JOURNAL OF BACTERIOLOGY, Sept. 1978, p. 1080-1090
0021-9193/78/0135-1080$02.00/0

Vol. 135, No. 3
Printed in U.S.A.

Porin Activity in the Osmotic Shock Fluid of *Escherichia coli*

ROLAND BENZ, BEVERLY A. BOEHLER-KOHLER, REGINA DIETERLE, AND WINFRIED BOOS*
Department of Biology, University of Konstanz, D-7750 Konstanz, West Germany

Received for publication 19 April 1978

Osmotic shock fluid of *Escherichia coli* exhibited pore-forming activity. This activity could be followed by an in vitro assay based on the conductivity increase for ions due to the presence of pores in black lipid membranes. The histogram (the distribution of conductivity increments in a single pore experiment) obtained with osmotic shock fluid from *E. coli* was identical to the histogram obtained by detergent-solubilized porin isolated from the outer membrane. The osmotic shock fluid from porin-negative mutants also exhibited pore activity, although the histogram and ion specificity were different from those of porin. Antibodies raised against detergent-solubilized porin were able to form precipitin lines by the Ouchterlony immunodiffusion technique when shock fluids, but not detergent-solubilized porin, were used. These antibodies prevented the formation of pores when shock fluids contained porin but not when shock fluids obtained from porin-negative mutants were used. Macroscopic membrane conductivity of shock fluids due to porin exhibited a concentration dependence, in contrast to detergent-solubilized porin. These results indicate that the hydrodynamic properties of periplasmic or "soluble" porin are different from those of the detergent-solubilized porin of the outer membrane. Periplasmic porin comprises about 0.7% of total protein in the osmotic shock fluid.

The cold osmotic shock procedure pioneered by the work of Neu and Heppel (44) has been used to operationally define the periplasmic space (39) and, thus, periplasmic proteins (21, 22, 47, 54). These proteins are water soluble and considered to be located outside the cytoplasmic membrane, even though some cytoplasmic proteins may be released under certain conditions (25). Most of the studies on the localization of periplasmic proteins have been done with alkaline phosphatase (9, 13, 27, 36, 38) as well as with substrate-binding proteins (43, 50) that are part of active transport systems (5, 47). In particular, studies on the biosynthesis of periplasmic proteins indicate that they are synthesized through the cytoplasmic membrane and are thus located exclusively outside the osmotic barrier of the *Escherichia coli* cell (51, 60, 62). The outer limit of the periplasmic space is less well defined (11, 37). In particular, it is not clear to what extent periplasmic proteins may interact or may even be part of the outer membrane (16, 37).

This outer membrane of gram-negative bacteria such as *E. coli* is rather complex, being composed of phospholipids, lipopolysaccharides, and proteins (15, 45, 56). Via one of the major outer membrane proteins, the murein lipoprotein, this structure is covalently linked to the underlying peptidoglycan network (8). However, this lipoprotein also occurs in a form not linked to murein (24), but it is still part of the outer membrane (8).

In recent years numerous studies have concentrated on the proteins that are contained in the outer membrane. The evidence for their location is essentially the separation of the outer membrane by density gradient centrifugation of lysed spheroplasts (46) or by solubility properties of total membranes in Triton X-100 (58, 59). By using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, the major outer membrane proteins have been analyzed by several groups of investigators (7, 10, 18, 34, 55, 59, 67). They are a class of closely related proteins that are strongly membrane bound and are only solubilized by detergents. One of these major outer membrane proteins, in particular, has been studied intensively. Various investigators have called this protein protein I (17), protein 1 (59), protein O-8,9 (67), protein A_1, A_2 (7), protein b,c (34), matrix protein (55), and porin (41). In *E. coli* B porin apparently consists of one polypeptide chain (35, 55), whereas in *E. coli* K-12 more than one closely related polypeptide may be present (20, 23, 26, 57). The relative amounts of these closely related polypeptide chains vary depending on growth conditions (35), as well as on mutations affecting the structure of the lipopolysaccharide (1, 28).

Porin spans the outer membrane and can function as a phage receptor (2, 12). Its position in the outer membrane has been described as a matrix based on a hexagonal lattice (63). Cross-link experiments with intact cells as well as with isolated cell walls indicate a dimeric (48) or trimeric structure (49, 53).

Porin exhibits a strong affinity towards peptidoglycan, since it is not released by treatment in SDS at temperatures up to 70°C (19, 33, 55). In addition, binding of porin to peptidoglycan is enhanced by lipopolysaccharide (69). Also, murein lipoprotein seems to play a role in the interaction of porin with peptidoglycan. Porin can be released by trypsin treatment (41). Under these conditions, murein lipoprotein is degraded (8), whereas porin remains resistant to trypsin (55). Also, direct interaction of murein lipoprotein with porin has been reported recently (30).

All these data demonstrate the tight interaction of porin with the other components within the outer membrane as well as with itself.

The outer membrane plays an important role in the diffusion of hydrophilic molecules into the periplasm (R. J. Kadner and P. Bassford, *in* B. P. Rosen, ed., *Bacterial Transport,* in press). Recently, porin has been shown in reconstitution experiments to function as a hydrophilic pore mediating the passive diffusion of small molecules (molecular weight, 600) through otherwise impermeable phospholipid vesicles (40–42). The effect of porin on increasing the permeability of these vesicles to small molecules is reflected in the pleiotropic effect that porin mutants have on the transport capacity in whole cells (3).

In another paper (R. Benz, K. Janko, W. Boos, and P. Läuger, Biochim. Biophys. Acta, in press), we have demonstrated that detergent-solubilized porin is able to increase the conductivity of black lipid membranes to ions by several orders of magnitude. Moreover, at low concentration of porin increases in single steps of conductance could be observed. The distribution in sizes of these individual conductivity increments is characteristic for porin. In the present paper we demonstrate that soluble periplasmic proteins isolated by the osmotic shock procedure contain pore-forming activity due to porin and other pore-forming proteins.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains are described in Table 1. They were grown in 500 ml of rich medium (10 g of yeast extract, 16 g of tryptone, 10 g of sodium chloride, and 2 g of glycerol per liter) overnight under aerobic conditions. The porin-negative phenotype of the porin-negative strains was routinely checked on minimal glucose plates (by the formation of small colonies).

