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Ionophore properties of OmpA of *Escherichia coli*

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Both porins OmpAl, from wild-strain K12 *Escherichia coli*, and OmpA2, from a K12 derivative deficient in both OmpF and OmpC, are able to form ion channels in virtually solvent-free membranes. The conductance has been shown to vary in a discrete fashion with different single increment values especially with OmpA2. This behaviour seems to indicate, beside monomers, the presence of aggregates of different sizes. The estimated small pore diameter (0.6–0.7 nm) for the monomeric would explain the weak permeability of this narrow channel toward different solutes. OmpA protein, from experiments of ion selectivity and zero-current potential, is determined weakly anion selective.

Introduction

OmpA protein is a major outer membrane protein of *Escherichia coli* with an apparent molecular monomeric weight of about 35 kDa [1]. This protein, rich in β-sheet structure [2,3], is believed to exist in monomeric form in its native state. A typical feature of OmpA is the heat-modifiability [2]: the mobility of this protein in SDS-PAGE strongly decreases when the sample is heated in SDS. For a long time, OmpA was not considered as a pore-forming protein. Our studies on the protein F of *Pseudomonas aeruginosa* [4] showed that this protein reincorporated into planar lipid bilayers exhibits only weak conductance levels (90 pS in 0.25 M KCl) whereas larger channels are not observed. The very strong homology of properties between OmpA and protein F [5] led us to think that OmpA protein could be a former of ion channels in spite of the previous literature conclusions. This idea was recently confirmed by an interesting study performed with OmpA extracted from a K12 derivative deficient in OmpC and OmpF porins [6]. Indeed, it was found that OmpA produced diffusion channels by the swelling liposome technique and that the pore-forming activity was destroyed by the heat-denaturation.

Nevertheless it is difficult to compare the permeability properties of proteins from the diffusion rate of different substrates in liposomes since these values are strongly dependent of the nature of the permeant and this technique only measures macroscopic phenomena. An alternative is to conduct reconstitution experiments in black lipid membrane since it is possible to distinguish the conductance of ion channels formed by one protein molecule in controlled electrolyte solutions and thus allows the access to the conductance of the monomer.

For these reasons, we tested OmpA protein from wild-strain (OmpA1) and ırom K12 derivative deficient in OmpC and OmpF porins (OmpA2) and found that this protein is able to form ion channels in virtually solvent-free bilayers of diphytanoylphosphocholine.

Materials and Methods

Outer membrane proteins

OmpA1 protein is a generous gift of Dr. Henning [7] while OmpA2 is a gift of Dr. Nikaido [6]. The samples of OmpA1 was lyophilized from 1% SDS, 10 mM Tris-HCl solution at pH 7.5 and 1 mM EDTA and OmpA2 from 0.1% LDS, 0.4 M LiCl and 10 mM Tris-HCl solution at pH 7.5. These sample (0.25 μg) were solubilized in 250 μl 10 m Tris-HCl at pH 8.0 containing 1% octyl-POE just before the conductance measurements.

For testing the role of the detergent and LDS, the OmpA2 lyophilisat (see above) was solubilized in water before dialysing it during 3 h at 4°C against solutions of octyl-POE (1%) in a ultrafiltration cell (10 ml, Amicon) with Diaflo membranes (YM10).

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Abbreviations: OmpA1, protein extracted from a wild-strain K12; and OmpA2, protein extracted from a K12 mutant deficient in OmpC and OmpF; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; LDS, lithium dodecyl sulfate; DPhPC, diphytanoylphosphocholine; POPC, palmitoyl-oleoylphosphocholine; DOPE, dioleoylphosphatidylethanolamine.

In order to control the purity of proteins, SDS-PAGE was performed on a PhastSystem (Pharmacia) and the gels (10–15% gradient) were stained with silver or Coomassie blue.

Reconstitution in planar lipid bilayers

From a DPhPC (Avanti Lipids) solution in hexane (1%), virtually solvent-free planar lipid bilayers were formed by the apposition of two monolayers [8] on a 125 μm diameter hole in a thin teflon film (25 μm) sandwiched between two half glass cells and pretreated with hexadecane/hexane (1:12, v/v). Bilayer formation was monitored by the capacitance response and the voltage and current sign conventions are the usual ones. The current fluctuations were recorded using a RK 300 amplifier (Biologic) and stored on a DTR 1200 (Biologic). The stored signals were transferred on a computer for different treatments (amplitude histogram, channel lifetime) using a software from Intracell (Royston, UK). The electrolyte solutions was KCl either 1 M or 0.25 M buffered with 10 mM Tris at pH 7.4 and the bulk concentration of reincorporated protein was about 10^{-9} M.

