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Voltage-Sensitive Ion Channel of *Escherichia coli*

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Summary. A voltage-sensitive, cation-selective ion channel of *Escherichia coli* has been reconstituted into liposomes and studied with the patch-clamp method. The single channel conductance was 91 pS in symmetric solutions of 150 mM KCl. Many channels were open most of the time, with frequent brief transitions to closed levels. Multiple conducting units could close and reopen simultaneously, and this apparent cooperativity in gating was increased with depolarizing voltages. Above a voltage threshold, the channels closed irreversibly, often in groups.

Key Words *Escherichia coli* · outer membrane · ion channel · patch clamp · porin

Introduction

Ion channels appear to be ubiquitous membrane proteins which are found in a great variety of membrane types, from procaryotic microorganisms to various kinds of eucaryotic cells and their organelles. Their function is often associated with regulation of transmembrane voltage and excitability, but some of them are also involved in signal transduction and osmotic regulation.

Escherichia coli, a Gram-negative bacterium, is surrounded by an asymmetric outer membrane, made of lipopolysaccharide and phospholipid, and a cytoplasmic (inner) membrane. Large water-filled channels, called porins, mediate the permeation of hydrophilic solutes of less than 600 daltons across the outer membrane (Benz & Bauer, 1988). It has been estimated that the outer membrane of *E. coli* contains 10^5 such pores (Rosenbusch, 1974). The electrical activity of porins has been mostly studied in planar lipid bilayers (Schindler & Rosenbusch, 1978, 1981; Dargent et al., 1986; Lakey, 1987; Benz, 1988). The two major types of porins, products of the *ompF* or the *ompC* genes, are general diffusion pores, with a slight cation selectivity and a conductance in the order of 2 nS in 1 M KCl (Benz et al., 1978). There is some debate on whether these two pores are gated, and whether the gating is voltage dependent (Schindler & Rosenbusch, 1978; Benz, 1988). The debated differences in porin behavior might have originated from the differences in the methods of porin purification and reconstitution in the lipid bilayer (Lakey, 1987). A voltage-dependent closure of the porins in reconstituted bilayers, as demonstrated by several groups (Schindler & Rosenbusch, 1978, 1981; Dargent et al., 1986; Xu et al., 1986; Mauro, Blake & Labarca, 1988) has not, however, received support in macroscopic measurements of outer membrane permeability in live cells (Sen, Hellman & Nikaido, 1988).

We have recently applied a modified reconstitution procedure to the patch-clamp study of bacterial ion channels (Delcour et al., 1989*a*,*b*). Native membrane vesicles are fused with exogenous lipids at the desired protein-to-lipid ratio in the absence of detergent. By this method, we have been able to observe the activity of a mechanosensitive channel and another voltage-dependent channel (Delcour et al., 1989*a*). These two channels were initially observed on the surface of live cells and appear to be outer membrane proteins (Martinac et al., 1987; M. Buechner, A.H. Delcour, B. Martinac, J. Adler and C. Kung, *in preparation*). We present here a detailed description of the nonmechanosensitive but voltage-dependent channel studied in a reconstituted system. Preliminary results have previously been reported in an abstract (Delcour et al., 1989*b*).

Materials and Methods

Preparation of Biological Material

The preparation of bacterial membranes and their fusion with exogenous lipids was essentially as described (Delcour et al., 1989*a*). Cells from *E. coli* strain AW405 (Armstrong, Adler & Dahl, 1967) were grown at 35°C in tryptone broth (1% tryptone

(Difco, St. Louis, MO) and 0.5% NaCl) to an OD_{590} of about 0.5. Cells were centrifuged and resuspended in 50 mM potassium phosphate buffer (pH 6.6) containing 5 mM $MgSO_4$ and 1 mM dithiothreitol. Membranes were collected by centrifugation after passage of the cells through a French press (American Instrument, Silver Spring, MD) either once at 4500 lb/in^2 (Hertzberg & Hinkle, 1974) or twice at 16,000 lb/in^2 (Smit, Kamio & Nikaido, 1975). In some cases, inner and outer membrane fractions were separated through sucrose gradient centrifugation, while in other preparations the membrane vesicles were not further purified (Delcour et al., 1989*a*). For fusion with azolectin (Sigma, St. Louis, MO), we used either unfractionated membranes or purified outer membranes.

