J. Membrane Biol. 133, 119–127 (1993)

The Journal of
Membrane
Biology

Voltage-Dependent Cationic Channel of *Escherichia coli*

Catherine Berrier†, Alain Coulombe‡, Christine Houssin†, and Alexandre Ghazi†

†Laboratoire des Biomembranes, URA CNRS 1116 and ‡Laboratoire de Physiologie Cellulaire, URA CNRS 1121, Université Paris-Sud, 91405 Orsay Cedex, France

Summary. A fraction highly enriched with inner membranes of *E. coli* was fused with liposomes, using the dehydration-rehydration technique, to produce giant liposomes amenable to patch-clamp recordings. Among the several channels present in this type of preparation, one was further characterized. The channel has a conductance of some 200 pS (in 0.1 M KCl) and is weakly selective for cations (PK/PCl = 4). The channel stays open at negative and low positive membrane potentials and shows an increasing probability of closure with increasing voltage. High positive membrane potentials favor transitions to a long-lived inactivated state, following slow kinetics. Voltage-dependent rapid flickerings of the same amplitude, between open state and other short-lived closed states, are superposed on these kinetics. The channel is presumed to be localized in the inner membrane, but its characteristics are also compatible with those of porins of the outer membrane. However, the major porins OmpF and OmpC, purified and reconstituted into giant liposomes, exhibited a marked different behavior.

Key Words bacteria · *Escherichia coli* · ion channel · porin · patch clamp · liposomes

Introduction

The Gram-negative bacterium *Escherichia coli* is delimited by two membranes, an outer membrane and an inner (cytoplasmic) membrane.

The outer membrane contains large, water-filled channels called porins, which are permeable to compounds with molecular weights up to 600–700 D (Benz et al., 1978; Schindler & Rosenbusch 1978; *see* Jap and Walian, 1990, for a review). Consequently, the outer membrane is generally viewed as a molecular sieve. The electrophysiological data gathered so far on porins have been mostly obtained using purified proteins reincorporated into planar bilayers. The two major porins, OmpF and OmpC, form trimers, whose conductance is in the order of 2 nS in 1 M KCl. The voltage dependence of porins is still debated. One group of laboratories found that porins could be closed, following slow kinetics, by increasing the membrane potential (Schindler & Rosenbusch, 1978, 1981; Dargent et al., 1986; Xu et al., 1986; Morgan, Lonsdale & Alder, 1990), while another group observed that porins are mostly open, and that the rare closure events are independent of the applied potential (Benz et al., 1978; Sutcliffe et al., 1983). This difference has been generally ascribed to differences in the reconstitution methods and/or the purification methods. Nevertheless, Lakey and Pattus (1989) purified OmpF in three different ways and reconstituted it by three different methods, and observed, in all cases, a voltage-dependent closure of the porins. Recently, patch-clamp techniques, performed either on *E. coli* giant spheroplasts (Martinac et al., 1987; Szabo' et al., 1990) or on giant liposomes into which bacterial membranes have been fused (Berrier et al., 1989; Delcour et al., 1989*a*) have started being used. Using this method, Delcour et al. (1989*b*) described a voltage-dependent channel, later identified as OmpC, on the basis of alterations in conductance and cooperative behavior in a strain carrying a single mutation in the *ompC* gene (Delcour, Adler & Kung, 1991). Large stretch-activated ion channels are also observed in these preparations; their localization is still debated (Berrier et al., 1989; Buechner et al., 1990).

The inner membrane, capable of energy transduction like that of mitochondria and chloroplasts, is highly polarized electrically, due to active expulsion of protons through the respiratory chain or the ATPase: the values of the membrane potential, as measured by the accumulation of cationic lipophilic probes, range from −100 to −150–180 mV, depending on the external pH (Padan, Zilberstein & Rottenberg, 1976; Booth, Mitchell & Hamilton, 1979). This membrane contains active transport systems (pumps and carriers), some of which have been the object of intense study, at the genetic, biochemical and biophysical levels (Harold, 1986). In contrast, very little is known about ion channels in the

inner membrane. In inner membranes fused into planar bilayers, Simon and co-workers (1989) reported the existence of a 115 pS channel (in 45 mM KCl) and, more recently, of a 220 pS channel which is opened by the addition of a signal sequence (Simon & Blobel, 1992). It is suggested that this last channel is involved in protein translocation. Although it is generally believed that the inner membrane is unlikely to contain ion channels because they would cause dissipation of the proton-motive force, several ion channels of unknown functions have recently been described in other energy-transducing membranes such as the inner membrane of mitochondria (Sorgato, Keller & Stühmer, 1987; Antonenko, Kinnally & Tedeschi, 1991; Szabo' & Zoratti, 1991) and of thylakoids (Schönknecht et al., 1988).

Prior separation of the inner and outer membranes on sucrose gradients, followed by fusion into giant liposomes by dehydration-rehydration, allows the two membrane fractions to be studied separately. Using this method, we observed in the inner membrane fraction the presence of mechanosensitive (stretch-activated) channels (Berrier et al., 1989), as well as several other, nonmechanosensitive channels. Among these latter channels, one was more frequently observed than were the others. We present a description of this channel.

Materials and Methods

Membrane Isolation and Reconstitution into Giant Liposomes

E. coli strains K12 and PAP1402 (K-12 W3110 carrying the mutations *ompR*::Tn5 and *gyrA*) were grown at 37 °C in M9 minimal medium containing 0.2% glucose, as the sole carbon source. Strain PAP1402 was a generous gift from Dr. A.P. Pugsley. Cells were harvested at $OD_{650} = 0.5$, and broken by passages through a French pressure cell. The different membrane fractions (inner membrane, outer membrane, contact zones) were isolated by sedimentation through a sucrose gradient, as previously described (Berrier et al., 1989). Inner membrane vesicles or outer membrane vesicles were then fused with liposomes of azolectin (from soybean, type II-S, Sigma) at a lipid-to-protein ratio of 6, by a cycle of dehydration-rehydration described previously (Berrier et al., 1989).

