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Characterisation of channels induced in planar bilayer membranes by detergent solubilised *Escherichia coli* porins

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Purified OmpF, OmpC, NmpC, PhoE and Lc (Protein 2) porins from the *Escherichia coli* outer membrane were incorporated into planar phospholipid bilayer membranes and the permeability properties of the pores studied. Triton X-100 solubilised porin samples showed large and reproducible increases in membrane conductivity composed of discreet single-channel events. The magnitude of the cation selectivity found for the porins was in the order OmpC > OmpF > NmpC = Lc; PhoE was anion selective. For the cation selective porins the cation/anion permeability ratios in a variety of solutes ranged from 6 to 35. Further information on the internal structure of the porins was obtained by examination of the single-channel conductance and this was used to interpret macroscopic observations and to estimate single-channel diameters. The same porins solubilised in SDS exhibited slight conductance increases with no observable single-channel activity. Use of on-line microcomputer techniques confirmed the ohmic current vs. voltage behaviour for all the single porin channels examined.

Introduction

The function of the outer membrane of Gram-negative bacteria may be regarded as that of a molecular sieve. The protein content of the outer membrane can be divided into two groups, the minor and the major outer membrane proteins. The former group exist in small copy numbers per cell and a number have been shown to facilitate the diffusion of specific nutrients into the cell. Included in this group is the LamB (λ, lambda phage receptor) protein, which shows channel forming activity when reconstituted into planar lipid bilayers [1]. Among the major proteins, produced in much higher copy numbers per cell, are the matrix proteins [2] or porins [3] which form hydrophilic, non-specific pores. In *Escherichia coli* such pores have an exclusion limit of 600 Da and are formed by trimers of identical polypeptide subunits with molecular weights of about 35 000 [4,5].

To date pore forming activity has been shown for OmpF [6,7], OmpC [7], NmpC [8], Lc (protein 2) [9], protein K [10,11] and PhoE [7,12] porins. With the exception of Lc, single-channel behaviour has been demonstrated in these porins by using a variety of preparative techniques and lipid bilayer compositions.

It was decided therefore to compare the single-channel characteristics of all the above porins using a single purification and reconstitution procedure. In particular it was hoped that the results would not only confirm the inferred channel forming capability of Lc but also provide additional data on the relationship between Lc and NmpC which have been shown to be almost identical by other techniques [13].

Also as there is considerable current interest in the reconstitution of relatively fragile (and often

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complex) eukaryotic membrane proteins into phospholipid bilayers by the use of a variety of detergents [14], we considered porins to provide a suitably simple and stable model system for comparing the effects of different detergents on membrane channel reconstitution.

Materials and Methods

Membrane experiments

Bilayer lipid membranes were formed from a solution of 0.25% (w/v) egg phosphatidylcholine [15] and 0.125% (w/v) cholesterol in *n*-decane by the brush technique of Mueller et al. [16] on the ends of five polyethylene tubes. The bathing solutions specified in the results section were contained within a glass thermostatic bath and all experiments were carried out at 26°C. When required, porins were added to the bathing solution, some of which were then sucked through the membrane tubes to ensure symmetry about the membrane aperture. Five minutes after formation the conductances and capacitances of the membranes were measured successively as previously described [17].

Measurements of single-channel fluctuations were carried out as previously described [18] with the following additions. Membranes were formed using the brush technique or by successive lowering and raising of the bathing solution past the membrane aperture following addition of 7.5 μl of the membrane forming solution to the surface of the bathing solution. The syringe for this operation was accessible from outside of the Faraday cage and so membrane formation could be achieved rapidly with least disturbance to the apparatus. Data were collected by an Apple II microcomputer via an AI13 12 bit A/D converter (Interactive Structures Inc Bala, Cynwyd, PA, U.S.A.) [19]. The current transients following voltage pulses from an A0ϕ3 D/A converter (Interactive Structures Inc) were sampled and integrated numerically by a subroutine to measure membrane capacitance. The data collection rate was varied between 5 Hz and 1000 Hz and data storage was as either a graphics printout or as a file on magnetic disc. In addition to current fluctuation analysis, the rapid data collection was used to generate current/voltage (I/V) graphs from individual porin channels. The membrane aperture was 0.45 mm^2 and by assuming a capacitance of $3.8 \cdot 10^{-3}$ $F \cdot m^{-2}$ the black film area was approx. 0.3 mm^2.