Results and Discussion

Before testing OmpA1 and OmpA2 proteins in planar lipid bilayers, their purity were checked by SDS-PAGE. The electrophoretic profile (Fig. 1) shows a good homogeneity for OmpA1 and OmpA2 (29 kDa). The heating of both OmpA samples at 100°C during 5 min gives the 'heat-modified' form (37 kDa) but also new bands in migration front, involving the presence of LPS in native proteins (the presence of LPS for OmpA2 is also observed but at higher concentrations).

OmpF protein

For testing our set-up, we performed control experiments with OmpF of *E. coli* (Fig. 2). No potential was applied for 15 min after addition of octyl-POE solubilised OmpF in DPhPC bilayer membrane (1 μg of protein in 2 ml of 1 M bath NaCl solution). Then, a 200 mV voltage induced closure steps indicating the presence of voltage-sensitive channels as described in the literature [9]. No channel activity was observed when the detergent 1% octyl-POE alone was added to the bathing solution.

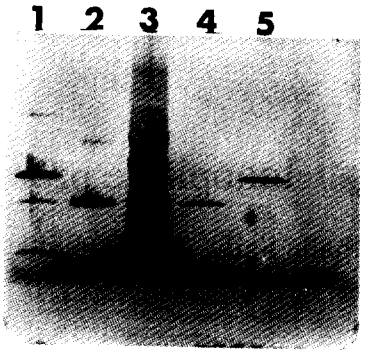

Fig. 1. SDS-PAGE patterns of OmpA1 and OmpA2. Lane 1 and 5, OmpA1 and OmpA2 heated 5 min in sample buffer at 100°C; lanes 2 and 4, non-heated OmpA1 and OmpA2; lane 3, molecular weight standards, phosphorylase *b* (94000), albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20100), α-lactalbumin (14400).

OmpA1 protein

The reincorporation of OmpA1 protein in DPhPC bilayers (0.25 M KCl) mainly induced long duration steps of 1200 pS conductance after application of 100 mV potential (Fig. 3). During channel insertion most steps were directed in the increasing current direction. Fast fluctuations can be observed on these steps and if the scale is enlarged the fast fluctuating levels become well-defined and can be estimated to 180 pS. It is noteworthy to see that these increments of current are fairly reproducible whereas for many other porins channels the single-conductance increments are distributed over a broad range of values [10,11].

Another experiment was performed in 1 M NaCl (not shown) and a similar behaviour was observed with different values of conductance levels which are mainly 600 pS. Occasionally other levels were also seen (1900, 2300 pS).

Fig. 2. Behaviour of octyl-POE solubilized OmpF in DPhPC in 1 M NaCl for a membrane potential of 200 mV at room temperature. OmpF concentration is about 0.6 μg/ml. The upper trace shows the enlargement of the delimited box. The records are acquired at 1000 Hz and filtered at 50 Hz.