Purification and Reconstitution of OmpF and OmpC Porins into Giant Liposomes

OmpF and OmpC porins were purified from the outer membrane fraction of *E. coli* K12, by salt extraction, using the procedure of Morgan et al. (1990). Solubilized porins (1 μg) resuspended in 20 μl of 50 mM Tris-HCl (pH 8) buffer containing the detergent lauryldimethylamine (0.1% w/v) 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 T1-100 Beckman centrifuge, and the pellet was resuspended in 15 μl of 10 mM Hepes KOH buffer pH 7.4. The liposomes were then fused into giant liposomes, by a cycle of dehydration-rehydration (Berrier et al., 1989).

Electrical Recordings and Data Analysis

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). All experiments were conducted at room temperature (19–25 °C). Single channel activity was measured using the methods of Hamill et al. (1981). Patch electrodes were pulled from pyrex capillaries (Corning code 7740) and were not fire-polished before use. Application of the pipette (5–10 MΩ) to the surface of a proteoliposome and gentle suction generally resulted in the formation of a giga-seal. The membrane patch was then excised and the internal face was continuously perfused by a flow of solution from one of a series of five piped outlets. The flow rate of the perfusion solutions was 50–100 μl/min. Unitary currents were recorded using a Dagan 8900 patch-clamp amplifier with a 10 GΩ feedback resistance, and stored either upon a beta video cassette recorder (Sony) after 16-bit digitization at 22 kHz with pulse code modulator (Sony PCM-701-ES), or upon digital audio tape (Biologic DTR 1200 DAT recorder). Records were subsequently filtered at 1 or 2 kHz (−3 dB point) through an 8-pole Bessel low-pass filter 920LPF (Frequency Devices), for data representation and data analysis, respectively. Filtered data were occasionally retrieved directly using a Gould 2400 pen-recorder. More frequently, after having been digitized at a rate of 2 or 10 kHz by Compaq, Deskpro 286 using an S200 interface (Cambridge Research System), data were analyzed and retrieved on a HP 7475A plotter or on a HP Laserjet printer. Unitary currents were measured using a computer to form histograms of baseline and open-level data points. These histograms were fitted to the sum of Gaussian functions using the nonlinear least-squares gradient-expansion algorithm of Marquardt (1963), to determine the amplitude, the mean and the dispersion of each peak. The mean unitary current corresponds to the means of fitted peaks. The open/closed current levels were determined by setting the level for threshold detection at 50% of the mean single-channel current level determined from the amplitude histograms. Open time, closed time histograms and *I-V* curves were fitted by the numerical method described above. F-tests were used to evaluate whether significant improvements of the fits were obtained with two *vs.* one exponential.

The contents of all the pipette, bath and perfusion solutions are listed in the figure legends.

Results

The channel investigated was observed in excised patches from giant liposomes into which *E. coli* inner membranes had been fused by dehydration-rehydration. Under our conditions of reconstitution (lipid to protein ratio of 6 : 1), the channel was observed in 49 out of approximately 350 patches. It was not observed in giant liposomes into which outer membranes, isolated from the same cells, had been recon-

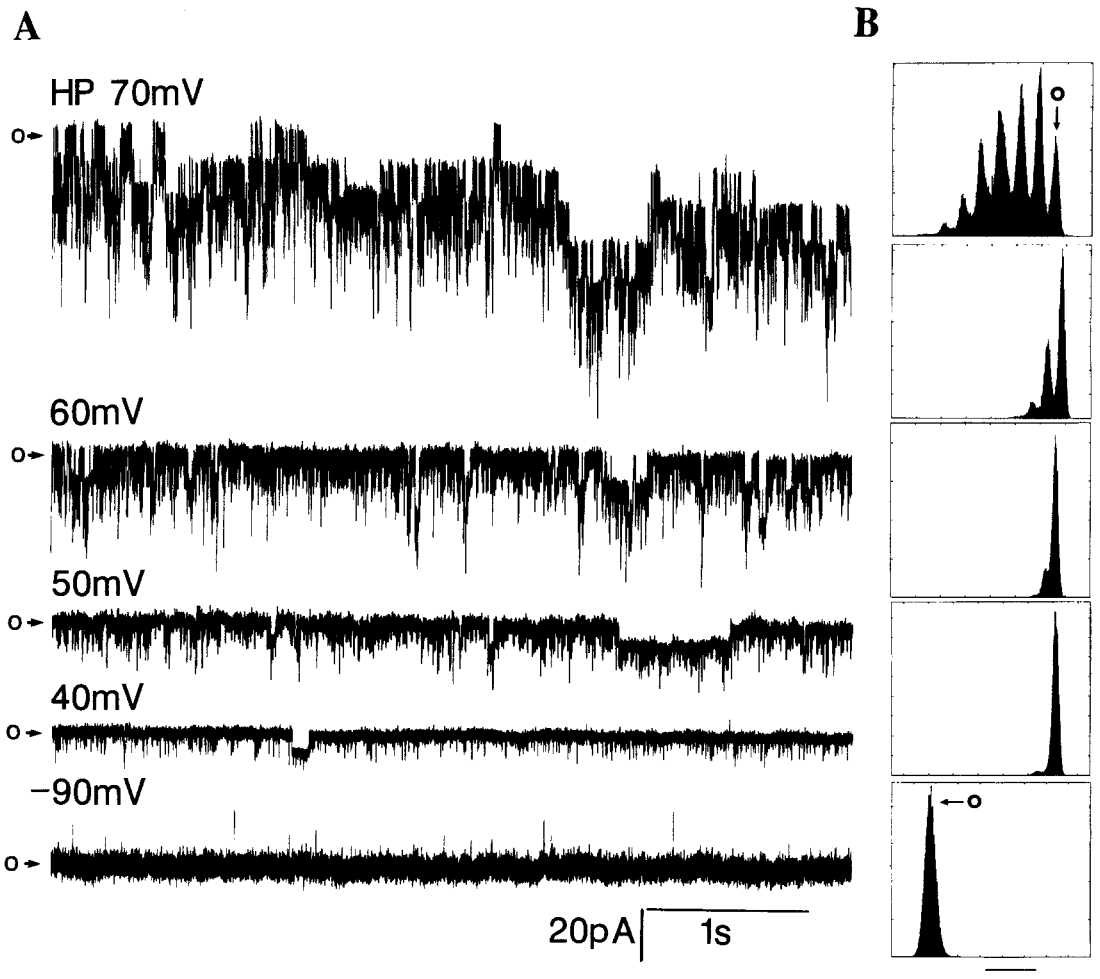

Fig. 1. Properties of the current fluctuations through the voltage-dependent channel. (*A*) Current traces recorded, at different holding potentials, from an inside-out excised patch of giant liposome fused with *E. coli* inner membrane (lipid/protein ratio: 6 : 1). Bath medium: 10 mM HEPES-KOH adjusted to pH 7.4, 100 mM KCl. Pipette medium: as bath medium, with the addition of 2 mM $CaCl_2$, 5 mM $MgCl_2$. *O*: open level. (*B*) Corresponding amplitude histograms (10 sec recordings), displaying multiple current peaks separated by equal intervals. The frequency of occurence is given in arbitrary units. Horizontal bar: 40 pA.

