High-conductance channel induced by the interaction of phage lambda with its receptor maltoporin

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Abstract Bacteriophage λ that binds to liposomes bears its receptor maltoporin (LamB) and is able to inject its DNA into the internal space. During this process, the liposomes are permeabilized, suggesting that a transmembrane channel has formed (Roessner and Ihler (1986) J. Biol. Chem. 261, 386–390). This pore possibly constitutes the pathway used by λ DNA to cross the membrane. We reconstituted purified LamB from *Shigella* in liposomes that were incubated with λ phages. Addition of this mixture to a bilayer chamber resulted in the incorporation in planar bilayers of high-conductance channels whose conductance, kinetics and voltage dependence were totally different from those of maltoporin channels.

Key words: Phage lambda; LamB; Porin; Ion channel

1. Introduction

The first stages of phage infection are binding of the phage to its receptor and injection of its DNA through the bacterial envelope. DNA is injected from the phage through its tail, the extremity of which interacts with the receptor. How phage DNA crosses the outer and inner membranes of Gram-negative bacteria is a process which is poorly understood.

When FhuA, an iron-chelator transporter of the *Escherichia coli* outer membrane, was reconstituted in planar lipid bilayers, addition of phage T5 for which it is a receptor, triggered the opening of high-conductance channels that were observed neither with the phage alone nor with the FhuA protein [1]. The electrophysiological characteristics of these channels were similar to those of the channel formed by an FhuA derivative whose surface-exposed loop that binds the phage had been removed [2]. Presumably, binding of T5 causes a conformational change that unmasks an inner channel in the protein. It has been speculated that this channel is involved in the process of DNA transfer across the outer membrane. When purified FhuA was reconstituted in large unilamellar liposomes, binding of phage T5 triggered the transfer of phage DNA into the liposomes [3,4].

The receptor of phage lambda (λ) is the maltoporin LamB [5]. LamB, which catalyzes the transport of maltosides across the outer membrane, on its own forms relatively low-conductance ion channels in lipid bilayers [6,7]. Its structure is known from X-ray diffraction studies. It is a trimer composed of three independent channels, each channel being formed by an 18-stranded antiparallel β barrel [8].

The solubilized *E. coli* LamB protein is able to bind phage λ. It causes DNA ejection, but this activity requires treatment with either chloroform or ethanol [5,9]. In contrast, the analogous protein from *Shigella sonnei* can trigger the ejection of DNA from λ in the absence of organic solvents [10]. The λ bacteriophages bind irreversibly to unilamellar liposomes carrying LamB from *Shigella*. Under these conditions, part of the phage DNA is injected into the aqueous compartment of the liposomes [11]. Upon addition of phage λ, the permeability of the liposomes increases, suggesting that a transmembrane pore had formed [12].

In this study, purified maltoporin from *S. sonnei* was reconstituted in liposomes that were incubated with λ phages. Addition of the complexes of λ phages and proteoliposomes to the cuvette of a bilayer set-up led to the incorporation of high-conductance channels that were different from the maltoporin channels.

2. Materials and methods

2.1. Production and purification of phage λ

λ Phages were produced using *E. coli* pop3, a derivative of *E. coli* K12. Cells were grown in Luria Broth supplemented with 10 mM $MgSO_4$. At $OD_{650\,nm} = 0.2$, cells were infected at a MOI of 10^{-2} phage/cell. After cell lysis and DNAse treatment (DNAse I, Boehringer Mannheim, 10 μg ml^{-1}, 2 h, 37°C), unlysed cells were eliminated by low-speed centrifugation (16 000 × *g*, 4°C, 10 min). Phages were precipitated twice with polyethylenglycol 6000 (100 g l^{-1}) and NaCl (30 g l^{-1}) overnight at 4°C, then gently resuspended in 10 mM Tris–HCl pH 7.2, 150 mM NaCl, 10 mM $MgSO_4$ and purified on a discontinuous cesium chloride gradient (densities 1.6, 1.5, 1.3 and 1.2). After centrifugation (180 000 × *g*, 4°C, 2.5 h), the phage suspension (density 1.5–1.6) was dialyzed against 10 mM Tris–HCl pH 7.2, 100 mM KCl, 10 mM $MgCl_2$. The final titer was 10^{12} phages ml^{-1}.