Electrical Recording

Patch-clamp experiments were carried out according to standard technique (Hamill et al., 1981). Gigaohm seals were formed on blisters induced from liposomes fused with bacterial membranes as previously described (Delcour et al., 1989*a*). The patches were excised, and the electrical activity was recorded in a bath solution of 150 mM KCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA, potassium salt), 10^{-5} M $CaCl_2$, 5 mM N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid (HEPES), pH 7.2 (solution A). The electrical recordings were performed at room temperature with an EPC-7 amplifier (List-Electronic, Darmstadt, West Germany). Data were recorded on chart (Gould, Cleveland, OH) and on tape (Indec, Sunnyvale, CA). They were filtered at 1 kHz (Frequency Devices, Haverhill, MA), digitized at a sampling rate of 1 per 0.1 msec and analyzed on computer (Indec Systems, Sunnyvale, CA) with a program developed by Dr. Yoshiro Saimi.

Data Analysis

The data points of the current-voltage (I/V) plots of Figs. 3 and 4 are averages of three experiments. For each experiment, we repeated a control I/V curve in symmetric 150 mM KCl solutions. Although we electronically corrected the offset potential during the experiment, we were not always able to obtain a perfect zero reversal potential in symmetric solutions because of the noise (rms value on the order of 0.7 pA). The recording voltages used in each experiment were therefore corrected by the deviation of the observed reversal potential of this control curve from zero. These deviations varied by up to 7 mV between experiments. Once the correction was made for this deviation in individual experiments, the current obtained in asymmetric solutions of 150 mM KCl in the pipette and 50 mM KCl in the bath always reverted at a smaller voltage than the expected reversal potential for K^+. This indicates some permeability for anions. The coordinates of the data points of Figs. 3 and 4 are the average of the recording voltages (X coordinate) and the average of the current amplitudes (Y coordinate) among the experiments.

The current steps of the most frequent transition, which was defined as the unit conductance, were measured by the Gaussian fit of amplitude histograms. The number of simultaneous closures of two or more units of conductance was too small to generate an amplitude histogram. Therefore, the current flowing through each of these larger steps was measured individually on an expanded trace and averaged. Current steps of amplitude ranging between 1.5 and 2.5 times the unit conductance were assigned to the simultaneous closure of two units, between 2.5 and 3.5 times the unit conductance were assigned to the simultaneous closure of three units, and so forth.

This algorithm was also used in counting the number of closures plotted in the histograms of Fig. 5. "Closures of n units of conductance" are defined as brief excursions from the fully open state (all channels open) to the current level corresponding to the simultaneous closure of n units of conductance. To obtain the data of the Table, we used a computer program to measure the duration of the closures at each conductance level. The sum of these closed times divided by the total recorded time was defined as the probability of each multiple of the unit conductance being closed (P_c^i). The overall probability of any unit being closed (p_c) was calculated by a computer program, which measures the total amount of current flowing through the observed open channels and divides it by the expected current through all channels of the patch for the recorded period. It is the probability of any single unit conductance being closed at any time.

Results

The channel to be described here in liposomes fused with bacterial membranes has also been encountered on the surface of live cells and appears to be an outer membrane protein (M. Buechner, A.H. Delcour, B. Martinac, J. Adler and C. Kung, *in preparation*). Since it displays voltage dependence of the same polarity in patches from the reconstituted membranes as from the surface of intact cells, it is likely that the reconstituted membranes have the same orientation as the native outer membrane. Thus, we have assigned positive pipette voltages as polarizing (cell negative inside) and negative pipette voltages as depolarizing (cell positive inside). The stability of the seals and the frequency of observing this channel in patches made on reconstituted membranes are, however, greater than in intact cells. This method was, therefore, chosen for a detailed investigation of this channel.

Figure 1 shows currents through several open channels of this type. The channels appear mostly open at all polarizing voltages (top trace, −50 mV; currents upon larger polarizations not shown) and at small depolarizing voltage (2nd and 3rd traces, +30 and +50 mV, respectively), but display frequent closures of a few milliseconds to levels of reduced overall current. At higher depolarizing voltages (bottom trace, +70 mV), longer closures are observed, followed by reopenings. We define the most frequent event as the closing of one unit of conductance. It is used as a reference in the measurements of reversal potentials in selectivity experiments (*see below*) and as the common denominator of all larger transitions. Transitions of larger current amplitude correspond to the simultaneous closures of two, three, four or more of the defined unit conductance. These closures appear as discrete events in our recording conditions (filtering at 1

kHz) and therefore suggest cooperativity among these ion-conducting units. All the patches exhibiting this kind of activity contain many multiples of the unit conductance, even when patches were taken from membranes reconstituted at low protein-to-lipid ratios. We do not know whether each unit corresponds to an individual channel protein or is a substate of a partially open larger structure. If each conductance unit in fact represents an individual channel, the observation of cooperativity of multiple units and their co-appearance in membranes from experiments at low protein-to-lipid ratios may indicate clustering of these proteins in vivo.

