature in sample buffer containing 2% SDS (final concentration) prior to electrophoresis, both proteins ran at the same lower molecular mass of 35.5 kDa (Fig. 2, lanes 1 and 2), as reported previously for *P. aeruginosa* (14). Additional structural information about *P. putida* OprB was obtained by CD spectroscopy of the protein. The CD spectrum had a single minimum ellipticity at 215 to 217 nm, which is consistent with the presence of a high content of β-sheet structure.

Whole-cell suspensions of glucose- and succinate-grown *P. putida* were surface-labeled with $Na^{125}I$ by using Iodobeads (20) and subsequently analyzed by SDS-PAGE autoradiography. OprB was surface labeled, and its position was confirmed by running an adjacent lane of purified ^{125}I-labeled OprB (data not shown).

The amino acid compositions of *P. putida* and *P. aeruginosa* OprB were determined. Similar compositions were obtained for both proteins, and indeed these were comparable to the maltose-inducible porin LamB of *E. coli* (Table 1) and not greatly different from other reported porins.

Single-channel conductance experiments. Single-channel conductance experiments were performed by adding small amounts of *P. putida* OprB to the aqueous phase bathing the black lipid bilayer membranes (for a final concentration of 10 ng/ml). Step increases in conductance were observed shortly after the addition of protein to one or both sides of the lipid bilayer membrane. The average single-channel conductance of the OprB channel was 35 pS in 1 M KCl. A histogram of the conductance steps (Fig. 3) showed that there was a narrow range of step sizes, indicating that the porin preparation was very homogeneous with respect to channel size. This along with the SDS-PAGE analysis demonstrated that the porin preparation was pure and that contaminating porins were not present to a significant degree. In addition, this distribution of conductance steps was not influenced by the addition of 1 mM maltohexaose (a concentration that completely blocks LamB of *E. coli* and of *Salmonella typhimurium* [5, 21]), suggesting that the large maltooligosaccharide did not bind to OprB.

Single-channel conductances for OprB (this study) and LamB (5) were measured in the presence of KCl at various concentrations and in the presence of 1 M LiCl and 1 M potassium acetate (Table 2). When various concentrations of KCl were used, an almost linear dependence of the conductance on salt concentration was observed for both OprB and LamB. The observed single-channel conductance for OprB was significantly lower than that of LamB for all concentrations of KCl. LiCl and potassium acetate were used to investigate whether the channel was selective for cations or for anions, since Li^+ and acetate on the one hand and K^+

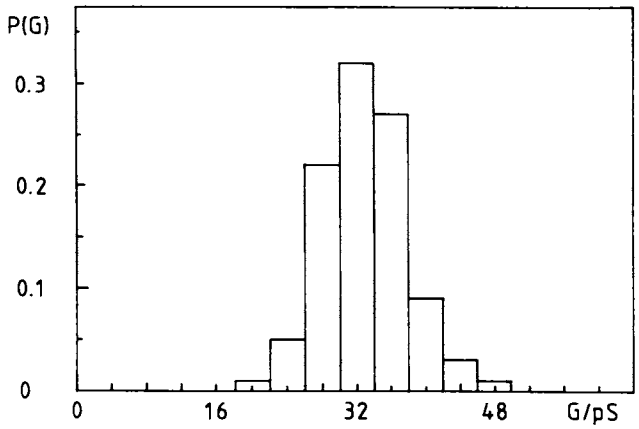

FIG. 3. Histogram of the conductance steps observed with diphytanoyl phosphatidylcholine-*n*-decane membranes in the presence of 10 ng of OprB protein from *P. putida* per ml. The average single-channel conductance was 35 pS for 148 steps. The aqueous phase contained 1 M KCl, the temperature was 25°C, and the applied voltage was 50 mV.

