The EMBO Journal vol.15 no.8 pp.1850–1856, 1996

FhuA, a transporter of the *Escherichia coli* outer membrane, is converted into a channel upon binding of bacteriophage T5

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The *Escherichia coli* outer membrane protein FhuA catalyzes the transport of Fe^{3+}-ferrichrome and is the receptor of phage T5 and Φ80. The purified protein inserted into planar lipid bilayers showed no channel activity. Binding of phage T5 to FhuA resulted in the appearance of high conductance ion channels. The electrophysiological characteristics of the channels (conductance, kinetic behavior, substates, ion selectivity including the effect of ferrichrome) showed similarities with those of the channel formed by a FhuA derivative from which the 'gating loop' (Δ322–355) had been removed. Binding of phage T5 to FhuA in *E.coli* cells conferred SDS sensitivity to the bacteria, suggesting that such channels also exist *in vivo*. These data suggest that binding of T5 to loop 322–355 of FhuA, which constitutes the T5 binding site, unmasks an inner channel in FhuA. Both T5 and ferrichrome bind to the closed state of the channel but only T5 can trigger its opening.

Keywords: carrier/ion channel/phage DNA/planar lipid bilayer/porin

Introduction

Channels or pores are classically distinguished from carriers or transporters on the basis of the mechanism of transport. Channels are generally viewed as tunnels in which the binding sites for the transported substrate are accessible from both sides of the membrane at the same time. Although a channel can undergo a conformational change from the closed state to the open state, no alteration of conformation is necessary to promote the transport of the solute once it has entered the channel. In contrast, solutes transported by carriers first bind from one side of the membrane and a conformational change is required for them to be released on the opposite side. Thus, the solute binding site of a carrier is accessible only from one side of the membrane at a given time (Gennis, 1989). However, since membrane proteins are unlikely to undergo large conformational changes, it is often assumed that carriers possess some kind of inner channel which allows for the translocation of the transported solute. The clearest experimental evidence in favor of such a channel in a transporter has been obtained recently by Killmann *et al.* (1993), who showed that deletion of a stretch of amino acid residues in FhuA, a transporter located in the *Escherichia coli* outer membrane, yielded a non-specific diffusion channel. This finding makes FhuA an attractive system to study the mechanisms of carrier transport, which are still poorly understood.

FhuA, a 78.9 kDa protein, catalyzes the high affinity transport of the ferric siderophore ferrichrome across the outer membrane of *E.coli*. While ferrichrome can bind to FhuA at the cell surface in de-energized cells, it is only transported across the outer membrane in energized cells. Transport of ferrichrome also requires the participation of TonB, a protein anchored in the cytoplasmic membrane which extends across the periplasm interacting with FhuA and two inner membrane proteins, ExbB and ExbD, that may form a complex with TonB (Postle and Skare, 1988; Kamfenkel and Braun, 1992, 1993; Braun, 1995). Since energizing the cell does not affect the outer membrane directly but does affect the inner (cytoplasmic) membrane in the form of a proton-motive force, TonB has been implicated in energy transduction between the cytoplasmic and outer membrane (reviewed in Kadner, 1990; Postle, 1990, 1993).

Modeling suggests that the FhuA protein contains membrane-spanning β strands and loop regions connecting these transmembrane domains which are involved in substrate binding. Killmann *et al.* (1993) deleted a cell surface loop which is part of the active site of FhuA and showed that the modified protein (FhuA Δ322–355) formed large conductance channels in black lipid membranes, in contrast to the wild-type FhuA which had no electrophysiological activity. *In vivo*, the uptake of [$^{55}Fe^{3+}$]ferrichrome through the FhuA derivative exhibited the characteristics of channel-mediated transport and was no longer dependent upon TonB, ExbB and ExbD. The FhuA derivative also conferred SDS sensitivity to bacteria whereas wild-type FhuA strains are resistant. These results are consistent with those obtained previously by Rutz *et al.* (1992) with FepA, another carrier in the *E.coli* outer membrane which transports enterobactin. In this protein, deletion of a large stretch of 140 amino acid residues resulted in TonB-independent uptake of [$^{59}Fe^{3+}$]enterobactin and ferrichrome and in the permeabilization of the outer membrane to SDS and several antibiotics. Although the electrophysiological activity of the modified protein has not been documented in this case, these results suggest the formation of a large channel in the modified protein.