At low voltages (<+40 mV), closures of one unit are predominant, but transitions of two (Fig. 1, second trace) and three (Fig. 5) units of conductance are also observed. More depolarizing voltages (>+40 mV) increase the number of transitions of two and three units of conductance and also induce closures of four (Fig. 1, bottom two traces) and five (Fig. 5) units of conductance. Occasionally, closures of half the unit conductance or multiples of half the unit conductance are also observed. These events are fairly infrequent and have been ignored in this analysis. They might reflect substates of the channel unit. Brief opening events are also occasionally encountered. Most of them are unresolved spikes, even when filtered at 3 kHz.

The patches contain, in fact, more units of conductance than the number revealed by the size of the largest transition observed. In most cases, a high depolarizing voltage (>+60 mV) will cause an irreversible inactivation of the open channels, seen as a stepwise decrease in the whole current (Fig. 2). In the experiment shown in Fig. 2, the difference between the beginning open-channel current and the leakage current of the patch after channel inactivation (labeled *all closed*) was 79 pA. The single channel current for this experiment was found to be 6.7 pA at +80 mV. The 79-pA current decrement thus corresponds to the closure of 12 conductance units. It is noteworthy that the current steps during inactivation are of unequal amplitude. In the case shown in Fig. 2, for example, the first current decrement is of 31.3 pA, equivalent to the simultaneous closure of about five units. It was followed by smaller decrements in current. Such inactivation is irreversible and is likely to represent a state different from the reversible closure. The channels cannot be reactivated by either hyperpolarizing or depolarizing voltages or the absence of a transmembrane voltage. The inactivation of all the open channels brings about an increase in the resistance of the patch. At the beginning of the experiment shown in Fig. 2, when all the channels are open, the pipette resistance is 0.87 GΩ; after channel inactivation, it becomes 6 GΩ. Values ranging between 5 and 15 GΩ have been obtained after channel inactivation. These pipette resistances are only slightly smaller than those obtained on pure azolectin vesicles. Because inactivation of these channels increases the resistance (i.e., decreases the conductance) of the patch to the level of pure lipid bilayer, we believe that the number of channels present in the patch as calculated from the decrement in whole current is valid. We used this calculation to determine the number of channels in the experiment presented in the Table (*see below*).

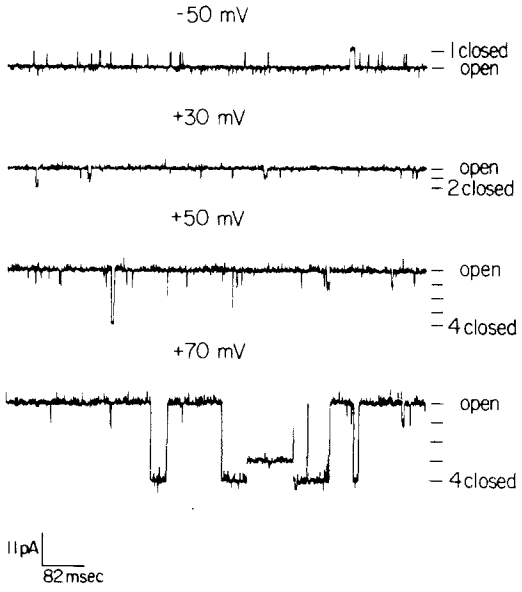

Fig. 1. Current traces of the voltage-dependent channel. Unfractionated membrane vesicles made at 4500 lb/in² were reconstituted at a protein-to-azolectin ratio of 1:75. The patch was excised and the activity recorded in symmetric solution A (*see* Materials and Methods). The current level corresponding to all units open and to the most units closed for this stretch of data are labeled. The other conductance levels are denoted by tick marks. Note that the channels are usually open. The probability of finding them in closed states increases with membrane voltage (stronger depolarization)

The unit conductance, i.e., that of the most frequent current step, is deduced from the current-voltage relationship represented in Fig. 3*A*. The slope conductance, calculated from the linear regression of the data points in the voltage range of −50 to +50 mV (linear regression coefficient $R > 0.99$) is 91 pS, in symmetric 150 mM KCl solutions. The channel appears to rectify slightly both at polarizing and depolarizing voltages. The chord conductance calculated by use of Ohm's law from the current value of the fitted curve at +100 mV (in the voltage range where rectification is observed) has a value of 68 pS. When asymmetric solutions of KCl

