J. Membrane Biol. 119, 267–275 (1991)
002226319100025M

The Journal of
Membrane Biology

A Single Amino Acid Substitution Alters Conductance and Gating of OmpC Porin of *Escherichia coli*

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Summary. We have reconstituted into liposomes outer-membrane fractions from *Escherichia coil* strains which express OmpC porins with altered pore properties. Single-channel experiments were performed with the patch-clamp technique on blisters generated from the reconstituted liposomes. Our goal was to identify positively the activity pattern of OmpC in our reconstituted system. The properties of the parent strain were compared to those of a strain whose OmpC porin has a single amino acid substitution in a postulated transmembrane segment. The parent and the mutant strain each exhibit a cation-selective channel of high open probability and gating to closed levels of various amplitudes. However, the mutant channel appeared to be 9 to 30% larger in unit conductance. It tended to close and reopen most often in groups of three units, as opposed to two units in the parent channel. The results are discussed in terms of the observed phenotype and of their implication as to the structure-function relationship of the porin channels.

Key Words *Escherichia coli* · ion channel · mutant · patch clamp · porin

Introduction

The Gram-negative enteric bacterium *Escherichia coli* has two membranes. The inner cytoplasmic membrane, made of phospholipid, is like the cytoplasmic membrane of eukaryotic cells in that it contains the protein machinery responsible for ion and metabolite transport and stimulus sensing, in addition to the enzymes of the respiratory chain. The outer membrane, which is made of phospholipid and lipopolysaccharide, plays the role of permeability barrier, protecting the cell against bile salts, digestive enzymes and antibiotics, and preventing the loss of periplasmic proteins, but allowing the passage of small hydrophylic solutes, nutrients and stimuli for chemotaxis. The molecular sieving properties of the outer membrane are due to the presence of large diffusion pores, called "porins," which act as specific or nonspecific channels (Nikaido & Vaara, 1985; Benz & Bauer, 1988).

The functional properties of porins have been studied by a variety of biochemical and electrophysiological techniques (Benz, 1985, 1988; Nikaido & Vaara, 1985). Purified proteins or outer membrane vesicles have been reconstituted into planar lipid bilayers and the electrical properties of the reconstituted membranes revealed that the porins are large channels, open for long periods of time (seconds), showing a voltage-dependent closing activity in most studies (Schindler & Rosenbusch, 1978, 1981; Dargent et al., 1986; Mauro, Blake & Labarca, 1988). The two major porins, the products of the *ompC* and the *ompF* genes, are called nonspecific porins. They form channels which favor cations over anions (Benz, Schmid & Hancock, 1985; Lakey, Watts & Lea, 1985), but do not allow the flow of compounds of molecular weight greater than 600 (Nakae, 1986). They are assembled into homo- or heterotrimers by the random mixing of the 33- to 36-kD polypeptides before polymerization (Gehring & Nikaido, 1989). It is not clear, either from structural or functional studies, whether the three channels of a trimer form a single functional pore, or whether individual monomers can open and close independently. That the trimer is the functional unit is suggested by the loss of permeability by dissociated porin oligomers (Nakae, Ishii & Tokunaga, 1979). However, individual channel closing events were also seen in the apparent trimeric gating of porin (Engel et al., 1985).

A minor porin, the product of the *lamB* gene, is maltoporin which is required for growth on maltose and maltodextrins. In planar lipid bilayers, LamB forms ion-conducting pores of conductance smaller than OmpF, which can be blocked completely by

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the addition of maltose and maltodextrins (Benz et al., 1986). The results suggest the existence of a saturable binding site inside the LamB pore, confering the specificity for transport (Benz & Bauer, 1988).

The *ompC* and *ompF* genes have been cloned and sequenced (Inokuchi et al., 1982; Mizuno, Chou & Inouye, 1983). Studies aimed at defining functional domains of these nonspecific porins, as well as of maltoporin LamB, have been carried out by the use of mutations in defined regions of the proteins (Heine et al., 1988; Misra & Benson, 1988*a,b;* Ferenci & Lee, 1989) and by the construction of hybrid proteins (Nogami, Mizuno & Mizushima, 1985; Tommassen et al., 1985; van der Ley et al., 1987, Benz et al., 1989). LamB$^-$ cells grow poorly on maltose and oligosaccharides, which are presumably too bulky to enter efficiently the cell *via* the nonspecific OmpF and OmpC porins. By selection for growth on maltose and maltodextrins in the absence of the LamB and OmpF porins, Misra and Benson (1988*a,b*) have isolated and characterized *E. coli* mutant strains whose OmpC porins are postulated to have an enlarged pore, now able to accommodate a flow of maltose or its oligomers sufficient for growth. They have identified four amino acid residues, mutations in each of which appear to affect the pore size of the OmpC channel.

One of these mutations is the substitution of an arginine for a cysteine at residue 37. We have investigated the electrophysiological properties of this mutated strain (RAM276) and compared them to those of its parent (RAM105). Our goal was to positively identify the activity pattern of OmpC in our reconstituted system, in order to compare it to a nonidentified channel of the outer membrane with properties similar to those documented for porins (Delcour et al., 1989*a,b*). We have prepared and reconstituted outer-membrane vesicles and performed patch-clamp experiments on blisters made from the reconstituted liposomes, according to a published method (Delcour et al., 1989*a*). Conductance, selectivity, and gating pattern of single channels were compared between the parent and the mutant strain. They each exhibit a channel with some common properties: it has a high open probability (>0.9), is cation selective and closes in current steps of various amplitudes. However, we found in the mutant channel an increase in the conductance as well as altered gating kinetics and cooperativity. This channel is absent in two different OmpC$^-$ OmpF$^-$ porin mutants and we believe it to represent OmpC porin. These observations have previously been presented in an abstract (Delcour et al., 1990).

