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Pore Formation by LamB of *Escherichia coli* in Lipid Bilayer Membranes

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Lipid bilayer experiments were performed in the presence of different *Escherichia coli* LamB preparations. These LamB preparations formed two types of pores in the membranes. Large pores, which had a single-channel conductance of 2.7 nS and comprised about 1 to 6% of the total pores, were presumably contaminants which might have been induced together with LamB. LamB itself formed small pores with a single-channel conductance of 160 pS in 1 M KCl. These pores could be completely blocked by the addition of maltose and maltodextrins. Titration of the pore conductance with maltotriose suggested that there was a binding site inside the pores with a K_s of 2.5×10^{-4} M for maltotriose. On the basis of our data we concluded that the structure of the LamB channels is quite different from the structures of the channels of general diffusion porins, such as OmpF and OmpC.

The outer membranes of gram-negative bacteria act as molecular filters which have defined exclusion limits for hydrophilic substrates (1a, 23). The molecular sieving properties of the outer membranes are due to a major class of proteins, called porins (19). Most porins, such as OmpF and OmpC from *Escherichia coli* K-12, exhibit little specificity for solutes (6, 22) and act as general diffusion pathways (1a, 23). Other porins are induced in the outer membranes if the organisms are grown under special conditions (17). For example, PhoE, a general diffusion porin with some specificity for anionic solutes, is induced in members of the *Enterobacteriaceae* under conditions of phosphate starvation (29), whereas LamB (maltoporin) is induced in *E. coli* (25) or *Salmonella typhimurium* (24) if the cells are grown on maltose or maltodextrins.

LamB is part of the maltose uptake system, and mutants lacking this protein are impaired in maltose transport when the concentration of maltose is below 0.1 mM (25). This suggests that the LamB pores are highly specific and have an important role in the maltose uptake system. Nevertheless, there has been some controversy concerning the precise function of LamB. For example, it has been suggested that LamB acts as a general diffusion pore for hydrophilic solutes in a manner similar to the OmpF and the OmpC pores (8, 13). On the other hand, LamB preferentially facilitates the diffusion of maltose and maltodextrins in whole cells (25, 26, 30).

A similar controversy exists for the function of purified LamB in reconstitution experiments. These experiments have shown that LamB forms large pores in lipid bilayer membranes and reconstituted vesicles with a very limited specificity for solute molecules (7, 20). In contrast, a considerably higher specificity of LamB channels for maltose and maltodextrins was observed when the liposome swelling assay was used (15). The results of this assay suggested that LamB facilitated the diffusion of maltose into liposomes 40 times faster than it facilitated the diffusion of sucrose despite the identical molecular weights of these sugars. The liposome permeability assay with entrapped glycosidases showed a similar solute specificity, although the transport rate of maltose through LamB channels was identical to the transport rate through OmpF pores (14, 27).

Despite the variability described above, aspects of the translocation of solutes through LamB channels are consistent with the assumption that the channels form a defined structure (i.e., open pores under all conditions). This contradicts the results of a recent study, in which general pore function for LamB was observed only in the presence of the maltose-binding protein (MBP) (21).

In this paper we describe lipid bilayer experiments performed in the presence of different LamB preparations. The addition of LamB protein resulted in the formation of two types of pores. One pore type was presumably a general diffusion pore with a larger cross-sectional area than OmpF pores. Our data suggest that these large pores are probably a porin-like impurity or, perhaps, an aberrant configuration of LamB pores. The other pore type had a much lower single-channel conductance and appeared to be more frequent than the large pore type. In contrast to the large pore type, the small pore type could be blocked by maltose and maltodextrins. Our data are consistent with the concept that the small pores are maltoporin.

MATERIALS AND METHODS

Purification of LamB. (i) Preparation I. *E. coli* K-12 T19 (F^- *tsx-354 sup-42 ompB*) (20) cells were grown in 5 liters of M9 medium supplemented with 0.5% maltose, 0.1% Casamino Acids, and 10^{-6} M ferric ammonium citrate overnight. The cells were harvested, washed once with 1 liter of distilled water, and suspended in 80 ml of 50 mM Tris hydrochloride (pH 7.5). The LamB protein was purified essentially by the procedure described previously for the purification of porin (28). Cells were passed through a French pressure cell twice at 1,500 kg/cm^2 at room temperature, and the envelope fraction was obtained by centrifugation at 100,000 × *g* for 60 min. A portion of the envelope fraction containing approximately 240 mg of protein was dissolved in 300 ml of a solution containing 2% sodium dodecyl sulfate (SDS), 0.05 M Tris, 0.05% β-mercaptoethanol, and 3 mM NaN_3 (pH 7.5), and this preparation was shaken gently at 37°C overnight. The SDS-insoluble material was obtained by centrifugation at

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100,000 × g for 30 min. This insoluble material was treated successively with 300 ml of the solution described above for 4 h and 300 ml of a solution containing 1% SDS, 0.05 M Tris, 0.05% β-mercaptoethanol, 5 mM EDTA, and 3 mM NaN_3 (pH 7.5) overnight as described above. The centrifuged pellets were treated with 50 ml of a solution containing 1% SDS, 0.05 M Tris, 0.05% β-mercaptoethanol, 5 mM EDTA, 0.4 M NaCl, and 3 mM NaN_3 (pH 7.5) at 37°C for 2 h, and the soluble fraction was obtained by centrifugation at 100,000 × g for 30 min. The soluble fraction was concentrated by using a Diaflow type PM30 membrane to an appropriate volume (about 6 ml) and was applied to a Sephacryl S-300 column (2.5 by 100 cm) that was equilibrated with the same solution used in the last step described above, and the column was eluted with the same solution. The eluates containing the LamB protein were pooled and subjected to gel filtration through a Sephacryl S-300 column (1 by 100 cm) with the same solution. A single protein peak eluted from the second column chromatography contained homogeneous LamB protein, as judged by SDS-acrylamide gel electrophoresis with and without heating at 100°C, except for a very faint band just below the LamB protein (Fig. 1). The yield of LamB protein at the final step was about 4 mg.