The FhuA protein is also the receptor for phage T1, T5, Φ80 and colicin M (Hantke and Braun, 1978). Domains of FhuA involved in binding of these ligands include the external 'gating loop' extending from residue 316 to 356 (Carmel and Coulton, 1991; Killmann and Braun, 1992; Koebnik and Braun, 1993). A more precise definition of the ligand binding sites was obtained recently using competitive peptide mapping (Killmann *et al.*, 1995). In particular, a hexapeptide belonging to the gating loop was

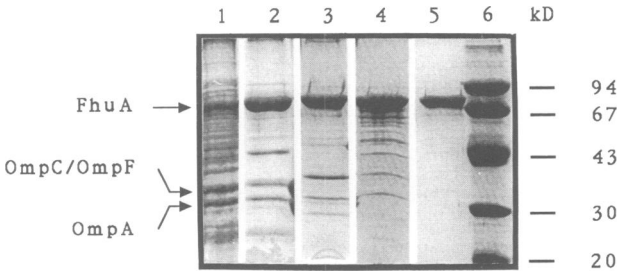

Fig. 1. Identification of outer membrane proteins and FhuA at different stages of the purification. Proteins were subjected to SDS–PAGE and stained with Coomassie Brilliant Blue. Lane 1: membrane vesicles isolated after passage of the cell suspension through a French pressure cell. Lane 2: soluble fraction obtained after an octylglucoside/EDTA treatment. Lane 3: anion exchange chromatography. Lane 4: chromatofocusing. Lane 5: gel filtration chromatography. Lane 6: standard proteins. The numbers on the right refer to the molecular mass (in kDa) of standard proteins.

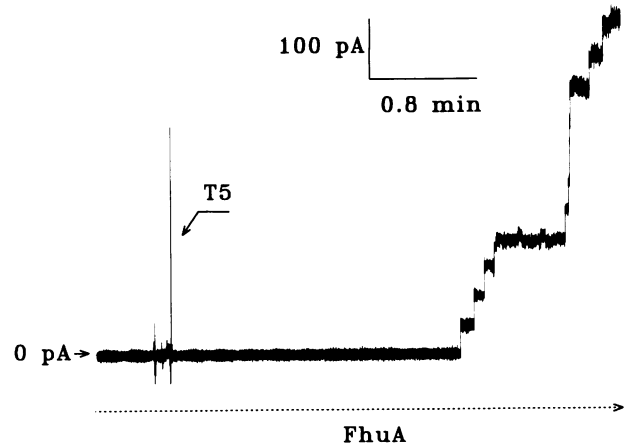

Fig. 2. Stepwise increase of current upon successive addition of FhuA and phage T5 to the bilayer chamber. FhuA (0.1 ng/ml) was added in the *cis* compartment. After 5 min, T5 (10 µl at 10^{13} phage per ml) was added to the same compartment. Both compartment solutions were 10 mM Tris–HCl, 100 mM KCl pH 7.3. The membrane potential was held at +50 mV. The experiment was performed at 20°C.

sufficient to inhibit phage T5 infection and to trigger *in vitro* the release of DNA from the phage capsid. The transfer of DNA from phage T1 and Φ80 through the *E.coli* envelope requires, in addition to FhuA, TonB, ExbB, ExbD and an energized cytoplasmic membrane. In contrast, infection by phage T5 neither requires an energized membrane nor TonB, ExbB or ExbD. In all cases, the mechanisms by which phage DNA crosses the outer and the inner membrane remain unclear.

In this study, we show that binding of phage T5 to purified wild-type FhuA incorporated into planar lipid bilayers converts the 'silent' protein into an ion channel. Single channel analysis indicates that the conductance of this channel is very similar to that of the FhuA derivative (Δ322–355) in which the 'gating loop' had been removed. Our data suggest that the interaction of the phage with FhuA triggers a conformational change in the loop that converts the transport protein into an open channel. The possible implications of this result upon the study of the transport mechanism of the FhuA carrier and also on the understanding of phage DNA transfer are discussed.