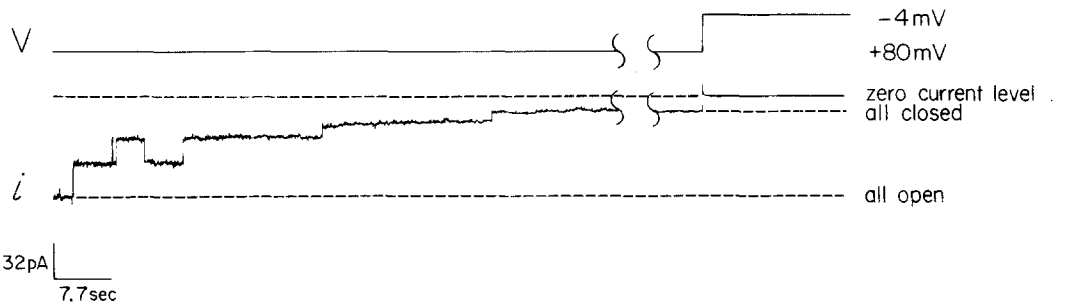

Fig. 2. Inactivation of the channels by +80 mV. Unfractionated membrane vesicles made at 4500 lb/in^2 were reconstituted at a protein-to-azolectin ratio of 1 : 75. The current trace is shown at low time resolution and small amplification. The events of one unit conductance are frequent at the beginning of the trace but are not resolved on these time and amplitude scales. After about 5 min, when the voltage is changed from +80 to −4 mV, there is a difference in current which corresponds to a seal of approximately 6 GΩ. The channels never re-open during the time spent at +80 or −4 mV (of the order of 5 to 15 min)

are applied on each side of the membrane, the observed reversal potential (+24.8 ± 1.2 mV) approaches the calculated reversal potential for K^+ (E_K = +27.7 mV), which indicates the cation selectivity of the channel. From the deviation of the reversal potential from E_K, one obtains by the Goldman-Hodgkin-Katz equation (Aidley, 1978) a ratio of permeability of chloride to potassium (P_{Cl}/P_K) of 0.05. The selectivity among cations is presented in Fig. 3*B*. The following series is obtained from the reversal potentials: $Rb^+ \cong Cs^+ \cong K^+ > Na^+ > Li^+$. A more pronounced rectification is also apparent in the presence of Na^+ and Li^+ at voltages where the current is carried by these ions (in our solutions, positive voltages).

Figure 4*A* and *B* represent the current-voltage relationship of transitions of all amplitudes in symmetric and asymmetric solutions. Data are shown only at positive (depolarizing) voltages, since current steps of amplitude larger than the unit conductance are not observed at negative (polarizing) voltages. Regression coefficients greater than 0.99 are obtained when the data points are fitted to curves intersecting the *X* axis at the same reversal potential as the one-unit conductance curve. The chord conductances of the events of larger current amplitude are multiples of the unit chord conductance. For example, in symmetric solutions, they are 2.1, 3.2 and 3.9 times the unit chord conductance (*see* Fig. 4 legend). These larger transitions, therefore, are likely not to be the closures and reopenings of other kinds of ion channels, but rather the simultaneous closures and reopenings of units of identical conductance and selectivity.

The open channels are sensitive to depolarizing voltages. The histograms plotted in Fig. 5 demonstrate an increase in the frequency of closures when the voltage is made more positive. The steepness of this voltage dependence is more pronounced for the closing of two or more units of conductance than for one unit. For example, in the experiment reported in Fig. 5, there is an increase in the number of one-unit conductance events by 42% between +20 and +90 mV. The increase in the number of closures of multiples of the unit conductance averages 280% between +20 and +90 mV.

The Table compares the observed probability of the unit conductance and each of its multiples being closed at three depolarizing voltages to the closed probability predicted by the binomial distribution. The binomial distribution calculates the probability of having 1, 2, . . . *N* channels closed at the same time in a patch containing *N* channels, on the assumption that these channels are independent and that their gating is random. The observed probabilities of finding three or more units closed at the same time are tens or hundreds time higher than predicted by the binomial distribution. In other words, the units gate together more frequently than by chance if they were independent. The cooperativity, already suggested by the simultaneity of the closures of many identical units, is substantiated by this deviation of the observed closed probabilities from the binomial distribution. However, this discrepancy between observed and predicted values is not apparent for one or two units of conductance. Gating apparently becomes cooperative when more than two units are involved.

