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Electrophysiological Characteristics of the PhoE Porin Channel from *Escherichia coli.* Implications for the Possible Existence of a Superfamily of Ion Channels

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Abstract. Purified PhoE porins from *Escherichia coli* were reconstituted in giant proteoliposomes obtained by dehydration-rehydration, and studied by the patch-clamp technique. The following electrophysiological characteristics were observed. (i) The channels for which the probability of opening is maximum around 0 mV, closed at positive and negative potentials, at voltages higher than ±120 mV. (ii) The channels behaved asymmetrically in response to positive and negative potentials. (iii) The channels exhibited two types of kinetics (fast and slow) on very different time scales. (iv) The channels had several closed states including a reversible inactivated state and a large number of substates. Similar characteristics have been described for channels other than bacterial porins, in particular mitochondrial porins and maxi-chloride channels of the plasma membrane of animal cells. These characteristics might constitute the electrophysiological fingerprint of a superfamily of ion channels for which the basic structure, rather than sequence, would have been conserved during evolution.

Key words: PhoE — *Escherichia coli* — Porin — Ion channel — VDAC — Maxi-chloride channels

Introduction

Porin channels are large water-filled pores which represent the main pathway for the passage of ions and nutrients through the bacterial outer membrane, a protective envelope present in Gram-negative bacteria. Porins have been extensively studied at the genetic, biochemical and biophysical level (for a review, *see* Nikaido, 1994). In particular, these recent years have seen the elucidation of the structure at the atomic level of different bacterial porins, making porins the first channels to be known at this resolution. There is a high degree of similarity between all known porin structures. These porins are trimeric with each monomer constituting a channel. A monomer channel is a β-barrel made of 16 β-strands for the porin from *R. capsulatus* (Weiss et al., 1991), OmpF and PhoE from *E. coli* (Cowan et al., 1992) and the porin from *R. blastica* (Kreusch et al., 1994), and of 18 β-strands for the maltoporin from *E. coli* (Schirmer et al., 1995). In all cases, one of the external loops folds back into the pore, thus forming a constriction zone that dictates the selectivity and size exclusion of the channel. Strikingly, this structural homology is achieved despite a lack of sequence homology.

The electrophysiological properties of porins have been studied by incorporation of the channels into artificial lipid bilayers. The voltage dependence of these channels has been debated, but it is now clear that, for instance, in the case of OmpF and PhoE from *E. coli*, they are open at low voltages but can be reversibly closed upon imposition of a relatively high membrane potential (Lakey & Pattus, 1989). The physiological significance of this property is, however, unclear (Sen, Hellman & Nikaido, 1988).

Electrophysiological studies of porin have focused on conductance measurements as a means of predicting the size of the pore, and on the problem of the voltage dependence. The dynamic behavior of porin channels has been the object of relatively little concern. Most of the studies have described porin channels as large conductance pores governed by a slow gating kinetics. This may be ascribed to the fact that porins have not generally been considered as ''ion channels'' in the usual sense of the term, but mostly as static open structures whose complex kinetic behavior has little physiological relevance. In studying the OmpF and OmpC porins of *E. coli*, we previously showed that these channels are governed by both fast and slow kinetics and we distinguished at least

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three different closed states of the channels (Berrier et al., 1992). We present here a more exhaustive study of the kinetics of the PhoE porin channels reconstituted in giant liposomes and studied by the patch-clamp technique. Our results allow us to distinguish four different electrophysiological characteristics of this channel. These same characteristics have been described for channels other than porins of Gram-negative bacteria, in particular mitochondrial porins and several ''maxi-chloride'' channels of the plasma membrane of animal cells. These characteristics might thus reflect a similar structural organization.

Materials and Methods

PhoE Protein

The PhoE protein was a generous gift of Dr. F. Pattus. The protein was extracted and purified from *E. coli* CE 1197 as described in Dargent et al. (1986).

Reconstitution of the PhoE Porin Channel in Giant Proteoliposomes

Ten to 100 ng of purified porins were added to 2.8 ml of an azolectin liposome suspension (1 mg lipids in 10 mM Hepes-KOH, pH 7, 100 mM KCl). The suspension was incubated for 1 hr at room temperature, centrifuged at 90,000 rpm for 30 min in a TL 100 Beckman centrifuge and the pellet was resuspended in 15 μl of 10 mM Hepes-KOH buffer pH 7. The liposomes were then fused into giant liposomes, using a cycle of dehydration-rehydration, as previously described (Berrier et al., 1989). A 2–5 μl drop of the giant proteoliposome suspension was deposited on a nunclon plastic tissue dish and diluted with 1.5 ml of the bath solution (as defined in the figure legends) for electrophysiological recording.

Single Channel Recording

Single-channel activity was measured using the method of Hamill et al. (1981). Patch electrodes were pulled from pyrex capillaries (Corning code 7740) and were not fire-polished before use. After seal formation on a giant liposome, the patch was excised and electrical recording was performed in this mode. The internal face of the membrane patch could be superfused by a flow of solution from one of a series of five piped outlets. Unitary currents were recorded using a Biologic RK-300 patch-clamp amplifier with a 10 GΩ feedback resistance, and stored on digital audio tape (Biologic DTR 1200 DAT recorder). Records were subsequently filtered at 1 kHz (−3 dB point) through a 4-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. The membrane potential refers to the potential in the bath minus the potential in the pipette.

Results

Purified PhoE porins were reconstituted in azolectin liposomes which were fused into giant proteoliposomes by dehydration followed by rehydration. After seal formation on a liposome, the patch was excised and the electrical activity recorded.