Results

Purification and characterization of FhuA

The protocol designed by Hoffmann *et al.* (1986) to purify FhuA from a strain overproducing the protein was used as the first stage of purification. Such a preparation is contaminated by several proteins and especially by porins (Figure 1), which renders it unsuitable for electrophysiological studies. Therefore, the protein was purified further by anion exchange chromatography, chromatofocusing and gel filtration chromatography, as described in Materials and methods. Highly purified protein was recovered (Figure 1). The purified protein solubilized in 0.9% (w/w) octyl β-D-glucopyranoside (OG) was active as judged by its capacity to bind to phage T5 and to induce the release of phage DNA (P.Boulanger, M.le Maire, S.Dubois, M.Desmadril and L.Letellier, manuscript submitted). Receptor activity of FhuA was preserved even after a 1000-fold dilution of the detergent. The protein stored at –20°C did not lose activity over several weeks.

Addition of phage T5 to FhuA results in the appearance of channels in planar lipid bilayers

FhuA protein added to the *cis* compartment of the bilayer setup, at concentrations ranging from 0.1 to 10 ng/ml, did not alter the permeability of the membrane. No channel activity could be recorded under these conditions with salt concentrations ranging from 100 mM to 1.5 M KCl. This result indicates that the purified FhuA protein is free from porin contamination and does not by itself form channels. This absence of channel activity was also observed by Killmann *et al.* (1993) on wild-type FhuA eluted from an SDS–PAGE gel. Addition of pronase (1 mg/ml, final concentration) to either of the two chambers had no effect, suggesting that loop 322–355, the deletion of which converts FhuA into a channel, is not accessible to the proteases. Similarily, phage T5 alone, up to 2.6×10^{11} phage per ml, did not induce any channel activity in planar lipid bilayers.

However, in the presence of FhuA, addition of phage T5 in the *cis* compartment induced, in all cases (>50 experiments), the appearance of ion channels in the membrane. Typically, FhuA was added to the *cis* chamber at a final concentration of 0.1 ng/ml. Phage T5 (10 µl at 10^{13} phage per ml, corresponding to 120 phage per FhuA protein) was added 5 min later. After a lag time varying from 30 s to 3 min (in 100 mM KCl or NaCl symmetric media) the membrane current started to increase in a stepwise fashion until breakage of the membrane (Figure 2). All channels had a similar conductance (0.65 and 0.9 nS in 100 mM NaCl and 100 mM KCl respectively, at room temperature) and no closure event could be observed, whatever the membrane potential between –80 and +80 mV. The increase in the ionic strength of the medium led to an increase in the lag time between phage addition and induction of channel activity, and reduction of the number of induced channels. At 1.5 M KCl concentration and for a phage to protein ratio of 120, the lag time was of the order of 20 min, and only a few (of the order of 4–10) channel openings could be recorded during the following 10 min. When T5 was added to the *cis* chamber before FhuA, no channel events were observed, suggesting first that the phage preparation is free of porin

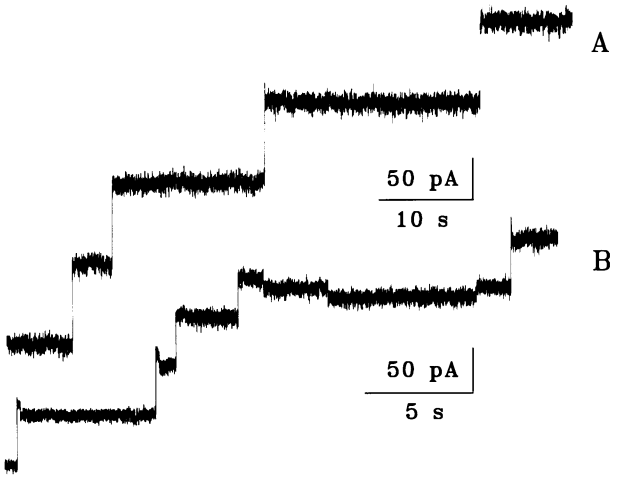

Fig. 3. Single channel current of the T5–FhuA complex. (**A**) Trace recording illustrating the uniformity of channel size. Both compartment solutions were 10 mM Tris–HCl, 400 mM KCl pH 7.3 and the applied potential was +50 mV. The experiment was performed at 28°C. Other conditions were as in Figure 2. (**B**) Trace recording illustrating the existence of substates. Both compartment solutions were 10 mM Tris–HCl, 100 mM KCl pH 7.3 and the applied potential was +50 mV. Other conditions were as in Figure 2.

