Receptor for Bacteriophage Lambda of *Escherichia coli* Forms Larger Pores in Black Lipid Membranes Than the Matrix Protein (Porin)

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The receptor for phage $\lambda$ in *Escherichia coli* was isolated by cholate extraction and purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein bands corresponding to the monomer and the dimer were eluted from the gel and tested for their activity to inactivate phage $\lambda$ and to form pores in black lipid membranes. It was found that only the dimer inactivated phage $\lambda$, whereas both the monomer and the dimer were active in forming pores. The pore characteristics were similar to those exhibited by the matrix protein (porin) (R. Benz, K. Janko, W. Boos, and P. Läuger, Biochim. Biophys. Acta 511:305-319, 1978). In comparison, the $\lambda$ receptor showed a somewhat higher degree of cation specificity, and its pore size was larger. Assuming that the thickness of the outer membrane is 7.5 nm and that the pore is an ideal hydrophilic channel, the pore diameter in vivo was estimated to be 1.6 nm for the $\lambda$ receptor and 1.2 nm for the matrix protein.

Transport of maltose and maltodextrins in *Escherichia coli* is mediated by a single transport system that is composed of several components (10, 29). Characteristics of this system are its sensitivity toward osmotic shock and its dependence on the periplasmic maltose-maltodextrin-binding protein (11). It was puzzling for a long time (13) that among the genes in the *ma1B* region, the only genes necessary for maltose transport, there was gene *lamB* that codes for the receptor of phage $\lambda$, a gene apparently unrelated to maltose transport (21). However, recently Szmelcman and Hofnung (24) found that *lamB* mutants were impaired in maltose transport when the concentration of this sugar in the uptake medium was less than 0.1 mM. Furthermore, treatment of wild-type bacteria with antibodies directed against purified $\lambda$ receptor strongly reduced the initial rate of maltose transport, thus demonstrating that the phage receptor is an element of the maltose uptake machinery.

Later, a comparison of the kinetic parameters of transport of maltose and maltotriose in wild type and $\lambda$-resistant mutants with the binding constant for both sugars to purified maltose-binding protein led to the conclusion that the $\lambda$ receptor passively facilitates the diffusion of maltose and maltodextrins through the outer membrane. The observation that *lamB* strains grew well on maltose at concentrations above 0.1 mM suggested that maltose was also able to enter via pores other than the $\lambda$ receptor (25).

Indeed, as first shown by Nakae (14-16), some major outer membrane proteins (then also called porins) from *Salmonella typhimurium* and *E. coli* are able to increase the permeability of phospholipid vesicles to molecules of molecular weight less than 600 and should therefore also accommodate maltose. The ability of porin to increase the permeability of vesicles to small molecules is also reflected in the pleiotropic effect that porin mutants have on the transport capacity in whole cells (2, 27). Mutants missing porin could be suppressed to a normal phenotype by introducing an intact $\lambda$ receptor (28). These findings once more indicated that both porin and $\lambda$ receptor are unspecific pores for small molecules and are interchangeable in their function. However, despite the presence of pore-forming proteins in the outer membrane due to porin, strains missing the $\lambda$ receptor exhibit an apparently specific defect in maltose and particularly maltodextrin transport (6, 24, 25). Therefore, the study of the pore characteristics of the $\lambda$ receptor in comparison with those of porin might clarify this discrepancy.

Recently, we demonstrated that detergent-solubilized porin is able to increase the conductivity of black lipid membranes to ions by several orders of magnitude (4). At low concentrations of porin, the conductivity increases in single steps of conductance. The statistical analysis for these steps, as well as other characteristics of conductance, permit the description of some properties of the pore-forming activity. Surpris-
ingly, we found that the cold osmotic shock procedure that had been used to isolate soluble periplasmic proteins (17) does release porin in a detergent-free solution. This preparation also forms pores that can be differentiated by their size distribution. The analysis of the osmotic shock fluids of wild type and lamB mutants indicated that receptor can also be released by osmotic shock and can form pores in black lipid membranes that can be recognized by their particular size distribution (3). In this paper we describe the characteristics of the pore-forming activity of detergent-solubilized and purified λ receptor and compare them with those of porin.