Materials and Methods

Preparation of Biological Material

Cells of the following *E. coli* K12 strains 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: RAM105 (OmpF$^-$ LamB$^-$ OmpC$^+$) (Misra & Benson, 1988*b*), RAM276 (OmpF$^-$ LamB$^-$ OmpC(Dex))(Misra & Benson, 1988*a*) and HN600 (OmpF$^-$ OmpC$^-$) (von Meyenburg & Nikaido, 1977) and HN705 (OmpF$^-$ OmpC$^-$) (H. Nikaido, *unpublished*). A fraction enriched in outer-membrane vesicles was prepared as described (Delcour et al., 1989*a*) after passage of the cells through a French press (American Instrument, Silver Spring, MD) at 4500 lb/in^2. These vesicles were fused with liposomes of azolectin (Sigma, St. Louis, MO) at a protein-to-lipid ratio of 1 : 750 for the RAM strains and 1 : 75 for the HN strains, and blisters were induced on these reconstituted liposomes according to the method of Delcour et al., (1989*a*). In some experiments with the HN strains, unfractionated membrane vesicles were fused with azolectin at a protein-to-lipid ratio of 1 : 30.

Electrical Recording

Patch-clamp experiments were carried out according to standard technique (Hamill et al., 1981) on the liposome blisters (Delcour et al., 1989*a*). The standard pipette and bath solution (solution A) was 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. The patches were excised by air exposure and the current was recorded with an EPC-7 amplifier (List-Electronic, Darmstadt, FRG) on chart (Gould, Cleveland, OH) and on tape (Indec, Sunnyvale, CA). The data were filtered at 1 kHz (Frequency Devices, Haverhill, MA), digitized at a sampling rate of 10 kHZ and analyzed on computer (Indec Systems, Sunnyvale, CA) with a program developed by Dr. Yoshiro Saimi.

Data Analysis

The *I/V* plots were corrected for offset potentials on the basis of the zero reversal potential expected for the control curve in symmetric 150 mM KCl solutions. The deviations of the observed reversal potentials from zero varied but were no more than 10 mV and judged insignificant. Because this correction varied among the experiments, the coordinates of the data points of the *I/V* plots are the average of the current amplitudes (*Y* coordinate) and of the corrected recording voltages (*X* coordinate). Since the orientation of the channel in the reconstituted membrane is unknown, the voltages are plotted as pipette voltages.

In most cases, the single-channel current measurements were obtained from the Gaussian fit of amplitude histograms. When the number of events was too small to generate a reliable amplitude histogram, the current amplitude of each event was measured individually on a time-expanded trace and an average was made of all the measurable events of the recorded trace. We define the unit conductance as the smallest transition measured. This level is rarely observed. The two most frequent transitions are two or three times that of the unit conductance and appear to represent the simultaneous closure of 2 or 3 units, respectively, as described for a voltage-dependent channel of a different *E. coli* strain (Delcour et al., 1989*b*). In order to average the current

Table 1. Conductance (G) and reversal potentials (E_{rev}) of the OmpC channel in RAM105 (parent) and RAM276 (mutant)

Buffer[a]	Strain	2 Units			3 Units		
		G[b] (pS)	$\%G$[c]	E_{rev}[d] (mV)	G[b] (pS)	$\%G$[c]	E_{rev}[d] (mV)
150/150	RAM105	116 ± 3	100	−0.1	162 ± 9	100	0.0
	RAM276	127 ± 3	109	−0.3	210 ± 6	130	1.9
150/50	RAM105	101 ± 3	100	−18.9	156 ± 9	100	−20.6
	RAM276	110 ± 2	109	−20.3	177 ± 7	113	−18.3
150/950	RAM105	213 ± 3	100	23.0	318 ± 8	100	21.0
	RAM276	253 ± 5	119	22.3	396 ± 7	124	19.9

[a] The buffer solutions used are given in mM of KCl in pipette/bath.
[b] Conductance values are given ± SE (*see* Materials and Methods).
[c] The percentage of the conductance with respect to that of RAM 105.
[d] Given as pipette voltages.

amplitudes, we assigned current steps of amplitude ranging between 1.5 and 2.5 times the unit conductance to the simultaneous closure of 2 units, between 2.5 and 3.5 times the unit conductance to the simultaneous closure of 3 units, and so forth. This algorithm was also used in counting the number of closures plotted in Fig. 2.

Regression analysis was performed on each I/V plot, according to a standard algorithm (Sokal & Rohlf, 1987), and the lines were drawn with the Sigma-Plot program (Jandel Scientific, Sausalito, CA). In all cases, regression coefficients were greater than 0.9. The slope and intercept of each curve, obtained from the regression analysis, are summarized in the Table and represent the conductance and reversal potential, respectively. The SE of the conductance was obtained from the regression analysis of all the data points collected at many different voltages in three separate experiments. A statistical analysis of the significance of the difference in the slopes obtained with RAM105 and RAM276 was performed with an F test (Sokal & Rohlf, 1987).