At low membrane potentials the channels were mostly open, except for fast transitions to more closed levels (*not shown*). Long time closures of the channels were rarely observed between −100 and +100 mV. Figure 1 illustrates the effect of voltage pulses of increasing magnitude, at negative membrane potentials, on the closure of the channels present in a given patch. Between pulses the membrane potential was held at 0 mV. The channels which were fully open at 0 mV, closed upon application of a negative potential above −100 mV. This closure followed the pattern of slow kinetics (in the second range) classically documented for porin channels. The slow kinetics of closure clearly implicated at least two different types of closed states: a closed state from which the channel can be reopened and an inactivated state. Once the channel has reached this state, reopening was rare or absent. The higher the magnitude of the membrane potential, the faster this last state was reached.

Superimposed on the slow kinetics of closure were also spikes, corresponding to fast closures, which were not fully resolved on this time scale (Fig. 1). The fast kinetics could appear or disappear during the recordings. In addition, the frequency of gating of the fast kinetics could also show striking variations. Two different modes of the fast kinetics, in the msec range, are illustrated in Fig. 2. In one case (Fig. 2, upper traces), the resolved events of the fast transitions were about one third of the slow transitions. In the other case (Fig. 2, lower traces), the frequency of gating was lower and the fast and the slow transitions had the same conductance.

After closure at a high negative membrane potential, all PhoE porins could generally be reopened almost instantaneously upon exposure to a low voltage as illustrated in Fig. 3. A patch containing a large number of porin channels was subjected to repeated steps of potential from −20 to −120 mV. The first step to −120 mV resulted in some 30 closing steps. All closed porins reopened at −20 mV as evidence from the fact that, upon a second step to −120 mV, the initial level of current reached a similar value to that of the first step. Reopening of all the porin channels took less than 150 msec, and only the very last opening events could be distinguished. However, in two cases (out of 16 similar experiments), after several successful cycles of closures and reopenings, all the porins apparently got locked in a different inactivated state which could not be reversed at low potentials.

Voltage dependence was markedly asymmetric as seen by the effect of voltage pulses of opposite polarity on a patch containing a few channels (Fig. 4). A negative membrane potential closed the channels completely. Yet, upon application of a positive membrane potential

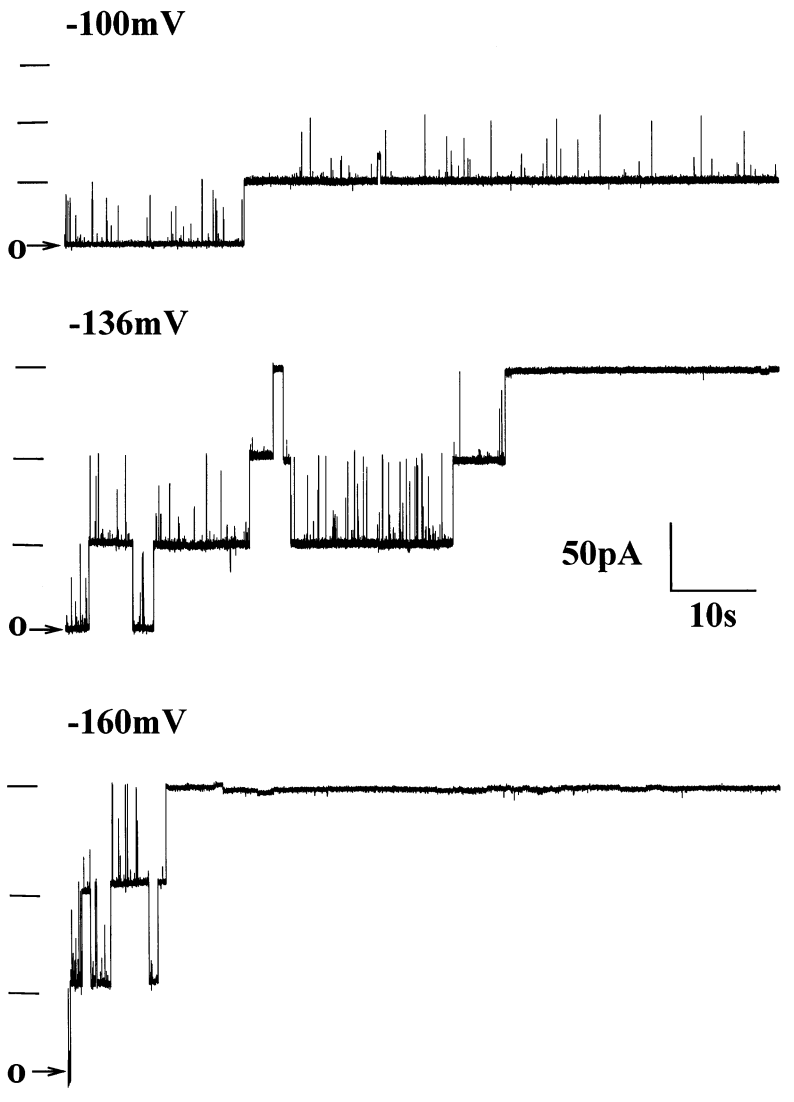

Fig. 1. Closure of PhoE porin channels at negative membrane potentials. Porins were reconstituted at a lipid to protein ratio of 100,000 (w/w). After excision, the patch was subjected to 2 min pulses from 0 mV to the various membrane potentials indicated on the figure. The beginning of each trace corresponds to the onset of each pulse. Between pulses, the patch was held at 0 mV for 30 sec. O: full open level of all channels in the patch. Bath and pipette media (in mM): 10 Hepes-KOH, pH 7, 600 KCl with in addition 2 $CaCl_2$, 5 $MgCl_2$ in the pipette.

