

NOTES

Outer Membrane Protein NmpC of *Escherichia coli*: Pore-Forming Properties in Black Lipid Bilayers

MICHAEL S. HINDAHL,^{1,2} GORDON W. K. CROCKFORD,¹ AND ROBERT E. W. HANCOCK^{1*}

Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1W5,¹ and Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon 97201²

Received 10 April 1984/Accepted 13 June 1984

The purified NmpC outer membrane protein from *Escherichia coli*, when incorporated into planar lipid bilayers, gave rise to channels with a single-channel conductance of 1.8 nS in 1 M KCl. This suggests that the NmpC protein is a porin.

The *Escherichia coli* outer membrane has been shown to contain a variety of polypeptides which function as transmembrane diffusion channels and bear the common name porins (10). All *E. coli* porin proteins studied to date share the following properties (10): the functional pore-forming unit is a trimer species which maintains its oligomeric association even in the presence of sodium dodecyl sulfate (SDS) at moderate (<60°C) temperatures; it displays tight noncovalent association with the peptidoglycan; the monomeric molecular weights are similar and range from 35,000 to 45,000; the porin monomers have acidic isoelectric points; porins are present in high copy numbers (>10⁵ polypeptides per cell) under the appropriate circumstances; and all form hydrophilic transmembrane pores with similar channel sizes (estimated as 1.2 to 1.5 nm in diameter [1, 1a, 2, 5]). In addition, there is considerable nucleotide and protein sequence homology among some of these porins (14). The porin proteins from *E. coli* whose function has been well documented include the constitutive porins OmpC, OmpF (1-3, 11), and protein K (15), and the inducible porins LamB (2) and PhoE (11; Benz et al., in press). In addition, Pugsley and Schnaitmar provided evidence that protein 2 (also called the Lc protein), which is encoded by the prophage PA2, can reverse some of the transport defects associated with loss of the constitutive porins OmpC and OmpF (13). Although this reversal of the transport defects was somewhat strain, growth medium, and substrate specific, it suggested that protein 2 might have a porin function. It has since been demonstrated that the NmpC protein which is produced in high levels in a pseudorevertant of an *E. coli* K-12 porin-deficient mutant (12) is extremely similar but not identical to protein 2 on the basis of proteolytic cleavage patterns (7). Furthermore, the NmpC protein appears closely related to the OmpD (38,000) porin protein of *Salmonella typhimurium* (2, 6). Although this has provided circumstantial evidence that the NmpC protein is a porin, direct evidence has been lacking to date.

The NmpC protein from *E. coli* K-12 strain CS483 (12) (obtained from Barbara Bachmann of the Coli Genetic Stock Center, Yale University, New Haven, Conn., as strain CGSC6066) was purified exactly as described previously for the protein K porin (15), except that the final solubilization step was performed at 60°C for 2 h rather than at 37°C for 2 h.

The basis of this purification procedure was the resistance to denaturation by SDS and strong noncovalent association with the peptidoglycan of the NmpC protein. The NmpC protein remained bound to the peptidoglycan even after treatment with 2% SDS, high salt concentration, and EDTA at 37°C for 2 h, although the same procedure when performed at 60°C resulted in nearly quantitative release of the NmpC protein from the peptidoglycan. The purified NmpC protein formed an oligomer with an apparent mass of 74,000 daltons when analyzed by SDS-polyacrylamide gel electrophoresis (5) after solubilization in SDS at temperatures less than 60°C (data not shown). However, after solubilization at 100°C for 5 min, the protein appeared in the gel electrophoretogram as a monomer of 39,500 daltons.

The methods used for black lipid bilayer experiments have been described previously in detail (1, 3). The apparatus consisted of a Teflon chamber with two compartments connected by a small hole (0.1 to 2 mm²). A membrane was formed across the hole by painting on a solution of 1 to 2% (wt/vol) oxidized cholesterol in *n*-decane. Bilayer formation was indicated by the membrane turning optically black to incident light. Conductance through the pores was measured after application of a given voltage, using a pair of Ag-AgCl electrodes inserted into the aqueous solutions on both sides of the membrane. The current through the pores was boosted by a preamplifier, monitored by a storage oscilloscope, and recorded on a strip-chart recorder.

Addition of moderate amounts (final concentration, 46.7 pg/ml) of the purified NmpC protein to the aqueous salt solution bathing a lipid bilayer membrane resulted in an increase in specific membrane conductance of 2 orders of magnitude. After the membrane turned black, conductance increased in a time-dependent fashion for more than 60 min and failed to reach a stationary level before the membrane broke. When a large number of NmpC channels (>50) were incorporated into the membrane, current measured through the channels was a linear function of applied voltage and extrapolated to zero current at zero voltage. This suggested that the NmpC channel is not voltage induced or regulated. In all, no significant differences were observed in macroscopic conductance experiments with the NmpC protein or other *E. coli* porins (1, 3, 4, 15).

When small amounts (3×10^{-11} M) of purified NmpC porin were added to the aqueous phase bathing a lipid bilayer membrane, membrane conductance increased in a stepwise

* Corresponding author.