Voltage does not affect the probability of the unit conductance being closed, although in some experiments like the one of Fig. 5, the number of closures of one unit is slightly increased when the voltage changes from +20 to +90 mV. Many of these events are short (<1 msec), and, although their number increases with voltage, they do not contribute significantly to an increase in the closed probability. However, the increase in the number of simultaneous closures of two, three and four units is

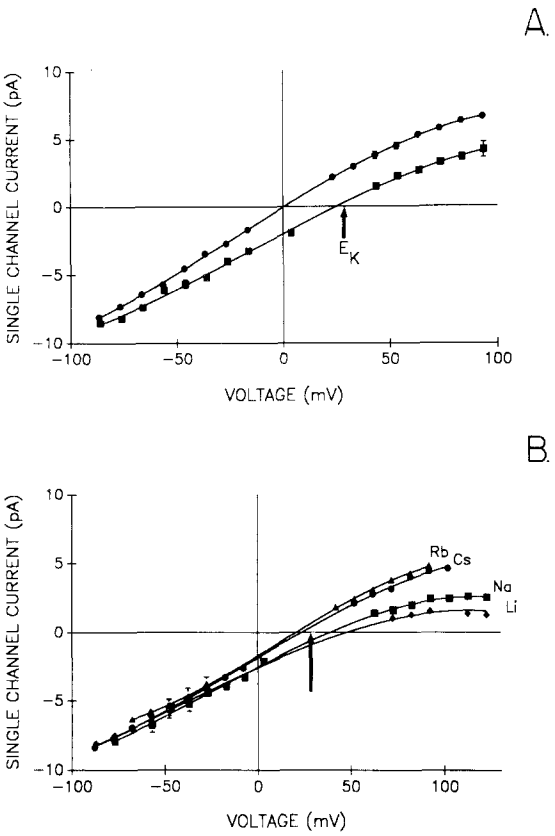

Fig. 3. Selectivity of the voltage-dependent channel as measured by the reversal potentials of current-voltage plots. (*A*) The current amplitude of the most frequent event (the unit conductance) is plotted *vs.* voltage in (●) symmetric solutions (150 mM KCl) and (■) asymmetric solutions (150 mM KCl in the pipette and 50 mM KCl in the bath). The equilibrium potential for K^+ is zero in symmetric solutions (not marked) and is calculated to be +27.7 mV in asymmetric solutions (marked by E_K). (*B*) The current amplitude of the unit conductance is plotted *vs.* voltage in asymmetric solutions containing 150 mM KCl in the pipette and (▲) 50 mM RbCl, (●) 50 mM CsCl, (■) 50 mM NaCl or (◆) 50 mM LiCl in the bath. The arrow indicates the expected reversal potential for K^+ when the bath solution contains 50 mM KCl. In both *A* and *B*, the other components are the same as in solution A (*see* Materials and Methods). The data points were collected from three experiments. Not all recording voltages were repeated in these three experiments. Only the data points obtained at three identical voltages have standard deviations. In some cases, the standard deviation lies within the thickness of the symbol. The data points are fitted to third order polynomials by use of the SigmaPlot program (Jandel Scientific, Sausalito, CA). The reversal potentials are calculated from the polynomial coefficients of the fitted curve: 20.8 ± 1.2 mV (Rb^+), 23.8 ± 1.2 mV (Cs^+), 39.0 ± 2.6 mV (Na^+), 48.0 ± 0.6 mV (Li^+). The standard deviations of the reversal potentials are those obtained from averaging the recording potentials of the experiments