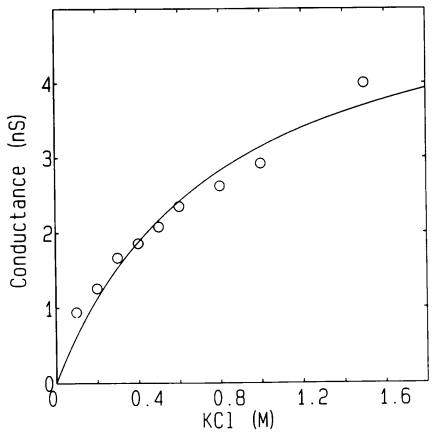

Fig. 4. Unitary chord conductance of the FhuA–T5 channel as a function of increasing KCl concentrations. Symmetrical solutions of KCl in 10 mM Tris–HCl pH 7.3 were used. The membrane potential was held at +50 mV. Other conditions were as in Figure 2.

contamination and second that the FhuA–T5 complex formed in solution cannot by itself insert into the membrane. Addition of T5 to the *trans* chamber, 15 min after the addition of FhuA to the *cis* chamber, did not modify the permeability of the membrane, suggesting that FhuA is incorporated into the lipid bilayer in only one orientation. Phage DNA ejection is temperature dependent (Tosi *et al.*, 1984; Boulanger and Letellier, 1992; Killmann *et al.*, 1995). However, channel activity of the FhuA–T5 complex was not significantly different when experiments were performed at 28°C instead of 20°C (data not shown).

In most cases, the conductance steps were found to be very uniform in size (Figure 3A). However, small current fluctuations were sometimes observed on top of channel openings. Relatively often one could observe a full opening of the channel, rapidly followed by the closure of a substate of ~100 pS (in 100 mM KCl) as shown in Figure 3B. Fluctuations of up to three similar substates are also documented in Figure 3B, but this sequence of events was relatively rare and the corresponding recording has been chosen to illustrate this case.

The conductance of the channel was measured for KCl concentrations ranging from 100 mM to 1.5 M. Although saturation was clearly observed, the conductance reached values as high as 4 nS at 1.5 M KCl (Figure 4). These values were obtained in solutions buffered with 10 mM Tris–HCl, pH 7.3. Killmann *et al.* (1993) reported a conductance of 0.6 nS for FhuA Δ322–355 in unbuffered solutions (~pH 6) of 100 mM KCl. Experiments performed under similar conditions for the sake of comparison indicated a conductance of 0.7 nS for the FhuA–T5 complex (data not shown). Figure 5 displays the I–V curve obtained for unitary currents under symmetric and asymmetric conditions. Under asymmetric conditions (428 mM and 100 mM KCl in the *cis* and *trans* side respectively), the reversal potential was −16 ± 0.4 mV (three determinations), indicating a weak cationic selectivity. From the Goldman–Hodgkin–Katz (GHK) equation, P_K/P_{Cl} was calculated to be 2.7. Both the high single channel conductance and the weak ion selectivity of the FhuA–T5 complex suggest that a large, water-filled channel is formed.

Effect of phage Φ80 and of Fe^{3+}-ferrichrome on channel activity

Phage Φ80, which also binds to FhuA, was added to the *cis* compartment after FhuA. Phage Φ80 did not trigger any channel activity whatever the number of phage added, ranging from 2.6×10^{10} to 10^{12} phage per ml. Moreover, Φ80 did not inhibit channel induction triggered by a subsequent addition of phage T5 (data not shown). The effect of Fe^{3+}-ferrichrome on the channel induction and conductance was also investigated. Fe^{3+}-ferrichrome added to the bilayer chamber after FhuA had no effect up to a concentration of 1 mM. However, in contrast to Φ80, Fe^{3+}-ferrichrome added after FhuA, but before T5 and at a concentration of 10 μM, completely inhibited the appearance of channels.

Killmann *et al.* (1993) reported that the conductance of the channel formed by FhuA Δ322–355 could be decreased by addition of high (compared with the K_T of transport of the native protein) concentrations of Fe^{3+}-ferrichrome. Similarily, we observed that, after induction of the FhuA–T5 channels, the conductance of the membrane could be decreased by addition of Fe^{3+}-ferrichrome. A reduction of 70–75% of the membrane current was observed upon addition of 1 mM Fe^{3+}-ferrichrome (final concentration) (data not shown).