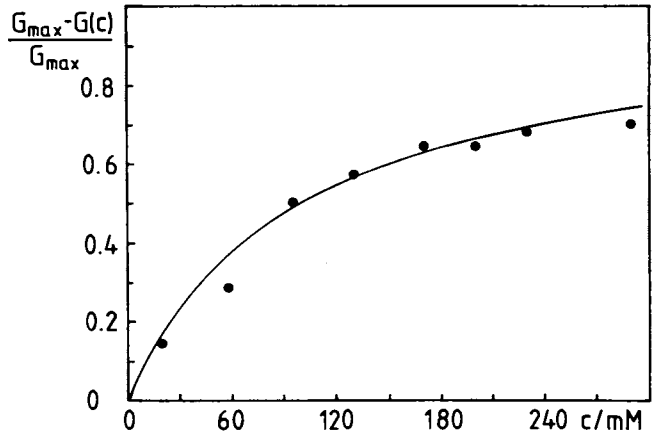

FIG. 4. Plot of the relative conductance inhibition, $[G_{max} - G(c)]/G_{max}$, as a function of the glucose concentration in the aqueous phase. The data were derived from a titration experiment in which the OprB-mediated membrane conductance, G(c), was measured as a function of increasing concentrations of glucose. The membrane was formed from diphytanoyl phosphatidylcholine-*n*-decane in an aqueous solution containing 1 M KCl and 100 ng of OprB per ml. The solid line was drawn from equation 1, assuming a stability constant for sugar binding of 10 liters/mol (corresponding to a half-saturation constant of 100 mM).

and Cl^- on the other hand have the same aqueous mobility (6). Interestingly, the single-channel conductance of the OprB channel was smaller in LiCl than in potassium acetate, which suggested that the channel was cation selective. It is interesting to note that a similar sequence has been observed for the single-channel conductances of LamB, although the absolute values of those for LamB were about a factor of three larger (Table 2) (6).

Binding of carbohydrates to OprB. Binding of [6-^{3}H]glucose to solubilized OprB was not detected by using equilibrium dialysis. Therefore, macroscopic conductance inhibition experiments were performed to determine whether the OprB porin channel was capable of binding carbohydrates with affinities unmeasurable by equilibrium dialysis methods. OprB from *P. putida* was added to the black lipid membrane in concentrations up to 100 ng/ml. The macroscopic conductance was allowed to reach its maximum within 30 to 40 min after the protein was added. At this point, increasing concentrations of glucose were added to the 1 M KCl solutions on both sides of the membrane to observe the effects on the macroscopic conductance. With increasing glucose concentration, we observed increasing inhibition of the macroscopic conductance. The addition of glucose up to a concentration of 300 mM resulted in a maximum inhibition of conductance at 70% (Fig. 4).

The results shown in Fig. 4 suggested that the addition of glucose led to a dose-dependent blockage of OprB-mediated membrane conductance, i.e., that the channel did not conduct ions when the binding site inside the channel was occupied. Assuming that the two-barrier, one-site model used for the description of sugar-transport through LamB of *E. coli* (6) and *S. typhimurium* (21) is valid, the conductance, G(c), at a given sugar concentration c, relative to the initial conductance, G_{max} (in the absence of sugar), is given by the following equation (6):

$$\frac{[G_{max} - G(c)]}{G_{max}} = \frac{K \cdot c}{(K \cdot c + 1)} \quad (1)$$

This equation indicates that the sugar-induced block of ion current through OprB can be used for the evaluation of the stability constant, K for sugar binding (half-saturation constant $K_S = 1/K$), from a plot of sugar binding as a function of sugar concentration (as given in Fig. 4) or by a Lineweaver-Burke plot. By using this equation, a half-saturation constant, K_S, of 100 mM ($K = 10\ M^{-1}$) could be calculated from the inhibition curve in Fig. 4 (this was close to the mean K_S of 110 mM obtained as a result of four experiments; Table 3).

Similarly, half-saturation constants and stability constants were calculated for several carbohydrates binding to OprB (Table 3). It is interesting to note that with the exception of glucose, the binding affinities of sugars to OprB were generally lower than they were to LamB (6). Furthermore, OprB bound glucose with higher affinity than did galactose (Table 3), whereas LamB had more than twofold-higher affinity for galactose than for glucose. OprB bound maltose with about three times greater affinity (K_S = 30 mM) than glucose, whereas LamB bound maltose with 11-fold greater affinity (6). With other disaccharides such as lactose and sucrose and higher maltooligosaccharides up to three residues, OprB had half-saturation constants at least 10 to 100 times higher

TABLE 2. Average single-channel conductance, G, of OprB of *P. putida* and LamB of *E. coli* in different salt solutions

Aqueous salt solution	G (pS)[a]	
	OprB	LamB[b]
0.1 M KCl	4	17
0.3 M KCl	11	57
1 M KCl	35	155
3 M KCl	140	570
1 M LiCl	15	40
1 M KCH_3COO (pH 7)	24	135

[a] The membranes were formed from diphytanoyl phosphatidylcholine dissolved in *n*-decane. The pH of the aqueous salt solutions was around 6 unless otherwise indicated. G was calculated from single-channel recordings by averaging at least 100 single events. The applied membrane potential was 50 mV; the temperature was 25°C.