Osmotic shock procedure. The cold osmotic shock procedure was done essentially according to Neu and Heppel (44). From 500 ml of culture, 100 ml of shock fluid was obtained. This solution was lyophilized and resuspended in 2 ml of 10 mM tris(hydroxymethyl)aminomethane(Tris)-hydrochloride, pH 7.3. It was dialyzed overnight against the same buffer and centrifuged at 100,000 × *g* for 1 h. The supernatant contained between 2 and 3 mg of total protein per ml. It was stored at −18°C.

Antiporin antibodies. Antiporin antibodies were obtained by injecting a rabbit subcutaneously with 2 mg of cholate-solubilized porin (Benz et al., in press) in a mixture of 1 ml of 0.1% cholate and 1 ml of complete Freund adjuvant. After 4 weeks this injection was repeated. Two weeks later serum containing antiporin antibodies was obtained. Immunodiffusion tests were done in preformed plates (Hyland-Immunoplate, pattern D). The plates were incubated for 4 h at 37°C and subsequently treated with a 2% sodium chloride solution for 2 days. They were stained with Coomassie brilliant blue as previously described (31).

Membrane experiments. Optically black lipid bilayer membranes were obtained in the usual way (4) from a 1 to 2% (wt/vol) solution of oxidized cholesterol in *n*-decane (Fluka, Buchs, Switzerland; purum). The cell used for bilayer formation was made from Teflon; the circular holes in the wall between the two aqueous compartments had an area of either 2 mm^2 (macroscopic conductance measurements) or 0.1 mm^2 (single fluctuation experiments). The temperature was kept constant at 25°C throughout all experiments.

The different salts (Merck, Darmstadt, West Germany; analytical grade) were dissolved in twice-distilled water without a buffer. The aqueous solutions had a pH of about 6. Small variations in the pH had no influence on the single fluctuations or on the macroscopic conductance. Oxidized cholesterol was prepared by boiling a 4% (wt/vol) suspension of choles-

TABLE 1. *Bacterial strains (E. coli K-12 derivatives)*

Strain	Parent	Genotype	Porin phenotype	Origin
pop 1730	Hfr G6	Hfr (*malK-lamB*) *his*	+ (Ia^+, Ib^+)	M. Schwartz (52)
LA 5001	MC 4100	F^- *araD lac rpsL glpR, Mucts:glpT nalA*	+ (Ia^+, Ib^+)	Parent described in T. J. Silhavy et al. (61)
CM 1068	AB 2847	F^+ *kmt*$^+$ *malT*	+ (Ia^+, Ib^+)	K. von Meyenburg (3)
CM 1070	AB 2847	F^+ *kmt*^{a} *malT*	− (Ia^-, Ib^-)	K. von Meyenburg (3)
CM 1072	AB 2847	F^+ *kmt malT*$^+$	− (Ia^-, Ib^-)	K. von Meyenburg (3)

[a] *kmt* and *ompB* are most likely identical genetic loci (3).

terol (Eastman; reagent grade) in *n*-octane (Merck; analytical grade) for 4 h under reflux and bubbling oxygen through the suspension (66). The membranes from oxidized cholesterol-*n*-decane had a specific capacity of 0.555 $\mu F/cm^2$ and, assuming a dielectric constant of 2.1, a corresponding thickness of 3.3 nm (R. Benz, unpublished data).

For the electrical measurements, Ag/AgCl electrodes were inserted in the aqueous compartments on both sides of the membrane. In series with the electrodes, a voltage source and a current amplifier were used. For the macroscopic conductance measurement a Keithley 150B microvolt ammeter or a Keithley 610C electrometer was used. For the conductance fluctuation experiments a Keithley 427 current amplifier and a Tectronix 5111/5A22 storage oscilloscope (as the detecting instrument) were used. The amplified signal at the output of the oscilloscope was recorded with a strip chart recorder or in some cases with a tape recorder. The bandwidth of the measurement was 300 Hz to 3 kHz. The rise time of one single step in the fluctuation measurement was faster than 300 μs, and within this time resolution the current rise did not contain smaller intermediate steps.

Small samples of the concentrated solution of the shock protein were added to the salt solutions bathing the membrane. In the case of macroscopic conductance experiments, a final concentration of 5 ng to 5 μg of the shock proteins per ml was used. The concentration used for the single fluctuation measurements was much smaller in order to obtain only a limited number of steps. With the shock proteins containing porin (strains pop 1730 and CM 1068) a concentration of 1 ng/ml was sufficient, whereas for the other shock proteins (strains CM 1070 and CM 1072) a concentration of 5 ng/ml was needed.

In the shock solution the pore-forming activity remained for at least 8 weeks, although it was necessary after some time to stir the solution prior to use. In the aqueous solutions of high ionic strength (0.1 to 1 M), however, the protein lost its activity continuously and became inactive after about 20 h. For this reason fresh solutions were used for the membrane experiments.

Affinity column. The immunoglobulin G fraction of serum (10 ml) was purified by the method of Livingston (32) and then coupled to cyanogen bromide-Sepharose (Pharmacia), using the instructions supplied by the manufacturer. The antibody-coupled cyanogen bromide-Sepharose (4 ml) was then poured into a column (0.9 by 6.0 cm) and equilibrated with BBS (0.2 M borate, 0.5 M NaCl) buffer, pH 8. Concentrated shock fluid was applied, and the column was washed with the equilibrating buffer until no more UV-absorbing material was present in the effluent. Upon addition of a 2-ml sample of BBS buffer, 7 M in guanidine-HCl, a sharp UV-absorbing peak was obtained in the effluent. The protein-containing fractions were pooled and dialyzed against 10 mM Tris, pH 7.3. SDS-gel electrophoresis was performed with the system described by Laemmli (29).