Binding of phage T5 to FhuA in E.coli cells confers sensitivity of the bacteria to SDS

The *E.coli* outer membrane shows strong resistance towards several detergents and in particular SDS (Nikaido and Vaara, 1987). SDS, however, gains access to the cytoplamic membrane and solubilizes it if the outer membrane permeability barrier is broken. This is indeed what was observed upon expression of the FhuA Δ322–355 derivative in *E.coli* (Killmann *et al.*, 1993). If the channel opening triggered by T5 upon binding to FhuA which is observed *in vitro* also takes place *in vivo*, one would expect that bacteria would become sensitive to

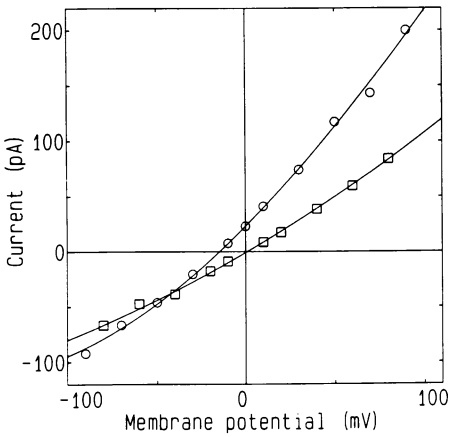

Fig. 5. Relationship between unitary current of the FhuA–T5 channel and membrane potential, under symmetrical and asymmetrical conditions. Squares: *cis* and *trans* solutions were 10 mM Tris–HCl, 100 mM KCl pH 7.3. Circles: *cis* KCl concentration was 428 mM; *trans* KCl concentration was 100 mM. Other conditions were as in Figure 2. The reversal potential was found to be −16.1 mV.

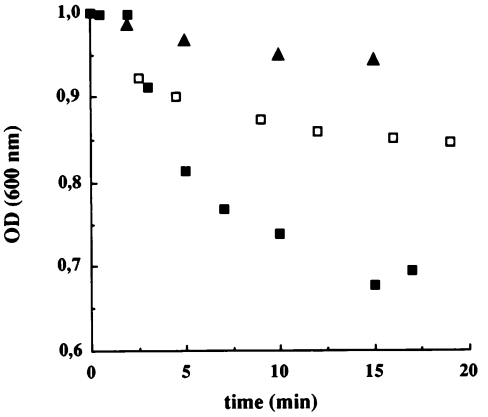

Fig. 6. Change of OD_{600} of *E.coli* cells upon binding of phage T5. *E.coli* cells (5×10^8 per ml) were incubated at 20°C in 10 mM HEPES, 150 mM NaCl, 0.2% glucose, 0.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, pH 7.2. T5 phage (m.o.i. = 50) was added at time zero and SDS (0.07%, final concentration) was added 2 min later. (■) cells + T5 + SDS; (▲) cells + T5; (□) cells + SDS.

SDS upon addition of the phage. Changes in the optical density of a cell suspension in the presence of phage T5 and of SDS were measured at 20°C and for a phage multiplicity (number of phage per bacterium) of 50. This is the highest multiplicity compatible with phage adsorption. It corresponds to 50 FhuA receptors occupied by the phage compared with 400–1000 present in the membrane. Under these conditions, binding of the phage to its receptor takes place within 2 min (Boulanger and Letellier, 1992). Figure 6 shows that addition of 0.07% SDS after phage T5 resulted 10 min later in a decrease in OD_{600} of ~30%. Addition of phage alone to the cell suspension or SDS to bacteria in the absence of phage did not result in such a decrease. These results suggest that SDS has gained access to the cytoplasmic membrane upon binding of the phage to its receptor.