2.2. Purification of LamB protein

The LamB from *S. sonnei* was produced using *E. coli* strain pop154, a derivative of *E. coli* K12 carrying the *malB* region of *S. sonnei* 3070 [13]. Cells were grown in M63 supplemented with 0.2% casamino acids, 10 μg ml^{-1} thiamine, 1 mM $MgSO_4$, 0.2% maltose and harvested at $OD_{650nm} = 1.2$ by low-speed centrifugation (10 000 × *g*, 4°C). The cell pellet was resuspended in 10 mM HEPES pH 7.4, 200 mM NaCl, 0.2 mM phenylmethylsulfonyl fluoride, 0.1% β-mercaptoethanol, 1 mM $MgSO_4$, 10 μg ml^{-1} DNAse (deoxyribonuclease I, Bovine pancreas, Amersham Pharmacia) and cells were broken using a French press cell (10 000 p.s.i.). The cell debris was eliminated by centrifugation at 10 000 × *g* and 4°C for 10 min. Membrane vesicles were recovered by centrifugation for 1 h at 130 000 × *g* at 4°C and the pellet was resuspended and incubated for 30 min at 60°C in 10 mM Tris–HCl pH 7.2, 2 mM $MgSO_4$, 10% glycerol, 2% SDS. Outer membrane proteins bound to the peptidoglycan were recovered by centrifugation (1 h at 130 000 × *g*, 4°C) and the pellet was resuspended in 10 mM Tris–HCl pH 7.2, 5 mM EDTA, 2% Triton X-100. After 2 h incubation at 30°C, the suspension was centrifuged (1 h at 130 000 × *g*,

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4°C) and the solubilized proteins were recovered from the supernatant by precipitation with 66% ethanol absolute and 100 mM NaCl final concentration for 1 h at −70°C followed by centrifugation (24 000×*g*, 30 min, 4°C). The pellet was resuspended in 100 mM $NaHCO_3$ pH 8.4, 1% Triton X-100 and the LamB protein was purified by a one-step affinity chromatographic procedure using starch Sepharose column [14]. After washing with 100 mM $NaHCO_3$ pH 8.4, 1% Triton X-100, 0.1% SDS, LamB was eluted with 100 mM $NaHCO_3$ pH 8.4, 1% Triton X-100, 20% maltose then dialyzed against 10 mM Tris–HCl pH 7.2, 150 mM KCl, 10 mM $MgS0_4$, 1% Triton X-100. The purity of the preparation was assessed by SDS–PAGE and the protein content was measured by the method of Lowry et al. [15].

2.3. Reconstitution of LamB in liposomes and interaction with λ phages

The purified protein (200 ng) was added to 1 ml of 10 mM HEPES–KOH pH 7.4, 300 mM KCl, 1% Triton X-100, containing 1 mg of sonicated lipids (asolectin from soybean, type IV-S, Sigma). After incubation for 20 min at room temperature, 160 mg wet weight of SM-2 Bio-Beads (Bio-Rad) were added to the suspension to remove the detergent [16]. Incubation was carried out overnight at 4°C; the Bio-Beads were discarded and the suspension was centrifuged for 30 min at 344 000×*g* at 4°C. The proteoliposomes were resuspended in 100 μl of 10 mM HEPES–KOH pH 7.4, 300 mM KCl, and stored at 4°C. Complexes of phages and proteoliposomes were prepared by incubating 5 μl of proteoliposomes (10 ng of protein) with 50–250 μl of phage suspension at 37°C for at least 30 min in 10 mM HEPES–KOH pH 7.4, 300 mM KCl, 10 mM $MgCl_2$. This corresponds to a phage to protein ratio of 1–5.