stituted at a similar lipid to protein ratio (52 patches). It is not known whether or not the reconstituted membranes have the same orientation as the native inner membranes. Membrane potentials are thus arbitrarily given in terms of potentials of bath solutions, assigning zero potential level to the pipette.

Figure 1*A* displays currents through channels of this kind at different holding potentials. The channels were mostly open at negative potential and low positive potential and closed as the potential is raised. In 48 out of 49 patches the channel presented the voltage-dependent polarity shown in Fig. 1*A*, while in only one patch, the inverse polarity (closure at negative membrane potential) was observed. Inspection of the traces displayed in Fig. 1 clearly shows the superposition of two kinetics: slow closure and reopening events on which are superposed rapid flickerings between open and closed states. These two kinetics belong to the same channel since: (i) they were always observed together, and (ii) they have the same elementary conductance. This can be seen from the corresponding amplitude distribution histograms (Fig. 1*B*) which display multiple current peaks separated by equal intervals. Also, segments of recordings plotted on an expanded time scale indicate that the resolved events of the fast kinetics have the same conductance as those of the slow kinetics (Fig. 2). This was true in symmetrical as well as in asymmetrical media (Fig. 2), indicating the same ionic specificity for the two types of events.

Despite the dilution resulting from the reconstitution, and despite these channels being only observed in about one patch out of 7, they were always observed in clusters. Fast kinetics was studied at

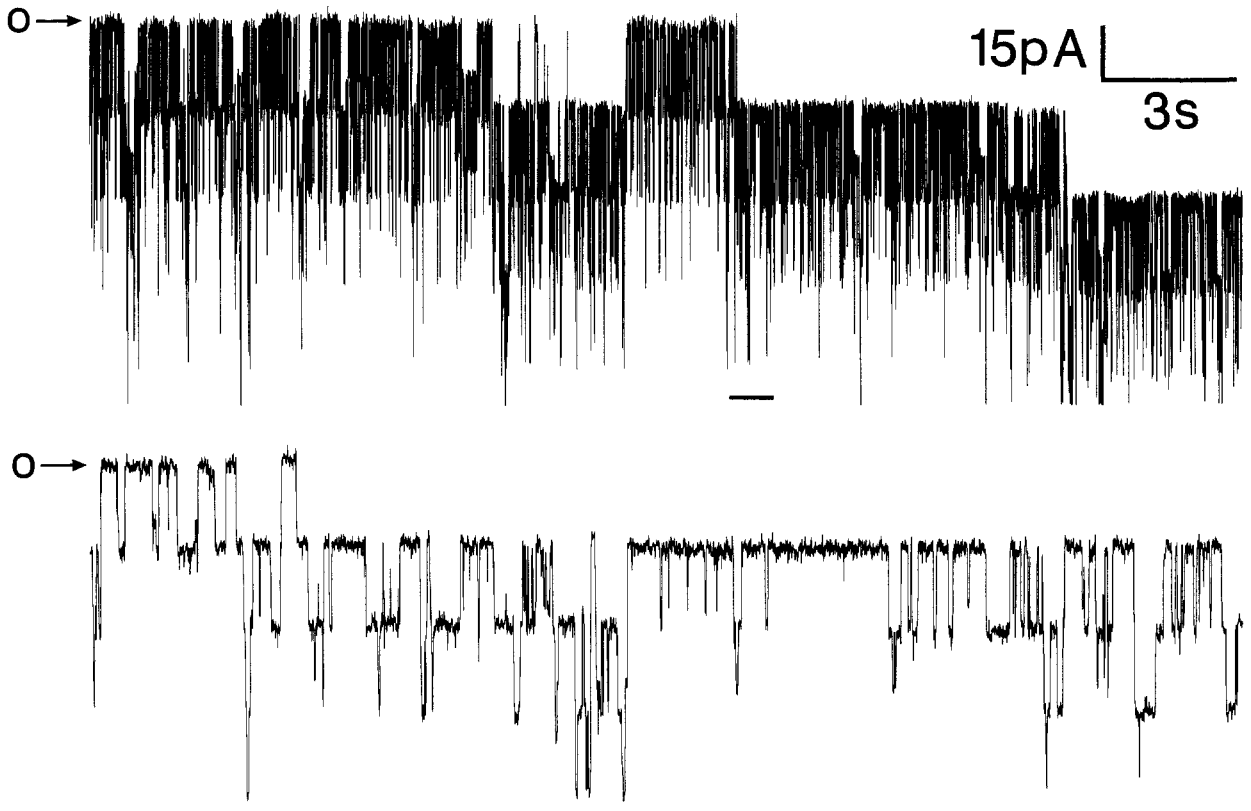

Fig. 2. The two kinetics of closure have the same elementary conductance. The recording was performed in asymmetrical media (pipette: 100 mM KCl, bath: 300 mM KCl). The pH was adjusted to 7.4 with 10 mM HEPES-KOH, pipette medium additionally contained 2 mM $CaCl_2$, 5 mM $MgCl_2$. The membrane potential was 56 mV. The lower trace shows 1 sec recording of the upper trace (corresponding to the horizontal bar) on an expanded time scale.

membrane potentials (up to 40 mV) for which one level of closure is largely predominant, so that the other levels can be neglected, and for which slow closure events are rare and can thus be avoided. Figure 3*A* shows a closed time histogram at 40 mV: the closed times are not distributed exponentially, a large component of very brief closures being superposed on smaller numbers of longer closures. At all potentials, the closed time histograms fitted the sum of two exponentials, the time constant of the first exponential being probably overestimated, due to missing very short events. None of the time constants of the two exponentials appeared to be significantly sensitive to the membrane potential (*not shown*). Thus, the channel would possess two closed states and the transition from closed states to open state would be largely voltage independent. Because of the presence of several channels in the patches, the open time distribution of one channel could not be determined. We measured, instead, the open time distribution of N channels, N being the largest number of elementary-current units present in one patch. Figure 3*B* shows such an open time histogram at 40 mV. The open times were observed to be exponentially distributed at all potentials and the mean open time (corresponding to $N \times$ mean open time of one channel) decreased with increasing membrane potential (Fig. 3*C*), implying that closures through fast kinetics implicate only one open state and are voltage dependent.