MATERIALS AND METHODS

Purification of λ receptor. Purification of λ receptor was performed by the method of Randall-Hazelbauer and Schwartz (18), with modifications. E. coli K-12 strain CM 1072 missing proteins Ia and Ib (5) was grown in 10 liters of rich medium (28) at 37°C in a shaking incubator. The cells were harvested in exponential phase at an optical density at 600 nm of 1. The pellet was suspended in 500 ml of 10 mM Tris-hydrochloride (pH 7.5) containing 2 mM EDTA and 1% cholate; after the suspension was shaken at 37°C for 30 min, it was centrifuged at 20,000 × g for 40 min at 15°C. The supernatant was collected and dialyzed against 10 mM Tris-hydrochloride (pH 7.5) overnight. Before chromatography the volume was reduced to 200 ml by placing the solution into a dialysis bag surrounded by solid sucrose. The concentrated sample containing 225 mg of protein was then dialyzed exhaustively against 10 mM Tris-hydrochloride, pH 7.5. The solution was then applied to a diethyl-2-hydroxypropyl)aminoethyl (QAE) Sephadex column (4 by 17 cm) equilibrated with 10 mM Tris-hydrochloride, pH 7.5. The column was eluted with a 1,200-ml linear salt gradient (0 to 0.8 M NaCl) in 10 mM Tris (pH 7.5), followed by a detergent salt wash (10 mM Tris-hydrochloride [pH 7.5] containing 1.5 M sodium chloride and 2% cholate). The fractions were dialyzed against 10 mM Tris-hydrochloride and assayed for λ receptor activity (18). Long exposure to high salt concentrations had to be avoided because it reduced the pore-forming activity of the λ receptor. A peak of protein containing λ receptor activity was obtained from the cholate–salt wash. Fractions containing λ receptor activity (6.4 mg) were pooled. Approximately 2 mg of the pooled protein in a volume of 1.5 ml was then applied to a 12.5% polyacrylamide slab gel containing 0.1% sodium dodecyl sulfate (SDS) according to the system of Studier (23). Electrophoresis was performed at 50 mA for 3.5 h. Slices 1 cm in width were cut off each side of the gel and stained with Coomassie brilliant blue. The rest of the gel was cut into 2-mm-thick slices and placed overnight in 1 ml of 10 mM Tris-hydrochloride (pH 7.5) containing 0.1% SDS. Samples (5 and 20 μl) were taken from these portions and assayed for λ receptor activity. Lambda receptor activity was localized in two consecutive slices of the gel which, when pooled, contained 60 μg of protein. Protein determination was done as described by Schaffner and Weissmann (20).

Membrane experiments. Optically black lipid membranes were formed as described previously (3–5, 26). The protein dissolved in 10 mM Tris-hydrochloride (pH 7.5) containing 0.1% SDS or cholate was added in small portions to give the final concentration indicated in the particular experiments. This stock solution was found to be active for about 4 weeks in membrane experiments. Membranes were formed from oxidized cholesterol (22, 26). The electrical equipment for the membrane measurements was described previously in detail (3).

RESULTS

Purification of the λ receptor. Cells of E. coli strain CM 1072 were used as the source of the λ receptor. This strain does not contain the porin proteins Ia and Ib of the outer membrane (28). The use of this strain was necessary because it was difficult to separate λ receptor from the porin proteins Ia and Ib. In addition, even small amounts of these porins are quite active in the membrane assay and would therefore overshadow the effects of λ receptor. Initial attempts to purify the λ receptor, analogous to the elegant procedure described by Rosenbusch for the matrix protein (19) and to the procedure described by Endermann et al. for the λ receptor (8), failed. They yielded pure but inactive protein. Finally, we adopted the method described by Randall-Hazelbauer and Schwartz that involves extraction of whole cells by cholate and subsequent chromatography on QAE Sephadex columns (18). Treatment with chloroform had to be omitted because it destroyed the pore-forming ability of the λ receptor. The final purification was done by slab gel electrophoresis in the presence of SDS. The position of the band that contained activity in the phage assay corresponded to a molecular weight of 130,000. The material eluted from this band was used for the membrane experiments. As Fig. 1 shows, rerunning this protein on the same gel system after heating at 100°C yielded one band with a molecular weight of approximately 68,000, a value that is somewhat higher than reported previously (18). The molecular organization of the material eluted from the first SDS gel at a position of molecular weight 130,000 was not clear. It might have been a “dimer” (12) or a conformation of λ receptor that bound less SDS. Rerunning this material on the same gel system after incubation in SDS or cholate at room temperature for several hours or at 4°C for several days also resulted in the appearance of the “monomer”. The material eluted from this band was inactive on the phage assay but did form pores, which behaved identically to the pores formed by the material from
Pore-forming activity of the λ receptor.