2.4. Electrophysiological recording

Bilayers were formed from a solution of asolectin lipids dissolved in *n*-decane at 30 mg ml^{-1} across a 250 μm diameter hole. Proteoliposomes (10 ng of protein) or complexes of phages and proteoliposomes were added to the *cis* compartment. Fusion was induced by imposing a salt gradient between the two chambers (500 mM KCl, 10 mM HEPES–KOH pH 7.4, 10 mM $MgCl_2$ in the *cis* compartment versus 100 mM KCl, 10 mM HEPES–KOH pH 7.4, 10 mM $MgCl_2$ in the *trans* compartment). The bilayer set-up was connected to the external circuit through salt bridges with Ag/AgCl electrodes. Unitary currents were recorded using an Axon 200B patch clamp amplifier and stored on digital audio tape (Biologic DTR 1200 recorder). Records were subsequently filtered at 300 Hz to 1 kHz through a 4-pole Bessel filter and digitized offline at 2 kHz. Data were plotted on an HP laser printer. Open probability was calculated using 30 s segment recording. The membrane potential refers to the potential of the *cis* side minus the potential of the *trans* side.

3. Results

Purified maltoporin from *Shigella* was reconstituted into liposomes. Fusion of the proteoliposomes to a planar lipid bilayer led to the electrical activity previously described for LamB channels [7]. The LamB channels were open most of the time but could occasionally gate (Fig. 1A). The channels had a relatively low conductance compared to other porins (25–30 pS in 500 mM versus 100 mM KCl media). The typical voltage dependence of porins [17] was not observed for LamB porins [6,7] whose opening was independent of voltage between +200 mV and −250 mV. The channels were weakly selective for cations and were blocked by addition of maltotriose (14 mM) or maltoheptose (5 mM) in the *trans* or the *cis* compartment, as previously described [6,7].

In the next series of experiments, we first incubated proteoliposomes containing LamB with λ phages (at a phage to receptor ratio of 1 to 5) at 37°C in the presence of 10 mM magnesium. Addition of this suspension to the *cis* compartment of the bilayer set-up led not only to the appearance of the LamB channels, but also to the insertion of novel high-conductance (700 pS) channels, with characteristic kinetics. The electrical activity of these channels consisted of two