We took advantage of the appearance of the channels in clusters to study the slow kinetics by voltage pulses. In Fig. 4, a 120 sec pulse, from -40 mV where channels were opened, to $+100$ mV, resulted in the closure of some 25 channels; return to -40 mV resulted in a level of current indicating that the closed channels had not reopened. In the 120 sec following the return to -40 mV, one closure followed by two reopenings can be observed. The remaining 24 channels remained closed, as confirmed by the limited current amplitude reached upon a second pulse to $+100$ mV (*not shown*). The number of channels closed and reopened is calculated on the basis of the single channel conductance of the channel at $+100$ and -40 mV, respectively. This pattern, i.e., immediate reopening of only a

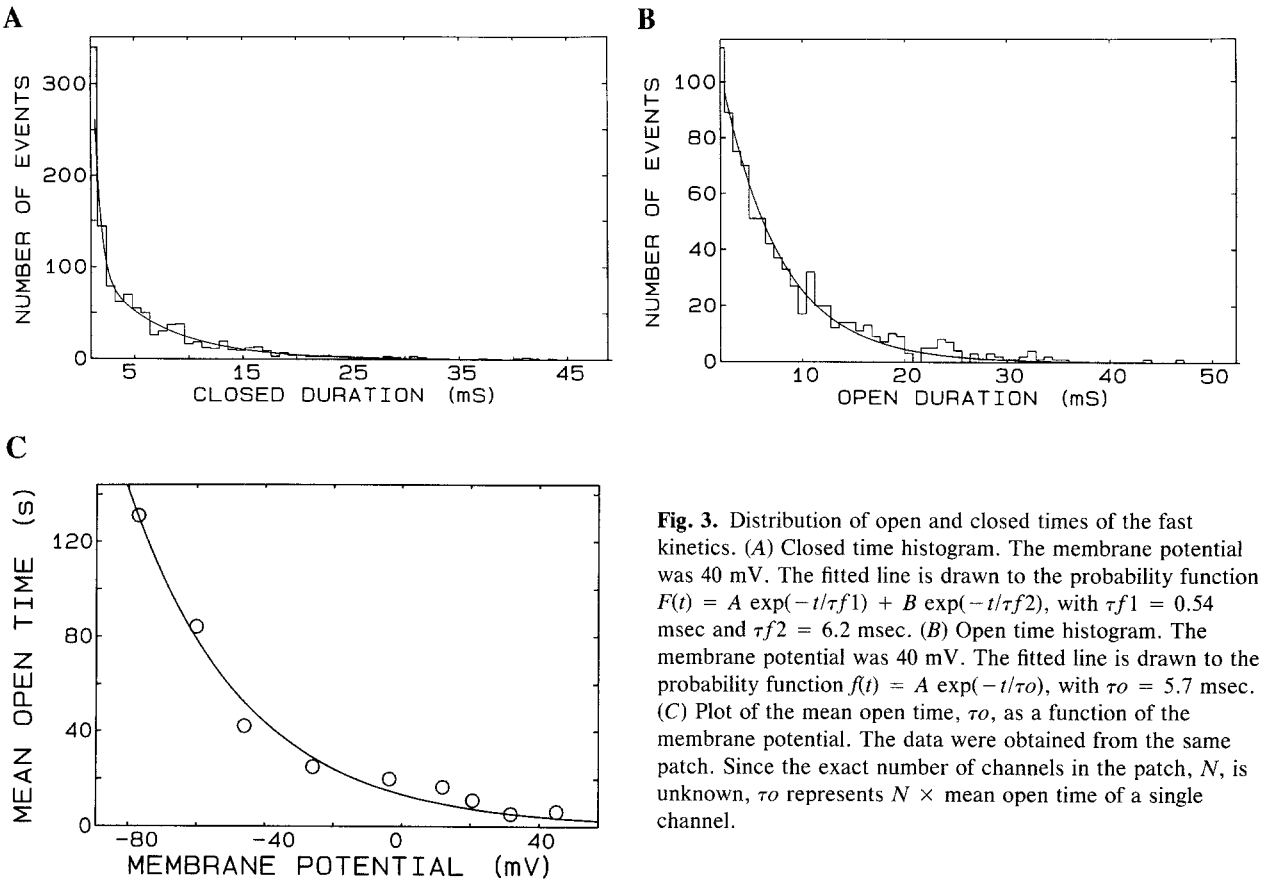

Fig. 3. Distribution of open and closed times of the fast kinetics. (*A*) Closed time histogram. The membrane potential was 40 mV. The fitted line is drawn to the probability function $F(t) = A \exp(-t/\tau f1) + B \exp(-t/\tau f2)$, with $\tau f1 = 0.54$ msec and $\tau f2 = 6.2$ msec. (*B*) Open time histogram. The membrane potential was 40 mV. The fitted line is drawn to the probability function $f(t) = A \exp(-t/\tau o)$, with $\tau o = 5.7$ msec. (*C*) Plot of the mean open time, τo, as a function of the membrane potential. The data were obtained from the same patch. Since the exact number of channels in the patch, N, is unknown, τo represents $N \times$ mean open time of a single channel.

fraction of the closed channels and prolonged closure of the other channels, was observed in all patches subjected to voltage pulses. Together with the observation that reopening events can be observed in the slow kinetics (*see* Figs. 1,2,5), this indicates that a high membrane potential triggers the transition to, at least, two closed states, one of which is an absorbing, inactivated state.