(ii) Preparation II. LamB was isolated from envelopes of maltose-grown cells of *E. coli* TK24, which lacks OmpC, OmpF, and OmpA. Details of the isolation procedure have been described elsewhere (31). Briefly, LamB was isolated by (i) extraction of envelopes with SDS at 60°C and (ii) release of LamB from the protein-peptidoglycan complex by salt treatment. The crude LamB fraction obtained was further purified over a QAE-Sephadex column run in the presence of Triton X-100. Preparation II contained about 95% pure LamB and was dissolved in 10 mM Tris hydrochloride (pH 7.5) containing 1% (vol/vol) Triton X-100 and 0.25 M NaCl at a concentration of 1 mg/ml. The protein was present in its trimeric form (Fig. 1).

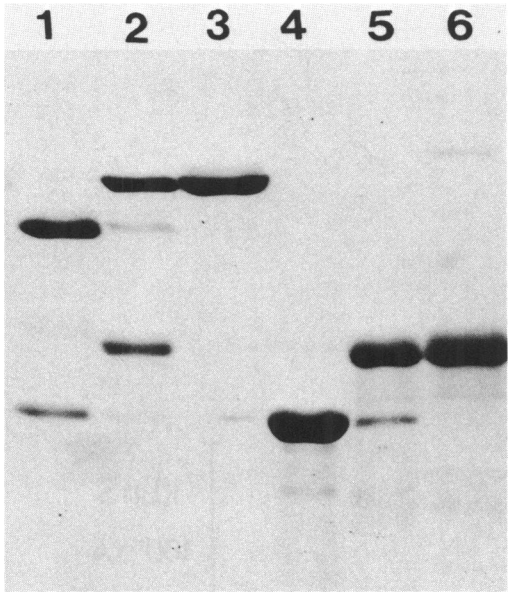

FIG. 1. SDS-polyacrylamide gel electrophoretogram of the two LamB preparations and OmpF from *E. coli* K-12. Lane 1, OmpF from *E. coli* K-12, 30°C; lane 2, LamB preparation I, 30°C; lane 3, LamB preparation II, 30°C; lane 4, OmpF from *E. coli* K-12, 100°C; lane 5, LamB preparation I, 100°C; lane 6, LamB preparation II, 100°C. The temperatures given above are the temperatures applied to the proteins before electrophoresis.

Preparation of LamB-specific antiserum. Antiserum was raised against highly purified LamB trimers (preparation II) as described previously (31). The specificity of the antiserum was checked by immunoprecipitation with [^{35}S]methionine-labeled cell lysates (32). The antiserum contained minor amounts of contaminating antibodies, which were removed by absorption with envelopes containing no LamB or other maltose-inducible proteins (32). The cleared antiserum was used in this study.

Membrane experiments. The methods used for black lipid bilayer experiments have been described previously (5). The apparatus consisted of a Teflon chamber with two aqueous compartments. Circular holes in the wall between the two compartments had an area of either 2 mm^2 (for macroscopic conductance measurements) or about 0.1 mm^2 (for single-channel experiments). Membranes were formed across the holes by painting on a 1% (wt/vol) solution of a 4:1 mixture of diphytanoyl phosphatidylcholine and phosphatidylserine (Avanti Biochemicals, Birmingham, Ala.) in *n*-decane. The temperature was kept at 25°C throughout.

All salts were obtained from E. Merck AG, Darmstadt, Federal Republic of Germany (analytical grade). Maltose and maltodextrins were purchased from Boehringer GmbH, Mannheim, Federal Republic of Germany. The aqueous solutions were unbuffered and had pHs of about 6. To prevent protein inactivation, the protein was added to the aqueous phase from 10-fold-diluted LamB preparations containing either 0.1% SDS or 0.1% Triton X-100 either prior to membrane formation or after the membranes had turned completely black.

The membrane current was measured at different voltages by using a pair of calomel electrodes with salt bridges, which were inserted into the aqueous solutions on both sides of the membrane. The current through the pores was boosted with a preamplifier (Keithley model 427), monitored with a storage oscilloscope (Tektronix model 5115), and recorded on a strip chart or a tape recorder. The macroscopic conductance measurements were performed with a Keithley model 610C electrometer.

RESULTS

Macroscopic conductance. When one of the two LamB preparations was added in small quantities (10 to 100 ng/ml) to the aqueous solutions bathing a lipid bilayer membrane, the conductance of the membrane increased by several orders of magnitude. The time course of the conductance increase was similar to that described previously for other bacterial porins (4). After an initial rapid increase for 15 to 20 min, the membrane conductance increased at a much slower rate, and this slow increase continued until membrane breakage. The conductance increase occurred regardless of whether the protein was added to only one side or to both sides of the membrane. The addition of detergent alone at the same concentration as the concentrations present in the protein solutions did not lead to any appreciable increase in the membrane conductance above the specific conductance in the absence of protein (10^{-8} to 10^{-7} S/cm^2).