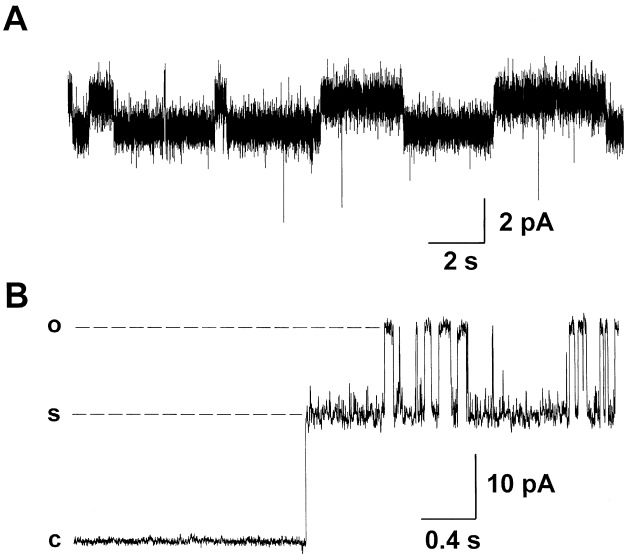

Fig. 1. Interaction of maltoporin with phage λ: identification of a novel channel. A: Channel activity of maltoporin channels. Proteoliposomes reconstituted with LamB porin from *Shigella* (10 ng protein) were added to the *cis* compartment and fused into bilayer under asymmetrical conditions (500 mM KCl, 10 mM HEPES–KOH pH 7.4, 10 mM $MgCl_2$ in the *cis* compartment *versus* 100 mM KCl, 10 mM HEPES–KOH pH 7.4, 10 mM $MgCl_2$ in the *trans* compartment) resulting in the insertion of several maltoporin channels. The membrane potential was −60 mV. B: Channel activity induced by the interaction of maltoporin with λ. Proteoliposomes containing LamB from *Shigella* (10 ng protein) were incubated with λ phages (at a phage to receptor ratio of 1) at 37°C in the presence of 10 mM magnesium. Addition of this mixture to the *cis* compartment under asymmetrical conditions (same ionic conditions as in A) led to the insertion of a high-conductance channel. The closed level is indicated by c. The channel displayed a permanently open substate (s) and gated to a fully open state (o). The membrane potential was +50 mV.

parts: a permanently open conductance (whatever the voltage between −150 mV and +150 mV) and a gating part that was voltage dependent, as described below (Fig. 1B). In all our experiments, the two parts always appeared together, therefore we assumed that the permanently open conductance was a substate of the full channel. This type of channel was observed in 52 independent experiments. In some cases up to 20 similar channels could be observed to insert together with LamB channels, until the channel gating blurred the electrical activity. This type of channel was also observed in two experiments in which the λ phages were added directly to the *cis* chamber containing LamB proteoliposomes in the presence of 10 mM magnesium.

Extensive control experiments were made to test whether the high-conductance channel formation required the presence of both λ and its receptor. In 22 independent experiments (totalling 24 h of recording) with LamB alone, this type of channel was never observed. In 56 independent experiments (totalling 36 h of recording) in which λ phages pre-incubated with pure lipid liposomes were used, this channel was observed four times. In each case, only one channel was observed at a time. We therefore ascribe this activity to contaminations.

The gating part of the channel displayed a characteristic voltage dependence. It was completely closed at negative potential and the probability of opening increased with positive

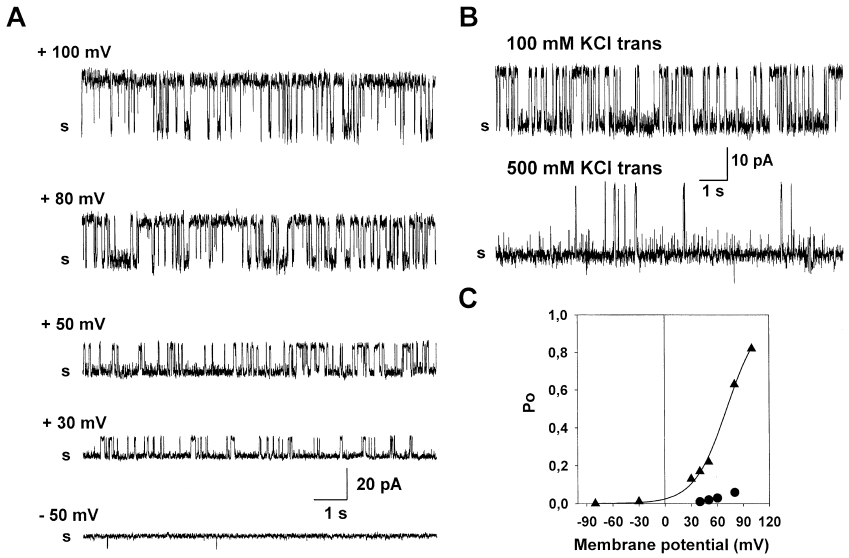

Fig. 2. Voltage dependence of the high-conductance channel. A: Recording of the gating activity of the channel at different membrane potentials. Only the gating part of the channel activity is shown. The channel was in the fully open state at high positive membrane potentials and was preferentially in the substate (s) at negative potentials. Same ionic conditions as in Fig. 1. B: The open probability of the gating part of the channel activity was sensitive to the salt concentration in the *trans* compartment. *Upper trace*: activity of the channel under asymmetrical condition (as defined in Fig. 1A). *Lower trace*: activity of the same channel after establishment of symmetrical conditions (500/500 mM KCl) by addition of concentrated KCl in the *trans* compartment. Membrane potential was +50 mV. C: Open probability of the gating part of the channel under asymmetrical (▲) and symmetrical (●) conditions. The open probability of the full channel versus the imposed potential could be fitted to a Boltzmann distribution of the form: $PO = 1/(1+\exp(-z{\cdot}e(V-V_{0.5})/kT))$, where e, k and T have their usual meaning, $z{\cdot}e$ is the gating charge and $V_{0.5}$ is the potential for which $PO = 0.5$. $V_{0.5}$ and z were calculated to be +71 mV and 1.3, respectively.