RESULTS

Antiporin antibodies. Porin was isolated from the outer membrane by SDS solubilization after trypsin treatment of SDS-extracted membranes (Benz et al., in press). This material was used to raise antibodies in a rabbit. The serum of the immunized rabbit was tested against detergent-solubilized porin in the Ouchterlony immunodiffusion tests. No precipitin lines were formed within the gel, but instead precipitation with the antibodies occurred on the border of the porin-containing well (Fig. 1). This indicates that the porin preparation consists of large aggregates that are unable to penetrate the agar. However, surprisingly, osmotic shock fluids that had been used as a supposedly porin-free control gave one strong precipitin band and a second, smaller band positioned closer to the antibody well. Since it was possible that one of these bands was caused by the presence of porin in the periplasmic proteins of a set of isogenic strains, one of them lacking porin (68) was tested. Figure 2 shows the corresponding immunodiffusion tests. As can be seen, the small precipitin line was present in all preparations, but the large band was missing in the mutant preparations lacking porin. This indicates that shock fluids of wild-type strains do in fact contain porin. The fact that the porin derived from the periplasm diffuses into the Ouchterlony plate is evidence for the solubility of this protein. Further evidence for solubility was obtained during the

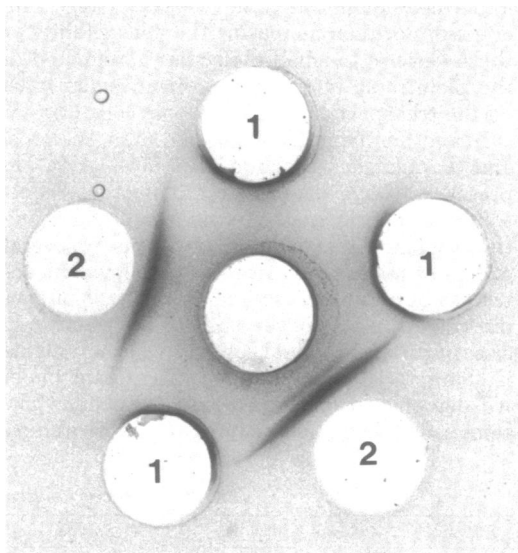

FIG. 1. *Immunodiffusion test with antiporin antibodies. The wells contained the following: 1, SDS-solubilized porin, 0.2 mg of protein per ml; 2, concentrated shock fluid (2 mg of protein per ml) of strain LA 5001. The center well contained antiporin antibodies. The precipitin bands were stained with Coomassie brilliant blue.*

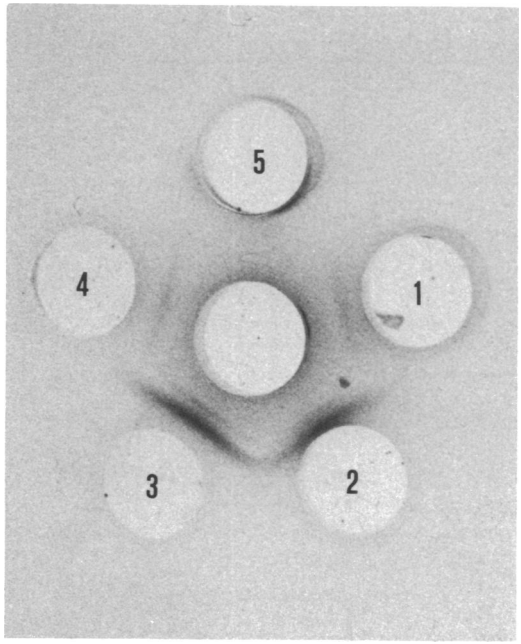

FIG. 2. *Immunodiffusion test with antiporin antibodies. The wells contained shock fluids (2 mg of protein per ml) from the following strains. 1, CM 1072; 2, CM 1068; 3, LA 5001; 4, CM 1070. Well 5 contained SDS-solubilized porin (0.2 mg/ml). The center well contained antiporin antibodies.*

preparation of the periplasmic proteins. Crude shock fluids were centrifuged at 100,000 × *g* for 1 h, and the porin activity remained in the supernatant under these conditions.

Pore-forming activity in osmotic shock fluids. Concentrated shock fluid of a wild-type strain was diluted to about 1 ng/ml and added to the aqueous solutions bathing a black lipid membrane. As can be seen in Fig. 3A, the membrane conductance given in nanosiemens (10^{-9} S) or picoamperes (10^{-12} A) starts to increase in a stepwise fashion. The analysis of the distribution in the size of the individual conductance increments (histogram) is shown in Fig. 4A. For comparison, the same analysis using detergent-solubilized porin (42; Benz et al., in press) is depicted in Fig. 4B. Assuming that this conductance is due to the formation of hydrophilic channels, the conductivity, Λ, of ions through these channels is given by $\Lambda = (\sigma \cdot \pi \cdot r^2)/l$ (σ = specific conductivity of the bathing solution; l = length of the channel). Thus, Λ is directly proportional to the cross section (πr^2) or the pore size. As can be seen, both preparations give rise to the formation of pores that are identical in their size distribution. Also, no significant difference can be seen between shock fluid preparations of strains LA 5001 and CM 1068 (not shown).

When antiporin antibodies were mixed with the osmotic shock prior to the addition to the membrane, only a few conductance steps could be observed, the histogram of which did not resemble porin. However, the antibodies completely prevented pore formation by detergent-solubilized and -purified porin (not shown). In some experiments the antibodies were added to one or both compartments of the cell after pores had been formed by porin. In these cases, no decrease in the conductance was observed. However, the membranes broke 5 to 10 min after the addition of antibodies. Apparently, this was caused by the antibodies or other components of the serum itself and also happened in the absence of porin.