Figure 5 shows the time course of inactivation triggered by voltage pulses, from a holding potential of −40 mV, to increasing positive potentials. The decaying part of the signal, for recordings of pulses above 80 mV, could be meaningfully fitted by a single exponential, with a time constant decreasing drastically with increasing voltage (Fig. 5). Nevertheless, a latency to the first closure, which decreases with increasing voltage, is clearly observed in the recordings. Following the classical treatment of Amstrong (1969), this would indicate that there are several open states through which the channel has to pass before the transition to the inactivated state. In keeping with this hypothesis, the normalized whole curves, latency to the first closure included, could be reasonably fitted by functions of the form $1\text{-}(1 - \exp(-t/\tau))^3$ (*not shown*).

In symmetrical 100 mM KCl solutions, the current-voltage relationship was non-ohmic (Fig. 6*A*). The chord conductance varied from 90 pS at −100 mV to 195 pS at +100 mV, and the slope conductance from 70 pS at −100 mV to 270 pS at +100 mV. In asymmetrical solutions, the observed reversal potential was −15 mV for a reversal potential for K^+ of −28 mV, corresponding to a ratio of permeability of potassium to chloride (PK/PCl), of 4. The selectivity among cations is shown in Fig. 6*B*. From the reversal potentials, the following order is obtained: $K^+ > Na^+ > Li^+$.

The channel was found in the inner membrane fraction fused into giant liposomes and never in the outer membrane fraction. We therefore presume that it is localized in the inner membrane. However, since there is a certain contamination (about 8%) of the inner membrane fraction by the outer membrane fraction, we considered the possibility that the channel could be a contaminating porin from the outer membrane. Indeed, OmpF and OmpC, the only known porins present in significant amounts under our conditions of culture, are major constituents of the outer membrane and the voltage dependence of the channel studied here is reminiscent of that of porins reconstituted into planar lipid bilayers, as reported by several groups (Schindler & Rosen-

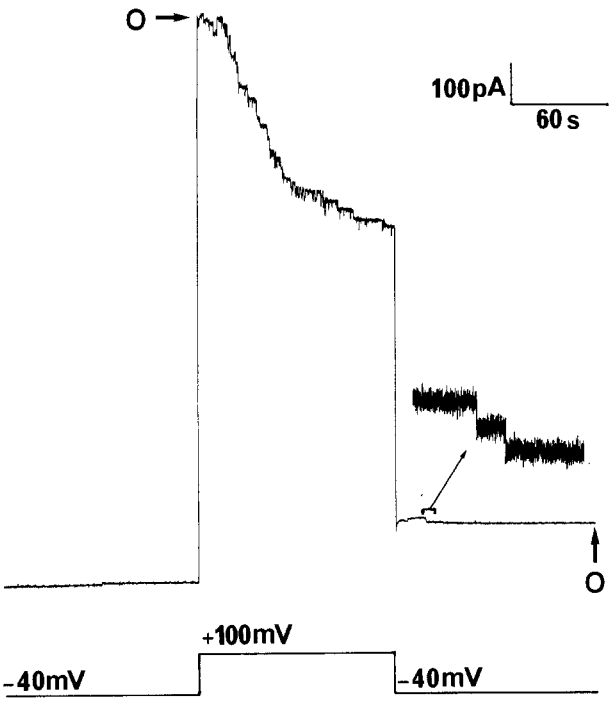

Fig. 4. Inactivation of the channels triggered by voltage pulse. The patch was subjected to a voltage pulse from a holding potential of −40 mV to +100 mV, as indicated. Other conditions as in Fig. 1.

busch, 1978, 1981; Dargent et al., 1986; Xu et al., 1986; Lakey & Pattus, 1989; Morgan et al., 1990). Although this channel and OmpF and OmpC porins apparently differ in other characteristics reported from planar bilayer experiments, we studied the behavior of porins reconstituted in giant liposomes.

OmpF and OmpC were purified by salt extraction, following the procedure of Morgan et al. (1990), from the outer membrane fraction of the *E. coli* K12 cells used in this study. The purified porins were reconstituted into azolectin liposomes, which were then fused into giant liposomes by a cycle of dehydration-rehydration and studied by patch clamp. The results of this study, which is detailed elsewhere (Berrier et al., 1992), indicate that porins behave differently from the channel described here. Porins could be reversibly closed by test pulses to negative as well as to positive membrane potentials (but only above 100 mV or under −100 mV). Negative potentials were more efficient in promoting closure. Even in this case, the kinetics of closure is much slower than for the channel described above. Figure 7 shows three 190 pS steps (in 0.1 M KCl) of closure, elicited by a test pulse to −160 mV applied to giant liposomes reconstituted with porins. From the data obtained in planar bilayers, we interpret these events as the cooperative closure of trimers. In most cases rapid fluctuations of approximately one third of the