Strains and growth conditions

Escherichia coli strains used for the preparation of biologically active porins, together with their final porin phenotypes, were B/r (Omp F^+), CSH57 (Omp F^-Omp C^+) [20] and CS483 ($NmpC^+$), CS484 (Pho E^+), CS485 (Pho E^+), CS492 (Lc^+) [21]. Strain CSH57 normally possesses both the Omp F and Omp C porins and the Omp F^- Omp C^+ mutant was created by selection for resistance to phage TuIa. Similarly mutants of strains CSH57, CS483, CS484, CS485 and CS492 lacking the peptidoglycan-associated λ receptor protein were created by selecting for resistance to λc phage. This was done in order to prevent the possibility of the λ receptor protein, which has been shown to form channels [1], from contaminating the final biologically active porin preparations. SDS-polyacrylamide gel electrophoresis, carried out in the presence and absence of urea, was used to check the porin phenotypes of all parent and mutant strains as well as for determining whether λ receptor protein was present. All strains used for preparation of biologically active porins were grown at 37°C in nutrient broth.

Preparation of biologically active porins

This was performed using a previously described method [22] with some modifications. Late-exponential phase cells were collected by low speed centrifugation at 4°C and the resuspended pellets sonicated in 10 mM phosphate buffer (pH 7.0) on ice. After removal of unbroken cells by another low speed centrifugation at 4°C the cell free supernatant was centrifuged at $100\,000 \times g$ for 40 min at 4°C to obtain crude total membrane pellets. This membrane fraction was shaken with 2% SDS in 10 mM Tris-HCl (pH 7.4) at 55°C until solubilised then centrifuged at $100\,000 \times g$ for 40 min at 20°C. The peptidoglycan-protein pellet was further purified by repeating this step another three times. Porin was freed from the peptidoglycan by rapid shaking of the pellet at 37°C for 40 min using the same extraction buffer at pH 7.4 containing 0.5 M NaCl and 0.7 M 2-mercaptoethanol, followed by centrifugation at

100 000 × *g* for 40 min at 20°C. The supernatant was dialysed twice for 18 h at 25°C against 5 mM $NaHCO_3$ containing 0.1% SDS (pH 7.2). Porin was precipitated from the solution with 90% acetone for 2 h at 4°C, washed twice with acetone and distilled water and resuspended either in buffer containing 2% (v/v) Triton X-100, 5 mM EDTA, 50 mM Tris-HCl (pH 7.2) or 2% SDS, 10 mM Tris-HCl (pH 7.4) in concentrations between 0.1 and 1.0 mg/ml. Unbound detergent was removed from the samples by dialysis for 2 × 12 h in 2 litres 5 mM EDTA, 50 mM Tris-HCl (pH 7.2). Aliquots of the stock solutions were stored at −20°C without loss of activity.

Estimation of total carbohydrate in Triton X-100- and SDS-solubilised porin samples

Carbohydrate contamination of the biologically active porin samples was determined using the phenol-sulphuric acid assay [23] with D-glucose as a standard. None of the samples were found to contain more than 0.7% (w/w) carbohydrate relative to protein and detection was at the limit of the method used.

SDS-polyacrylamide gel electrophoresis

Slab gel electrophoresis in SDS was performed using the Tris-glycine buffer system [24]. Resolution between different porin bands was enhanced by the use of 10% acrylamide gels containing either 4 M or 6 M urea. Only single bands, characteristic for each porin, were obtained when samples of the SDS-solubilised biologically active porins were boiled in sample buffer prior to loading on the gel. No traces of contaminant proteins were observed.