of the same magnitude, the channels reached an apparent steady state characterized by gating of the channels on up to three similar levels without total closure. This polarity effect was documented for a patch containing a much larger number of channels, due to reconstitution at a lower lipid to protein ratio (Fig. 5). A high positive membrane potential induced closure of some of the channels, but at a slower rate than the opposite voltage. The inactivated state observed at negative potential was never observed at a positive potential (up to 160 mV). This phenomenon was quantified by plotting $I/I\text{max}$ *vs.* the applied potential (Fig. 5, inset), where I is the total current 120 sec after application of the pulse and $I\text{max}$ is the total current at the onset of the pulse. I was measured after an apparent steady state had been reached, so that $I/I\text{max}$ should reflect the probability of opening at steady state. The data shown in Fig. 5 (inset) are representative of four similar experiments. Maximum opening was obtained at zero mV or low membrane potential, and a decrease in membrane current was observed for both polarities, but the curve was clearly asymmetric. This pattern, complete closure at a high negative potential but not at the positive potential, was repeatedly observed, indicating that the protein was incorporated asymmetrically during reconstitution.

The relationship between $I/I\text{max}$ and membrane potential could be fitted at negative potentials and at positive potentials, by the equation:

$$I/I\text{max} = 1/[1 + \exp(-z.e\ (V - V_{0.5})/kT],$$

where e, k and T have their usual meaning, $z.e$ is the gating charge and $V_{0.5}$ is the potential for which $I/I\text{max}$ is 0.5. From the data in Fig. 5, at negative potentials, z and $V_{0.5}$ were calculated to be 2.3 and −92 mV, respectively. At positive potentials z and $V_{0.5}$ were −1.2 and

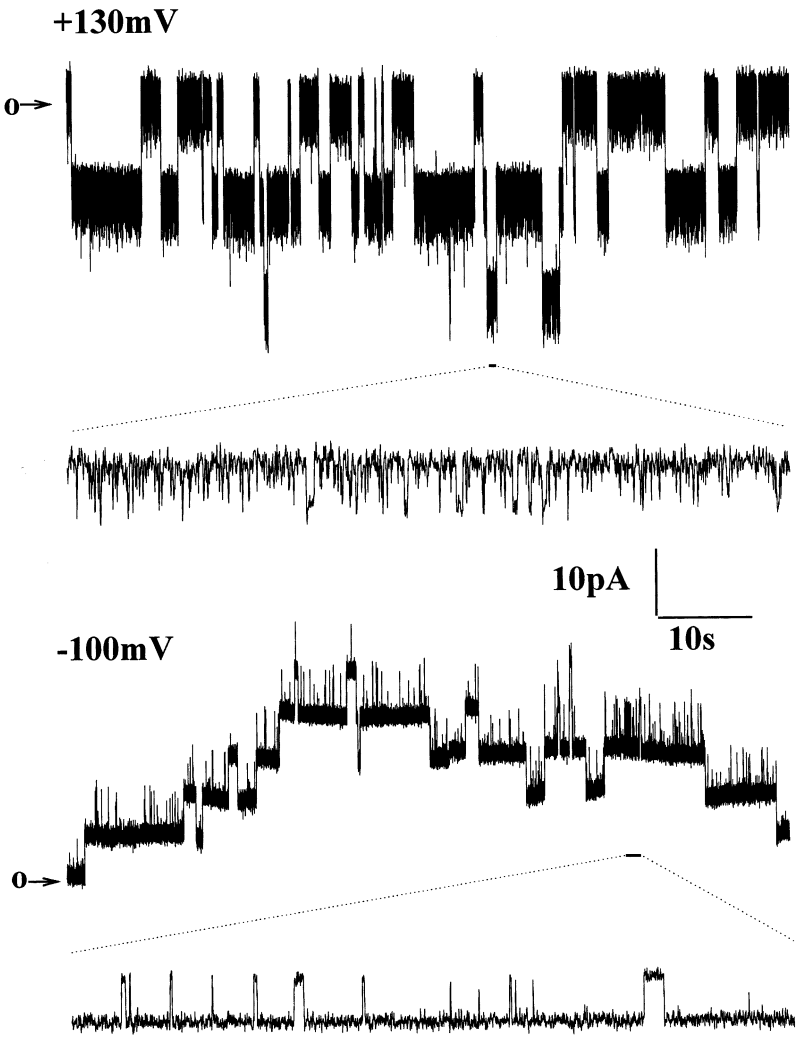

Fig. 2. Fast kinetics of PhoE porin channels. The figure illustrates two different modes of gating of the fast kinetics. Porins were reconstituted at a lipid to protein ratio of 100,000 (w/w). Bath and pipette media (in mM): 10 Hepes-KOH, pH 7, 100 KCl with in addition 2 $CaCl_2$, 5 $MgCl_2$ in the pipette. Upper traces: segments of recording at +130 mV; the second trace represents 0.5-sec recording. Lower traces: segments of recordings at −100 mV; the second trace represents 1.5-sec recordings.

+156 mV, respectively. The equation above describes the voltage dependence of a channel with only two states, open and closed (Hille, 1992). Clearly the PhoE channel has several closed states, thus the above values for z and $V_{0.5}$ should be considered only as phenomenological parameters, useful for comparisons with other channels.