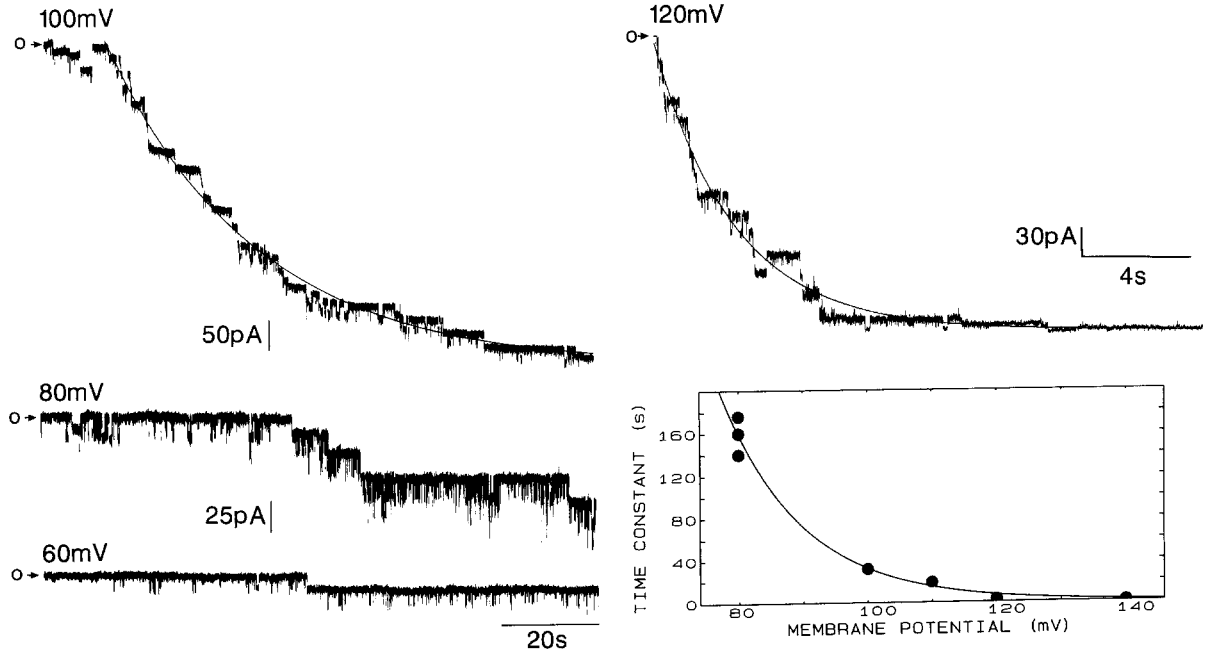

Fig. 5. Kinetics of inactivation at different positive potentials. Current traces elicited by 120 sec voltage pulses from a holding potential of −40 mV to increasing positive potentials: 60, 80, 100 and 120 mV (total pulse period: 240 sec). The traces at 60, 80 and 100 mV are from the same patch. The decaying part of the signal was fitted to a single exponential curve which is shown superposed on the traces at 100 and 120 mV. The bottom figure shows the variation of the time constant of inactivation with the membrane potential. Other conditions as in Fig. 1.

large steps (60–70 pS) were observed, superposed on the slow kinetics of closure (Fig. 7). These fast events, previously not detected by the planar bilayer technique, were always observed at lower membrane potentials (between −90 mV and +90 mV) and were the only manifestation of the porins at these potentials. They probably represent the flickering of monomers and may correspond to the electrical activity described by Delcour et al. (1989*b*) in patch-clamp experiments performed on giant liposomes fused with outer membranes, and recently ascribed to OmpC (Delcour et al., 1991). This pattern was never observed in the channel described in this paper. Alternatively, rapid fluctuations of the same amplitude as that of the slow closing events, were not observed with porins. Moreover, OmpF and OmpC channels exhibited a cooperative behavior which increased with increasing membrane potential

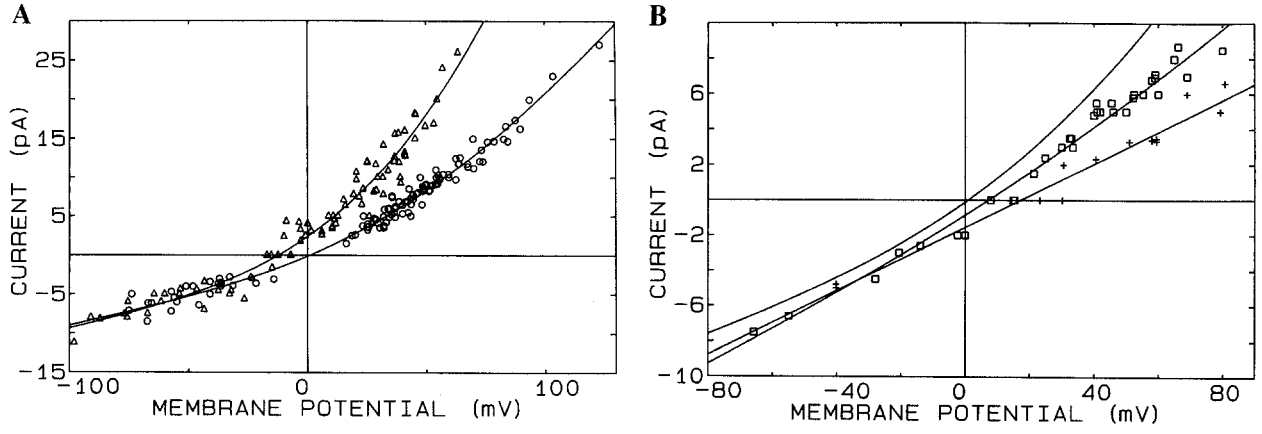

Fig. 6 Current-voltage relationship and ionic selectivity. (*A*) *I-V* curve obtained in (○) symmetrical (pipette and bath: 100 mM KCl) and (△) asymmetrical media (pipette: 100 mM KCl, bath: 300 mM KCl). (*B*) *I-V* curve in asymmetrical K/Na media (□) (Pipette: 100 mM KCl, bath: 100 mM NaCl) and K/Li media (+) (pipette: 100 mM KCl, bath: 100 mM LiCl). The curve in symmetrical KCl media is given as a reference. In both *A* and *B*, the pH was adjusted to 7.4 with 10 mM HEPES-KOH, pipette media additionally contained 2 mM $CaCl_2$, 5 mM $MgCl_2$.