FIG. 1. Stepwise increases in the membrane conductance after addition of 3×10^{-11} M purified NmpC protein to the aqueous phase (1 M NaCl) bathing a lipid bilayer membrane. The membrane was formed from 1.5% oxidized cholesterol in *n*-decane, the applied voltage was 20 mV, and the temperature was maintained at 25°C. The record begins at the left.

fashion (Fig. 1). By analogy with other lipid bilayer experiments, these increases probably represented the time-dependent incorporation of single NmpC channels into the membrane. Occasionally, increments were observed which were twofold larger than the most common increments, especially in the presence of 1 M NH_4Cl , probably due to the simultaneous incorporation of two channels into the membrane. Most of the conductance increments were directed upwards although downwards-directed conductance steps of similar magnitude were also observed, presumably due to loss or inactivation of a previously incorporated channel.

As shown previously for other porins (1–4, 15), the single-channel increments were not of uniform size but were distributed around a mean. For example, in 1 M KCl the most common conductance increment was 1.5 nS (33% of measured channels), but a substantial number of channels with conductances of 1.0 nS (18%), 2.0 nS (23%), and 2.5 nS (19%) were observed in the 213 recorded single-channel conductance steps. Analogous observations were made for all other salts studied.

The average single-channel conductances were recorded for a variety of salts (Table 1). Despite substantial variations in the bulk conductance (i.e., the conductance σ of the salt

solution in the absence of a membrane) and in the average single-channel conductance $\bar{\Lambda}$, the ratio of $\bar{\Lambda}$ to σ varied only 1.7-fold. This suggested that the NmpC channel is filled with water. In agreement with this possibility, single-channel conductance was a linear function of salt concentration, a result expected for a large water-filled channel (Table 1).

The data presented here confirm previous hypotheses that the NmpC protein from *E. coli* is a porin and provide the first direct evidence for this. The purified NmpC porin forms large water-filled channels as judged by the linear relationship between KCl concentration in the aqueous phase and single-channel conductance, the relatively constant $\bar{\Lambda}/\sigma$ ratios for a variety of salts, and the linear relationship between macroscopic current and applied voltage. Thus, the NmpC channel has properties similar to those of the well-studied porins of *E. coli* and other bacteria. Many other physical properties of the NmpC channel resemble those of other *E. coli* porins, including an oligomeric (presumably trimeric) association of subunits that is stable to SDS (9), a strong noncovalent association with the peptidoglycan (7, 10), a monomeric molecular weight in the 35,000 to 45,000 range, an acidic isoelectric point and a tendency to form multiple isoelectric focusing bands (10), expression dependent on the *tolC* locus (8), and a similar conductance (1.8 nS) in 1 M KCl (1, 1a, 2, 15).

Single-channel conductance experiments suggested that the size of the NmpC channel is similar to that of the PhoE and protein K pores but somewhat smaller than the OmpC, OmpF, and LamB pores. On the basis of the data in Table 1 for 1 M KCl and assuming a pore length of 7.5 nm (2), we could estimate the effective pore diameter as 1.2 nm. This small decrease in size over the OmpC (1.3 nm) and OmpF (1.4 nm) porin channels (2) could be quite significant when considering the effectiveness of NmpC in the uptake of larger molecules such as nucleotides, β -lactams, and di- or trisaccharides, given the substantial differences in the permeability of OmpC and OmpF toward such compounds (11).

The clear demonstration here that NmpC is a porin brings the known number of porins in *E. coli* to six, i.e., OmpC, OmpF, NmpC, PhoE, LamB, and protein K. Even given the conditional production of the PhoE and LamB porins and the fact that NmpC is a silent gene in wild-type *E. coli* K-12, a major unanswered question concerns the reason for so many

TABLE 1. Average single-channel conductance increments in different salt solutions^a

Salt	Concn (M)	Avg single-channel conductance $\bar{\Lambda}$ (nS)	Bulk phase conductance of salt solution (mS/cm)	$\bar{\Lambda}/\sigma$ (10^{-8} cm)	No. of increments measured
KCl	0.03	0.058	3.4	1.7	104
	0.1	0.19	11	1.7	178
	0.3	0.49	34	1.5	174
	1.0	1.80	110	1.6	213
NaCl	1.0	1.12	84	1.3	258
NH_4Cl	1.0	1.39	112	1.2	122
MgCl_2	0.5	0.62	64	1.0	249
Tris-chloride	0.5	0.33	30	1.1	132
K_2SO_4	0.5	1.32	76	1.7	110

^a The aqueous phase contained the indicated concentration of salt and 3×10^{-11} M NmpC protein was added to initiate single-channel measurements. The applied voltage was 10 mV; membranes were made from 1 to 2% oxidized cholesterol; the temperature of the aqueous phase was 25°C. $\bar{\Lambda}$ was averaged for a large number of conductance increments. The bulk conductance for each salt solution was the measured conductance in the absence of a membrane. Control experiments demonstrated that the small amount of SDS added to the aqueous phase together with NmpC protein did not cause membrane conductance to increase.

genes coding for very similar proteins in the same species of bacterium.

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