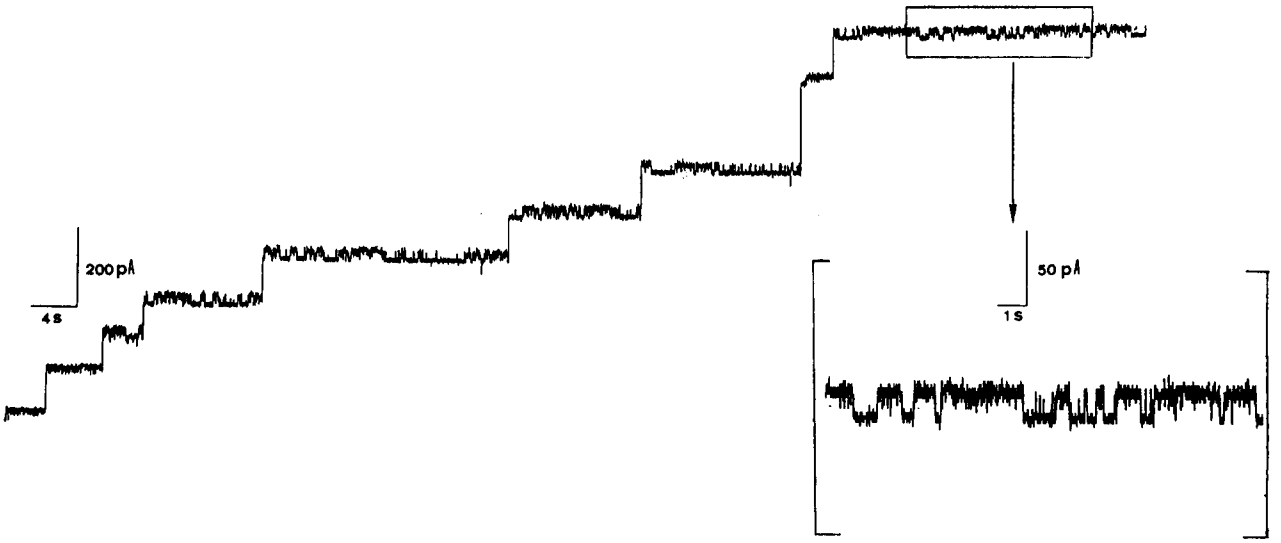

Fig. 3. Single-channel current records of OmpA1 protein (10^{-9} M) in 0.25 M KCl under a 100 mV potential. Digitization rate: 500 Hz and filter: 100 Hz.

From the lower conductance values we observed in 0.25 M KCl (180 pS) and in 1 M NaCl (600 pS), it is possible to estimate the channel diameter, d, from the equation:

$$\Lambda/\sigma = \pi d^2/4l$$

where Λ is the single-channel conductance, l the channel length (the thickness bilayer) and σ the conductivity of a 0.25 M KCl (27.5 mS/cm) or a 1 M NaCl solution (84 mS/cm).

Whereas this equation would become unvalid for small diameters, it can be seen in Table I that different experiments conducted in KCl and NaCl led to similar diameters.

TABLE I

Diameter values (nm) of OmpA1 in KCl 0.25 M and NaCl 1 M for two lenghts of the pore (6 and 4,5 nm)

	d_6	$d_{4.5}$
0.25 M KCl	0.71	0.61
1 M NaCl	0.74	0.63

To avoid eventual artefacts, i.e., the action of possible contaminants which could be responsible for the

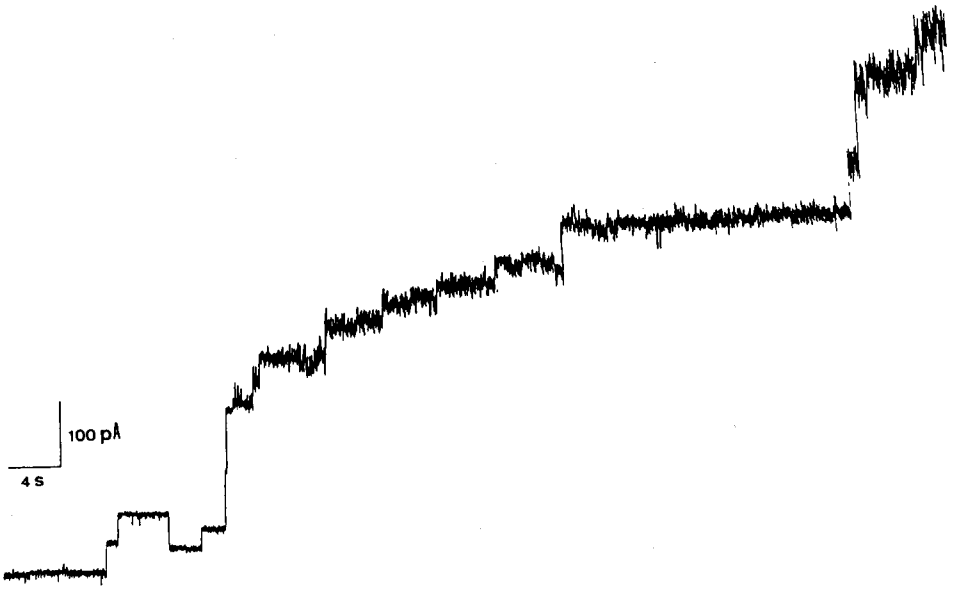

Fig. 4. Single-channel current records of OmpA2 protein (10^{-9} M) in 0.25 M KCl under a 80 mV potential. Digitization rate: 500 Hz and filter: 100 Hz.

Fig. 5. Trace of current fluctuations of OmpA2 protein (10^{-9} M) in 0.25 M KCl. Applied voltage $V = 50$ mV. Digitization rate: 5000 Hz and filter: 100 Hz.

ion channels (OmpF and OmpC have nearly a 100-fold higher pore forming activity), experiments were performed with OmpA2 (protein extracted from a K12 derivative deficient in OmpC and OmpF).