Results

When outer-membrane vesicles of either RAM105 (the parental strain with wild-type OmpC) or RAM276 (with a mutated OmpC presumed to make a larger pore) are fused with artificial liposomes, the most frequently observed channel is a cation-selective channel with a typical gating pattern; the channel is open most of the time at all voltages, but displays brief closures of various amplitudes (Fig. 1*A* and *B*). The frequency of occurrence of this kind of channel is markedly reduced in two different double porin mutants: HN705 which lacks OmpF and OmpC and HN600 which expresses reduced amount of the two major porins (E.Y. Rosenberg & H. Nikaido, *personal communication*). Between the parental and the mutant strains, the channel shows some variations, to be described here. For these reasons, we believe that it represents the OmpC porin. This channel has been encountered in more than 20 experiments and its activity has been studied at many different voltages and for long periods of time (some of them lasting for more than 1 hr).

The current traces at low time resolution (top traces of Fig. 1*A* and 1*B*) demonstrate the difference in gating kinetics between the two strains. Closures are observed as brief spikes, which occur about 10 times more frequently in the mutant strain than in its parent. The mean open time ranged from 3.6 to 10.0 sec in RAM105 and from 0.3 to 0.8 sec in RAM276, in two separate experiments each. This corresponds to the channels being closed 0.15–0.25% of the time in RAM105 and 0.9–2.2% of the time in RAM276. This difference in kinetics is not due to a difference in the number of open channels, because it exists even when we compare patches with more channels in RAM105 than in RAM276 (the number of channels was estimated by dividing the leakage current change by the single-channel current, when solutions of 950 mM KCl were exchanged for 150 mM KCl in patches of RAM105 and RAM276 which had a similar seal resistance).

The current traces displayed at a high time resolution (bottom traces of Fig. 1*A* and 1*B*) show that, in both strains, closing events appear as square-pulse transitions of two main amplitudes. The larger one is about 1.5 times as great as the smaller one. Figure 1*C* is an amplitude histogram of the current trace obtained at +50 mV on liposomes reconstituted with RAM276 outer membrane. Similar amplitude histograms could be routinely generated with RAM276, thanks to the large number of closures. It shows that the amplitudes of current decrease during closures are distributed as two distinct, well-resolved, Gaussian curves corresponding to the two main types of conductances.This histogram of cur-

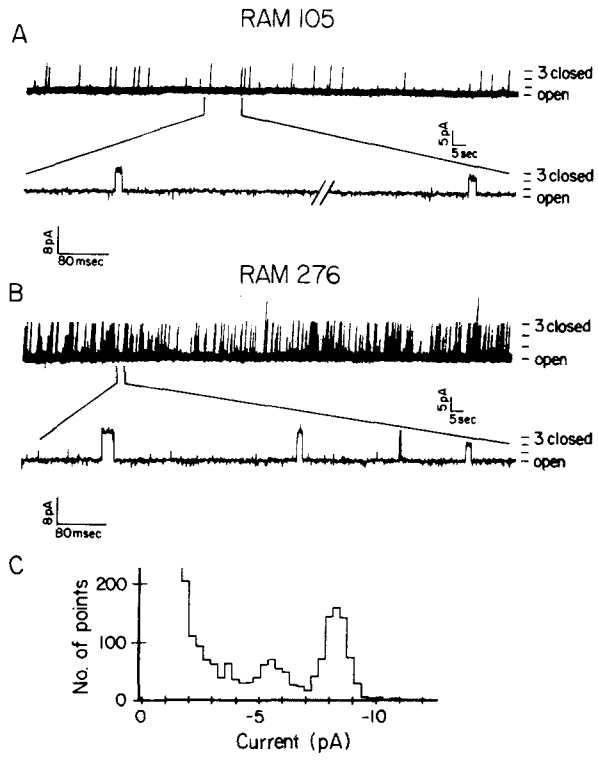

Fig. 1. (*A*) Current traces of the OmpC channel from parental strain (RAM105) in symmetric KCl solution A. Since we could not find two contiguous closures within 1 sec showing the two main conductance levels because of low frequency of gating, the bottom trace is a composite of two separate regions of the same 20-sec stretch of continuous recording. (*B*) Current traces of the OmpC channel from the mutant strain RAM276 in symmetric KCl solution A. In both *A* and *B*, the recording pipette voltage was −40 mV. The current levels corresponding to all units open and to 3 units closed are labeled. The other conductance levels are indicated by tick marks only. Because the top traces of *A* and *B* were recorded with a chart recorder, the amplitude of the spikes cannot be measured accurately and might show some apparent discrepancy with a more accurate measurement made from the expanded traces. (*C*) Amplitude histogram of the current recorded at a pipette voltage of +50 mV on liposomes reconstituted with RAM276 outer membrane. The fully open level was set as the zero current level and current amplitude was measured as a decrease in the whole current; this is why the current is plotted on the *x*-axis as negative numbers. The amplitude histogram was generated with a bin size of 0.3 pA. The Gaussian fit of the peaks gave two main conductance levels of absolute value 5.4 and 8.1 pA

rent amplitude is consistent with the histogram of frequency of closures (Fig. 2*C* and 2*D*) since it shows that the peak of lower conductance (2-unit closures) has a smaller area than the peak of larger conductance (3-unit closures). The large number of points of amplitude less than 5 pA in the histogram of Fig. 1*C* corresponds to spikes and open-channel noise which are not resolved from the current fluctuations around the baseline (which was set at the fully open level, *see* figure legend). Since these two major conductance levels have the same reversal potentials in asymmetric solutions (*see* Table), it is unlikely that they represent two different types of channels, but are rather the result of the cooperative closing, followed by cooperative reopening, of 2 or 3 identical units of conductance. This gating pattern has also been described in another strain of *E. coli*, although the identity of that voltage-dependent channel of the outer membrane was not known (Delcour et al., 1989*b*). Because we do not know whether each conductance level corresponds to an individual channel protein or is a substate of a larger structure, we refer to the conductance levels as multiples of a "unit," which refers the smallest conductance observed.