potentials. At 100 mV the channel was almost fully open (Fig. 2A, C). The open probability was drastically reduced when the ionic strength was increased in the *trans* compartment (Fig. 2B, C).

The current–voltage relationships of both the gating part of the channel and the permanently open substate were studied in asymmetrical media (500 mM/100 mM) and in symmetrical media (500 mM KCl), (Fig. 3A and B). In both cases the curves were linear. The conductances of the gating part and of the substate were 380 and 580 pS respectively (in 500 mM symmetrical KCl). In both cases, the reversal potential in asymmetrical media was 0 mV, indicating that neither state of the channel discriminates between cations and anions.

Maltotriose (up to 14 mM) or starch (up to 12.5 mg ml^{-1}), added to either side of the bilayer, did not inhibit either the fluctuating part of the high-conductance channel or its permanently open part.

Van Gelder et al. have recently shown, in planar bilayer experiments, that the presence of λ in the *cis* chamber prevented maltosides, added on the same side, from blocking *E. coli* LamB channels [18]. This was also observed in our experiments with LamB from *Shigella*. In the experiment displayed in Fig. 4, at the onset of the trace the bilayer contained one high-conductance channel, as evidenced from its gating

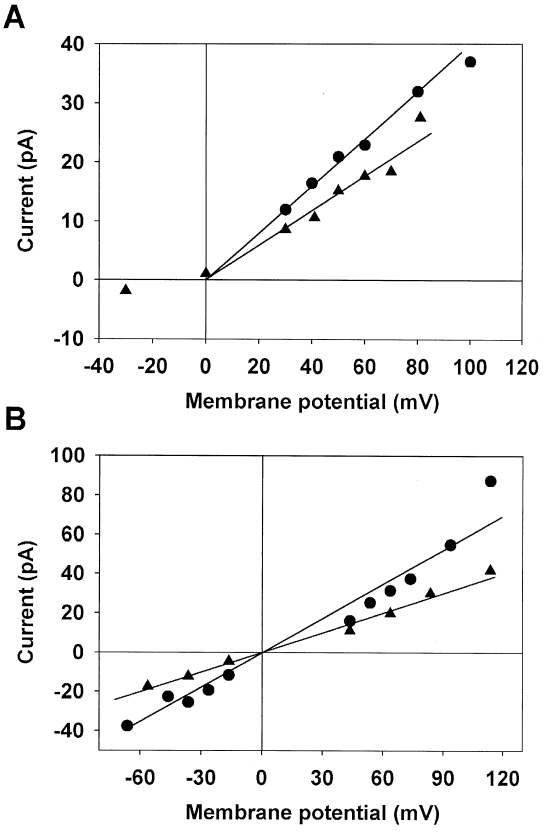

Fig. 3. *I–V* curve of the high-conductance channel. A: Unitary current of the gating part of the channel as a function of membrane potential, under symmetrical (●) (500 mM *cis*/500 mM *trans* KCl) and asymmetrical (▲) (500 mM *cis*/100 mM *trans* KCl) conditions. B: Unitary current of the permanently open substate of the channel as a function of membrane potential, under symmetrical (●) and asymmetrical (▲) conditions.