[b] Taken from reference 7.

TABLE 3. Half-saturation constants, K_S and stability constants, K, for the binding of different sugars to the OprB channel[a]

Sugar	K (1/mol)	K_S (mmol/liter)
Glucose	9.1	110
Maltose	33	30
Maltotriose	17	60
Maltotetraose	≤1	≥1,000
Galactose	3.3	300
Sucrose	2.5	400
Lactose	1.7	600

[a] The membranes were formed from diphytanoyl phosphatidylcholine-*n*-decane. The unbuffered aqueous solutions (pH around 6) contained 100 ng of OprB per ml and 1 M KCl; the temperature was 25°C; V_m = 20 mV. The stability constant K is given as the mean of at least three experiments. K was calculated from titration experiments similar to that shown in Fig. 4. K_S is the half-saturation constant.

than those of LamB. Unlike LamB, the affinity for maltooligosaccharides did not increase for OprB with increasing number of glucose residues (Fig. 5). For maltotetraose, we could only give a lower limit for K, which was 1 M^{-1} (K_S = 1 M), since it bound extremely poorly.

Selectivity measurements. The single-channel measurements suggested that OprB was cation selective. To study the channel selectivity of OprB in more detail, we measured the zero-current membrane potential in the presence of salt gradients. After incorporation of 100 to 1,000 channels into the membrane, the salt concentration on one side of the membrane was raised in small steps from 50 to 250 mM. For each gradient, the zero-current potential was measured and the permeability ratio P_{cation}/P_{anion} was calculated according to the Goldman-Hodgkin-Katz equation (4). The results representing the means from at least three different membranes at a given experimental condition are presented in Table 4. For all three salts used in these experiments, the more dilute side was positive, which indicated a preferential movement of cations through the pore, i.e., that OprB was cation selective.

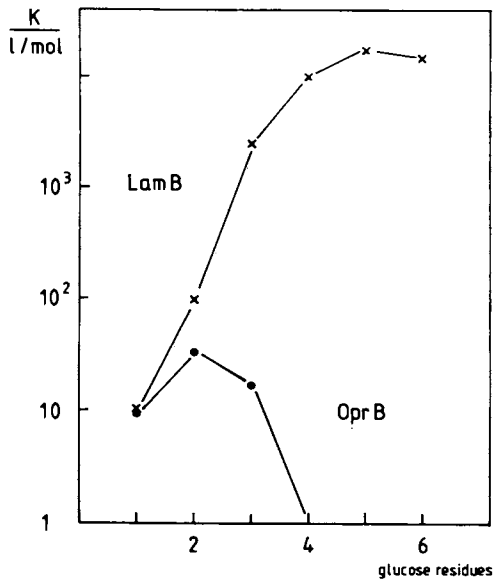

FIG. 5. Stability constants for the binding of glucose, maltose, and maltooligosaccharides to the binding site inside the LamB channel of *E. coli* (circles, taken from reference 5) and of the OprB channel of *P. putida* (crosses, this study). As described in Table 3, the stability constant for maltotetraose ($glucose_4$) was below the limits of detection (i.e., we could not deliver sufficient maltotetraose to our chambers to inhibit chloride conductance).

TABLE 4. Zero-current membrane potentials, V_m, of membranes from diphytanoyl phosphatidylcholine-*n*-decane in the presence of OprB measured for a fivefold gradient of different salts[a]

Salt	V_m (mV)	P_{cation}/P_{anion}
KCl	35	17
LiCl	34	14
KCH_3COO (pH 7)	36	19

[a] V_m is defined as the difference between the potential on the dilute side (50 mM) and the potential at the concentrated side (250 mM). The pH of the aqueous salt solutions was around 6 unless otherwise indicated; the temperature was 25°C. P_{cation}/P_{anion} was calculated from the Goldman-Hodgkin-Katz equation from at least three individual experiments (4).