Consistent with the notion of cooperative gating, rarer events of 1 unit or more than 3 units of conductance do occur. The histograms of Fig. 2 show the number of closures of different amplitudes which were counted during a 100-sec recording at six different voltages, in two separate experiments for each strain. In RAM105, no events of amplitude larger than 5 units were observed in those two experiments. However, a small number of such events, as well as the closures of only 1 unit, were seen at some voltages in RAM276. These numbers are small and do not appear clearly on the scale used in Fig. 2*C* and *D* (*see* figure legend for detailed description). There are two striking differences between the two strains: (*i*) the absolute number of closing-reopening events per 100 sec is much larger in the mutant RAM276 than in the parent RAM105. This increased activity is obvious even in Fig. 1*A* and *B* without any quantitative analysis. (*ii*) Although the two most frequent transitions are, for both strains, the cooperative closures and reopenings of 2 and 3 units, the distribution of those transitions is reversed in the two strains. In the mutant strain (RAM276), 3 units tend to gate cooperatively more frequently than 2 units, and this is reversed in the parent strain (RAM105). Therefore, the most frequent transition in the mutant is of a larger size than that of the parental strain. This might have some implication on other known phenotypes of the mutant, as discussed below.

A clear voltage dependence in the gating frequency has been documented in a similar channel of a different *E. coli* strain, AW405 (Delcour et al., 1989*b*). In the study reported here, a voltage dependence is clearly observed for the simultaneous closures of 3 units in the mutant RAM276 in three out of four experiments. One of these three experiments is shown in Fig. 2*C*; the number of closures increases when the pipette voltage is made more positive (or less negative) from −90 to +70 mV. Figure 2*D* shows the experiment in which the voltage depen-

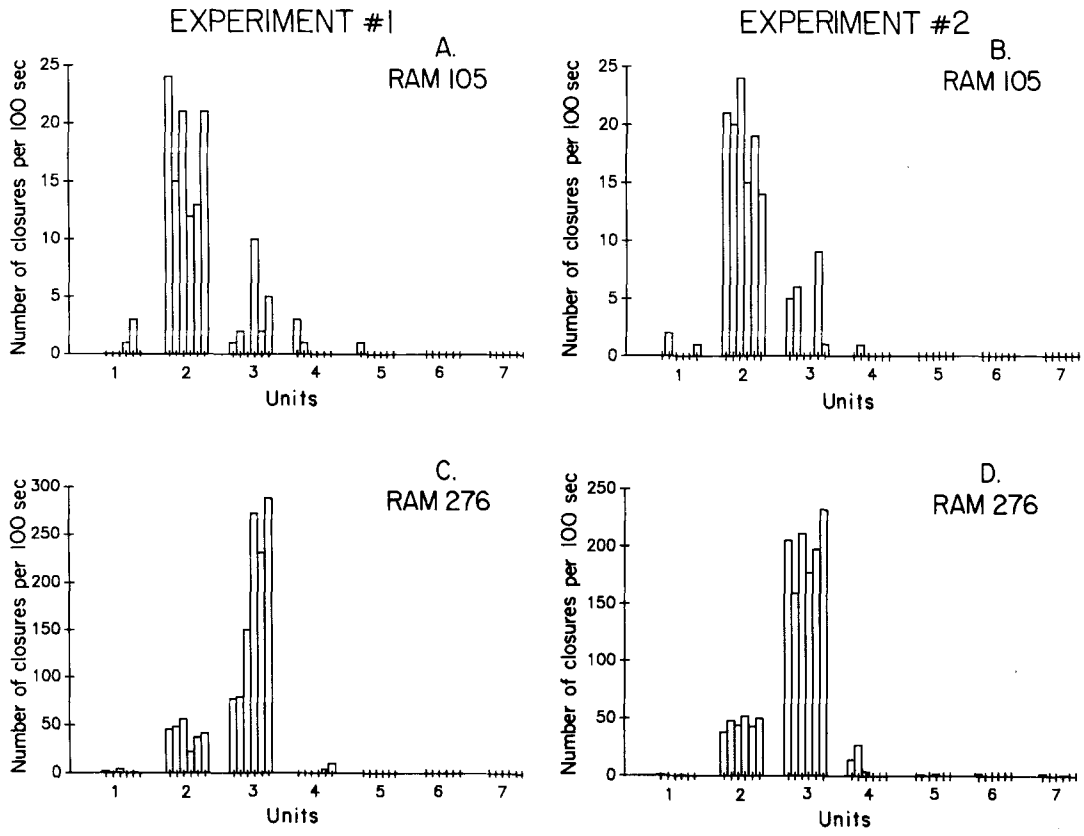

Fig. 2. Histograms of the number of closures of single and multiples of the unit conductance obtained from two separate experiments for each strain. In both experiments, the parent channel (RAM105) tends to gate frequently at the 2-unit level, while the mutant channel (RAM276) tends to gate at the 3-unit level. The bars of each histogram represent the number of events at pipette voltages indicated by tick marks, which are from left to right −90, −70, −40, +30, +50, and +70 mV. The events are counted as described in Materials and Methods. The absence of a bar means that no event was detected. This method of plotting clustered bars is to ease the detection of systematic increase or decrease of the gating probability with voltage within each cluster. Voltage dependence is best seen in *C* for 3-unit events of the mutant RAM276. Although they do not appear clearly on the graph because of the scale, a small number of events were detected for RAM276 for 1 unit (at −90, −40, +30, and +50 mV on *C*; at −90 and +30 mV, on *D*), 5 units (at −70 and +30 mV on *D*), 6 units (at −90 mV on *D*) and 7 units (at −90 and +30 mV on *D*)

dence of the gating of 3 units is not as pronounced. In all experiments with RAM276, a voltage dependence was not clearly observed for the gating of 2 units.