OmpA2 protein

Preliminary trials with 0.1% LDS-solubilized OmpA2 in DPhPC membranes showed a weak incorporation. Adding LDS at a final concentration of 0.5% slightly improved the incorporation in planar lipid bilayers and conductance levels at about 350–400 pS are observed in 0.25 M KCl as well as fast small levels of 150–200 pS (not shown). The substitution of DPhPC by a mixture of POPC/DOPE (7:3) did not improve the incorporation. The nature of these lipids and detergent (LDS) did not seem very compatible and could explain this difficulty for inserting the LDS-protein aggregates in the membrane. For decreasing the LDS concentration and in order to compare the OmpA2 behaviour with the one of OmpA1 solubilized in octyl-POE, the solution of OmpA2 protein was dialysed against a solution of 1% octyl-POE (3 h at 4°C) and an another set of experiments in lipid bilayer (0.25 M KCl) was performed.

In a first experiment (at 80 mV), the current increases in a stepwise fashion (Fig. 4) as it was already observed with wild OmpA but the trace became noisy after several increments. Different conductance values might be determined: 400, 600 and 1200 pS. Additionally small channels about 180–200 pS can be observed. In a second experiment (Fig. 5), different long duration steps could also be seen but with fast fluctuations which become well-defined if the acquisition frequency is increased. Their conductance values at 50 mV are 1200–1300 pS. Finally after breaking and reforming the membrane several times, long steps with fast fluctuations (760 pS) are mainly observed at 100 mV (Fig 6). In this case, other conductance values were found: 300, 400, 600 pS.

These experiments showed that the lower conductance value found with OmpA2 is 180 pS. This value agrees well with the one already observed with OmpA1. The conductance increments are distributed over a large range of values involving different sizes of the ion channels.

Finally, the ionic specificity was investigated in the case of OmpA2 from the reversal potential resulting from a 0.1 M KCl/0.7 M KCl (cis/trans) gradient. The observed shift (-8 mV) allowed to estimate from the application of the Hodgkin-Goldman-Katz equation [12] the ratio P_{Cl}/P_K and to show a weak anionic selectivity.

In a recent swelling proteoliposome study, it was observed that the permeability of liposomes toward

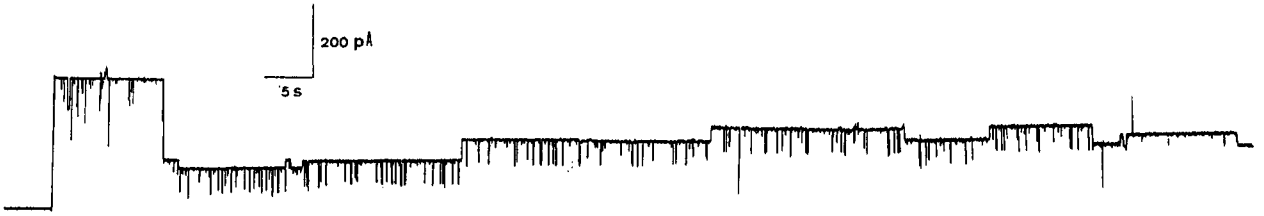

Fig. 6. Single-channel current records of OmpA2 protein (10^{-9} M) in 0.25 M KCl under a 100 mV potential. Digitization rate: 500 Hz and filter: 100 Hz.

L-arabinose is proportional to the amount of OmpA2 added [6]. The authors assumed that if the OmpA2 produces pores only by oligomer formation this proportionality should not be observed and concluded that the active form would be monomeric. If we take as an hypothesis that the value of 180 to 200 pS is the conductance of the monomer, the calculation of pore diameter with a length of channel about 4.5 nm or 6 nm gives, respectively, 0.6 nm or 0.7 nm. It is noteworthy that this value is similar to the one observed from Omp43 protein of *Wolinella recta* [13] in black lipid membranes experiments. Furthermore this Omp43 protein presents a strong analogy with OmpA of *E. coli* since Omp43 is heat-modified, active under monomeric form and weakly anion selective.

This diameter value is lower than the one found by swelling liposome technic [6]. The difference is not surprising because it is known that estimating pore diameters from conductance measurements would be undervalued [14]. Nevertheless the pore size found for OmpA2 by conductance measurement could explain the small rate of diffusion of solutes into the liposomes [6]; the pore is too small to allow the transfer of solutes as arabinose across the membrane.

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