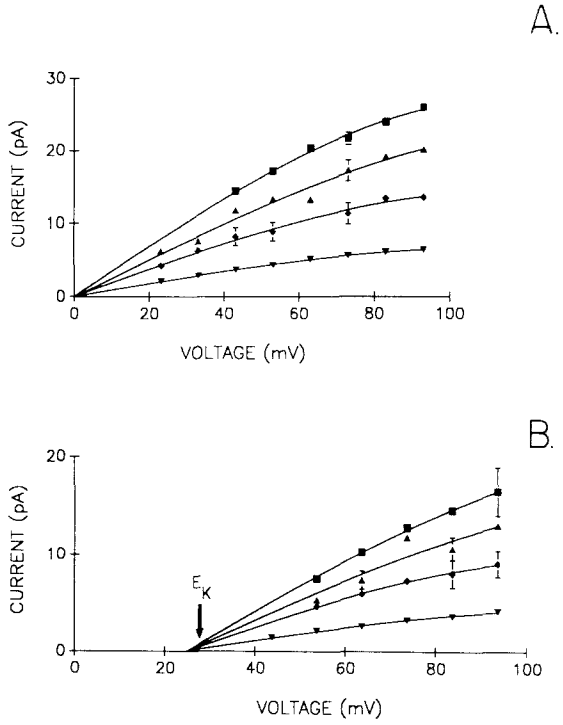

Fig. 4. Current-voltage plots of single and multiples of the unit conductance showing uniform unit size and strong selectivity for K^+ ion. The current amplitude is plotted *vs.* voltage in (*A*) symmetric solutions (150 mM KCl) and (*B*) asymmetric solutions (150 mM KCl in the pipette and 50 mM KCl in the bath). The other components are the same as in solution A (*see* Materials and Methods). The data points are fitted to third-order polynomials. Four units of conductance are represented: (▼) one unit, (◆) two units, (▲) three units, (■) four units. The chord conductances are in symmetric solutions 68 pS (one unit), 142 pS (two units), 215 pS (three units), 266 pS (four units), and in asymmetric solutions 42 pS (one unit), 92 pS (two units), 137 pS (three units) and 174 pS (four units). E_K is the calculated reversal potential for K^+ in asymmetric solutions

paralleled by an increase in the closed probability of these levels, especially when the values at +30 and +70 mV are compared. This suggests that the voltage has both increased the frequency of gating and the time spent in the closed state. This is reflected in the mean closed times: for three units of conductance, they are 5.1 and 26.7 msec at +30 and +70 mV, respectively; for four units of conductance, they are 4.7 and 36.8 msec at +50 and +70 mV, respectively.

We emphasize that the results reported here are intended to illustrate voltage-dependent trends in the behavior of this channel. However, there is some variability from patch to patch. The variability is not apparent when the frequency of closures of the single unit conductance is compared. However, the absolute number of closures of two or more units of conductance for a defined time period shows some variation. The smallest voltage at which closures of a defined amplitude appear varies also between experiments: closures of five units are first observed at +90 mV in Fig. 5, but in other experiments they already appear at +60 mV (*data*

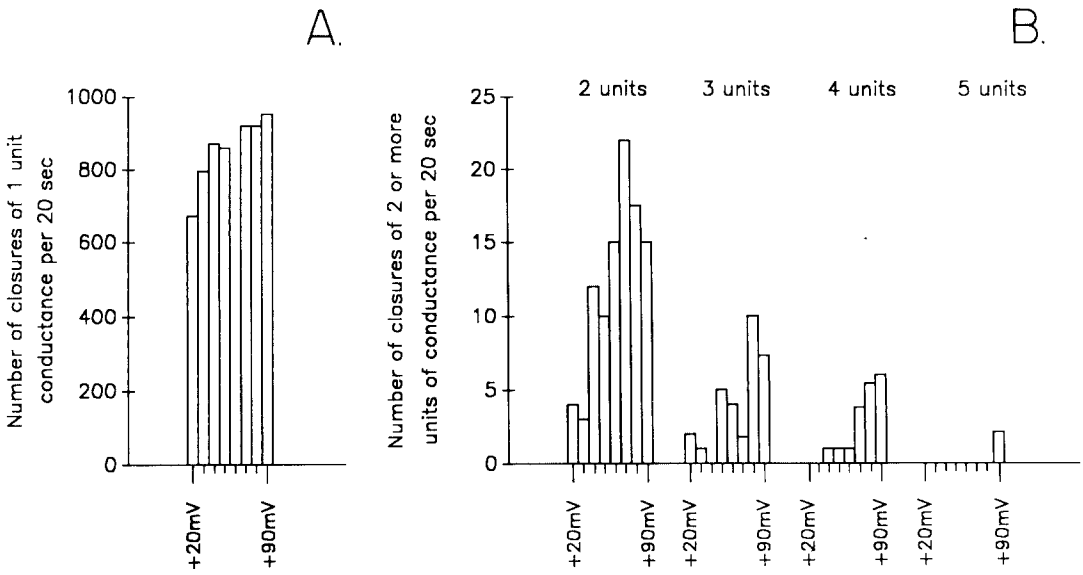

Fig. 5. Histograms of the number of closures of single (*A*) and multiples (*B*) of the unit conductance at depolarizing voltages. Each histogram refers to a defined multiple of the unit conductance. The bars of each histogram represent the number of events at voltages ranging from +20 to +90 mV in 10-mV increments (indicated by tick marks). The absence of a bar at a particular voltage means that no event was detected, except in *A* where the number of closures of one unit conductance was not determined at +60 mV. The data were obtained from one patch

not shown). Finally, the largest transition encountered can be smaller or bigger than five units: for example, the experiment reported in the Table shows a closure of six units simultaneously. We do not know why this variability exists. It might be due to the loss of regulatory factors during reconstitution or to an altered "communication" between the units which would affect their cooperativity.