Shock fluids of two strains that lack porin (CM 1070 and CM 1072) also contained pore-forming activity, although this activity was only obtained at protein concentrations fivefold higher than those of shock fluids from a porin-positive strain. The conductance steps of the preparation of one of these strains (CM 1070) is shown in Fig. 3B. The histograms for both strains, CM 1070 and CM 1072, are shown in Fig. 5B and C in comparison to the histogram of the isogenic porin-positive strain, CM 1068 (Fig. 5A). As can be seen, both shock fluids from the porin-negative strain contain pores that are smaller (CM 1070) and both smaller and larger (CM 1072) than those from porin-positive strain CM 1068. The only difference in strains CM 1070 and 1072 is the presence of the receptor of phage λ in CM 1072.

The differences in the pore size are also reflected in the average pore conductivity exhibited by the different shock protein preparations, in 1 M NaCl as well as in other alkali chlorides (Table 2).

Differences between the pores of the different preparations are most seen with lithium and sodium. Here, the pores of the porin-negative strain are on the average smaller than those from the porin-positive strains (LA 5001 and CM 1068).

Addition of anti-porin antibodies to the shock fluids of the porin-negative strains, CM 1070 and CM 1072, did not alter their pore-forming capacity.

Macroscopic conductivity. At higher concentrations of osmotic shock fluids single steps of conductance increase are no longer observable, but the conductance rise with time is smooth. With detergent-solubilized porin this increase in conductance is largely independent of porin concentration (Benz et al., in press). At a concentration of 1 μg/ml and measured 10 min

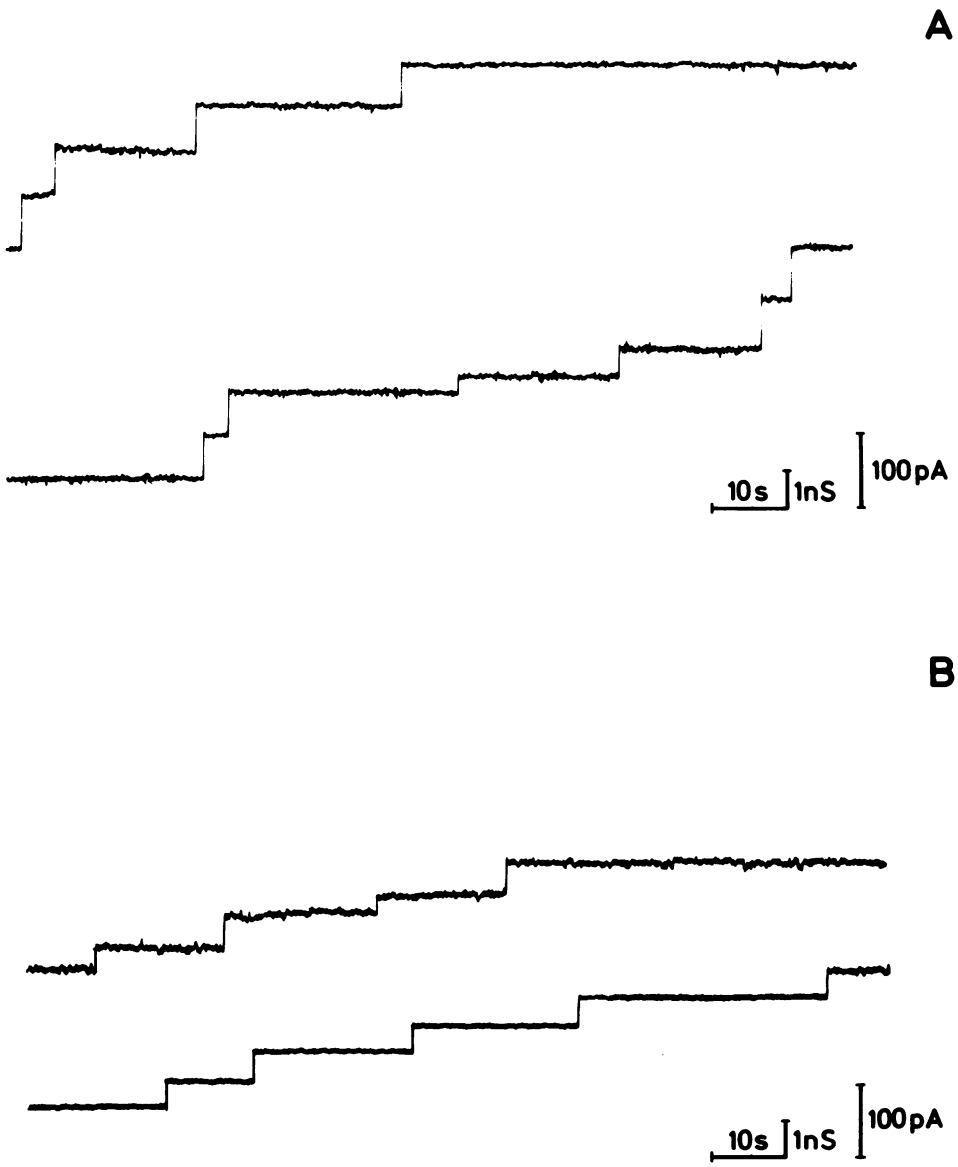

FIG. 3. *Conductance fluctuations of membranes from oxidized cholesterol-n-decane in the presence of shock protein from strain CM 1068 (1 ng of protein per ml) (A) and from strain CM 1070 (5 ng of protein per ml) (B). The aqueous phase in both cases contained 1 M NaCl, pH 6. The temperature was 25°C. The applied voltage was 50 mV; the current prior to the addition of shock protein was about 1 pA. The record starts in both cases at the left ends of the lower traces and continues in the upper traces.*

after black membrane formation, the shock fluids conferred different conductivity upon the membrane (Table 3). Here again, shock fluids of the porin-positive strains were 5- to 10-fold more active than those from the porin-negative strains. When different concentrations of shock fluids from porin-positive strains were tested, a linear dependence between conductance and concentration was observed in a log/log plot, the slope of which is between 1.3 and 1.4 (Fig. 6). Fresh preparations that had been centrifuged but neither lyophilized nor dialyzed exhibited slopes up to 2 (not shown).