When λ receptor from a stock solution in SDS was added in small quantities to the aqueous solutions bathing a membrane composed of oxidized cholesterol, the membrane conductance started to increase in a stepwise fashion (Fig. 2). The concentration of λ receptor needed to observe the same number of steps in a given time interval was considerably higher than was the case with porin (20 versus 0.5 ng/ml). This shows that incorporation into the bilayer in a functional state is less difficult for porin than it is for the λ receptor. As in the case of porin, the conductance steps were observed irrespective of whether the protein was added to the aqueous solution on one side of the membrane or on both sides. The lifetime of the pores was longer than 1 min. Because of the increasing high noise level of the current trace, it could not be determined with higher accuracy. An increasing noise level of the membrane current with increasing numbers of pores was observed (Fig. 2). Therefore, only a limited number (5 to 10) of λ receptor pores could be observed with one single membrane. This finding is in contrast to experiments with detergent-solubilized porin (4, 4a) and with detergent-free porin from cold osmotic shock fluid (3), where usually more than 30 pores could be observed with one single membrane. This difficult behavior of the two pore-forming proteins in single-channel experiments may be caused by different binding properties of the two proteins toward detergent, particularly because the porin has been shown to exhibit only weak binding to SDS (19).

The conductance increments were not uniform in size but were distributed over a certain range. Histograms of the conductance steps with potassium chloride as the permeant ion and the λ receptor, compared with those with porin, are shown in Fig. 3. The majority of conductance steps and thus of the pores were larger with the λ receptor than with porin. This was also reflected in the average conductance increment (Δ) in different electrolyte solutions (Table 1). Δ measured with all alkaline chlorides, including ammonium, was larger for the λ receptor than for porin. Table 1 also shows the ratio of Δ to σ, where σ is the specific conductance of a 1 M solution of the corresponding salt in water. The ratio of Δ to σ was reasonably constant, indicating that the λ receptor indeed forms a large water-filled pore in the membrane.

To test whether there was a difference in anion and cation specificity, the zero-current membrane potential (V0) in the presence of a potassium chloride gradient was determined. At a gradient of 10 (10^-2 versus 10^-1 M) a potential of 30 mV was obtained, with the positive side in the dilute solution. The observed value of V0 corresponded to a permeability ratio (ratio of permeability of K to permeability of Cl) of 4.5 (calculated by the Goldman-Hodgkin-Katz equation), whereas under the same conditions porin exhibited a ratio of 3.5. Thus, both types of pores exhibited slight cation selectivity that was more pronounced in the λ receptor than in porin.

Figure 4 shows the average single-pore conductance of λ receptor at different concentrations of potassium chloride. Δ was proportional to the salt concentration. This again demonstrates that the λ receptor is best characterized as a water-filled pore and that its incorporation
Fig. 2. Pore-forming activity of the λ receptor. A small amount of λ receptor in 0.1% SDS was added to a solution of 1 M potassium chloride on both sides of the membrane to give a final concentration of 20 ng/ml. The applied voltage was 50 mV. The current before the addition of λ receptor was 1.8 pA. All membrane experiments were performed at 25°C, pH 5.5 to 6.0.

Fig. 3. Probability of the occurrence of a conductance step \( P(\Lambda) \) in 1 M potassium chloride. \( P(\Lambda) \) is the number of observed steps within an interval of width ±ΔΛ of 0.22 nS centered at Λ and divided by the total number of steps (n). (A) λ receptor at 20 ng/ml (n = 181); (B) porin at 0.5 ng/ml (n = 249); other details were as described in the legend to Fig. 2.

into the membrane is not dependent on ionic interactions. The average conductivity \( \langle Λ \rangle \) of single pores in 1 M potassium chloride remained constant when different potentials (ranging from 20 to 100 mV) were applied. Thus, the λ receptor pore behaves as an ohmic resistance that is independent of the applied potential.

**Macroscopic conductance measurements with λ receptor.** When larger concentrations of λ receptor were present in the membrane assay, the conductance quickly rose after formation of the bilayer and reached values two to three orders of magnitude above the level of untreated membranes. The time course of the specific conductance after protein addition is shown in Fig. 5; the rate of conductance increases rose with time.