Discussion

The results presented in this work show that addition of phage T5 to the purified wild-type FhuA protein inserted into planar lipid bilayers triggers the opening of high conductance ion channels. Our results rule out the possibility that this channel activity could be the result of porin contamination, since no channel activity was observed when only T5 or FhuA were added to the bilayer chamber. Furthermore, the ion channels described here show no similarities to known porins in conductance or kinetic behavior. We consider that two main hypotheses may explain our data. One possibility is that the observed channel is part of the phage T5. Since T5 by itself did not induce channel activity, this activity would be triggered upon anchoring of the phage in the lipid bilayer as a result of its interaction with FhuA. The second possibility is that binding of phage T5 to FhuA unmasks an inner channel in the FhuA protein. This second hypothesis is consistent with the recent finding that deletion in the FhuA protein of loop Δ322–355, which constitutes precisely the binding site for T5, converts this carrier into a high conductance channel (Killmann *et al.*, 1993). Both the *in vitro* and *in vivo* observations presented here argue in favor of the second hypothesis. The electrophysiological characteristics of the channel formed by FhuA Δ322–355 and by the FhuA–T5 complex were similar. The conductance of the two channels was close but not identical. However, it is noteworthy that FhuA derivatives carrying different deletions in the loop yield ion channels of close but different conductances, varying between 1.5 and 3 nS in 100 mM KCl (Braun *et al.*, 1994). Both channels had preferences for cations and exhibited the same kinetic behavior: once open, they remained in this state without gating to the closed state. The conductance of the FhuA Δ322–355 channel showed small fluctuations very similar to the substates documented here for the FhuA–T5 complex. Finally, the current carried by the two channels could be reduced in the presence of high concentrations of Fe^{3+}-ferrichrome, suggesting that a binding site of low affinity for this species is still present in both channels. Moreover, the fact that the conductance of the FhuA–T5 channel could be reduced whether Fe^{3+}-ferrichrome was added in the *cis* or *trans* compartment of the bilayer set suggests that Fe^{3+}-ferrichrome can diffuse through the channel. The similarity of the FhuA–T5 and FhuA Δ322–355 channels is also supported by *in vivo* experiments. Indeed, the *E.coli* outer membrane which normally shows strong resistance towards SDS, became permeable to the detergent both upon addition of phage T5 (this work) and upon expression of the FhuA Δ322–355 derivative (Killmann *et al.*, 1993). While none of the arguments listed above is in itself a definitive proof, taken as a whole they give very strong support to the proposal that FhuA–T5 and FhuA Δ322–355 channels are similar and they are consistent with the following model. Binding of T5 to loop 322–355 would trigger a conformational change resulting in the tilting of the loop which constitutes the gate of an inner channel in FhuA. The total absence of gating to a closed state observed here, which is unusual for an ion channel, can be understood if the presence of T5 locks the loop in a conformation corresponding to the open state. It is thus likely that the change of conformation of the loop caused by T5 is equivalent to the removal of the loop. Importantly, this result indicates that the channel observed upon removal of loop 322–355 is not a mere

artefact corresponding to the formation of a hole in the protein, but that the channel is present in the wild-type protein and can be observed under physiological conditions.

Killmann *et al.* (1993) proposed that the FhuA channel opens through interaction with the TonB–ExbB–ExbD complex. Our experiments indicate that there is no requirement for TonB or for the TonB–ExbB–ExbD complex in order to open the FhuA channel, at least in the case of binding of phage T5 to FhuA. However, the TonB complex might be necessary to open this channel in the case of binding of phage Φ80 and Fe^{3+}-ferrichrome, since neither of these ligands induced channel opening. These data are consistent with the fact that irreversible binding of phage Φ80 as well as transport of Fe^{3+}-ferrichrome require the TonB complex and an energized cytoplasmic membrane (Hantke and Braun, 1978; Eick-Helmerich and Braun, 1989; Schöffler and Braun, 1989), whereas infection by phage T5 neither requires an energized membrane nor TonB. Fe^{3+}-ferrichrome but not phage Φ80 prevented channel induction by phage T5. This is also consistent with the fact that, *in vivo*, Fe^{3+}-ferrichrome binds to FhuA (even in de-energized cells) and prevents phage T5 binding independently of TonB, whereas Φ80 does not bind to FhuA in the absence of TonB. Taken together, our results suggest the following model for the functioning of the FhuA channel: T5 and Fe^{3+}-ferrichrome would bind to the closed state of the channel but only T5 would be able to trigger its opening. The fact that addition of Fe^{3+}-ferrichrome decreases the conductance of the FhuA channel suggests that the siderophore is transported through the channel.