To study the conductance of the channel, recordings were performed at different membrane potentials under symmetrical conditions (100 mM KCl) and at a high lipid-to-protein ratio to decrease the number of channels per patch. The detailed analysis of 11 patches for establishment of *I-V* curves revealed a complex pattern. At each membrane potential, several different conductance steps of the slow kinetics were observed, ranging from 50 to 200 pS. However, predominant conductances were observed, which could be different from one patch to another, ranging from 60 to 115 pS at positive potentials. Figure 6 illustrates one of these cases. In the segment of recording of Fig. 6*A*, 3 different steps are observed, but one step (115 pS at 120 mV) represented more than 90% of all observed transitions. Report of the predominant transition at each membrane potential gave the *I-V* relationship (Fig. 6*B*) which was markedly nonlinear. Under asymmetrical transitions (300 mM KCl in the bath *vs.* 100 mM KCl in the pipette), the *I-V* curve was shifted to positive potentials. The reversal potential of 18 mV indicated a 4.9-fold preference for chloride over potassium, consistent with the previously reported weak selectivity of the PhoE porin for anions (Benz, Darveau & Hancock, 1984; Dargent et al., 1986). Figure 7 illustrates the case of another patch in which two predominant conductances were observed: 85 and 115 pS (at 140 mV, Fig. 7*A*). The 85 pS transition represented around 80% (*vs.* 20% for the 115 pS transitions) of the transitions. The number of other transitions was negligible in this patch. All these results indicate the existence of several different substates which sometimes differed slightly in conductance but which could be nevertheless clearly distinguished when observed on the same patch (Fig. 6*A* and 7*A*).

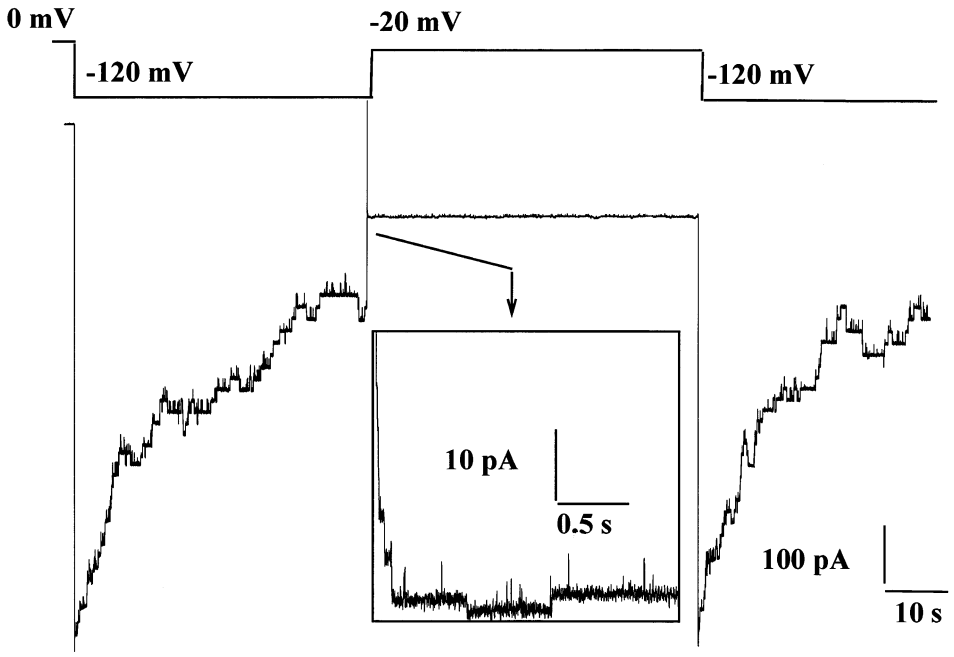

Fig. 3. Reversibility of PhoE channel closure at high negative potential. Porins were reconstituted at a lipid to protein ratio of 10,000 (w/w). The patch was subjected to voltage steps as indicated on the figure. After closure at −120 mV, the channels were reopened at −20 mV. Upon a second step to −120 mV, a full open level similar to that of the first step was reached, indicating that all channels had reopened at −20 mV. Inset: reopening of the PhoE channels on an enlarged scale. Bath medium (in mM): 10 Hepes-KOH, pH 7, 300 KCl. Pipette medium (in mM): 10 Hepes-KOH, pH 7, 100 KCl, 2 $CaCl_2$, 5 $MgCl_2$.

In recordings performed at a higher ionic strength (600 mM KCl symmetrical media) three predominant conductances were observed which were the same for all patches ($n = 3$). In these cases, the corresponding *I-V* curves were linear, and the corresponding slopes were 430, 160 and 40 pS, respectively (*not shown*).

Discussion

Here we have aimed at defining several characteristics of the complex electrophysiological behavior of the PhoE porin channel.

A first feature was the coexistence of very slow and fast gating kinetics. The fast kinetics was not always present and could appear or disappear during the recordings. While the characteristically slow gating kinetics were reported for the first studies of porin channels, the fast kinetics have only been more recently described by Delcour et al. for OmpC porins (Delcour et al., 1989; Delcour, Adler & Kung, 1991). Slow kinetics were not observed in these studies performed at low membrane potentials. We subsequently reported the existence of both slow and fast kinetics for OmpC and OmpF channels at higher membrane potentials (Berrier et al., 1992). The discrepancy between these studies and others is not likely due to the technique used (patch-clamp of liposomes for our studies and those of Delcour et al. *vs.* planar lipid bilayers). Probably the filtering and, in particular, the use of chart recorders that act as a low pass filter were mainly responsible for the lack of documentation of fast kinetics in earlier studies. Clearly, however, the fast kinetics described for OmpC and OmpF porins and here for PhoE porins are part of the dynamics of these channels and should not be overlooked