Stability of periplasmic porin. After storage for several weeks at 4°C in 10 mM Tris buffer, a precipitate formed and the shock fluids lost their ability to form precipitin lines in the immunodiffusion test against antiporin antibod-

ies. The precipitate but not the supernatants remained active in the membrane conductivity test. Apparently, "soluble" porin is unstable and tends to aggregate in aqueous solutions without loss of activity. As with detergent-solubilized porin, periplasmic porin became irreversibly inactivated by incubation for more than 1 day in 1 M sodium chloride.

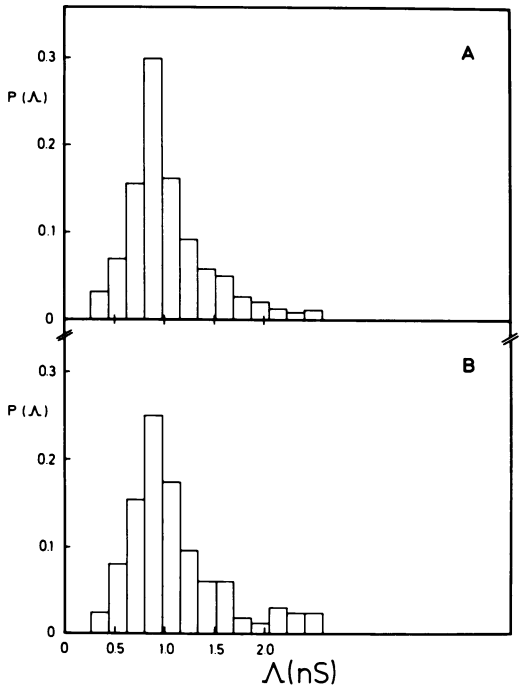

FIG. 4. *Probability, P (Λ), of the occurrence of a conductance step of magnitude Λ. P (Λ) is the number of observed steps within an interval of width ΔΛ = ±89 pS centered at Λ, divided by the total number of steps. The membranes were made from 2% (wt/vol) oxidized cholesterol in n-decane. The aqueous phase contained 1 M NaCl, pH 6; the temperature was 25°C. The applied voltage was 50 mV. The aqueous phases contained the following: A, shock protein from strain LA 5001 (1 ng of protein per ml), Λ = 1.1 nS (n = 345); B, porin solubilized in SDS (0.5 ng/ml), Λ = 1.2 nS (n = 241).*

Isolation of periplasmic porin. Shock fluid obtained from the porin-positive strain LA 5001, containing about 10 mg of total protein, was poured over an affinity column with covalently fixed antiporin antibodies. After being washed, the column was eluted with 7 M guanidinium chloride, releasing about 0.5 mg of protein in a sharp peak. The protein-containing fractions were dialyzed against 10 mM Tris buffer, pH 7.2. It contained pore-forming activity. Its histogram is identical to that of porin (not shown). However, the initially clear solution forms precipitates much faster than crude shock fluids. SDS-polyacrylamide gel electrophoresis of this material in comparison to detergent-solubilized porin as well as to shock fluid prior to and after passage through the column is shown in Fig. 7. The eluted material does contain porin, even though other proteins are also present. From the intensity of the Coomassie brilliant blue stain one can estimate that porin constitutes at least 15% of the eluted material. Therefore, one would calculate that approximately 0.7% of the total protein present in the shock fluid is porin.

DISCUSSION

Osmotic shock fluids of *E. coli* contain at least one protein that is usually found exclusively as an intrinsic membrane protein, closely associated with the outer membrane. In particular, they contain porin (41), a protein that has been shown to overcome the permeability barrier of the outer membrane for small hydrophilic molecules (3). Evidence for this statement is the following. (i) Shock fluids are able to form a precipitin line in an immunodiffusion test with antiporin antibodies that is absent in shock fluids from porin-negative strains. (ii) Shock

TABLE 2. *Average pore conductivity (Λ) in single pore experiments with different shock fluids*[a]

Conducting ion (1 M)	Conductivity (nS)				
	SDS-solubilized porin from outer membrane, strain 1730 (<1 ng/ml)	Crude osmotic shock fluid			
		G8,1 *nal* (1 ng/ml)	CM 1068 (1 ng/ml)	CM1070 porin⁻, λʳ (5 ng/ml)	CM1072 porin⁻, λˢ (5 ng/ml)
LiCl	0.72	0.69	0.68	0.38	0.54
NaCl	1.2	1.1	1.1	0.61	0.84
KCl	1.9	1.8	1.7	2.0	1.7
RbCl	2.1	2.0	1.6	1.7	1.8
CsCl	2.3	2.1	1.8	2.0	1.8

[a] A potential of 50 mV was applied. For the calculation of Λ, 200 to 400 conductance increments were averaged.

[b] Data from Benz et al. (in press).