Discussion

We present here the biophysical properties of a voltage-dependent ion channel of *E. coli*. This channel is found on both the surface of live *E. coli* cells (M. Buechner, A.H. Delcour, B. Martinac, J. Adler and C. Kung, *in preparation*) and in liposomes into which an outer membrane fraction has been reconstituted. The fingerprint of the channel is a high open probability (greater than 0.9) and rapid gating to closed levels. These brief excursions to closed levels are very frequent: in the experiment of the Table, the mean open time is on the order of 37 msec. The size of the major current step is 91 pS in symmetric solutions of 150 mM KCl.

An unusual characteristic of this channel is the high degree of cooperativity among the ion-conducting units. This cooperativity appears to be enhanced by depolarizing voltages, which bring about not only more frequent transitions of the multiples of the unit conductance, but also an increase in the number of units that gate simultaneously. The voltage, however, does not significantly affect the probability of the unit conductance being closed nor the overall closed probability. It seems that it does not increase the probability of each individual channel being closed, but rather the tendency of the units to close simultaneously.

The voltage dependence of this channel is asymmetric. The channel does not seem sensitive to hyperpolarizing voltages, where multiples of the unit conductance are only occasionally seen. The voltage dependence is reversible when the membrane is maintained depolarized under a certain threshold, which varies somewhat from experiment to experiment. Above the threshold, there is an irreversible inactivation of all the channels. The channels appear to be locked into a long-lived stable closed state different from the closed state from which they can reopen.

We believe that this channel resides in the outer membrane for reasons detailed elsewhere (M. Buechner, A.H. Delcour, B. Martinac, J. Adler and C. Kung, *in preparation*). In the growth conditions used with this strain of *E. coli* K12, the OmpF and OmpC porins are expressed, as judged by the presence of these porins bands on SDS/urea gels and from the sensitivity of the strain to colicin A and phage SS-4 (*data not shown*). The levels of porins PhoE and LamB should be minimal, since their expression is induced by phosphate starvation and presence of maltose, respectively (Nikaido &

Table. Comparison of the observed and expected probability of single and multiples of the unit conductance being closed at three depolarizing voltages[a]

		+30 mV	+50 mV	+70 mV
	p_c	3.1×10^{-3}	3.6×10^{-3}	1.1×10^{-2}
	n	1971	1949	2009
1 unit	$P'_{c(obs)}$	3.4×10^{-2}	3.4×10^{-2}	3.8×10^{-2}
	$P'_{c(exp)}$	3.6×10^{-2}	4.1×10^{-2}	1.2×10^{-1}
	n	30	73	55
2 units	$P'_{c(obs)}$	3.4×10^{-4}	4.3×10^{-4}	5.3×10^{-3}
	$P'_{c(exp)}$	6.1×10^{-4}	8.2×10^{-4}	7.1×10^{-3}
	n	7	21	13
3 units	$P'_{c(obs)}$	3.6×10^{-4}	3.9×10^{-4}	3.7×10^{-3}
	$P'_{c(exp)}$	6.4×10^{-6}	9.9×10^{-6}	2.6×10^{-4}
	n	0	31	49
4 units	$P'_{c(obs)}$	—	1.0×10^{-3}	1.8×10^{-2}
	$P'_{c(exp)}$	—	8.1×10^{-8}	6.6×10^{-6}
	n	0	0	0
5 units	$P'_{c(obs)}$	—	—	—
	$P'_{c(exp)}$	—	—	—
	n	0	0	1
6 units	$P'_{c(obs)}$	—	—	2.9×10^{-5}
	$P'_{c(exp)}$	—	—	1.5×10^{-9}

[a] The observed probabilities were obtained from an experiment in which stretches of data were collected for 100 sec at each voltage. The expected probability of being closed, assuming no cooperativity, was calculated from the binomial equation:

$$P'_c = [N!/\{K!(N - K)!\}]\, p_c^K\, (1 - p_c)^{N-K},$$

where p_c is the overall probability of being closed, P'_c is the probability of the Kth level being closed, and N is the total number of channels in the patch. The number of channels was found to be 12 in this experiment, from the measurement of the current decrease during and after channel inactivation (*see* Fig. 2). The observed number of closures at each voltage is represented by n. When no closures were detected ($n = 0$), we did not make the comparison between observed and expected probabilities (indicated by a dash mark). The significant to immense differences between the expected and the observed probabilities of multiple units being closed indicate cooperativity.

Vaara, 1987), both conditions not used in our study. Although the purpose of this paper is to describe the properties of this channel and not to find its structural identity, a comparison of its properties with those of the well-known outer membrane channels, porins, is of some interest, nonetheless.