Results

Conductance as function of porin concentration and detergent type

At concentrations below 10^{-9} M, SDS-solubilised porin did not significantly increase the specific membrane conductance, whilst at concentrations between 10^{-9} M and 10^{-8} M these samples caused a marked increase in specific membrane conductance. When $\log_{10}G$ (specific membrane conductance) was plotted as a function of $\log_{10} C$ (molar porin concentration) this region was linear with an approximate slope of one saturating at around 10^{-7} M (Fig. 1). All the Triton X-100-solubilised porin samples tested caused large and highly reproducible increases in membrane conductance. Between 10^{-11} M and 10^{-8} M a plot of $\log_{10}G$ versus $\log_{10}C$ was linear (Fig. 1) with a slope that varied between 1.1 and 2.6 depending on the porin used. At concentrations of porin greater than 10^{-8} the membranes exhibited no resistance as measured by the apparatus used. Below 10^{-3} S · m^{-2} specific conductivity (i.e., approx. 10^{-11} M porin) the control conductance of the lipid bilayer became significant and a non-linear relationship between $\log_{10} G$ and $\log_{10} C$ was observed.

Ion selectivity measurements

For each of the porin/salt combinations listed in Table I, the concentration of salt inside the membrane tube was fixed at 25 mM, while aliquots of 2.5 M salt were added to increase the salt concentration outside to 250 mM and after each concentration change the steady membrane PD was measured. The porin concentration used was that which gave a conductivity of between $5 \cdot 10^{-2}$ S · m^{-2} and 1.0 S · m^{-2}. This gave a conductivity

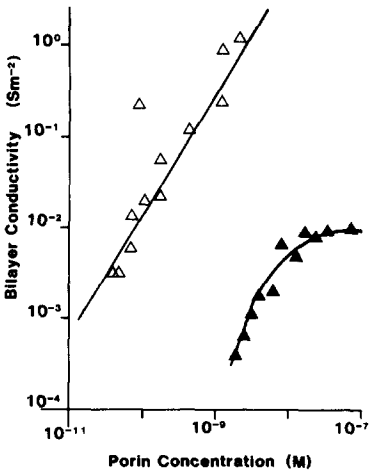

Fig. 1. Bilayer conductivity as a function of porin concentration and detergent type. Bilayers were formed from egg phosphatidylcholine-cholesterol- *n*-decane in 0.1 M NaCl at 26°C. Each point is the average of at least ten membranes measured five minutes after formation. Δ, Triton X-100-solubilised OmpC porin. Line fitted by method of least squares has a gradient of 1.31 ± 0.17. ▲ SDS-solubilised OmpC porin. Curve fitted by eye.

TABLE I

PERMEABILITY RATIOS FROM BEST FIT OF GOLDMAN-HODGKIN-KATZ EQUATION

For each measurement the concentration on the inside of the membrane tube was fixed at 25 mM while the concentration outside was increased in a series of steps. After each change the membrane PD was recorded. All membranes were formed from egg phosphatidylcholine/cholesterol in decane and Triton X-100-solubilised porin was added so that the membrane conductivity was 10^{-2} S·m^{-2}. The tabulated values of $P_{cation}/P_{chloride}$ are taken from the best fitting line produced by Eqn. 1. Each value is based upon the readings from at least 20 membranes.

Porin	$\frac{P_{Li}}{P_{Cl}}$	$\frac{P_{Na}}{P_{Cl}}$	$\frac{P_{K}}{P_{Cl}}$	$\frac{P_{TMA}}{P_{Cl}}$	$\frac{P_{Choline}}{P_{Cl}}$
OmpF	15	15	16	10	7
OmpC	35	35	30	30	25
PhoE	1/7	1/6	1/2.8	1/15	1/15
NmpC	11	18	6.5	6.5	10
Lc	12	13	-	7	14