Since a steady conductance level could not be reached in the experiments with LamB preparations, the dependence of the membrane conductance on the protein concentration was difficult to determine. However, meaningful comparison was possible when we used the conductance value at a fixed time after the addition of the protein. Figure 2 shows the

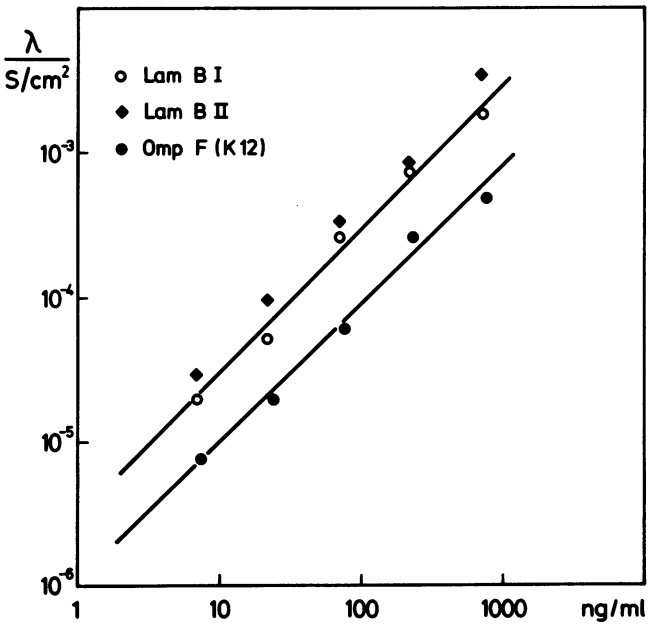

FIG. 2. Specific membrane conductance as a function of the concentrations of the different proteins in the aqueous phase. The membranes were formed from a 4:1 mixture of diphytanoyl phosphatidylcholine and phosphatidylserine dissolved in *n*-decane. The aqueous phase contained 1 M KCl and less than 1 μg of SDS or Triton X-100 per ml. Each point represents the mean of at least three membranes 20 min after blackening of the membranes or after the addition of the protein. The temperature was 25°C and the applied voltage was 25 mV.

dependence of the membrane conductance on the LamB concentration in the aqueous phase measured 20 min after the addition of the protein. Both LamB preparations used in this study gave virtually identical results, indicating that the two isolation and purification procedures led to proteins with similar membrane activities. The membrane activity of LamB was three times greater than that of the OmpF porin from *E. coli* K-12 under otherwise identical conditions (Fig. 2). The current through the LamB-containing membranes was a linear function of the applied voltage up to 150 mV. This result suggested that no voltage was required to initiate the single conductance unit; i.e., the pores formed by the two different LamB preparations (see below) were not voltage gated.

Single-channel experiments. The addition of smaller amounts of LamB protein to lipid bilayer membranes having small surface areas (0.1 mm^2) allowed the resolution of step increases in conductance (Fig. 3). Surprisingly, the steps were connected by zones of current "drift," where discrete steps could not be resolved at the level of conductance resolution used for this experiment (Fig. 3). The drift observed in the presence of LamB preparation I was about 60% of the total signal, whereas the use of preparation II resulted in 80 to 90% drift and a much smaller number of steps. Figure 4 shows a histogram of the step conductance increases in 1 M KCl. Unlike *E. coli* porins OmpF, OmpC, and PhoE, the large steps were relatively homogeneous in size and had a single-channel conductance of 2.7 nS in 1 M KCl (Fig. 4). This single-channel conductance was considerably higher than the values observed for any other *E. coli* porin (6), but it was very similar to the value which was reported previously from lipid bilayer experiments in the presence of a LamB preparation of uncertain purity (7).

LamB channels have been shown to be blocked for the penetration of glucose in the presence of maltose or