Assessing the extent of a voltage dependence is more difficult in the parent RAM105 than in the mutant RAM276. This is in part due to the slower kinetics of the wild-type channel which yields a smaller number of events for the same length of recording. A slight voltage dependence might exist for the closure of 3 units, but appears not to be as strong as in the mutant. That a voltage dependence is more pronounced in mutant than in wild type has also been observed for a deletion mutant of OmpC (Rocque & McGroarty, 1990). Some voltage dependence in the closures of 2 units could be discerned in two out of six experiments with RAM105 (one of them is shown in Fig. 2*B*). It is noteworthy that the number of closures increases when the pipette voltage is made more negative (or less positive), as opposed to what is observed for the gating of 3 units.

Occasionally, the channels would become inactivated or lost when pipette voltage was made more negative than −50 mV. This was also seen, in a more consistent fashion, in AW405 (Delcour et al., 1989*b*). In addition to the closing pattern we have studied here, the channels also show very frequent rapidly flickering spikes of opening and reclosure. Although these channel activities can be seen distinctly (*see* expanded traces of Fig. 1*A* and *B*), they cannot be resolved given our recording system, even when filtered at 3 kHz. We, therefore, could not obtain conductance measurements or kinetic information from them. They account for the thickness of the open level in the top trace of Fig. 1*A* and *B*.

Figure 3 shows the current-voltage relationships of the two main transitions, i.e. the cooperative clo-

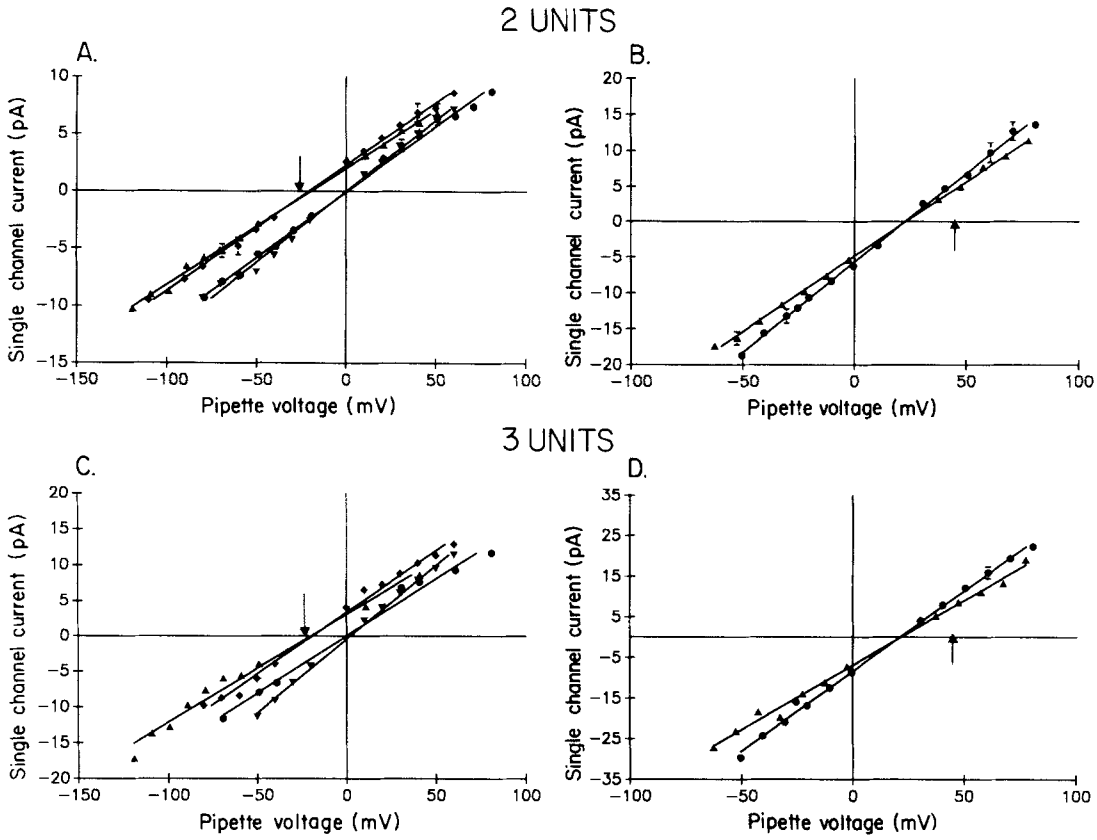

Fig. 3. Current-voltage relationships of OmpC in parent and mutant strains. The current amplitude of the two most frequent events, i.e., the simultaneous closures of 2 units (*A* and *B*) and 3 units (*C* and *D*) are plotted *versus* voltage. *A* and *C* show the results in symmetric solutions of 150 mM KCl (● for RAM105; ▼ for RAM276) and in asymmetric solutions of 150 mM KCl in the pipette and 50 mM KCl in the bath (▲ for RAM105; ◆ for RAM276). *B* and *D* show the data obtained in asymmetric solutions of 150 mM KCl in the pipette and 950 mM KCl in the bath (▲ for RAM105; ● for RAM276). In all cases, the line of the mutant RAM276, from regression analysis, has a higher slope than that of the parent RAM105 (*see* Table). The other components of the solutions are the same as in solution A (*see* Materials and Methods). The equilibrium potential for K^+ is zero in symmetric solutions (not marked) and −27.7 mV for asymmetric solutions of 150 mM KCl in the pipette and 50 mM KCl in the bath, and +46.5 mV in those of 150 mM KCl in the pipette and 950 mM KCl in the bath (marked by arrows). The data points were collected from three experiments but not all recording voltages were repeated. Only the data points obtained at three identical voltages have SD. In some cases, the SD lie within the thickness of the symbol