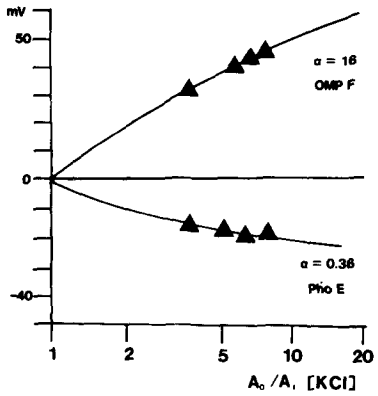

Fig. 2. Zero-current membrane potentials across egg phosphatidylcholine-cholesterol-*n*-decane bilayers containing OmpF or PhoE porins previously solubilised in Triton X-100. The potentials (ordinate) are represented as a function of the ratio of activities of the KCl concentrations outside and inside the membrane tube. (A_o/A_i, abscissa). The potentials correspond to $V_o - V_i$ of Eqn. 1 and each point is the average of five membranes. The lines were drawn according to Eqn. 1 using the specified values of the permeability ratio (α).

of 100-times that of the control value whilst limiting the effects of diffusion polarisation [25]. The results are illustrated in Fig. 2. Such a transmembrane zero-current potential difference can be described by the Goldman equation [26–28]

$$V_o - V_i = -\frac{RT}{F}\ln\frac{\alpha_x[X_o]+[Cl_i]}{\alpha_x[X_i]+[Cl_o]} \quad (1)$$

where [X] and [Cl] are the activities of the cation and chloride, respectively, subscripts refer to opposite sides of the membrane and α_x is the ratio of the permeability coefficients P_{X^+}/P_{Cl^-} for X^+ and Cl^-, respectively. Selectivities were thus measured using a line of best fit calculated using the Goldman equation.

With most of the porins studied the resultant potential was positive on the dilute solution side indicating that the porins were mainly cation selective. The size of the cation selectivity was in the order OmpC > OmpF > NmpC = Lc. PhoE, which has a specialised role in the phosphate uptake system [29], showed a distinct anion selectivity by producing a potential of the opposite sign (Fig. 2). OmpF and PhoE were the only two porins to show the selectivity order K > Na > Li > tetramethylammonium > choline expected from bulk solution conductivity. This result is discussed later.

In addition to the porins listed in Table I, three other porin preparations were tested (results not shown). One sample previously shown to be a probable mutant OmpC porin [30] showed a significantly greater cation selectivity than OmpC. A similarly greater selectivity was observed using a porin preparation from Klebsiella strain N.C.T.C. 204 [31]. Finally, a protein K preparation from strain N63 [32] exhibited cation selectivity midway between that of OmpF and OmpC [31] but though a single porin band was observed by SDS gel electrophoresis in the absence of urea, multiple bands appeared in the presence of urea leading to doubts as to whether only one porin was present [30].

Single-channel experiments

The amount of porin present in the 20 ml of solution bathing the Teflon pot in all experiments was 0.20 ± 0.1 ng (2.5 ± 1.25 pmol). Bilayers formed in this concentration of Triton solubilised porin showed stepwise increases in conductivity assumed to be porin channel opening events (Fig. 3) and average rate of channel opening varied considerably (0.01–1.0 Hz). With less than 2% of opening events did closing events occur and both events were unaffected by the sign or size of the

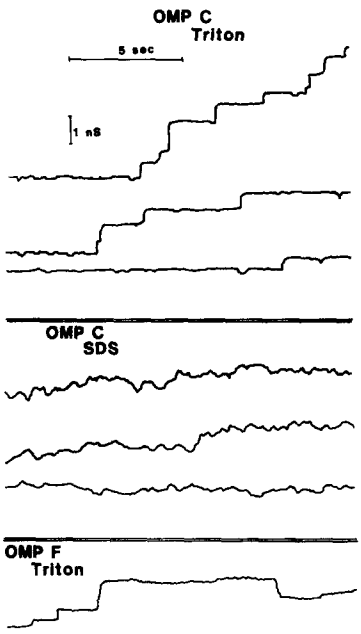

Fig. 3. Single-channel records of egg phosphatidylcholine-cholesterol-*n*-decane bilayers formed in 0.1 M NaCl at 26°C in the presence of a small amount of porin. Data was recorded at 10 Hz and the applied membrane potential was 50 mV. Upper trace: 0.20 ± 0.1 ng Triton-solubilised OmpC porin; centre trace: 2.0 ± 0.5 ng SDS-solubilised OmpC porin; lower trace: 0.20 ± 0.1 ng Triton-solubilised OmpF porin. Such closing events, whilst rare were not limited to this porin alone. Each trace begins at the bottom left and corresponds to the scales depicted at the top of the figure.