DISCUSSION

In this study, we have demonstrated that the glucose-inducible, organic acid-repressible OprB of *P. putida* has glucose-specific pore-forming activity. Taken together with previously published information (14, 27), the data suggest a functional homology between OprB from *P. aeruginosa* and *P. putida*, although this study adds substantially to our knowledge of the channel-forming characteristics and substrate-binding specificity of this porin. These results suggest that OprB behaves as a glucose-specific porin in the outer membrane and may form an important part of the high-affinity glucose uptake system of *P. putida*. Consistent with this, OprB appears to be coregulated with the high-affinity glucose transport system observed in *P. putida*, as indeed it is in *P. aeruginosa* (10, 14).

OprB of *P. putida* was compared with *P. aeruginosa* OprB by using a number of methods. OprB from both pseudomonads demonstrated heat-modifiable mobility on SDS-PAGE. Heat modifiability has been attributed to the high β-sheet content of outer membrane proteins (15). The single minimum ellipticity at 215 to 217 nm for nondenatured *P. putida* OprB observed by CD spectroscopy suggested a high β-sheet content. This compared favorably with recently obtained quantitative CD spectra for OprF and OprP of *P. aeruginosa*, which were calculated to contain 62 and 65% β-sheet, respectively (23). Despite differences in apparent monomer molecular weights, the OprB proteins had similar amino acid compositions (Table 1) and similar mobilities in the heat-unmodified form, and in preliminary studies with polyclonal sera, the two proteins were immunologically cross-reactive (1a). Thus, although *P. aeruginosa* and *P. putida* have minimal DNA homology (19), the OprB proteins appear analogous. Previous studies on OprB from *P. aeruginosa* indicated that it was a porin that permitted the passage of sugars with sizes as large as dissaccharides (14, 27) and that there was an apparent preference of the channel for glucose and related monosaccharides (27). However, these studies did not measure the binding affinity for glucose and did not formally demonstrate the existence of a glucose-binding site within the channel (although the results were consistent with the existence of such a site). Indeed, it could be implied from the data that these channels were moderately large, since substantial permeabilities were measured by liposome swelling for substances larger than dissaccharides. In contrast, the results obtained here for OprB of *P.*

putida demonstrate that this porin has quite a low permeability for small ions (Table 2). This indicates a rather narrow channel, a proposal consistent with the observation that even monosaccharides (Fig. 3) substantially blocked ion conductance through the channel (presumably by occupying the glucose-binding site within the channel). Therefore, the OprB channel bears some similarities to the well-characterized maltodextrin-specific porin LamB of *E. coli* (5, 6) and *S. typhimurium* (21). Several other similarities in channel properties between LamB and OprB were observed, including a linear relationship between salt concentration and conductance (implying that these channels are water-filled and do not contain strong ion-binding sites) and a strong selectivity for cations (implying the existence of anionic amino acids surrounding the mouths of the channels of LamB and OprB). Nevertheless, some significant differences were observed between LamB and OprB. For example, the lower ion conductance of OprB might be consistent with a somewhat narrower channel. Alternatively, the path that ions must follow might be more tortuous for OprB or the channel might be more poorly shielded from the low dielectric constant of the hydrocarbon core of the membrane interior.

The most profound difference between LamB and OprB, however, was in the sugar-binding characteristics. Previous measurements of the binding of different maltodextrins to the LamB channel have demonstrated that the binding affinity increases as the size of the maltodextrin increases from 2 to 5 glucose units and then remains constant for maltodextrins with 6 and 7 glucose units (Fig. 5) (5, 6). In contrast, although OprB bound glucose and maltose with affinities similar to those measured for LamB, it bound maltotriose more poorly than maltose and maltotetraose extremely poorly (Fig. 5). Consistent with this, maltohexaose substantially depressed the single-channel conductance of individual LamB channels (6) but had no effect on the single-channel conductance of OprB channels. The data for LamB have been interpreted as implying an elongated binding site for maltodextrins that can accommodate 5 glucose units (6). In contrast, the binding site for OprB must be much shorter, with a binding site corresponding to 2 glucose molecules (approximately 1.2 nm long). There are also some differences in the specificity of these two channels. For example, LamB actually binds galactose 2.5-fold better than glucose and binds sucrose almost as well as maltose (6), whereas OprB has a threefold preference for glucose over galactose and a 13-fold preference for maltose over sucrose (Table 3). Thus, despite the previously described similarities between LamB and OprB (14), they seem to form channels with quite different properties.

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