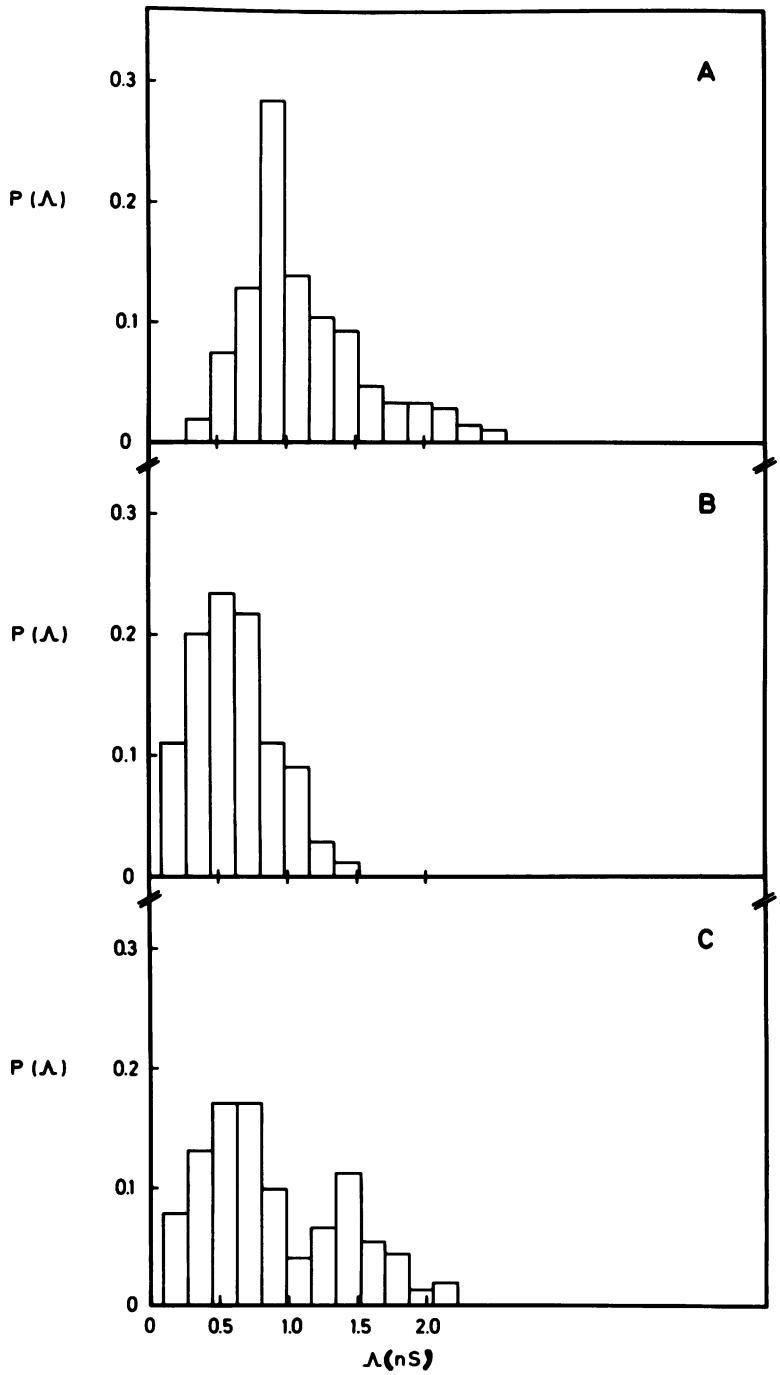

FIG. 5. *Probability, P (Λ), of the occurrence of a conductance step of magnitude Λ. P (Λ) is the number of observed steps within an interval of width ΔΛ = ±89 pS centered at Λ, divided by the total number (n) of steps. The membranes were made from 2% (wt/vol) oxidized cholesterol in n-decane. The aqueous phase contained 1 M NaCl, pH 6; the temperature was 25°C. The applied voltage was 50 mV. Shock proteins from the following strains were used: A, shock protein CM 1068 (1 ng/ml), Λ = 1.1 nS (n = 282); B, shock protein CM 1070 (5 ng/ml), Λ = 0.61 nS (n = 180); C, shock protein CM 1072 (5 ng/ml), Λ = 0.84 nS (n = 152).*

TABLE 3. *Macroscopic conductivity through black lipid membranes in the presence of osmotic shock fluid*[a]

Strain	Conductivity, λ^b ($\mu S/cm^{-2}$)
LA 5001	80
CM 1068	30
CM 1070	6
CM 1072	3

[a] Protein concentration was 1 μg/ml; bathing solution was 0.1 M NaCl, pH 6; temperature was 25°C; and 10 mV was applied.

[b] Macroscopic conductivity was measured after 10 min of addition of shock fluid protein. Since conductivity increases with time, the given values reflect: the concentration of pore-forming activity, the rate of their incorporation into the bilayer membrane, and the final conducting state.

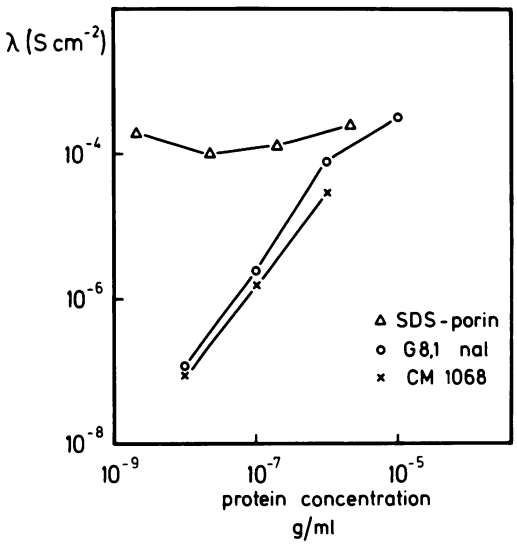

FIG. 6. *Specific membrane conductance, Λ, as a function of the protein concentration in the aqueous phase: 0.1 M NaCl, pH 6; temperature = 25°C. The membranes were formed from oxidized cholesterol in n-decane. The conductance was measured 10 min after the membrane was completely black (blackening time, 1 to 2 min). The applied voltage was 10 mV. Each point represents the average of at least three different experiments.*

fluid proteins exhibit pore-forming activity in black lipid membranes. The histogram of the pore conductance is identical to that obtained with detergent-solubilized porin from outer membranes. (iii) The formation of pores due to porin is prevented by antiporin antibodies. (iv) Porin isolated from the osmotic shock fluid is identical on SDS-polyacrylamide gel electrophoresis to that isolated from the outer membranes.

The soluble nature of periplasmic porin is given by the observation that porin activity remains in the supernatant of preparations that were centrifuged at 100,000 × *g* for 1 h. Also, the observation that the periplasmic porin is able to diffuse into the agar gel in the immunodiffusion technique is indicative of its soluble nature. In addition, periplasmic porin exhibits a concentration dependence of macroscopic conductance that is approximately proportional to the second power of protein concentration. This indicates that a polymeric structure is formed from smaller subunits.

The amount of porin present in the osmotic shock fluids is estimated by affinity chromatography, using columns containing covalently linked antiporin antibodies. From these experiments it is estimated that porin constitutes approximately 0.7% of the periplasmic proteins. Periplasmic proteins comprise about 3.5% (21) and total porin constitutes 7% of the cellular protein (55). Thus, only about 0.3% of total envelope porin is found in the osmotic shock fluid.