In contrast, porin measured under the same conditions exhibits a conductance that is about two orders of magnitude higher than that obtained with λ receptor. In addition, its time course is different. Conductance increases decline with time (4). As in the case of porin, the conductance of membranes due to λ receptor was largely independent of protein concentration. Since the conductance rises with time, a comparison for dif-

<table>
<thead>
<tr>
<th>Salt</th>
<th>( \langle Λ \rangle ) (nS)</th>
<th>( Λ/σ ) (( \times 10^{-10} ) m)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>1.3 (0.72)</td>
<td>1.8</td>
<td>84</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.1 (1.2)</td>
<td>2.4</td>
<td>137</td>
</tr>
<tr>
<td>KCl</td>
<td>2.7 (1.9)</td>
<td>2.4</td>
<td>181</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>2.5 (1.8)</td>
<td>2.2</td>
<td>157</td>
</tr>
<tr>
<td>RbCl</td>
<td>2.7 (1.6)</td>
<td>2.3</td>
<td>175</td>
</tr>
<tr>
<td>CsCl</td>
<td>3.0 (2.0)</td>
<td>2.6</td>
<td>98</td>
</tr>
</tbody>
</table>

*Lambda receptor (20 ng/ml) in a solution containing 0.1 μg of SDS per ml was used; the applied voltage was 50 mV. The salt solutions were 1 M, \( σ \) is the specific conductance \( \langle Λ \rangle \) of a 1 M salt solution at 25°C and \( n \) is the number of pores measured. The numbers given in parentheses are the corresponding values for porin.
Fig. 4. Average conductance ($\bar{\lambda}$) of single pores of $\lambda$ receptor at different potassium chloride concentrations. Protein concentration was 20 ng/ml, and applied voltage was 50 mV. $c_{M}$ is the molar concentration of potassium chloride. Other details were as described in the legend to Fig. 2.

![Graph showing conductance vs. concentration](image1)

Fig. 5. Specific conductance increase (macroscopic) after addition of $\lambda$ receptor. Formation of the membrane from 1% oxidized cholesterol in n-decan at zero time; after 2 min the membrane became black. The applied potential was 10 mV. The salt solution was 0.1 M potassium chloride, and the protein concentration was 200 ng/ml a solution containing 1 $\mu$g of SDS per ml. The conductance without $\lambda$ receptor in a solution containing 0.1 M potassium chloride and 1 $\mu$g of cholate per ml was about 10 nS/cm$^{-2}$. The points shown are the averages of five measurements. The reproducibility of each point was about ±25%.

![Graph showing conductance vs. time](image2)

Different protein concentrations can only be made by measuring the conductivity at a constant time. With different membranes these values are reproducible to ±50%. Table 2 shows the values of conductance obtained at different concentrations of phage $\lambda$ receptor. Although the protein concentration varied by a factor of 100, the conductivity remained largely constant.

Figure 6 shows the macroscopic conductance in the presence of different potassium chloride concentrations (Fig. 6A) and the volt-current dependence of a pore formed by the $\lambda$ receptor (Fig. 6B). As was the case for a single pore, there was a linear dependence of conductance on the salt concentration. This indicates that the incorporation of the $\lambda$ receptor is not dependent on the salt concentration in the aqueous phase. Also, the volt-current dependence exhibited the characteristics of an ohmic resistance. No potential-dependent closing or opening of pores could be observed.

Table 2. Macroscopic conductivity measured in dependence of protein concentration

<table>
<thead>
<tr>
<th>Protein concn (ng/ml)</th>
<th>$\lambda$ (S·cm$^{-2}$)</th>
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</thead>
<tbody>
<tr>
<td>200</td>
<td>$2 \times 10^{-6}$</td>
</tr>
<tr>
<td>50</td>
<td>$5 \times 10^{-6}$</td>
</tr>
<tr>
<td>20</td>
<td>$6 \times 10^{-6}$</td>
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<tr>
<td>5</td>
<td>$3.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>2</td>
<td>$4.5 \times 10^{-6}$</td>
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</table>

$^a$ Lambda receptor in a solution containing 0.1 $\mu$g of SDS per ml was used. The bathing solution was 0.1 M potassium chloride. Measurements were done 30 min after the membrane had become black.