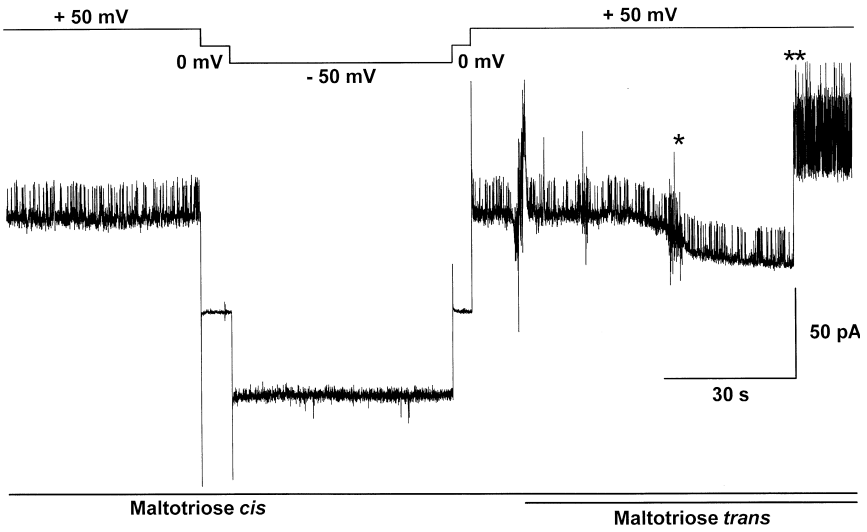

Fig. 4. Effect of maltotriose on maltoporin channels interacting with λ phages. Proteoliposomes containing LamB from *Shigella* were first incubated with λ phages and the mixture was then added to the *cis* compartment under asymmetrical conditions, as defined in Fig. 1B. At the onset of the trace, one high-conductance channel and several maltoporin channels are present in the bilayer. Maltotriose (14 mM final concentration) present on the *cis* side did not inhibit either the maltoporin channels or the maltoporin/λ channel. Maltotriose, added on the *trans* side, inhibited the maltoporin channels, thus inducing a decrease in the overall current (*), but not the high-conductance channel. See text for discussion. At the end of the recording, two new high-conductance channels inserted in the bilayer (**). Membrane potential as indicated.

part. The non-gating part of the current was higher than that of the permanently open substate, indicating the presence, in addition, of maltoporin channels. However, these maltoporin channels, as well as the high-conductance channel, were insensitive to the presence of maltotriose in the *cis* compartment. Addition of maltotriose to the *trans* compartment resulted in a decrease in current, corresponding to the inhibition of LamB porin channels. The non-gating part of the current then reduced to the value corresponding to one permanently open substate of the high-conductance channel, before the subsequent simultaneous insertion of two additional high-conductance channels. This and similar experiments indicate that λ could bind to *Shigella* LamB without inhibiting ion flow, and that this binding prevented maltotriose from reaching the mouth of the channels, as observed for *E. coli* LamB. This type of binding was clearly different from that which induced the formation of a high-conductance channel.

4. Discussion

Lambda phages can inject their DNA into liposomes in which the *Shigella* maltoporin has been reconstituted [11]. As shown by Ihler and Roessner in earlier experiments [12] the permeability of the liposomes increases during this process. When ATP was trapped in proteoliposomes reconstituted with the *Shigella* maltoporin, addition of bacteriophages λ to the suspension resulted in a time-dependent release of ATP from the liposomes. In contrast, trapped proteins were not released. When alkaline phosphatase was entrapped in the proteoliposomes, it was found to be active for at least 1 h on its substrate *p*-nitrophenyl phosphate added to the external medium, after addition of λ phages. These experiments suggested that interaction between bacteriophage λ and its receptor results in the formation of a transmembrane pore that remains open after its formation and the entry of DNA. Our results suggest that we have observed this channel in electrophysiology experiments. Indeed, the high-conductance channel activity described in this paper is observed when both the phage and its *Shigella* receptor are incubated together and its characteristics are unlike those of any known channel of the outer membrane.