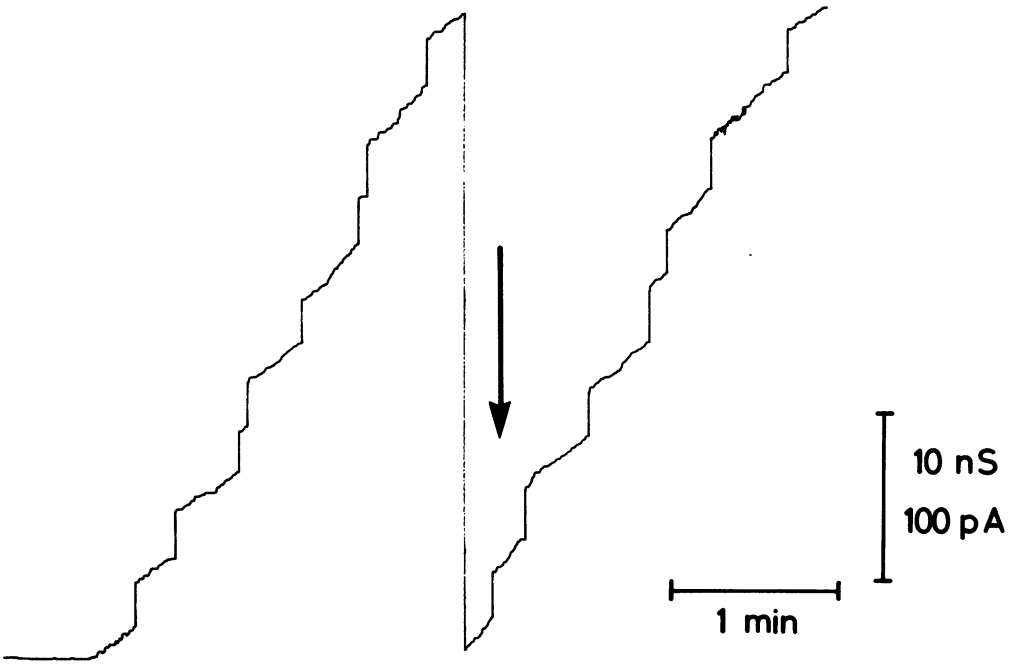

FIG. 3. Single-channel record of a membrane formed from a 4:1 mixture of diphytanoyl phosphatidylcholine and phosphatidylserine dissolved in *n*-decane. The aqueous phase contained 1 M KCl and 5 ng of LamB preparation I per ml. The applied voltage was 10 mV, and the current prior to the addition of maltoporin was 1 pA. The arrow indicates the shift of the base line.

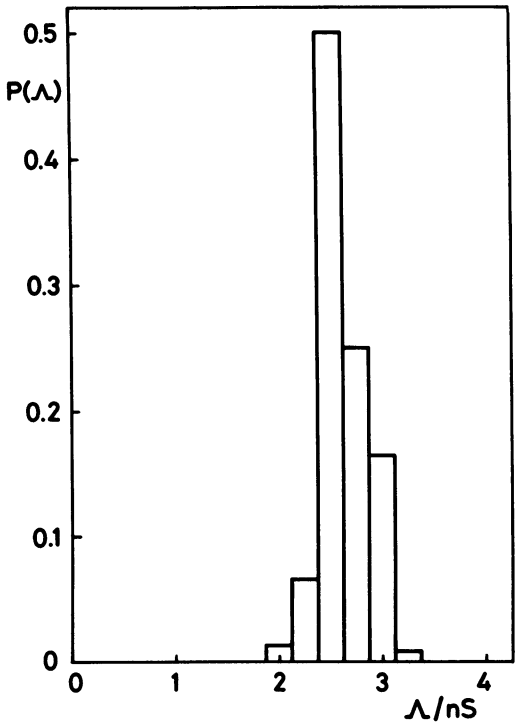

FIG. 4. Histogram of the large conductance steps observed with membranes formed from diphytanoyl phosphatidylcholine and phosphatidylserine in the presence of LamB preparation I. The average single-channel conductance was 2.7 nS for 253 steps. The aqueous phase contained 1 M KCl, the temperature was 25°C, and the applied voltage was 10 mV.

maltodextrins (15, 16). In order to measure a similar influence on the conductance induced by LamB in lipid bilayer membranes, single-channel experiments were performed in the presence of maltose and maltodextrins. Figure 5 shows the results of such an experiment, in which 10^{-2} M maltotriose was added; otherwise, conditions identical to those used for the experiment shown in Fig. 3 were used. The larger conductance steps shown in Fig. 3 were very similar, if not identical, to those shown in Fig. 5. This result indicated that the large steps were not influenced by the presence of maltotriose. On the other hand, it was also evident from a comparison of Fig. 3 and 5 that the presence of 10^{-2} M maltotriose completely abolished the current drift between the steps. The addition of maltotriose after considerable membrane conductance was established by the incorporation of many pores into the membrane resulted in a strong decrease in conductance (see below). This indicated that part of the conductance induced by the LamB preparations in lipid bilayer membranes could be blocked by maltose and maltodextrins.

In order to measure directly the single-channel conductance of the maltose- and maltodextrin-blockable pathway, the current resolution of the electrical instrumentation was increased considerably. Figure 6 shows such an experiment in the presence of LamB preparation II, for which the drift was predominant over the large steps. As Fig. 6 shows, at high current resolution the drift consisted of small steps which had a 20-fold lower single-channel conductance than the large steps. Figure 7 shows a histogram of the small conductance steps, which had a mean value of about 160 pS in 1 M KCl. It is interesting that no small steps were observed in the presence of 10^{-2} M maltotriose when we used a conductance resolution of the electrical instrumentation of about 1 to 2 pS. This result indicated that the binding of maltotriose to LamB decreased the flux of ions through the channels at least 100-fold.