membrane potential. The fluctuations in each membrane were recorded until the conductivity was beyond the range of the recording apparatus after which the membrane was reformed and further data recorded. Removal and replacement of the lipid/decane surface layer on the bathing solution by a new layer drastically reduced the channel activity whilst replacement of the aqueous phase had little effect even though the porin was always added to the aqueous phase. These repeated observations indicate that the oil/water partition coefficient of Triton X-100 solubilised porins is very large.

The stepwise increases in current were not restricted to a characteristic size for each porin but varied about a mean [33,34]. The results using 0.1 M sodium chloride (NaCl), tetramethylammonium chloride (TMAC) and sodium benzenesulphonate (NaBS) are thus presented as histograms of probability vs. channel conductance (Figs. 4–6).

Current vs. voltage behaviour of porin channels

By reducing the concentration of porin in the bathing solution it was possible to achieve long periods when only one porin channel was open. In such circumstances the application of a steady PD ramp across the membrane allowed a computer subroutine to make a series of current/voltage readings during the life of one channel. Normally the voltage was changed from −100 to +100 mV during which 250 readings of current and voltage were taken. Hence it was possible to examine the current voltage behaviour of one porin channel at a time, the results (Fig. 7) show that, due to the bilayer capacitance C, current flowed according to the relation:

$$I_c = C\frac{dV}{dt} \qquad (2)$$

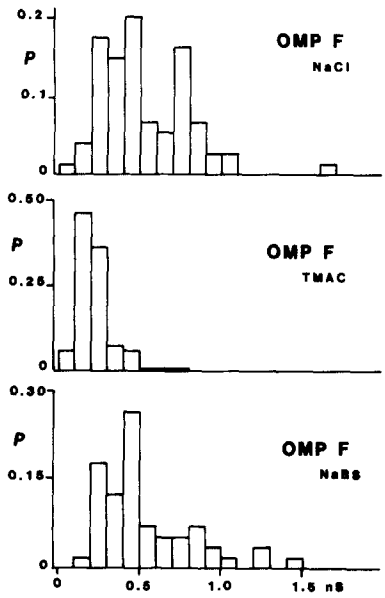

Fig. 4. Histograms of the conductance fluctuations observed in egg phosphatidylcholine-cholesterol-*n*-decane bilayers in the presence of 0.20 ± 0.1 ng Triton-solubilised OmpF porin. The applied voltage was 50 mV, the temperature was 20°C and the aqueous phase contained 125 ml of 0.1 M NaCl ($n = 74$) or TMAC (tetramethylammonium chloride) ($n = 154$) or NaBS (sodium benzenesulphonate) ($n = 74$). P is the probability (ordinate) of a conductance step of magnitude 0–100 pS, 101–200 pS, ... etc.

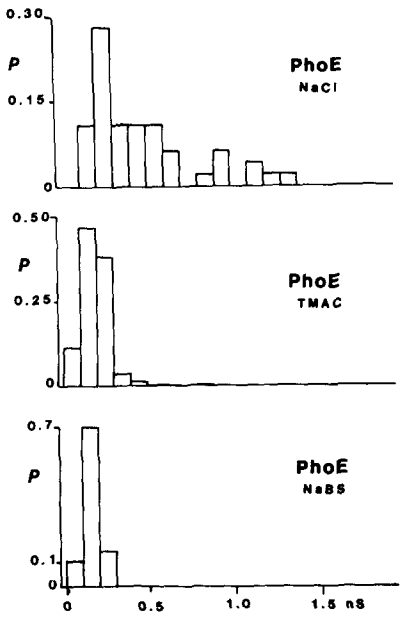

Fig. 5. Histograms of the conductance fluctations observed in egg phosphatidylcholine-cholesterol-*n*-decane bilayers in the presence of 0.20 ± 0.1 ng Triton-solubilised PhoE porin. The applied voltage was 50 mV, the temperature was 20°C and the aqueous phase contained 125 ml of 0.1M NaCl ($n = 46$) or TMAC (tetramethylammonium chloride) ($n = 193$) or NaBS (sodium benzenesulphonate) ($n = 70$). P is the probability (ordinate) of a conductance step of magnitude 0–100 pS, 101–200 pS, ... etc.