Is opening of the FhuA channel by T5 a mere coincidence or is it relevant to the physiological process of phage infection? The route used by phage DNA to cross the outer membrane is unknown. Binding of the phage to FhuA is triggered by the minor tail protein pb5, which is located at the distal end of the conical part of the tail, upstream of the central straight tail fiber (Heller and Schwartz, 1985). Killmann *et al.* (1995) showed that a synthetic hexapeptide derived from the gating loop of FhuA triggers DNA release from phage T5 head *in vitro*. They proposed that binding of the peptide to pb5 triggers a conformational change in T5 that results in phage DNA ejection. Such a conformational change might also occur *in vivo*. How then would the linear double-stranded DNA (121 kb, ~2 nm in diameter) be transferred through the outer membrane? The simplest model supposes that the naked DNA diffuses through the FhuA channel. Data on the channel size are not available presently. However, considering the data of Liu *et al.* (1993) on a FepA derivative, it is likely that such a large sized channel exists in the outer membrane. From permeability experiments in liposomes, containing mutated FepA in which the external loop had been removed, they estimated the diameter of the channel to be 2 nm. Crossing the outer membrane through the FhuA channel only solves part of the problem of the transport of the phage DNA, since this molecule has still to cross the periplasmic space (which contains nucleases) and the cytoplasmic membrane. Fractionation experiments previously have suggested that pb2, the unique protein forming the straight fiber (McCorquodale and Warner, 1988), is involved in the transfer of phage T5 DNA through the bacterial envelope (Guihard *et al.*, 1992). Given the length of the straight fiber in electron micrographs (50 nm) (Saigo, 1978), it was proposed that this protein spans the whole envelope and would form the DNA channel (Guihard *et al.*, 1992). A role for pb2 in channeling DNA was also proposed independently by Feucht *et al.* (1990). An alternative to the model of transport of naked DNA through FhuA could be that the interaction of the phage protein pb5 with FhuA induces a conformational change in the phage, allowing the insertion of pb2 either directly through the envelope or inside the FhuA channel. Whatever the mechanism of DNA transfer, if the FhuA channel is involved directly in the process, one would expect to observe an alteration in the conductance of the channel or even a transient block, corresponding to the transfer of DNA or to the insertion of pb2. No indication for such phenomena can be found in our recordings. It is possible, however, that the observed channel activity is that of a small proportion of channels for which DNA has been mistargeted and released to the *cis* compartment while the majority of channels transferring DNA are electrically silent. Alternatively, the events that we recorded might correspond to an unblocking of the FhuA channels after DNA transfer and release to the *trans* compartment. Clearly the mechanism of phage DNA transfer across the outer membrane remains an open question.

A next step in this study will be to determine which of the proteins of T5 triggers the opening of the FhuA channel. The protein pb5, which has been shown to bind to FhuA (Mondigler *et al.*, 1995), is a likely candidate. The finding of an 'opener' of the FhuA channel would be of interest for future structural studies. Modeling suggests for FhuA a β-barrel structure similar to that of bacterial porins (Koebnik and Braun, 1993). The three-dimensional structure of several porins at atomic resolution has been obtained recently by X-ray diffraction (Weiss *et al.*, 1991; Cowan *et al.*, 1992; Kreusch *et al.*, 1994; Schirmer *et al.*, 1995). It is therefore not unrealistic to expect that the structure of the FhuA protein could also be determined, a result that would be of extreme importance for the understanding of carrier mechanisms. The possibility of obtaining the structure of the FhuA protein (complexed with a T5 protein), in the open channel conformation, would be an additional asset for future studies of the transport mechanism.

Finally, it has to be noted that several phages bind to external loops of porins (Heller, 1992). It is unclear as yet whether the fact that these receptors normally form open ion channels in the outer membrane is relevant to phage DNA transfer, but the similarity with the case described here is worth noting. In all cases, a study of the possible effects of phage binding on porin channel activity could be of interest for the study of the mechanism of porin gating.

Materials and methods

Preparation and purification of phages

T5st*am*N5 (T5) phages were produced on *E.coli* Fsuβ$^+$, a permissive host (Hendrickson and McCorquodale, 1971; Zweig and Cummings, 1973). Phage Φ80 was propagated in *E.coli* K12. For phage production, host cells were grown and infected in LB medium (per liter: 10 g