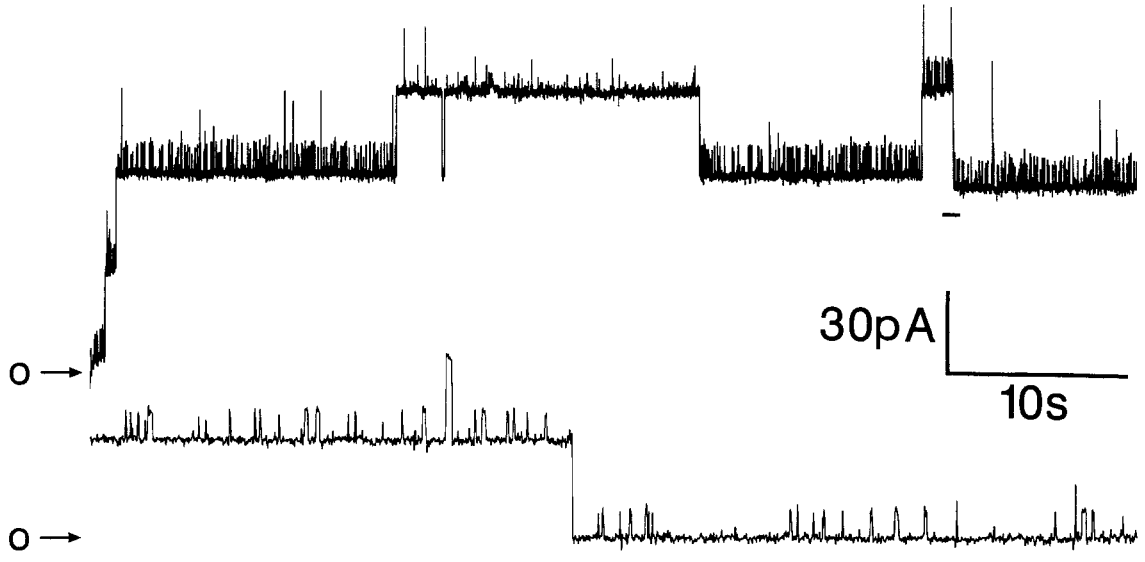

Fig. 7. Closure of porins (OmpC and OmpF) elicited by a 60 sec test pulse from 0 to −160 mV. Porins were purified by salt extraction from the outer membrane of *E. coli* K-12, and reconstituted into azolectin liposomes (lipid/protein ratio = 1000:1). The liposomes were then fused by dehydration-rehydration into giant liposomes amenable to patch-clamp recording. The recording was performed in asymmetrical media (pipette: 100 mM KCl, bath: 300 mM KCl). The pH was adjusted to 7.4 with 10 mM HEPES-KOH, pipette medium additionally contained 2 mM $CaCl_2$, 5 mM $MgCl_2$. The lower trace shows 1 sec recording of the upper trace (corresponding to the horizontal bar) on an expanded time scale.

and which was never observed for this channel. Also, the selectivity of the purified porins reconstituted into giant liposomes (PK/PCl = 25), was different from that of the channel described here.

Finally, a similar channel was observed (7 times out of 98 patches *vs.* 49 times out of 350 patches in *E. coli* K-12, under similar conditions of reconstitution) in the inner membrane fraction isolated from strain PAP1402 which is derived from strain K-12W3110 by deletion of the *ompR* gene. Deletion of the *ompR* gene decreases the level of OmpC and OmpF expression to extremely low levels (Hall & Silhavy, 1981), conferring to the bacteria an $OmpF^-$, $OmpC^-$ phenotype characterized by resistance to colicin A.

Discussion

This work investigates an ion channel found in the inner membrane fraction of *E. coli* fused into giant liposomes. The main characteristics of this channel are a large conductance (around 200 pS in 0.1 M KCl at positive potential), a weak selectivity for cations, and a high open probability at negative or low positive membrane potential. The voltage dependence of the channel is complex and was only analyzed in part. Increasing membrane potential favors the transition to two closed states of very short lifetimes. High positive membrane potentials also favor the transition to one, or more probably two, long-lived closed states, one of which is an absorbing, inactivated state.

We attempted to localize the channel by looking for it in inner and outer membrane fractions. Both fractions, reconstituted into giant liposomes display electrical activity (Berrier et al., 1989), but the channel described here is only observed in the inner membrane fraction. Here, the quantitative determination of a biochemical activity, used in the localization of membrane protein with enzymatic activity, is replaced by the frequency of occurence of the channel in the different fractions. This approach can be criticized on various grounds. While the outer membrane fraction is nearly devoid of inner membrane vesicles, there is significant contamination of inner membrane fractions by outer membranes (about 8%) (Berrier et al., 1989). It may also be argued that the efficiencies of reconstitution of the two fractions, which are unknown, may be different. Indeed, fusion of bacterial membranes into giant liposomes involves lipid-lipid interactions and since the lipid compositions of the two membranes are very different, one can expect differences in the efficiency of reconstitution. This, however, does not, in itself, invalidate the approach: there is no reason to suppose that outer membrane vesicles contaminating the inner membrane fractions would be reconstituted with a higher yield than outer membrane vesicles from the outer membrane fractions. The approach is unsatisfactory, nevertheless, if the contaminating fraction is a subpopulation which is selectively enriched in the protein under study. However, this is a difficulty which is also encountered in determining the localization of a protein by enzymatic assays. Consequently, conclusions derived from this approach, the only possible ones at this first stage, should be considered with caution, until a function can be clearly assigned to the channel.

Despite superficial resemblance to the voltage dependence of porins reconstituted in planar lipid bilayers, we do not think that the channel described here is one of the known porins, contaminating the inner membrane fraction. Under the growth conditions used here, only OmpF and OmpC are produced in significant amount, the expression of *phoE* and *lamB* requiring a low level of phosphate and the presence of maltose, respectively (Nikaido & Vaara, 1987). OmpC and OmpF reconstituted into liposomes had completely different characteristics, in particular the kinetics and ion specificity. Nevertheless, for the reasons given above, a localization of this channel in the outer membrane cannot be totally excluded. In this case, the channel would be a novel, minor porin. Its large conductance, and the fact that it is open at low or zero membrane potential—the situation found in the outer membrane under normal conditions—would be consistent with such a role. It would then remain to be explained why this channel coisolates preferentially with the inner membrane fraction.

If, as suggested by our experiments, the channel is present in the inner membrane, knowledge of its native orientation is important, since the inner membrane is electrically polarized. Inner membrane vesicles obtained by passage through a French press are inside out as compared to the native orientation, but it is unknown how fusion into giant liposomes by dehydration-rehydration affects the orientation. The data presented here show at least that it is not random, since the channel, which has an asymmetric voltage dependence, was observed in 48 out of 49 cases to have the same orientation. If the inner membrane was reconstituted right-side out (the cytoplasmic surface facing the bath), the channel would be constantly open under physiological conditions. Moreover, in this case, closure at positive potentials would have no physiological significance, since these potentials are never observed in vivo. If, however, the inner membrane was reconstituted inside-out, the channel would be normally closed at physiological potentials, and could open upon depolariza-

tion. In all cases, given the high conductance of the channel and its relative abundance, its presence in the inner membrane probably requires that it be regulated by soluble elements present in the cytoplasm or the periplasm to avoid dissipation of the transmembrane proton-motive force. The same consideration applies to some of the ion channels recently described in the inner membrane of mitochondria, another energy-transducing membrane (Sorgato et al., 1987; Szabo' & Zoratti, 1991). At this stage, given its lack of specificity, it is premature to speculate about the physiological function of this new channel.