sures of 2 and 3 units, in three different solutions. The reversal potentials in asymmetric KCl solutions approached E_K, demonstrating the cation preference of the channel. No significant difference in reversal potentials was seen between the two strains, indicating no large change in the K^+ *vs.* Cl^- preference in the solutions tested. The Table summarizes the values of the conductance obtained from the linear regression of these plots. A 9 to 30% increase in the conductance of the mutant channel over that of the parent was found in various solutions. This difference is significant at a confidence level of 2.5% or lower, in all cases except for 3 units in 150 mM/50 mM KCl solutions, where the confidence level is greater than 5%. However, for this condition, the small number of entries which went in the averaged current amplitude, especially for RAM105, might have accounted for a larger scatter of the data from the regression slopes than in other experiments. We believe that this small but significant difference between the conductances in five out of six plots represents a change in the channel properties of the mutated OmpC, as already suggested by Misra and Benson (1988*a*).

Discussion

We have previously described a voltage-sensitive channel in the *E. coli* outer membrane, with properties similar to those reported for porins (Delcour et al., 1989*a*,*b*). In an attempt to identify that channel

as one of the porins, we needed to positively assign channel activities recorded in our reconstituted system as representing specific porin activities. We have used the comparison of channel properties of wild-type and mutant porins to achieve that goal. Although Misra and Benson (1988*a*,*b*) have described a series of porin mutants, the analysis of only one of them was sufficient to identify positively channel activities as belonging to OmpC. Analyses of a large number of mutants to the detail given in this paper are impractical using the present technique, which is rather laborious. On the other hand, similar experiments can also be performed with a selected OmpF mutant in the future to identify the electric activities of OmpF.

We describe here a cation-selective channel with different properties in an OmpC mutant of *E. coli* than in its parent. We feel that this channel is the OmpC porin because its activity is absent in an $OmpC^-$ mutant and because some of the differences observed between the parent and the mutant channel activities are expected on the basis of the cellular phenotypes. Misra and Benson (1988*b*) suggested that the OmpC mutants which they isolated have enlarged pores that allow passage of maltose and maltodextrins. They suggested that the mutation might have altered intra- and intermolecular interactions. These modified intramolecular interactions (i.e., within a monomer) could create a change in the single-channel conductance. We have found that the mutant channel has indeed a larger conductance. It is, however, difficult to ascertain whether the measured 9 to 30% increase in KCl solutions could account for the flow of bulky molecules, such as maltotetrose and maltopentose shown to support growth of the mutant, but not its parent, as sole carbon sources (Misra & Benson, 1988*a*). A direct test with our method is not possible since maltose is not charged.

It is also possible that the pores are made larger because of modifications in the intermolecular interactions, e.g., among monomers of a trimer. We have found that such modifications have indeed occurred since the mutant shows a marked preference for gating 3 units simultaneously, while in the parent, the most frequent transition involves 2 units only. It is not clear whether the porin trimers have three individual ion pathways. A three-dimensional map of OmpF porin proposes that the trimeric pores merge to a single outlet as they traverse the membrane (Engel et al., 1985), but that of PhoE porin suggests that the trimeric channels converge but do not merge into a single large pore (Jap, 1989). A speculative model which would account for our data and the observed phenotypes would be that three monomers merge to form a single pore but have retained the ability to gate individually as well as cooperatively. The cooperativity is such that when 2 units gate preferentially at the same time, as in the parent strain, the size of the pore is smaller than when 3 units gate cooperatively, as in the mutant strain. The passage of maltodextrins would be allowed in the mutant only because the pore size made by the cooperative gating of 3 units is now large enough, and because the mutant channel gates 3 units together most of the time. This model is based on the ideal that, although the transitions we are studying are closures followed by reopenings, they still give an accurate description of the dynamics of the channel at the molecular level. In other words, although most of the porins of the patch are constantly open and do not show closures, we could argue that the size of the functional pore of these open porins in vivo is reflected by the size of the most frequent transition of the porins that do gate in our reconstituted patches. In that case, most of the porins of the parent strain would have 2 units open, while those of the mutant would have 3 units open. The KCl conductance of the functional pore would then increase, as measured, from 116 pS in the parent to 210 pS in the mutant, close to a twofold change (this change takes into account also the 30% increase in monomer conductance, *see* Table). Therefore, we propose that the functional modifications which could account for the observed growth phenotypes would include a small but significant increase in single-channel conductance (possibly monomer conductance) and the formation of an enlarged pore by the preferential gating of 3 units of conductance rather than 2.

The model we propose could reconcile the conclusions from the observations of Nakae et al. (1979) that dissociated porin monomers do not have permeability activities, with those of Engel et al. (1985) who saw individual channel closing events during the trimeric gating of porin. The oligomeric structure needs to be retained for functionality because each monomer contributes to the formation of a large functional pore, but gating of each monomer is seen in electrophysiological experiments as substates of identical conductance, which can open and close individually as well as cooperatively. Some cooperativity may also exist between trimers and would cause the occasional appearance of closures of larger amplitudes than 3 units of conductance.