The voltage dependence of bacterial porins has been debated. However, there currently seems to be a consensus as to the reality of this phenomenon, although its physiological significance is unclear. Voltage-dependent closures have been shown to be independent of the mode of preparation of the porin proteins OmpF and PhoE and of the different planar lipid bilayer techniques (Lakey & Pattus, 1989). Voltage dependence was also observed when OmpC, OmpF porins (Berrier et al., 1992) and PhoE porins (this study) were reconstituted in liposomes and studied by patch clamp. Here the voltage dependence was clearly polarity dependent since complete closure was only observed at negative membrane potentials. This is in contrast with the earlier studies which reported a voltage dependence for OmpC, OmpF and PhoE porins. In those studies, it was considered that the porins responded symmetrically to the membrane potential (Schindler & Rosenbush, 1981; Dargent et al., 1986). However, polarity dependence has been reported

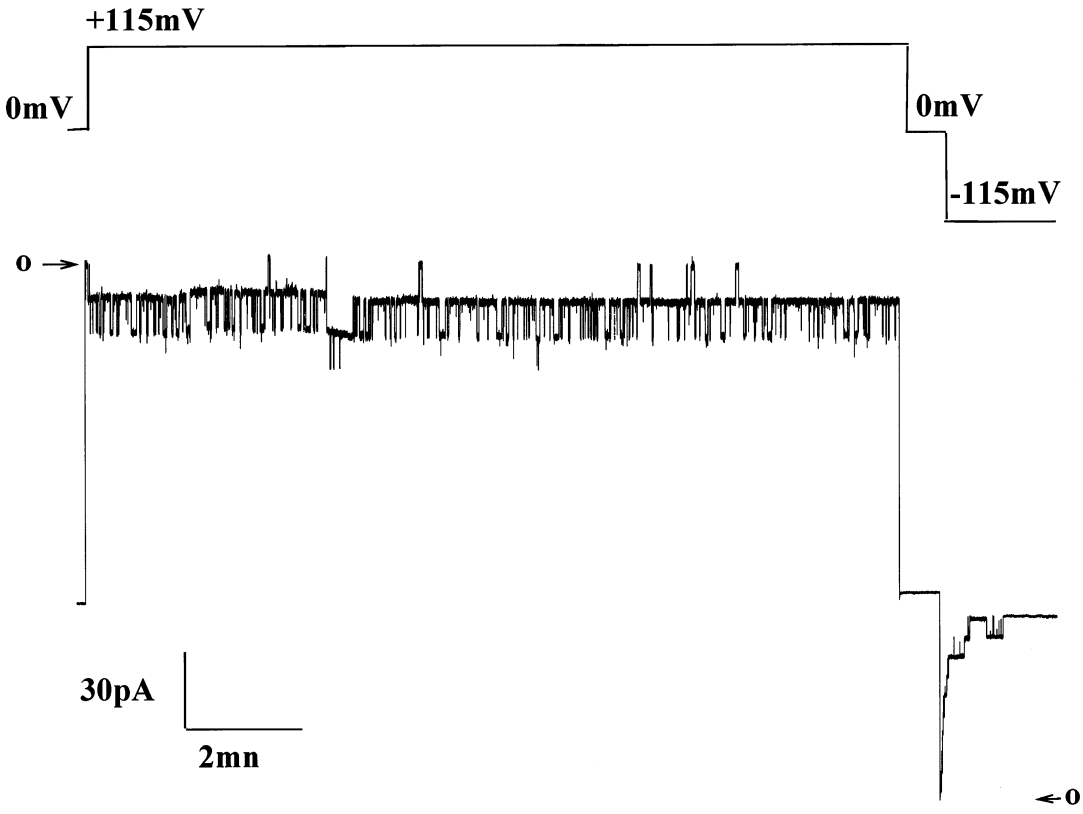

Fig. 4. Voltage dependence of PhoE channels was asymmetric. Porins were reconstituted at a lipid to protein ratio of 100,000 (w/w). The patch was subjected to opposite voltage steps as indicated. O: full open level of all channels in the patch. Other conditions as in Fig. 2. Note the asymmetry in single channel conductance at the positive and negative potentials.

more recently for OmpF and OmpC (Morgan, Lonsdale & Alder, 1990; Berrier et al., 1992) for the porin of *H. influenzae* (Dahan et al., 1994) and for *Acidovorax delafiedii* porin (Brunen & Engelhardt, 1993). This behavior is consistent with the asymmetry in the structure of the molecule (Cowan et al., 1992).

We observed that PhoE porins, as OmpF and OmpC porins (Berrier et al., 1992), possess more than one closed state. On the basis of its kinetics, and independently of the existence of substates discussed below, we distinguished at least a closed state of the fast kinetics, a closed state of the slow kinetics, and an inactivated state which is reached only at high membrane potential, and which is fully and rapidly reversible when the potential is lowered.