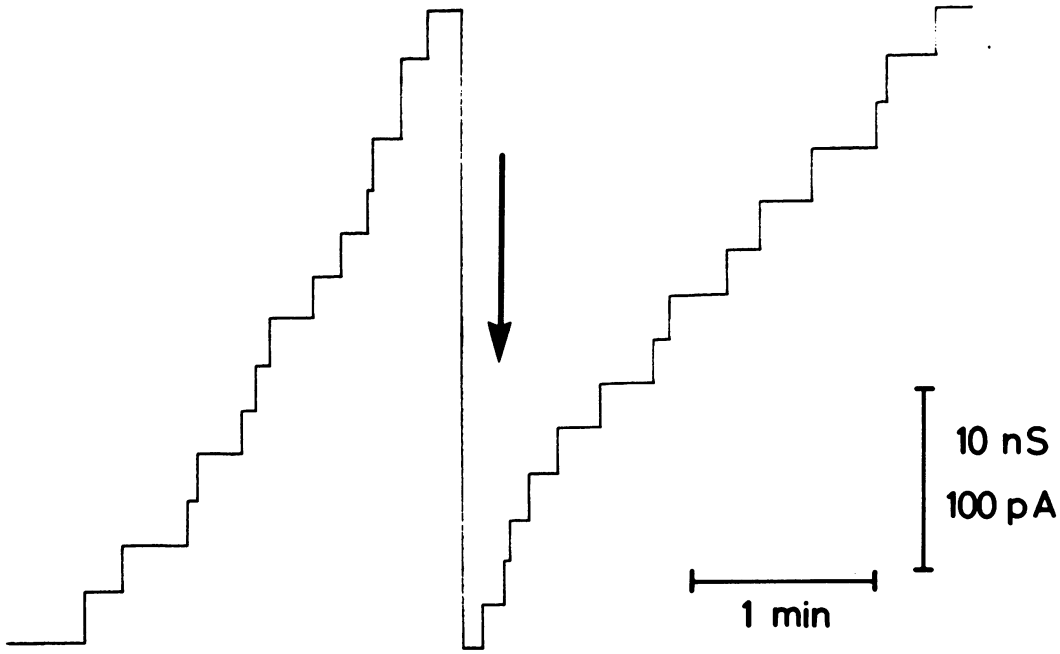

FIG. 5. Single-channel record of LamB preparation I in 1 M KCl–10^{-2} M maltotriose. For other experimental conditions see the legend to Fig. 3. The arrow indicates the shift of the base line.

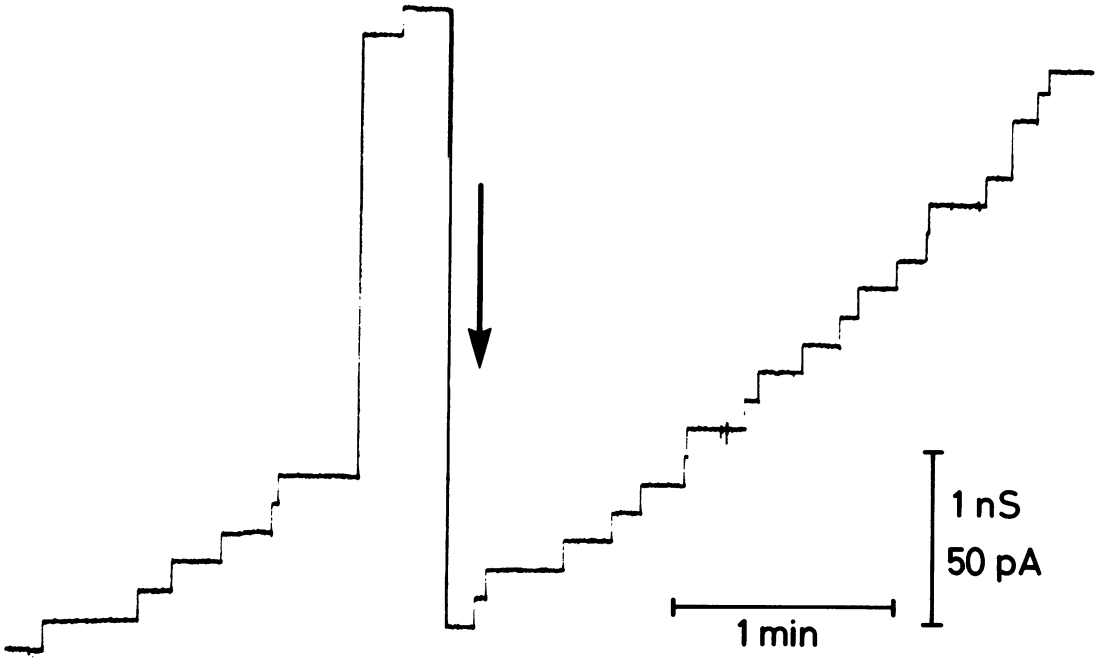

FIG. 6. Single-channel record of a membrane formed from a 4:1 mixture of diphytanoyl phosphatidylcholine and phosphatidylserine dissolved in *n*-decane. The aqueous phase contained 1 M KCl and 5 ng of LamB preparation II per ml. The applied voltage was 50 mV, and the current prior to the addition of maltoporin was less than 1 pA. The arrow indicates the shift of the base line. Note that the conductance resolution was considerably increased compared with Fig. 3 and 5.

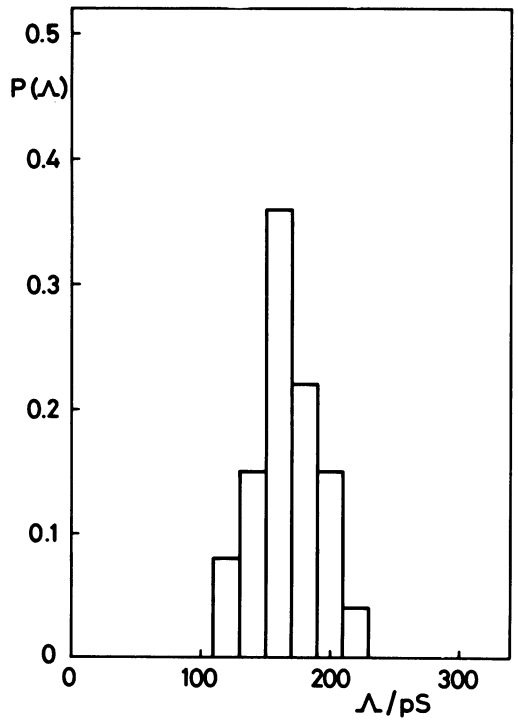

FIG. 7. Histogram of the small conductance steps observed with diphytanoyl phosphatidylcholine-phosphatidylserine membranes in the presence of LamB preparation II. The average single-channel conductance was 160 pS for 179 steps. The aqueous phase contained 1 M KCl, the temperature was 25°C, and the applied voltage was 50 mV.