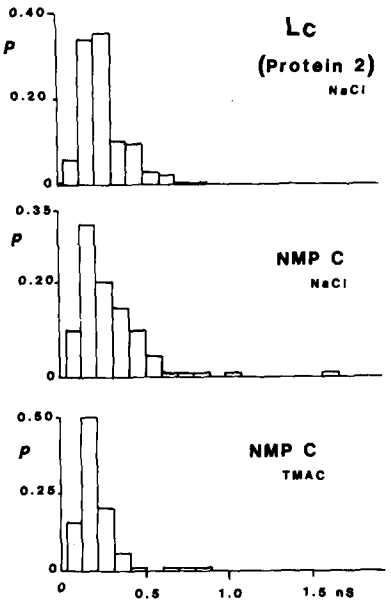

Fig. 6. Histograms of the conductance fluctuations observed in egg phosphatidylcholine-cholesterol-*n*-decane bilayers in the presence of 0.20 ± 0.1 ng Triton-solubilised porin. The applied voltage was 50 mV, the temperature was 20°C and the aqueous phase contained 125 ml of 0.1 M NaCl (Lc; $n = 171$) (NmpC; $n = 107$) or TMAC (tetramethylammonium chloride) (NmpC; $n = 89$). P is the probability (ordinate) of a conductance step of magnitude 0–100 pS, 101–200 pS, ... etc.

(I_c = capacitative current; dV/dt = rate of change of PD) resulting in lines that did not pass through the origin. As dV/dt is constant and the rate of change of C very low ten or more minutes after formation, I_c is constant and does not affect the shape of the I/V curve.

For all the porin channels mentioned in this paper Ohms law appears to apply. The differences in slope of the I/V lines in Fig. 7 are due to differences in the sizes of the conductance steps investigated. In no cases did channels switch off when the potential sign was changed and experiments over larger voltage ranges were similar except for membrane breakage effects above 250 mV.

Discussion

At the macroscopic level both SDS- and Triton-solubilised porins increase the conductivity of phosphatidylcholine (PC) bilayers though the effect of the latter is much greater than the former. At the microscopic level channel events were ob-

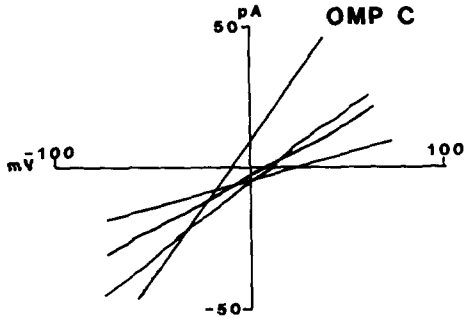

Fig. 7. Single-channel current/voltage relationships of Triton-solubilised OmpC porin channels in egg phosphatidylcholine-cholesterol-*n*-decane bilayers formed in 0.1 M NaCl at 20°C. Data were recorded at 50 Hz by microcomputer whilst the applied membrane voltage was changed gradually from +100 mV to −100 mV (or in one case −100 mV to +100 mV) using a voltage ramp. Each trace was obtained using a single porin channel.

served when using Triton-solubilised porins on PC membranes whereas no channels were observed when using SDS-solubilised porins on such membranes. SDS-solubilised porins did, however, produce single-channel activity in glycerylmonooleate membranes (results not shown). This observation agrees with that of Benz et al. [33] who found that SDS-solubilised porins only produced channels in oxidised cholesterol or glycerylmonooleate bilayers. Coupled with the observation [34] that Triton-solubilised F porin from *Pseudomonas aeruginosa* was more active in oxidised cholesterol membranes than PC membranes, it appears that porin incorporation is easier in membranes composed of lipids with small headgroups whilst SDS entirely prevents successful incorporation of *E. coli* porins into phospholipid membranes.