The existence of multiple conductance steps for porins has been noted since the onset of electrophysiological studies of these channels (Benz et al., 1978). In this and subsequent studies, the conductance histograms were based on stepwise increases in membrane currents reflecting insertion of the channels in the planar lipid bilayers. The wide range of values thus obtained might have reflected the existence of different states of aggregation of the porins before insertion as well as different substates. Other studies have reported the existence of only two types of conductances: one observed upon insertion of the porin trimer in the open state and one observed upon voltage-dependent closure of the channels and corresponding to one-third of the trimer conductance (Schindler & Rosenbusch, 1981; Dargent et al., 1986). The latter conductance would thus correspond to that of a monomer channel. More recently, Buehler et al. (1991) reported that the number of substates increased when the ionic strength was decreased. Our present study indicated that the PhoE porin channel could gate between different substates sometimes with close conductances. From patch to patch, and for the same preparation, the most frequent conductances may be different. This suggests that the channels can sometimes be locked preferentially in a given conductance state. Under these conditions, it is difficult to determine what the full conductance of the porin monomer channel is. It is not necessarily the more frequent conductance nor the higher recorded conductance, since cooperative transitions of two or more porin monomers cannot be excluded. Previously, the conductance of porin channels has been used to estimate the diameter of the pore. As discussed by Nikaido (1992), this procedure is not valid. Uncertainty

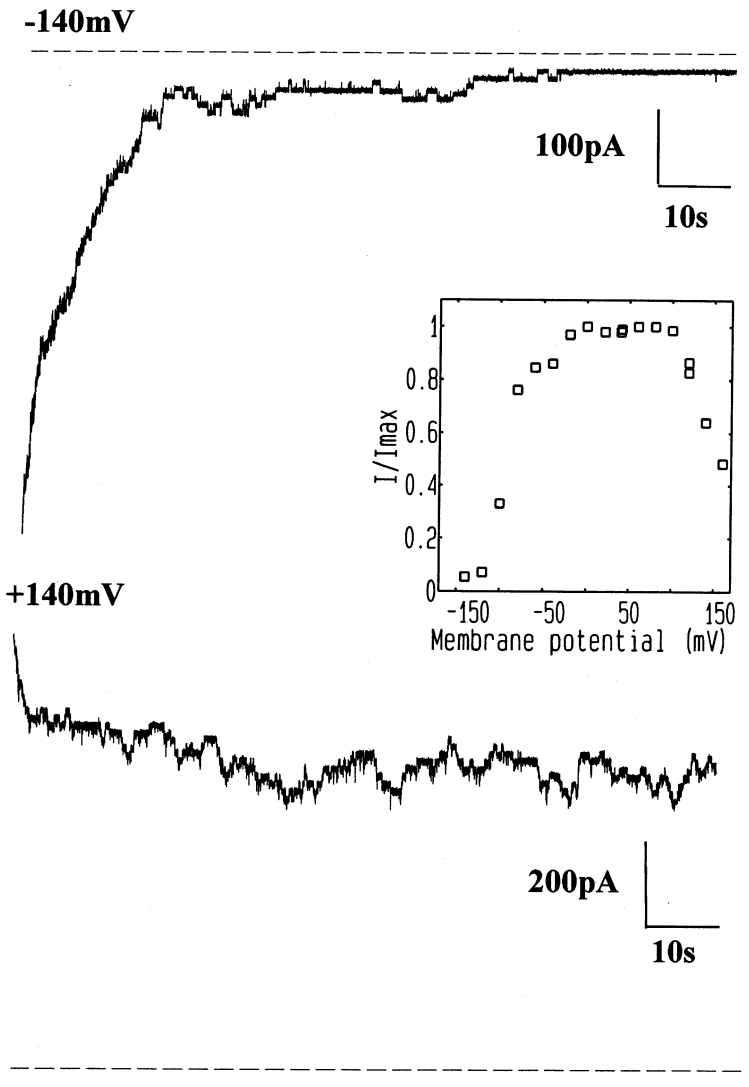

Fig. 5. Polarity dependence of PhoE channels. Porins were reconstituted at a lipid to protein ratio of 10,000 (w/w). Other conditions as in Fig. 2. The patch was subjected to voltage pulses to various positive and negative membrane potentials for 120 sec. Between pulses the patch was held at −40 mV for 40 sec. The traces shown on the figure correspond to pulses to −140 and +140 mV. The beginning of each trace corresponds to the onset of each pulse. Broken lines: zero current levels. The inset shows a plot of *I*/*I*max as a function of the applied membrane potential, where *I* is the steady-state current 120 sec after application of the pulse and *I*max is the peak current.

as to the real conductance of the full open channel is one more reason to discontinue this approach.

The electrophysiological characteristics described here are summarized as follows. (i) PhoE porins are large conductance channels which have a maximal probability of opening around 0 mV and which close at positive and negative potentials. (ii) The voltage gating is polarity dependent. (iii) The channels exhibit two types of kinetics (fast and slow) on very different time scales. (iv) The channels possess several closed states including a reversible inactivated state and a large number of substates.

As noted earlier, like bacterial porins, mitochondrial porins and the ''maxi-chloride'' channels of animal cells, are large conductance channels which are open at 0 mV, and which close at positive and negative voltages. Strikingly, the similarity between these channels extends to the more detailed electrophysiological characteristics described here for the bacterial PhoE porin.

The VDAC (voltage dependent anion channel) is a large conductance channel located in the outer membrane of mitochondria (reviewed by Benz, 1994; Colombini, 1994). Although the voltage dependence of VDAC in bilayers is often considered to be symmetrical about zero, asymmetry of the channel has been clearly documented (Szabo', De Pinto & Zoratti, 1993; Benz, 1994). In most reports, closure is described as following a pattern of slow kinetics (in the second range). However, using high frequency digitalization of the data, in contrast to previous studies, Mirzabekov et al. (1993) have reported the existence of a fast kinetics superimposed on the slow kinetics. The existence of several different substates has been documented by most studies on this channel (Benz, 1994). Maxi-chloride channels are channels found in the plasma membrane of animal cells. They have been documented in different tissues including rat skeletal muscle, neonatal rat cardiac myocytes, rabbit urinary bladder, pulmonary epithelia, and T and B lym-