![Graph showing conductivity vs. concentration](image3)

![Graph showing conductivity vs. time](image4)

![Graph showing current vs. voltage](image5)

Fig. 6. Dependence of macroscopic conductivity of $\lambda$ receptor on salt concentration (A) and volt-current relationship (B). Protein concentration was 200 ng/ml, and the solution contained 1 $\mu$g of SDS per ml. (A) Conductivity in different concentrations of potassium chloride ($c_{M}$ = molar concentration); conductivity was measured 30 min after the membrane had become black. Applied voltage was 10 mV. The points represent the average of four to five independent measurements. (B) Measurements started 20 min after the membrane had become black; potassium chloride concentration was 1 M. Voltage pulses of 5 ms duration were applied, and the current was measured after the decay of the capacitive transient. $V_{m}$. Voltage across membrane.
DISCUSSION

In this paper we have demonstrated that the receptor for phage λ can increase the conductance of ions in black lipid membranes. At low protein concentrations the incorporation of the protein molecules into the membrane can be followed directly by the stepwise increase of the membrane conductance to ions. From the histograms of conductance fluctuations (Fig. 3) it is obvious that the channel that is formed can assume a broad range of conductance values. The reason for this heterogeneity is not clear, but it may indicate that the channel is formed by subunits, the number of which is not fixed. The ohmic characteristics of conductance, the non-saturability of conductance with increasing salt concentrations, and the low anion-cation selectivity all indicate that the λ receptor is an unsensitive and hydrophilic pore.

Apparently there is no dependence of conductance on the amount of protein added. This indicates that the formation of pores occurs from a protein species, the amount of which is constant. This species might be a monomer that is in equilibrium with higher aggregates of λ receptor, or alternatively the membrane might be covered by a layer of λ receptor from which occasional protein incorporation into the membrane occurs.

All of these observations are similar to the effects observed with porins (3, 4), a group of closely related proteins of the outer membrane from E. coli that have been connected to the passive permeability properties of the cell wall (7). The differences that can be determined by the membrane assay may be significant for the biological function of these proteins. The most obvious difference is the magnitude of the single-pore conductance. This can clearly be seen by comparing the histograms of single-pore conductance for potassium chloride (Fig. 3) and the average single-pore conductance (A) for the different alkaline ions (Table 1). In all cases the conductance caused by the λ receptor pore is larger by 50 to 80% than that caused by porin. Assuming that the pores are cylindrical channels that span the membrane, the conductivity of this channel is given by: $A = \sigma l^2/\eta$, where $\sigma$ is the specific conductivity of the bathing solution and $l$ and $r$ are the length and the radius of the channel, respectively. By using the average single-pore conductivity (A) obtained with potassium chloride and 4 nm for the thickness of the membrane, the pore diameter can be estimated to be 0.9 nm for porin and 1.1 nm for the λ receptor. By using a larger estimate for the thickness of the outer membrane of 7.5 nm (8), the corresponding values are 1.2 and 1.6 nm. In contrast to the wild type, λamB strains that lack the λ receptor but still contain porin do not transport and do not grow on long maltodextrins. From the analysis of the pore characteristic of the λ receptor we propose that it is only the slightly larger pore size that allows maltodextrins to penetrate the λ receptor but not porin.

From the growth properties of wild-type and λamB strains it must be concluded that maltose, the smallest maltodextrin that is composed of only two glucose units, is able to penetrate porin, but apparently its diffusion through the λ receptor is less restricted. Thus, transport of maltose into a λamB strain at a low concentration of maltose is limited by its diffusion through the outer membrane via porin; only at concentrations above 0.1 mM does this passive diffusion become faster than the subsequent active transport mediated by the specific transport components localized in the periplasmic space and the cytoplasmic membrane. In contrast, a wild-type strain does not show this restriction at a low concentration of maltose (24, 25).

An obvious difference between porin and λ receptor in the formation of pores in black lipid membranes is the observation that the macroscopic conductivity obtained with porin is two orders of magnitude higher than that obtained with the λ receptor. This must mean that the successful incorporation of λ receptor into the bilayer is much rarer than is the incorporation of porin. One factor that might influence this incorporation is the difference in size of these two proteins (38,000 daltons for porin [9] and 55,000 daltons for the λ receptor [18] in their subunit forms). A second factor might be a higher number of inactive molecules in the λ receptor preparation. The possibility also exists that the λ receptor pore might have more bound detergent molecules, causing the lower activity and the higher noise level in the current traces obtained in single-channel experiments, as well as the larger cation selectivity.

The structural requirements for the λ receptor to form pores in black lipid membranes are apparently different from those needed to inactivate phage λ. The treatment of λ receptor with chloroform or methanol that is needed for the phage assay (18) completely abolishes the pore-forming activity. On the other hand, only λ receptor eluted from the SDS gel at the dimer position was active in the phage assay, whereas both the dimer and the monomer were active in the membrane assay.

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LITERATURE CITED