Shock fluid isolated from strains that lack porin also exhibit pore-forming activity. However, the histograms of these preparations are clearly different from those of the porin-containing preparations. The histogram of strain CM 1072 apparently contains two types of pores (smaller and larger than porin), whereas strain CM 1070 contains only the smaller type of pores. Strain CM 1072 contains the λ receptor, whereas its isogenic derivative, CM 1070, does not. Recently, the λ receptor has been implicated in the maltose and maltodextrin transport system (64) by overcoming the diffusion barrier for these sugars through the outer membrane (65). However, the λ receptor is also able to accommodate small molecules other than maltodextrins (68). Indeed, detergent-solubilized and -purified λ receptor is also able to form pores in black lipid membranes (manuscript in preparation). The histogram obtained by this preparation is very similar to the population of the large pores in the shock fluid of strain CM 1072 (Fig. 5), indicating its identity with the λ receptor.

The origin of the population of small pores in the shock fluid of strains CM 1070 and CM 1072 is not clear. One possible candidate is protein II* (17) since this protein has been shown to span the outer membrane (14).

Shock fluids from porin-positive strains also contain the pores present in porin-negative strains (CM 1070, CM 1072). However, these proteins are present in the shock fluid in smaller amounts than porin. Therefore, they do not contribute significantly to the respective histogram. They become apparent only when porin is inactivated by the antiporin antibodies.

It is not clear what the hydrodynamic prop-

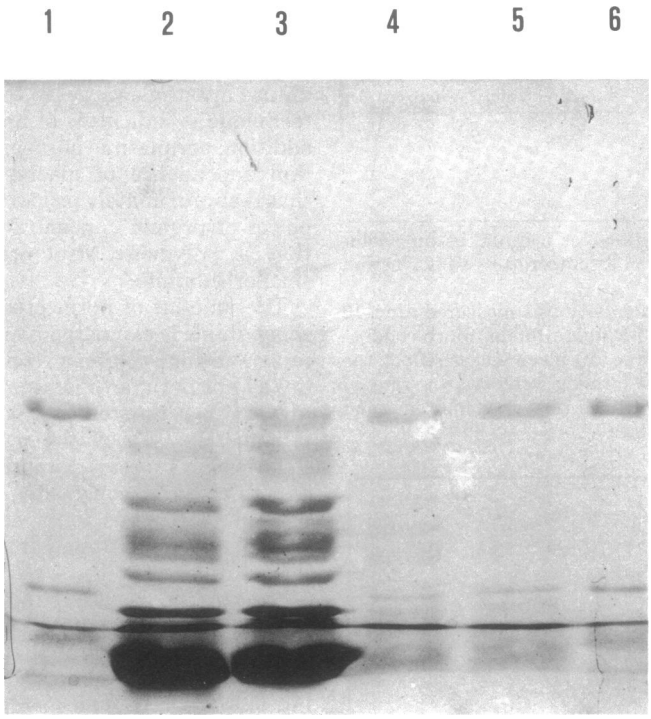

FIG. 7. *Polyacrylamide slab gel electrophoresis in the presence of SDS. The different positions contained the following preparations: 1, SDS-solubilized porin (20 μg of protein) (this preparation had been isolated by trypsin treatment [Benz et al., in press]; the fresh preparation only exhibited a single band by this gel technique; storage in 0.1% SDS for several months apparently results in considerable proteolytic breakdown, probably due to remaining trypsin); 2, concentrated shock fluid of strain CM 1068 (100 μg of protein) after passage through a Sepharose column containing covalently linked antiporin antibodies; 3, concentrated shock fluid of strain CM 1068 (100 μg of protein); 4, material eluted from the antibody-carrying column by 7 M guanidinium hydrochloride (20 μg of protein); 5, same as (4) at a higher protein concentration (25 μg of protein); 6, same as in (1).*

erties of porin in the shock fluids are. Since this protein appears to be soluble by means of immunodiffusion and ultracentrifugation, it seems unlikely that it is present as part of small outer membrane fragments. Our working hypothesis is based on the interaction of porin (and the other pore-forming proteins) with "free" lipoprotein (30). The latter may act as a detergent and keep the strongly hydrophobic outer membrane proteins in solution. At present, the free lipoprotein has not yet been identified to be present in osmotic shock fluids (V. Braun, personal communication). Yet, it is our experience that nearly all periplasmic shock fluids that we have tested so far by SDS-polyacrylamide gel electrophoresis, or by two-dimensional electrophoresis, do contain substantial amounts of a protein that migrates in close proximity to the tracking dye (bromophenol blue) and may in fact be free lipoprotein.

The next step in the understanding of the hydrodynamic properties of periplasmic porin will therefore be the possible identification of lipoprotein in osmotic shock fluids and the examination of its interaction with porin.

ACKNOWLEDGMENTS

We thank Peter Läuger for helpful suggestions and critically reading the manuscript. We are grateful to Kaspar von Meyenburg for supplying us with the right bacterial strains and K. Janko for the supply of porin.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 138) and the Verband der Chemischen Industrie. B.A.B.-K. was supported by a fellowship from the Cystic Fibrosis Foundation.