Interaction with antibodies. The LamB preparations used in this study formed two types of pores in lipid bilayer membranes. One of the two types of pores had a low single-channel conductance (160 pS) in 1 M KCl and could be blocked by maltose and maltodextrins. The small pores were about 15 to 100 times more frequently incorporated into the lipid bilayer membranes than the large 2.7-nS pores. These results suggested that the large pores could be an impurity in the LamB preparations, especially since the number of large pores was much reduced in the purest LamB protein (preparation II). In order to answer this question, we performed lipid bilayer experiments with LamB and antibodies raised against LamB preparation II. The addition of the antibodies did not close the pores already inserted into the membranes, but it inhibited further insertion of pores (data not shown).

Single-channel experiments in the presence of antibodies against LamB protein showed that the formation of both types of pores was inhibited. Low antibody concentrations blocked the formation of the small pores first, whereas large antibody concentrations were needed to block the appearance of the large steps. If the large pores were an impurity, as suggested above, the blocking of the incorporation of these pores was interesting since the antiserum was cleared by absorption onto envelopes of noninduced cells. In fact, this result implies that the large pores can either be an abnormal LamB configuration or an impurity induced together with LamB.

The known general diffusion pores in *E. coli* outer membranes are closely related to one another (18). We checked the possibility that the porin responsible for the formation of the large steps might be identical or related to one of these general diffusion pores. In fact, only a minor influence of the LamB antibodies on *E. coli* K-12 OmpF pores was observed, whereas at the same concentration the antibodies almost completely inhibited pore formation by LamB.

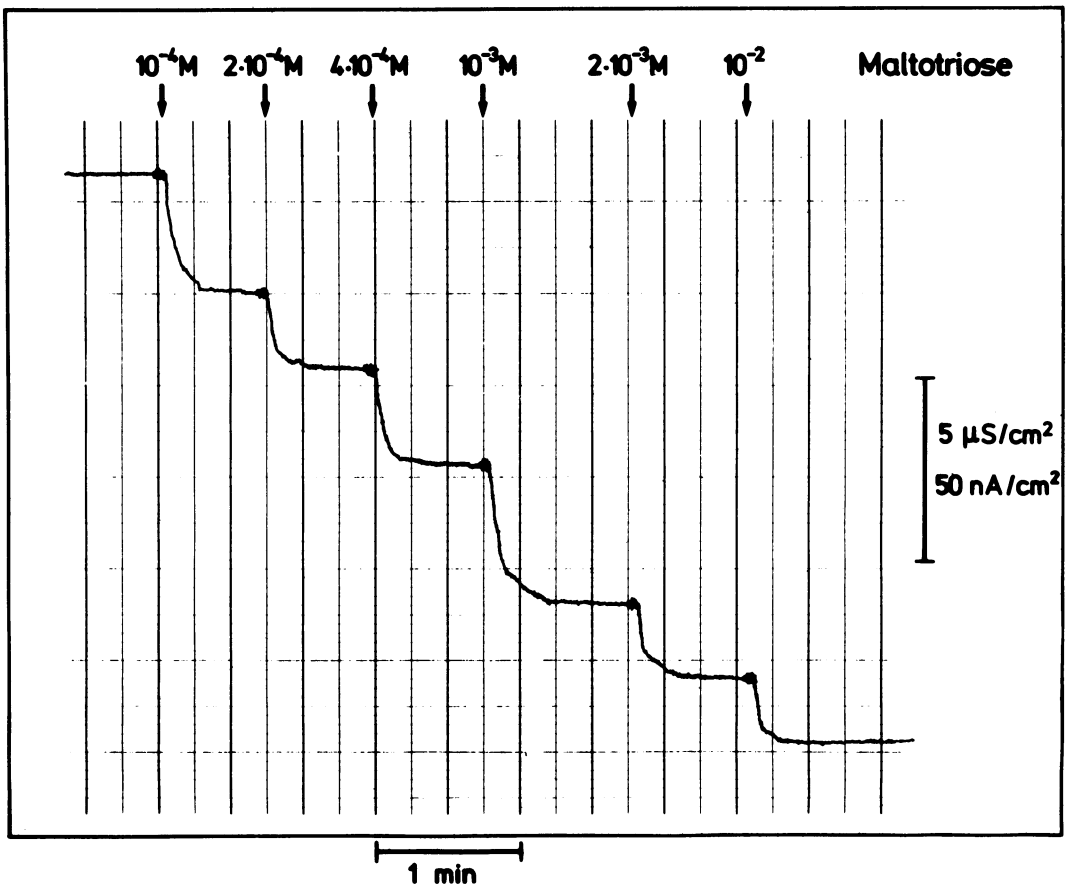

FIG. 8. Titration of LamB-induced membrane conductance with maltotriose. The membrane was formed from a 4:1 mixture of diphytanoyl phosphatidylcholine and phosphatidylserine dissolved in *n*-decane. The aqueous phases contained 70 ng of LamB per ml, 1 M KCl, and maltotriose at the concentrations shown at the top. The temperature was 25°C and the applied voltage was 10 mV.