Rocque and McGroarty (1989) have shown that stable and functional dimers of OmpC can be isolated from strains with defective OmpF. Although RAM105 differs from the strains used by Rocque and McGroarty (1989) in that the OmpF porin is not made at all, it is possible that dimers of OmpC are assembled, perhaps more frequently than trimers,

which would account for the dominance of 2-unit closures in RAM105. An effect of the mutation in the OmpC protein of RAM276 would be to re-establish a more stable trimeric structure, reflected in the dominance of cooperative closures of 3 units. In that case, one would have to postulate that separate ionic pathways exist for each monomer, and that the observed growth phenotype of the mutant is solely due to an increase in the single-channel conductance.

The model of OmpC folding which Misra and Benson presented (1988*a*) predicts a transmembrane location for arginine residue 37 which is mutated to cysteine in RAM276. The increase in conductance we observed confirms the suggestion of these authors that this membrane segment is part of the channel wall. It is also possible that the hydrophobic region containing arginine 37 plays some role in the communication between subunits. A mutation in this region would alter the cooperativity between conducting units, resulting in the more frequent gating of 3 units seen in RAM276. The suggestion of a possible role of this residue in gating is supported by the observation of a decreased mean open time of the mutant channel.

We have not found a consistent pattern in the small deviations in the reversal potentials reported here between the parent and the mutant channels. Presumably, the substitution of a charged amino acid (arginine) for a neutral one (cysteine) is not drastic enough a change to affect reversal potentials. We found that in asymmetric solutions of 150 mM KCl in the pipette and 50 mM KCl in the bath, the P_{Cl}/P_K of the wild-type channel is about 0.14, while with 950 mM KCl in the bath, it is 0.26. These values are much larger than those reported by Benz et al. (1985) and Lakey et al. (1985) for OmpC from their experiments with planar lipid bilayers, as well as those of the voltage-dependent channel of similar gating pattern described in *E. coli* AW405 by use of the present method (Delcour et al., 1989*b*). At this point, we cannot explain these different observations, except that we are using different strains where the membrane proteins might be in a different lipid or cell-wall environment, or which might have slightly different OmpC proteins. It is also possible that the activities measured in AW405 which express both major porins reflect largely the behavior of heterotrimers of OmpF and OmpC (Gehring & Nikaido, 1989), while only homotrimers of OmpC were present in the strains we used here.

The electrophysiological properties of porins have been studied mostly in planar lipid bilayers (Benz, 1985). The single-channel conductance varies between about 200 and 300 pS when calculated for 150 mM KCl either from the measurements of current increments (Benz et al., 1978, 1985; Lakey et al., 1985) or of current fluctuations at steady state (Schindler & Rosenbusch, 1978). In the work reported here, the unit conductance of wild-type OmpC is calculated to be about 56 pS in 150 mM KCl. This value is much smaller than reported by others. Since porins tend to gate cooperatively, it is possible that the values previously measured correspond to the conductance of several units opening or closing simultaneously, while the discrete events corresponding to monomer gating are unresolved due to their fast kinetics and small amplitude.

The voltage-sensitive channel of AW405 has a similar but not identical behavior as wild-type OmpC. Both channels are mostly open but the AW405 channel closes more frequently than OmpC. The cooperativity pattern is also different since the AW405 channel gates as 1 unit most of the time but shows a voltage-dependent increase in the frequency of closures of many units simultaneously. The single-channel conductance is 91 pS in AW405, larger than that of OmpC, but the assignment of that conductance as corresponding to 1 unit might be misleading since rare events of smaller amplitude had also been observed. In conclusion, we cannot at this time, ascertain that the voltage-sensitive channel of AW405 is OmpC, but it appears to reflect the activities of a protein belonging to the porin family, or maybe of heterotrimers of OmpC and OmpF. This latter possibility is presently being explored.

The work presented here also confirms the observations made by others (Schindler & Rosenbusch, 1978, 1981; Dargent et al., 1986; Xu et al., 1986; Mauro et al., 1988; Buechner et al., 1990) that the porins are not static, permanently open pores. They can indeed open for long periods of time (seconds) — although our mean open-time values are significantly smaller than previously reported — but they do show gating. It is not unlikely that the porins behave much more like eukaryotic channels and, possibly, are regulated by as yet unknown factors. This would confer to the outer membrane a much more dynamic nature than previously believed, as already suggested (Buechner et al., 1990).

We acknowledge Dr. Spencer Benson for the gift of the RAM105 and RAM276 strains, and Dr. Hiroshi Nikaido for the supply of strains HN600 and HN705. We thank Christine Hirscher for excellent technical assistance, Dr. Yoshiro Saimi for computer instruction and software, Leanne Olds for making the illsutrations, and Dr. Boris Martinac and Matthew Buechner for critical reading of the manuscript. This work was supported by grants to J.A. and C.K. from US Public Health Service (DK 39121) and the Lucille P. Markey Charitable Trust. A.H.D. was supported by a NIH postdoctoral training grant from the Laboratory of Genetics.