Because of its high probability of being open, this channel reminds us of porins. In some experiments, porins have shown voltage-dependent closures (Schindler & Rosenbusch, 1978, 1981; Dargent et al., 1986; Xu et al., 1986; Mauro et al., 1988). The pattern of porin closures is, however, different from the one of this channel. In general, porins are open for long periods of time and appear to close in three steps, behavior which has been correlated to their trimeric structure. The channel described here gates very frequently and has one major step. The size of this transition, 91 pS, is also smaller than the conductance reported for porins, calculated to be about 300 pS in our experimental conditions (Benz et al., 1978).

The voltage-dependent channel is similar to porins in terms of ionic selectivity. The porins are usually considered as nonspecific channels. However, a P_{Cl}/P_K of about 0.04 has been reported for OmpC by Benz, Schmid and Hancock (1985) and Lakey, Watts and Lea (1985). This value is close to the one we find for the voltage-sensitive channel (0.05) and would predict a reversal potential of +25.1 mV for OmpC in our asymmetric conditions. This value is close to the one observed with the voltage-sensitive channel (+24.8 mV, Fig. 3*A*). A specificity among cations similar to the one we found here has been reported for porins (Benz, Janko & Läuger, 1979). In that report, the conductances found in RbCl, CsCl and KCl solutions are also close to each other, with a slight preference for RbCl, while the conductances in NaCl and LiCl deviate more from the KCl conductance. This trend is also exemplified in the reversal potentials of the voltage-dependent channel in various cation solutions (*see* legend of Fig. 3).

The voltage dependence of the channel described here contrasts, however, with the behavior of porins. In planar lipid bilayers, the latter need first to be activated by an initial voltage step, then relax to a steady state of opening and closing events. They can then be re-activated by application of an electrical potential (Schindler & Rosenbusch, 1981). The voltage-dependent channel described here is open at rest; its activity can be observed at 0 mV in asymmetric solutions, but after voltage-dependent closure, it cannot be re-activated. Schindler and Rosenbusch (1981) observed hysteresis delays of up to 6 hr for the re-opening of voltage-inactivated channels from *E. coli* outer membrane. However, this hysteresis behavior occurs only when more than 30 channels have been activated by large initiation voltages; when the cluster size is smaller (<30 channels), there is no hysteresis. We were able to study only patches that contain a small (<15) number of channels, because the presence of too many channels would yield unfavorable seals. The difference of re-activation kinetics seen between our study and that of Schindler and Rosenbusch may reside in the different identity of the channel under study or in the reconstitution method.

We do not believe that the porins have been destroyed during membrane purification or the fu-

sion procedure, since they are known to be robust. In over 200 patches we have analyzed, we have never seen a channel behavior as described for porins in planar lipid bilayer studies: large conductance, open time in the minute-time region, trimeric gating (Schindler & Rosenbusch, 1981; Dargent et al., 1986; Mauro et al., 1988). The comparison should, however, be viewed with great caution since there are very important methodological differences in our study and those before us. Even the behavior of porins might depend on experimental conditions. For example, Schindler and Rosenbusch (1981) found that the porin conductance changes with lipid composition of the planar lipid bilayer and the size of the channel cluster. The voltage dependence of porin activity might also be affected by experimental conditions (Lakey, 1987).

All the studies on the electrical activity of porins have been done with planar lipid membranes (Benz, 1985). The size of the bilayer under study is much larger than in patch clamp. This leads to large capacitative effects and slow rise time, which might obscure the detection of fast events. The development of the vesicle fusion technique has allowed us to use the patch-clamp technique rather than planar lipid bilayer electrophysiology for the study of bacterial membranes in vitro. The patch-clamp method has the advantage of resolving fast events detected on the membrane of live cells or membranes which have not received any harsh treatment and might give a more accurate account of the channel activity *in vivo*. Although our method of preparing the biological material is very similar to that used by others (Schindler & Rosenbusch, 1981), the electrical properties of the bacterial membranes may be highly sensitive to the reconstitution method and to the lipid environment. This might explain, in part, the differences in patterns of electrical activity observed in vesicles studied by patch clamp or fused with planar lipid bilayers. To avoid the drawbacks of a seemingly difficult comparison between the properties of this channel and porins, we are attempting to identify this channel with porin(s) by use of porin mutants (A.H. Delcour, B. Martinac, J. Adler and C. Kung, *in preparation*).

The outer membrane of *E. coli* has been considered as a static structure, full of permanently open pores. The discovery of a mechanosensitive channel (Martinac et al., 1987) and the voltage-dependent channel reported here suggests that the outer membrane might be a more dynamic entity, whose role would not be limited to a permeability barrier. The voltage- and mechano-sensitivity of these proteins might confer to this membrane a role in cell growth, osmosensing and immediate response to environmental conditions.

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