Binding of maltotriose to LamB. The small pores could be completely closed by the addition of maltose and maltodextrins. This finding allowed the derivation of binding constants of these substrates to the pore interior. The experiments were performed in the following way. LamB was added to a black lipid bilayer membrane formed from diphytanoyl phosphatidylcholine and phosphatidylserine at a concentration of 70 ng/ml. At 30 min after the addition of LamB, the rate of conductance increase had slowed considerably. At this time, small amounts of concentrated maltose or maltodextrin solutions at final concentrations of between 10 µM and 50 mM were added to the aqueous solutions with stirring to allow equilibration. Figure 8 shows the results of an experiment of this type in which increasing concentrations of maltotriose were added (arrows). The membrane conductance decreased as a function of the maltotriose concentration. By assuming that during the binding of a maltotriose molecule inside a channel no ions can pass through the channel, the binding constant could be calculated from a Lineweaver-Burke plot (Fig. 9). The straight line in Fig. 9 corresponded to a stability constant of 2.5×10^3 M^{-1} (K_s = 0.4 mM). This value showed excellent agreement with the binding constant of maltotriose to whole cells (1.5×10^3 M^{-1} [11]). It should be noted that the binding of maltose and maltodextrins (i.e., the blocking of the pores) was reversible. Removing the maltotriose in experiments similar to the experiment shown in Fig. 8 led to a restoration of the initial membrane conductance (before the addition of maltotriose).

DISCUSSION

In this study we confirmed that LamB is able to form pores in lipid bilayer membranes. The addition of 70 ng of LamB per ml to the aqueous phase resulted in the formation of more than 10^6 pores per cm^2 in membranes. The pores had a long lifetime, and they were open in the absence of MBP, as indeed they are in vivo (8). This result is in sharp contrast to the results of a recent study (21), in which the pore-forming activity of LamB in lipid bilayer membranes was observed only in the presence of MBP concentrations between 10^{-9} and 10^{-5} M. We tested the influence of similar MBP concentrations on pore formation by LamB in preliminary experiments (Benz, Schmid, Nakae, and Vos-Scheperkeuter, unpublished data). So far we have not measured any influence of MBP on the pores. This result is consistent with the in vivo situation (8). We do not have a good explanation for the discrepancy between the data of Neuhaus et al. (21) and our data, but we stress the fact that the maltotriose binding (which was not found by Neuhaus et al. [21]) is identical to the binding found in vivo (11) and in vitro (16).

The LamB preparations used in this study formed two types of pores in lipid bilayer membranes. The large pores, which had a single-channel conductance of 2.7 nS in 1 M KCl, comprised between about 1% (preparation II) and

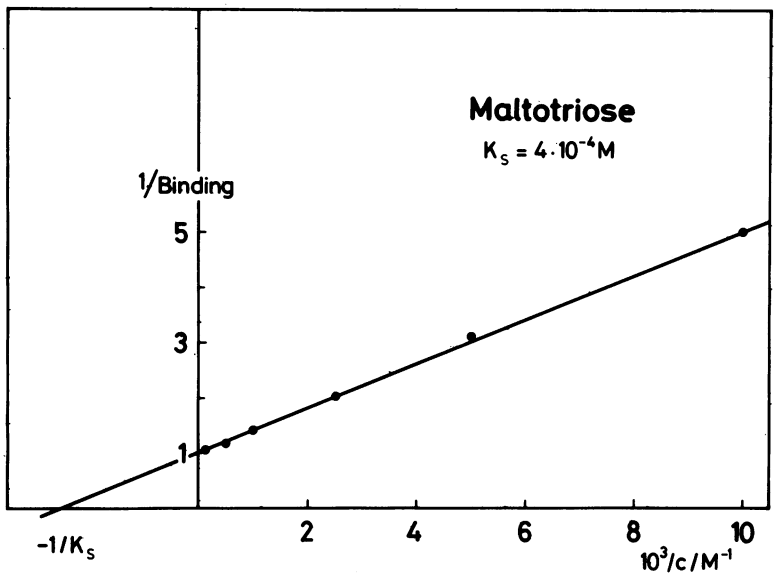

FIG. 9. Lineweaver-Burke plot of the inhibition of LamB-induced membrane conductance by maltotriose (see Fig. 8). We assumed that the binding of maltotriose to the binding site completely inhibited the ion flux through the pores.

about 6% (preparation I) of the total number of pores. This means that the large pores were a minor fraction of the total pores, although they had a 15-fold higher single-channel conductance than the small pores and thus contributed a substantial fraction of the total membrane conductance. The 2.7-nS pores had the features of general diffusion pores like OmpF and OmpC. The presence of these large pores in the outer membrane of *E. coli* could account for the general diffusion pore properties which have been ascribed to LamB itself (8, 13). On the other hand, the presence of the large pores in the LamB preparations may also explain the results of reconstitution experiments, in which it was found that maltoporin forms larger pores than OmpF (7, 20). With respect to this, it is interesting that mutations of OmpF and OmpC resulting in larger effective diameters have recently been discovered (1).

Our data suggest that the large pores are simply caused by a contaminant of LamB protein which may or may not be coregulated with LamB. Preparations of higher purity produced a smaller fraction of larger steps in the lipid bilayer experiments. On the other hand, we could not exclude the possibility that the large pores represented the LamB protein in a different conformation. In any case, it seems quite obvious from our data that the large pores are not identical to any porin channel from the outer membrane of *E. coli* which has been characterized to date (6). For example, the large pores had a substantially higher single-channel conductance Λ than the OmpF, OmpC, PhoE, protein K, and NmpC pores (6). By assuming that the pores are cylindrical channels with length l and that they are filled with an aqueous solution of specific conductance σ, the diameter ($d = 2r$) of the pores may be calculated as follows (5): $r^2 = \Lambda \cdot l / \sigma \cdot \pi$. Using the specific conductance of the aqueous KCl solution (112 mS/cm) and $l = 6$ nm, the pore diameter would be about 1.4 nm, whereas the largest Omp pores (OmpF of *E. coli* B) have a diameter of 1.2 nm (6).