tryptone, 5 g yeast extract, 10 g NaCl, pH 7.0). In the case of phage T5, the medium was supplemented with 1 mM $CaCl_2$. After treatment of the lysate with DNase (DNase I, grade II from Boehringer) (10 μg/ml, 3 h at 37°C), phage stocks were prepared and purified by polyethylene glycol (PEG) precipitation followed by a CsCl step gradient (Yamamoto and Alberts, 1970). Phages and cellular fragments were precipitated by 10% PEG 6000 (w/v) and 0.5 M NaCl, overnight at 4°C. Precipitated material was recovered by centrifugation at 23 000 *g* for 20 min at 4°C. In order to eliminate most of the membrane fragments, the pellet resuspended in 'phage buffer' (10 mM Tris–HCl, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, pH 7.4) was centrifuged at 4000 *g* for 20 min at 20°C. Phages, present in the supernatant, were again precipitated overnight by PEG and NaCl and centrifuged. Phages were purified further on a three-step gradient of CsCl (densities 1.3, 1.5 and 1.6 g/cm^3). The gradients were centrifuged at 261 000 *g* for 3 h at 4°C. Phages form a sharp band above the 1.6 g/cm^3 layer, well separated from the remaining few membrane fragments (density 1.15–1.25 g/cm^3) (Ishidate *et al.*, 1986). The phage fraction was collected from the top after having carefully removed the membrane fragments using an LKB autodensiflow. Phage were dialyzed in 'phage buffer'. They were stored in the same buffer containing 0.05% NaN_3 at 4°C and at 10^{13} phage per ml. Only those polypeptides characteristic of phage T5 structural proteins were detected on an SDS–PAGE of the phage particles (data not shown).

Purification of the FhuA protein

Escherichia coli AB2847 (pHK232), a strain overproducing FhuA, was used for purification (Hoffman *et al.*, 1986). The first steps of purification of FhuA involving solubilization of the envelopes and treatment with OG/EDTA were as described by Hoffman *et al.* (1986). The 'FhuA extract' in 50 mM Tris pH 7.8, 1 mM EDTA, 0.9% OG was purified further by anion exchange chromatography, chromatofocusing and gel filtration chromatography. All purification steps were carried out in the presence 0.9% OG. The 'FhuA extract' was loaded onto a MonoQ column (Pharmacia) previously equilibrated with buffer A (25 mM Tris pH 7.8, 20 mM EDTA), and eluted using a gradient of NaCl (0–0.3 M) in buffer A. Fractions containing the FhuA protein were pooled, diluted in buffer B (25 mM Bis-Tris pH 6.3), concentrated by ultrafiltration and loaded onto a MonoP column (Pharmacia) equilibrated with buffer B. The pH gradient was generated with polybuffer 74 (1/10), pH 4. Fractions containing FhuA were concentrated by ultrafiltration and further purified by gel filtration on a Superose 12 column (Pharmacia) equilibrated with buffer C (25 mM Tris pH 7.2, 150 mM NaCl). The eluted protein was stored at –20°C in buffer C containing 0.9% OG. Its purity was controlled on 7.5% SDS–PAGE.

Planar lipid bilayers experiments

Membranes were formed from a solution of soybean L-α-phosphatidylcholine phospholipids (Sigma) dissolved in *n*-decane at 30 mg/ml. Bilayers were formed across a 250 μm diameter hole by presenting a bubble of lipids in decane in front of the hole. FhuA (0.1–10 ng/ml, final concentration) and phages (10 μl of stock solution at 10^{13}/ml) were added to 3 ml of 10 mM Tris–HCl, pH 7.3, 100 mM KCl in the *cis* compartment. Experiments were carried at 20 or 28°C. All solutions were filtered and sterilized. Fe^{3+}-ferrichrome (Sigma) was added in the *cis* or *trans* compartment at variable concentrations. For determination of reversal potential, the buffer in the *trans* compartment was held at 100 mM KCl, but was 428 mM KCl in the *cis* side. The *trans* compartment was held at virtual ground potential. Recordings were stored on digital audio tape (Biologic DTR 1200 DAT recorder). Records subsequently were filtered at 1 kHz (–3 dB point) through a four-pole Bessel low pass filter and digitized off-line at a rate of 2 kHz on a personal computer. Data were plotted on a HP Laserjet printer.

SDS sensitivity tests

Cells from the *E.coli* F strain (F–, *tonB, mal, lam, lambda*) were grown at 37°C in LB medium to an OD_{600} of 0.5. They were washed and resuspended at 20°C in 10 mM HEPES, 150 mM NaCl, 0.2% glucose, 0.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, pH 7.2 and to an OD_{600} of 1. T5 phage (m.o.i. = 50) was added at time zero and SDS (0.07%, final concentration) was added 2 min later. The OD_{600} of the cell suspension was measured at different times.

Acknowledgements

We thank V.Braun for the generous gift of the *E.coli* strain overproducing FhuA. B.Martinac and M.Blight are acknowledged for critical reading of the manuscript. A.G. thanks Daniel Wolff for teaching him the planar bilayer technique. M.B. was a recipient of an MESR fellowship.

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Received on October 13, 1995; revised on December 19, 1995