We thank Prof. E. Coraboeuf and Prof. E. Shechter for support, and Dr. A.P. Pugsley for the gift of a strain. The assistance of Mr. G. Sadoc in computer programming is gratefully acknowledged. This work was financed in part by the European Economic Community under the program "Science."

References

Antonenko, Y.N., Kinnally, K.W., Tedeschi, H. 1991. Identification of anion and cation pathways in the inner mitochondrial membrane by patch clamping of mouse liver mitoplasts. *J. Membrane Biol.* **124:**151–158

Armstrong, C.M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injected in squid axons. *J. Gen. Physiol.* **54:**553–575

Benz, R., Janko, K., Boos, W., Laüger, P. 1978. Formation of large ion-permeable membrane channels by the matrix protein (Porin) of *Escherichia coli*. *Biochim. Biophys. Acta* **511:**305–319

Berrier, C., Coulombe, A., Houssin, C., Ghazi, A. 1989. A patch-clamp study of ion channels of inner and outer membranes and of contact zones of *E. coli*, fused into giant liposomes. Pressure-activated channels are localized in the inner membrane. *FEBS Lett.* **259:**27–32

Berrier, C., Coulombe, A., Houssin, C., Ghazi, A. 1992. Fast and slow kinetics of porin channels from *Escherichia coli* reconstituted into giant liposomes and studied by patch-clamp. *FEBS Lett.* **306:**251–256

Booth, I.R., Mitchell, W.J., Hamilton, W.A. 1979. Quantitative analysis of proton-linked systems. *Biochem. J.* **182:**687–696

Buechner, M., Delcour, A.H., Martinac, B., Adler, J., Kung, C. 1990. Ion channel activities in the *Escherichia coli* outer membrane. *Biochim. Biophys. Acta* **1024(1):**111–121

Dargent, B., Hofmann, W., Pattus, F., Rosenbusch, J.P. 1986. The selectivity filter of voltage-dependent channels formed by phosphoporin (PhoE protein) from *E. coli*. *EMBO J.* **5:**773–778

Delcour, A.H., Martinac, B., Adler, J., Kung, C. 1989*a*. A modified reconstitution method used in patch-clamp studies of *Escherichia coli* ion channels. *Biophys. J.* **56:**631–636

Delcour, A.H., Martinac, B., Adler, J., Kung, C. 1989*b*. Voltage-sensitive ion channel of *Escherichia coli*. *J. Membrane Biol.* **112:**267–275

Delcour, A.H., Adler, J., Kung, C. 1991. A single amino acid substitution alters conductance and gating of OmpC porin of *Escherichia coli*. *J. Membrane Biol.* **119:**267–275

Hall, M.N., Silhavy, T.J. 1981. The ompB locus and the regulation of the major outer membrane porin protein of *Escherichia coli* K-12. *J. Mol. Biol.* **146:**23–43

Hamill, O.P., Marty, A. Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Harold, F.M. 1986. The Vital Force: A Study of Bioenergetics. W.H. Freeman, New York

Jap, B.K., Walian, P.J. 1990. Biophysics of the structure and functions of porins. *Q. Rev. Biophys.* **23:**367–403

Lakey, J.H., Pattus, F. 1989. The voltage-dependent activity of *Escherichia coli* porins in different planar bilayer reconstitutions. *Eur. J. Biochem.* **186:**303–308

Marquardt, D.W. 1963. An algorithm for least-squares estimation of nonlinear parameters. *J. Ind. Appl. Math.* **11:**431–441

Martinac, B., Buechner, M., Delcour, A.H., Adler, J., Kung, C. 1987. Pressure-sensitive ion channel in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA.* **84:**2297–2301

Morgan, H., Lonsdale, J.T., Alder, G. 1990. Polarity-dependent voltage-gated porin channels from *Escherichia coli* in lipid bilayer membranes. *Biochim. Biophys. Acta* **1021:**175–181

Nikaido, H., Vaara, M. 1987. Outer membrane. *In: Escherichia coli* and *Salmonella typhimurium*. Cellular and Molecular Biology. F.C. Neidhart, editor. American Society for Microbiology. Washington, DC

Padan, E., Zilberstein, D., Rottenberg, H. 1976. The proton electrochemical gradient in *Escherichia coli*. *Eur. J. Biochem.* **63:**533–541

Schindler, H., Rosenbusch, J.P. 1978. Matrix protein from *Escherichia coli* outer membranes forms voltage-controlled channels in lipid bilayers. *Proc. Natl. Acad. Sci. USA* **75:**3751–3755

Schindler, H., Rosenbusch, J.P. 1981. Matrix protein in planar membranes: clusters of channels in their native environment and their functional reassembly. *Proc. Natl. Acad. Sci. USA* **78:**2302–2306

Schönknecht, G., Hedrich, R., Junge, W., Raschke, K. 1988. A voltage-dependent chloride channel in the photosynthetic membrane of a higher plant. *Nature* **336:**589–592

Simon, S.M., Blobel, G., Zimmerberg, B. 1989. Large aqueous channels in membrane vesicles derived from the rough endoplasmic reticulum of canine pancreas or the plasma membrane of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **86:**6176–6180

Simon, S.M., Blobel, G. 1992. Signal peptides open protein-conducting channels in *E. coli*. *Cell* **69:**677–684

Sorgato, C., Keller, K.U., Stühmer, W. 1987. Patch-clamping of the inner mitochondrial membrane reveals a voltage-dependent ion channel. *Nature* **330:**498–500

Sutcliffe, J., Blumenthal, R., Walter, A., Foulds, J. 1983. *Escherichia coli* outer membrane protien K is a porin *J. Bacteriol.* **156:**867–872

Szabó, I., Petronilli, V., Guerra, L., Zoratti, M. 1990. Cooperative mechanosensitive ion channels in *Escherichia coli*. *Biochem. Biophys. Res. Comm.* **171:**280–286

Szabó, I., Zoratti, M. 1991. The giant channel of the inner mitochondrial membrane is inhibited by cyclosporin-A. *J. Biol. Chem.* **266(6):**3376–3379

Xu, G.Z., Shi, B., MacGroartry, E.J., Tien, H.T. 1986. Channel-closing activity of porins from *Escherichia coli* in bilayer lipid membranes. *Biochim. Biophys. Acta* **862:**57–64

Received 11 March 1992; revised 25 November 1992