References

Benz, R. 1985. Porin from bacterial and mitochondrial outer membranes. *CRC Crit. Rev. Biochem.* **19:**145–190

Benz, R. 1988. Structure and function of porins from Gram-negative bacteria. *Annu. Rev. Microbiol.* **42:**359–393

Benz, R., Bauer, K. 1988. Permeation of hydrophilic molecules through the outer membrane of Gram-negative bacteria. *Eur. J. Biochem.* **176:**1–19

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

Benz, R., Schmid, A., Hancock, R.E.W. 1985. Ion selectivity of Gram-negative bacterial porins. *J. Bacteriol.* **162:**722–727

Benz, R., Schmid, A., Nakae, T., Vos-Scheperkeuter, G.H. 1986. Pore formation by LamB of *Escherichia coli* in lipid bilayer membranes. *J. Bacteriol.* **165:**978–986

Benz, R., Schmid, A., van der Ley, P., Tommassen, J. 1989. Molecular basis of porin selectivity: Membrane experiments with OmpC-PhoE and OmpF-PhoE hybrid proteins of *Escherichia coli* K-12. *Biochim. Biophys. Acta* **981:**8–14

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:**111–121

Dargent, B., Hofmann, W., Pattus, F., Rosenbusch, J.P. 1986. The selectivity of voltage-dependent channels formed by phosphoporin (PhoE porin) 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., Martinac, B., Adler, J., Kung, C. 1990. Effect of mutation on the channel properties of bacterial porin studied by patch-clamp. *Biophys. J.* **57:**322a

Engel, A., Massalski, A., Schindler, H., Dorset, D.L., Rosenbusch, J.P., 1985. Porin channel triplets merge into single outlets in *Escherichia coli* outer membranes. *Nature (London)* **317:**643–645

Ferenci, T., Lee, K.-S. 1989. Channel architecture in maltoporin: Dominance studies with *lamB* mutations influencing maltodextrin binding provide evidence for independent selectivity filters in each subunit. *J. Bacteriol.* **171:**855–861

Gehring, K.B., Nikaido, H. 1989. Existence and purification of porin heterotrimers *Escherichia coli* K12 OmpC, OmpF and PhoE proteins. *J. Biol. Chem.* **264:**2810–2815

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

Heine, H.-G., Francis, G., Lee, K.-S., Ferenci, T. 1988. Genetic analysis of sequences in maltoporin that contribute to binding domain and pore structure. *J. Bacteriol.* **170:**1730–1738

Inokuchi, K., Mutoh, N., Matsuyama, S., Mizushima, S. 1982. Primary structure of the *ompF* gene that codes for a major outer membrane protein of *Escherichia coli* K-12. *Nucleic Acids Res.* **10:**6957–6968

Jap, B.K. 1989. Molecular design of PhoE porin and its functional consequences. *J. Mol. Biol.* **205:**407–419

Lakey, J.H., Watts, J.P., Lea E.J.A. 1985. Characterisation of channels induced in planar bilayer membranes by detergent solubilised *Escherichia coli* porins. *Biochim. Biophys. Acta* **817:**208–216

Mauro, A., Blake, M., Labarca, P. 1988. Voltage gating of conductance in lipid bilayers induced by porin from outer membrane of *Neisseria gonorrhoeae*. *Proc. Natal. Acad. Sci. USA* **85:**1071–1075

Misra, R., Benson, S.A. 1988*a*. Genetic identification of the pore domain of the OmpC porin of *Escherichia coli* K-12. *J. Bacteriol.* **170:**3611–3617

Misra, R., Benson, S.A. 1988*b*. Isolation and characterization of OmpC porin mutants with altered pore properties. *J. Bacteriol.* **170:**528–533

Mizuno, T., Chou, M.-Y., Inouye, M. 1983. A comparative study on the genes for three porins of the *Escherichia coli* outer membrane. *J. Biol.Chem.* **258:**6932–6940

Nakae, T. 1986. Outer-membrane permeability of bacteria. *CRC Crit. Rev. Microbiol.* **13:**1–62

Nakae, T., Ishii, J., Tokunaga, M. 1979. Subunit structure of functional porin oligomers that form permeability channels in the outer membrane of *Escherichia coli*. *J. Biol. Chem.* **254:**1457–1461

Nikaido, H., Vaara, M. 1985. Molecular basis of bacteria outer membrane permeability. *Microbiol. Rev.* **49:**1–32

Nogami, T., Mizuno, T., Mizushima, S. 1985. Construction of a series of *ompF-ompC* chimeric genes by in vivo homologous recombination in *Escherichia coli* and characterization of the translational products. *J. Bacteriol.* **164:**797–801

Rocque, W.J., McGroarty, E.J. 1989. Isolation and preliminary characterization of wild-type OmpC porin dimers from *Escherichia coli* K-12. *Biochemistry* **28:**3738–3743

Rocque, W.J., McGroarty, E.J. 1990. Structure and function of an OmpC deletion mutant porin from *Escherichia coli* K-12. *Biochemistry* **29:**5344–5351

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 a native environment and their functional reassembly. *Proc. Natl. Acad. Sci. USA* **78:**2302–2306

Sokal, R.R., Rohlf, F.J. 1987. *Introduction to Biostatistics*. W.H. Freeman, New York

Tommassen, J., van der Ley, P., van Zeijl, M., Agtenberg, M. 1985. Localization of functional domains in *E. coli* K-12 outer membrane porins. *EMBO J.* **4:**1583–1587

van der Ley, P., Burm, P., Agtenberg, M., van Meersbergen, J., Tommassen, J. 1987. Analysis of structure-function relationships in *Escherichia coli* K12 outer membrane porins with the aid of *ompC-phoE* and *phoE-ompC* hybrid genes. *Mol. Gen. Genet.* **209:**585–591

von Meyenburg, K., Nikaido, H. 1977. Outer membrane of Gram-negative bacteria. XVII. Specificity of transport process catalyzed by the λ-receptor protein in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **78:**1100–1107

Xu, G., Shi, B., McGroarty, 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 25 May 1990; revised 7 August 1990