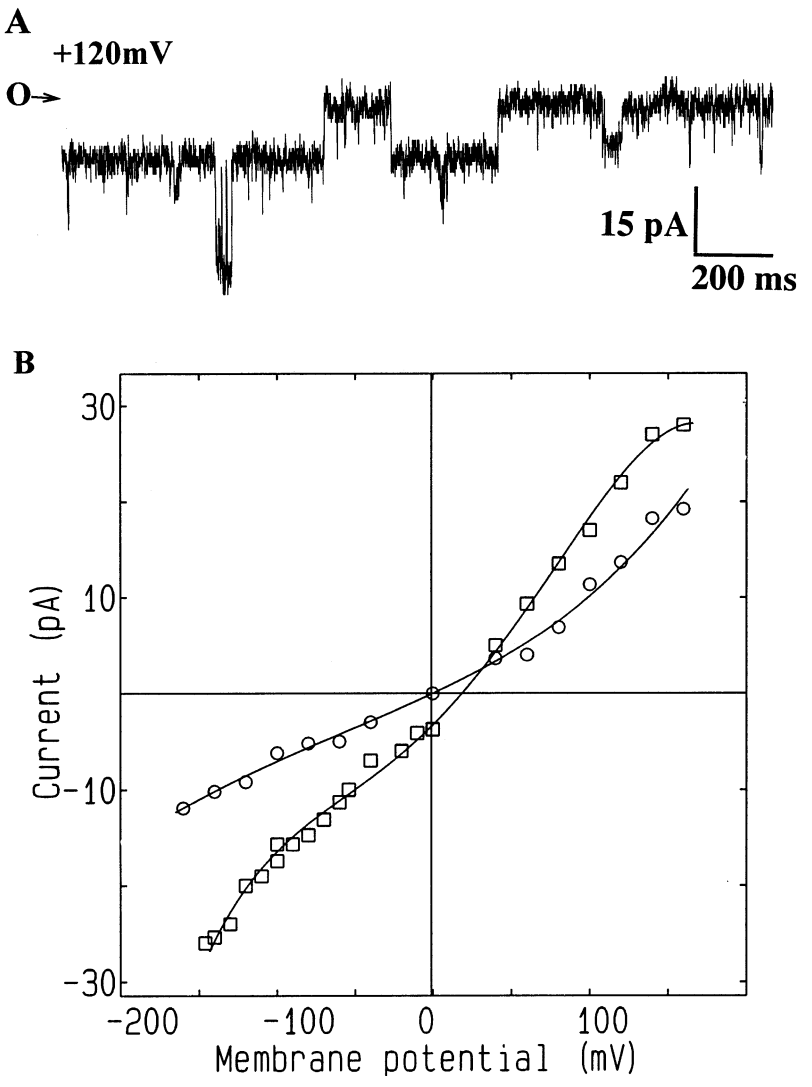

Fig. 6. Conductances of PhoE porin channels. Example of a patch with one predominant conductance. Porins were reconstituted at a lipid to protein ratio of 100,000 (w/w). (*A*) the chosen segment of recording at +120 mV shows different transitions (50, 115 and 200 pS), under symmetrical conditions (100 mM KCl). The 115 pS transition represented more than 90% of all transitions observed in the patch. (*B*) Current-voltage relationship for the predominant transition. (○): symmetrical conditions, bath and pipette media (in mM): 10 Hepes-KOH, pH 7, 100 KCl with in addition 2 $CaCl_2$, 5 $MgCl_2$ in the pipette. (□): asymmetrical conditions; bath medium (in mM): 10 Hepes-KOH, pH 7, 300 KCl; pipette medium (in mM): 10 Hepes-KOH, pH 7, 100 KCl, 2 $CaCl_2$, 5 $MgCl_2$.

phocytes (reviewed by Blatz, 1994; Cahalan & Lewis, 1994). Their physiological role is unclear. Electrophysiological characteristics similar to those listed above for PhoE have been described in the very first reports on maxi-chloride channels (Blatz & Magleby, 1983; Gray, Bevan & Richtie, 1984) and in subsequent reports. Finally, similar characteristics have also been found for channels found in the cell wall of certain Gram-positive bacteria such a Mycobacteria (Trias & Benz, 1993) and recently in an Archaebacterium (Besnard, Martinac & Ghazi, 1996).

The functions of bacterial and mitochondrial porins, in the outer membrane of bacteria and mitochondria respectively, are clearly similar. According to the endosymbiotic theory of evolution, they should have evolved from a common ancestor. Very little identity exists between the known sequences of VDAC and bacterial porins but they are generally believed to share a similar structure. The high resolution structure of VDAC is not known, but a β-structure is postulated on the basis of secondary structure prediction, with the number of β-strands varying from 12 to 16 (Benz, 1994). Yet, despite a lack of sequence homology, the structures are very similar between the *Rhodobacter capsulatus* porin and the *Rhodopseudomonas blastica* porin, and between the OmpF and PhoE porins from *E. coli*, as deduced from X-ray diffraction studies. This is also true for the maltoporin from *E. coli* for which the primary structure is also different from that of the other porins. As indicated by Schiltz et al. (1991), the porin chain fold probably does not require a particular structure so that there exist different porins that cannot be related to each other on the basis of sequence. As to the maxi-chloride channels, their molecular identity is still unknown. Thinnes and coworkers have been strong advocates of the hypothesis that maxi-chloride channels could be in fact VDAC, or one of the isoforms of VDAC, targeted to the plasma membrane (Thinnes, 1992). This hypothesis is based