The equation given above cannot be used to calculate the diameter of the small pores. The reason for this is the low single-channel conductance of these channels and their small diameter (see below). The LamB channels are completely blocked by the binding of maltose and maltodextrins to the channel interior. The binding constant increases with the number of glucose units (11). This indicates that the LamB protein forms a long channel which contains many binding sites for glucose. Presumably, its diameter does not exceed 0.7 to 0.8 nm, because potassium or chloride ions could not penetrate the channel after the binding of maltose and maltodextrins. It is interesting that both in vivo and in vitro the LamB channel has been found to be blocked in the uptake of substrates by the addition of maltodextrins (8, 16).

It is evident that the data presented here provide a good explanation for the controversial question of the function of LamB channels (14, 15). The presence of the large channels in vivo and in vitro leads to an apparent lower solute specificity of LamB and to the observation that LamB acts as a general diffusion pore (13). LamB itself is very likely a channel with a small cross section and an exclusion limit for nonsugar substrates with molecular weights of less than 300.

So far, only one other substrate-specific porin besides LamB is known (12). Protein P from *Pseudomonas aeruginosa* outer membranes contains a narrow selectivity filter (diameter, 0.6 to 0.7 nm) with a binding site for anions (3). This binding site is responsible for the high single-channel conductance of protein P at low anion concentrations. In fact, for a 10 mM KCl solution protein P (diameter, 0.6 to 0.7 nm) has a threefold-higher single-channel conductance than PhoE from *E. coli* (diameter, 1.1 nm [2]) and almost the same single-channel conductance as protein F from *P. aeruginosa* (diameter, 2.0 nm [4]). On the other hand, the values for single-channel conductance of protein P in 1 M KCl are about 10 and 40 times smaller than the values for PhoE (2) and protein F (4), respectively. A similar dependence on the substrate concentration may be expected for the penetration of substrates through LamB channels. The dependence of the flux [$\phi_{(c)}$] of maltotriose through these channels on the substrate concentration (c) at a given driving force (gradient) may be described by the following equation: $\phi_{(c)} = \phi_{max}(Kc/[1 + Kc])$, where ϕ_{max} is the maximum flux (at very

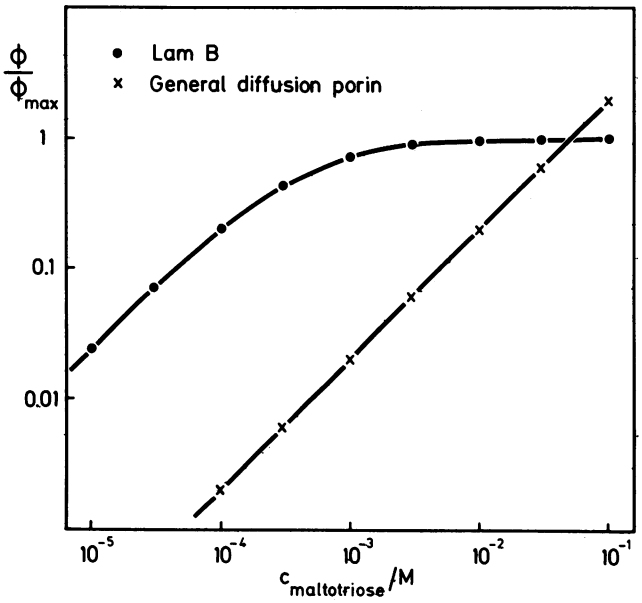

FIG. 10. Concentration dependence of maltotriose flux through maltoporin, given as the ratio of ϕ to ϕ_{max}. For the flux of maltotriose through a general diffusion porin it was assumed that the flux at a maltotriose concentration of 10^{-4} M was 1% of the flux through maltoporin.

high substrate concentrations) and K ($= 1/K_s$) is the stability constant for the binding of the substrate to the binding site. The flux saturates at high substrate concentrations, as shown in Fig. 10 for maltotriose. The flux of substrates through general diffusion pores without a binding site is linearly dependent on the concentration (Fig. 10). This means that a comparison between the permeabilities of maltoporin and a general diffusion porin for a given substrate is dependent on the substrate concentration. Figure 10 clearly shows that at high substrate concentrations the flux through a general diffusion porin could exceed that through the specific LamB channels even if it was considerably lower at low substrate concentrations (e.g., only 1% of the flux through LamB at a substrate concentration of 10^{-4} M in Fig. 10). This is consistent with the in vivo situation, in which the general diffusion pores become rate limiting in LamB mutants at maltose concentrations below 0.1 mM (25).

The data presented here show that the lipid bilayer assay is a powerful method for the characterization of channel-forming proteins. The main advantage of this assay is the evaluation of molecular events (i.e., the measurement of the conductance of single units, which allowed us to separate the LamB channels from other [presumably contaminant] pores). The binding of neutral substrates to a specific channel type was studied here. As the primary sequence of LamB is known (9) and mutant LamB proteins are available (10, 33), it would be of some interest to study the structure-function relationships of this channel type.

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