After incubation with λ phages, maltoporin channels were still observed in addition to the high-conductance channels, despite the fact that the experiments were conducted at a phage to receptor ratio of about 5. Each trimer, composed of three separate channels, can bind one phage, but, on average, we observed one high-conductance channel for 3–4 maltoporin trimers. However, these trimers must have bound a phage since they became insensitive to maltotriose added to the *cis* side, in contrast with free maltoporins, which can be blocked by maltotriose added to either side of the bilayer. If we assume that a high-conductance channel results from the interaction between a phage and its receptor, this implies the existence of two populations of complexes of λ and maltoporin. In one case the phage binds to the receptor without changing its electrical activity but its presence shields the maltoporin from maltotriose. This phenomenon, which has also been observed by Van Gelder et al. with *E. coli* maltoporin, implies a unidirectional insertion of the protein in the bilayer [18]. In the second case, the interaction results in the formation of a different, high-conductance, channel. It has previously been shown by Schwartz [19] that the number of λ phages that can infect bacteria (plaque-forming units) represents only 15% of the total number of phage particles, as measured from absorbance at 260 nm. Yet, all the phage particles thus numbered can bind to their receptor. It is tempting to speculate that the subpopulation of phages that is able to infect bacteria is also that which induces high-conductance channels. Electron microscopy experiments showed that two types of complexes can be formed between λ and its receptor. Type 1 complexes involve binding between the tip of the phage tail fiber and the receptor, so that the hollow tail is 17 nm from the surface of the liposome. In type 2 complexes, whose formation is believed to be necessary for DNA injec-

tion, the hollow tail is in direct contact with the membrane of the liposome [20]. Again, it may be speculated that type 2 complexes correspond to the high-conductance channels.

Does the high-conductance channel activity that we observed result from the modification of the maltoporin channel or is it due to the insertion of the phage tail into the membrane, upon binding to its receptor? We encountered a similar problem when we studied the interaction of phage T5 with its receptor FhuA. FhuA, the transporter of ferrichrome, is electrically silent when reconstituted in planar bilayers. Addition of phage T5 triggers the opening of high-conductance channels [1]. However, when the protein loop L4, which constitutes the binding site for T5, is deleted in FhuA, the carrier is converted into a high-conductance channel whose characteristics are similar to those observed upon binding of the phage [2]. FhuA is a β barrel composed of 22 antiparallel β strands which is obstructed by a structurally distinct domain, the 'cork' or 'plug', consisting in a four-stranded β sheet and four α helices [21,22]. Removal of this plug seems to be necessary for the opening of the large channel observed in planar lipid bilayer. Since loop L4, which binds the phage T5, interacts with the top of the plug, its deletion or the binding of T5 would indirectly result in the displacement of the plug. The maltoporin channel is an 18-stranded antiparallel β barrel. Its conductance is dictated by the constriction zone defined by residues from loop L3 and from the juxtaposed barrel wall. In principle, a mechanism similar to that postulated for the FhuA–T5 interaction could hold. Binding of the phage to LamB could trigger a conformational change resulting in the removal of loop L3, and possibly other loops that restrict the channel entrance. An 18-stranded β barrel totally or partially cleared of these loops could display the high conductance detected in our experiments. The high-conductance channel is not blocked by maltosides but this may not be a decisive argument. Although binding of maltosides to the 30 pS LamB porin channel results in channel blockade, its effect on the high-conductance channel might be negligible. However, and in contrast to the case of the FhuA protein, there is no experimental evidence available to tell us how such a modified LamB channel would behave. Therefore, it cannot be ruled out that the channel is formed by insertion of phage proteins. Interestingly, Roessner and Ihler have suggested that protein pH* might be part of a transmembrane hole for DNA entry [23].

A final question concerns the function of this channel. Is it an epiphenomenon related to phage binding or does it constitute the route used by DNA to cross the outer membrane? As in the case of the T5–FhuA channel, our data do not allow us to reach a conclusion on this point. It is however striking that, in both cases, binding of a phage to this β-barreled structure results in the formation of high-conductance channels.

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