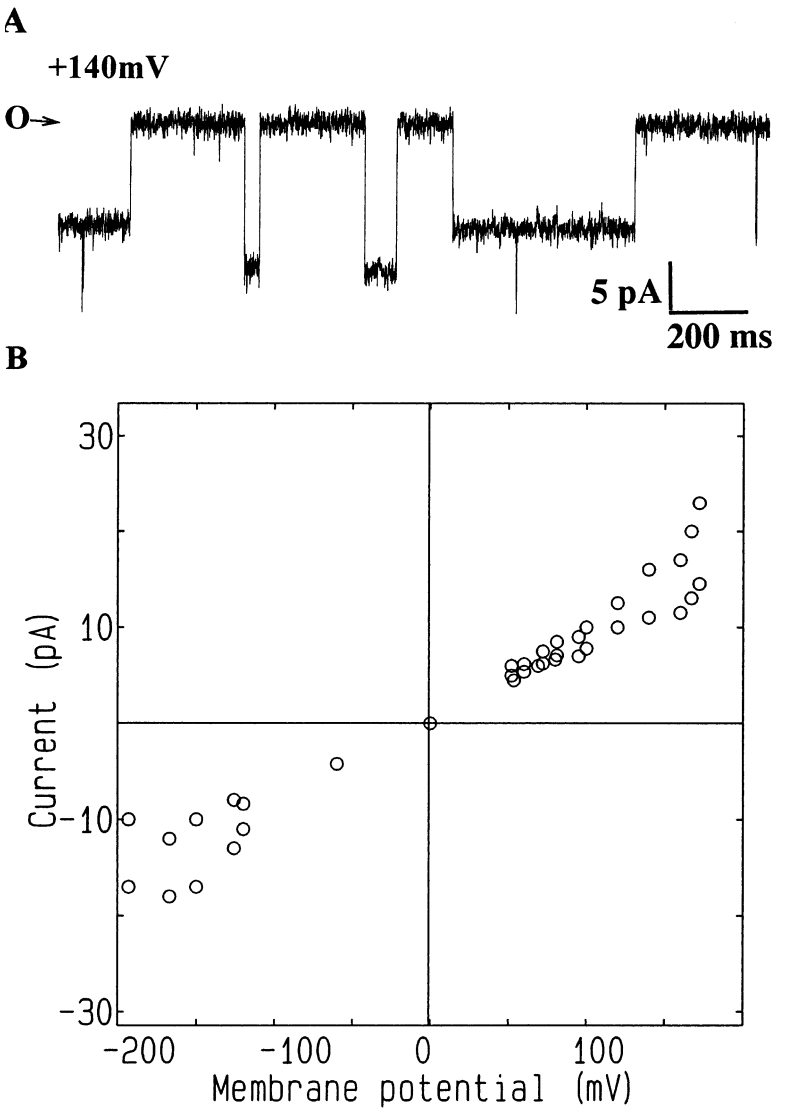

Fig. 7. Conductances of PhoE porin channels. Example of a patch with two predominant conductances. Porins were reconstituted at a lipid to protein ratio of 100,000 (w/w). (*A*) segment of recording at +140 mV showing the two predominant transitions (85 pS and 115 pS), under symmetrical conditions (100 mM KCl). (*B*) Current-voltage relationship for the two predominant conductances. Bath and pipette media (in mM): 10 Hepes-KOH, pH 7, 100 KCl with in addition 2 $CaCl_2$, 5 $MgCl_2$ in the pipette.

mainly on experiments using polyclonal and monoclonal antibodies against VDAC. In bovine astrocytes, a monoclonal antibody labeling the plasma membrane is able to block the maxi-chloride channels (Dermietzel et al., 1994). This view, however, has recently been challenged by Yu et al. (1995) who showed that three isoforms of VDAC are localized exclusively in mitochondria. Thus, possibly, maxi-chloride channels are only related, but not identical, to mitochondrial porins.

Considering the above, we wonder whether the similarity between the electrophysiological characteristics described here for PhoE and that of other channels is coincidental. We suggest that these characteristics may constitute a manner of fingerprint that would reflect a similar structural organization in different channels. The porin channels would constitute a superfamily comprising porins from Gram-negative and certain Gram-positive eubacteria, Archaebacterial porins and channels found in organelles as well as in the plasma membrane of eukaryotic cells. In this superfamily, the three-dimensional structure rather than the sequence would have been conserved during evolution.

What is the physiological significance of these electrophysiological characteristics and in particular of the voltage-dependence? The bacterial porins are localized to the outer membrane, which is not believed to be normally electrically polarized. Only under particular circumstances (low concentration of salts in the outside medium) can a Donnan potential exist across this membrane. But even under these conditions, its magnitude, measured or calculated, seems to be lower than the threshold necessary to close the channels (Sen, Hellman & Nikaido, 1988). Moreover, under conditions where a Donnan potential exists, the permeability of the outer membrane in vivo, does not appear to be altered (Sen et al., 1988). It is therefore possible that the voltage-depen-

dence of bacterial porins is a mere consequence of the structure of the protein, without biological significance. In contrast, for eukaryotic maxi-chloride channels, the voltage dependence must be important to control these high conductance channels present in the electrically polarized plasma membrane. It is noteworthy that the threshold of the membrane potential at which channels close is considerably lower for maxi-chloride channels (20–30 mV) than for bacterial porins. This low threshold might thus reflect a selective pressure.

Future work should aim at understanding the electrical properties of porins in terms of structure. The existence of a high-resolution structure for bacterial porins, as well as the simplicity of the genetic approach in bacteria could be important assets for such studies. To be insightful, these studies must consider the full electrophysiological complexity of these channels, especially if bacterial porins are representative of a large class of ion channels present in all domains of the phylogenetic tree.

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