For Triton-solubilised porins the plots of log conductivity vs. log concentration give gradients characteristic of particular porins and in general, similar porins from different strains give similar gradients. In particular, OmpF porins from three different strains (results not shown) all give gradients of greater than 2, i.e. conductance rises by at least the square of concentration. It has been reported [35] that porins solubilised in SDS give gradients of approximately unity and this was confirmed in this study. Porin preparations produced by osmotic shock procedures give slope values of between 1.3 and 2 [36]. Hence a variety of gradients can be produced in various conditions and there is no simple correlation between gradient and the number of monomers per conducting unit as has been suggested for gramicidin [37]. Nevertheless, similarity between gradients from similar porins from different strains suggests that whatever the cause, the relation between log conductivity and log concentration is porin specific.

The results of the zero current membrane potential experiments show that all the porins except one are cation selective. The exception is PhoE which like its *P. aeruginosa* analogue Protein P is anion selective [12,38]. Similar conclusions were reached by others [7,39,40].

OmpC has a higher cation selectivity than OmpF and this may be related to differences in their isoelectric points [7,13,41]. The PhoE porin is normally produced in response to low external phosphate concentrations and its anion selectivity facilitates the uptake of negatively charged phosphate across the outer membrane.

Although the cation or anion selectivities of the porins agree with previous results, the magnitudes of the selectivities presented here and those reported previously [12,39] differ significantly. Whilst Benz and his co-workers report permeability ratios, for a range of solutes, of between 2.0 and 3.6 for cation selective and 1.5–9.0 for anion selective *E. coli* porins, the results presented here (for the solutes in Table I) give values of 6–35 and 2.8–15, respectively. It is difficult to explain this behaviour as being due to the presence of SDS in Benz's experiments, since negatively charged SDS molecules adhering to the bilayer would cause a higher cation selectivity to occur [42]. The degree of selectivity of both the anion and cation selective pores is greater in this study and it is possible that the methods of porin preparation may have caused the differences between our results and those of Benz. This latter possibility is given more credence by the results of Benz and Hancock [34] who using PC or oxidised cholesterol membranes and Triton-solubilised F porin from *P. aeruginosa* found a selectivity similar to that of their SDS-solubilised *E. coli* porins.

All the Triton-solubilised porins used, showed the stepwise changes in membrane conductivity characteristic of the opening of large water filled channels across the hydrophobic region of the bilayer. The results agree with previous studies [1,33–36,38] in that the conductance steps occur in a broad distribution. Such a distribution cannot be due to the presence of a variety of porins as only purified extracts from single porin mutants were used in this study. The most probable explanation [38] is that an observed event may be composed of 1, 2, 3 or more channels. This may explain the occasional very large conductance steps observed even when the rate of channel opening is very low and hence they are unlikely to be coincidental events. Thus some prior association of the porin trimers into multiple conducting units is the likely cause. The limitation to this hypothesis is that with the exception of OmpC and OmpF, clear multimodal distributions do not occur regularly in this study or any other published results. The exception occurs in the work of Schindler and Rosenbusch [6,43] who using a quite different method of

reconstitution found clearly defined conductivity values, the lack of which in our results makes calculation of the single channel conductivity uncertain. It was decided, therefore, to choose the modal or lowest modal value (in the case of multimodal distributions) as a measure of the size of the single-channel conductance.

What is clear, though, is that the zero current selectivity measurements are supported by the investigation of OmpC, OmpF, PhoE and NmpC at the single-channel level, summarised in Table II. At the single-channel level all the Triton-solubilised channels studied show smaller conductances in tetramethylammonium chloride (TMAC) than in NaCl. Although Na^+ has a lower conductivity at infinite dilution the channels discriminate between the inherently large ion TMA^+ and Na^+ which appears larger by virtue of its hydration shell. OmpF is shown to be sensitive to the increase in cation size caused by the replacement of Na^+ by tetramethylammonium (TMA^+), whilst the large anion benzenesulphonate (BS^-) has little effect on these strongly cation selective channels. This indicates that the large anion does not enter the channel or channel mouth thus not affecting the single-channel conductivity based upon the movement of Na^+. The PhoE pore was shown by zero-current measurements to be less discriminating than OmpF and in single-channel measurements the conductance of this porin is reduced both by TMA^+ and benzenesulphonate. The greater effect of the increase in anion size confirms the measured selectivity of the pore whilst the effect of TMA^+ means that this ion must enter the pore or pore mouth, a conclusion in keeping with the magnitude of the anion selectivity. The peak of the PhoE distribution gives the single-channel conductivity in 0.1 M sodium chloride (Λ_{NaCl}) to be 0.25 nS which using Eqn. 3 gives a channel diameter of 1.6 nm. This is slightly larger than previously suggested [12] and a lot larger than the 0.7 nm estimated for protein P of *P. aeruginosa* [38] which although analagous to PhoE has a much higher anion selectivity.

TABLE II

SINGLE-CHANNEL CONDUCTANCE VALUES AND INFERRED CHANNEL DIAMETERS

Single-channel increments of Triton-solubilised porins in egg phosphatidylcholine-cholesterol bilayers were measured in 0.1 M salt solution at a PD of 50 mV. The conductances were plotted on a histogram and the lowest modal conductance taken as the single channel conductance (Λ). The conductance of the bulk solution (σ) was measured using a Radiometer conductivity meter and the channel diameters (d) calculated according to Eqn. 3 assuming a channel length of 7.5 nm. TMAC, tetramethylammonium chloride.

Porin	Salt	Λ (nS)	σ (mS·cm^{-1})	Λ/σ (10^{-8} cm)	d (nm)
OmpF	NaCl	0.25	9.67	2.59	1.6
	TMAC	0.16	8.86	1.81	1.3
OmpC	NaCl	0.30	9.67	3.10	1.7
	TMAC	0.07	8.86	0.75	0.9
PhoE	NaCl	0.25	9.67	2.59	1.6
	TMAC	0.18	8.86	2.03	1.4
NmpC	NaCl	0.21	9.67	2.18	1.4
	TMAC	0.15	8.86	1.69	1.27

$$r^2 = \frac{\Lambda l}{\sigma \pi} \tag{3}$$

where r is the channel radius, Λ single-channel conductivity, l the width of the outer membrane (7.5 nm) and σ the specific conductivity of the bathing solution.

The OmpC channel is affected more by the change from Na^+ to TMA^+ than the less selective OmpF channel. Also, whereas in NaCl the two channels appear of similar size, when Λ_{TMAC} values are used the channel diameter of OmpC (0.9 nm) appears significantly smaller than that of OmpF (1.3 nm). All of these results agree with Nikaido and Rosenberg [7] who concluded that the OmpC pore has a smaller diameter and, although not truly selective for any particular group of compounds, it is a more discriminating pore than OmpF.

NmpC which is less cation selective than OmpF also showed a smaller conductance decrease when in TMAC solution. The distributions of Λ_{NaCl} for NmpC and Lc together with their cation selectivities and log concentration/log conductivity slopes are very similar to each other. Both channels show zero-current selectivity results that are unexpected from bulk solution conductivities. This is unlikely to be due to pH effects as none of the solutions used was in the pH region of significant selectivity change shown by Benz et al. [39] and the agreement between the two proteins indicates that this

anomaly is a property of these porins. These results support the conclusion [13] that these two porins are closely related. The channel conductivities indicate a channel diameter smaller than that of OmpC and OmpF. This is in agreement with other recent results [8].

Previously all current/voltage measurements of porins have involved the use of macroscopic measurements of membranes containing many channels. Although this method is invaluable for measuring the effect that the membrane potential has upon the channel kinetics, it does tend to mask any measurement of rectifying effects in each individual channel. The method employed in this study, whereby the current/voltage characteristic of individual channels may be measured, has confirmed that all the porin channels obey Ohm's Law in the range -100 to $+100$ mV. No channels switched off when the PD changed sign and in only a tiny minority of experiments did the gradient change during the life of one channel.

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