LITERATURE CITED

1. **Ames Ferro-Luzzi, G., E. N. Spudich, and H. Nikaido.** 1974. Protein composition of the outer membrane of *Salmonella typhimurium*: effect of lipopolysaccharide mutations. J. Bacteriol. **117**:406–416.
2. **Bassford, P. J., D. L. Diedrich, C. L. Schnaitman, and P. Reeves.** 1977. Outer membranes of *Escherichia coli*. VI. Protein alteration in bacteriophage-resistant mutants. J. Bacteriol. **131**:608–622.
3. **Bavoil, P., H. Nikaido, and K. von Meyenburg.** 1977. Pleiotropic transport mutants of *Escherichia coli* lack

porin, a major outer membrane protein. Mol. Gen. Genet. **158**:23–33.
4. **Benz, R., G. Stark, K. Janko, and P. Läuger.** 1973. Valinomycin-mediated transport through neutral lipid membranes: influence of hydrocarbon chain length and temperature. J. Membrane Biol. **14**:339–364.
5. **Boos, W.** 1974. Bacterial transport. Annu. Rev. Biochem. **43**:123–146.
6. **Bosch, V., and V. Braun.** 1973. Distribution of murein lipoprotein between the cytoplasmic and outer membrane of *Escherichia coli*. FEBS Lett. **34**:307–310.
7. **Bragg, P. D., and C. Hou.** 1972. Organization of proteins in the native and reformed outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **274**:478–488.
8. **Braun, V.** 1975. Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **415**:335–377.
9. **Brockman, R. W., and L. A. Heppel.** 1968. On the localization of alkaline phosphatase and cyclic phosphodiesterase in *Escherichia coli*. Biochemistry **7**:2554–2562.
10. **Chai, T. J., and J. Foulds.** 1977. Purification of protein A, an outer membrane component missing in *Escherichia coli K-12 ompA* mutants. Biochim. Biophys. Acta **493**:210–215.
11. **Costerton, J. W., J. M. Ingram, and K. J. Cheng.** 1974. Structure and function of the cell envelope of gram-negative bacteria. Bacteriol. Rev. **38**:87–110.
12. **Datta, D. R., B. Arden, and U. Henning.** 1977. Major proteins of the *Escherichia coli* outer cell envelope membrane as bacteriophage receptors. J. Bacteriol. **131**:821–829.
13. **Dvorak, H. F., B. K. Wetzel, and L. A. Heppel.** 1970. Biochemical and cytochemical evidence for polar concentration of periplasmid enzymes in a minicell strain of *Escherichia coli*. J. Bacteriol. **104**:543–548.
14. **Endermann, R., C. Krämer, and U. Henning.** 1978. Major outer membrane proteins of *Escherichia coli K-12*: evidence for protein II* being a transmembrane protein. FEBS Lett. **86**:21–24.
15. **Freer, J. H., and M. R. Salton.** 1971. The anatomy and chemistry of gram-negative cell envelopes, p. 67–122. *In* G. Weinbaum, S. Kadis, and S. J. Ajl (ed.), Microbial toxins, vol. 4. Academic Press Inc., New York.
16. **Garrard, W. T.** 1972. Synthesis, assembly and localization of periplasmic cytochrome C*. J. Biol. Chem. **247**:5935–5943.
17. **Garten, W., and U. Henning.** 1974. Cell envelope and shape of *Escherichia coli K-12*: isolation and preliminary characterization of the major ghost-membrane proteins. Eur. J. Biochem. **47**:343–352.
18. **Garten, W., D. Hindennach, and U. Henning.** 1975. The major proteins of the *Escherichia coli* outer cell envelope membrane. Eur. J. Biochem. **59**:215–221.
19. **Hasegawa, Y., H. Yamada, and S. Mizushima.** 1976. Interactions of outer membrane proteins O-8 and O-9 with peptidoglycan sacculus of *Escherichia coli K-12*. J. Biochem. **80**:1401–1409.
20. **Henning, U., W. Schmidmayr, and D. Hindennach.** 1977. Major proteins of the outer cell envelope membrane of *Escherichia coli K-12*: multiple species of protein I. Mol. Gen. Genet. **154**:293–298.
21. **Heppel, L. A.** 1969. The effect of osmotic shock on release of bacterial proteins and on active transport. J. Gen. Physiol. **54**:95S–109S.
22. **Heppel, L. A.** 1971. The concept of periplasmic enzymes, p. 224–247. *In* L. I. Rothfield (ed.), Structure and function of biological membranes. Academic Press Inc., New York.
23. **Ichihara, S., and S. Mizushima.** 1977. Strain specificity of outer membrane proteins in *Escherichia coli*. J. Biochem. **81**:1525–1530.
24. **Inouye, M., J. Shaw, and C. Shen.** 1972. The assembly of a structural lipoprotein in the envelope of *Escherichia coli*. J. Biol. Chem. **247**:8154–8159.
25. **Jacobson, G. R., and J. P. Rosenbusch.** 1976. Abundance and membrane association of elongation factor Tu in *Escherichia coli*. Nature (London) **261**:23–26.
26. **Johnson, W. C., T. J. Sílhavy, and W. Boos.** 1975. Two-dimensional polyacrylamide gel electrophoresis of envelope proteins of *Escherichia coli*. Appl. Microbiol. **29**:405–413.
27. **Kashnarev, V. M., and T. S. Smirnova.** 1966. Electron microscopy of alkaline phosphatase of *Escherichia coli*. Can. J. Microbiol. **12**:605–608.
28. **Koplow, J., and H. Goldfine.** 1974. Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. **117**:527–543.
29. **Laemmli, U. K.** 1970. Cleavage of structural protein during the assembly of the head bacteriophage. Nature (London) **227**:680–685.
30. **Lee, N., C. Scandella, and M. Inouye.** 1978. Spin labelling of a cysteine residue of the *Escherichia coli* outer membrane lipoprotein in its membrane environment. Proc. Natl. Acad. Sci. U.S.A. **75**:127–130.
31. **Lengeler, J., K. O. Herman, H. S. Unsöld, and W. Boos.** 1971. The regulation of the β-methylgalactoside transport system and of the galactose binding protein of *Escherichia coli K-12*. Eur. J. Biochem. **19**:457–470.
32. **Livingston, D. M.** 1974. Immunoaffinity chromatography of proteins. Methods Enzymol. **34**:723–731.
33. **Lutgenberg, B., H. Bronstein, N. van Selm, and R. Peters.** 1977. Peptidoglycan associated outer membrane proteins in gram-negative bacteria. Biochim. Biophys. Acta **465**:571–578.
34. **Lutgenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane protein of *Escherichia coli K-12* into four bands. FEBS Lett. **58**:254–258.
35. **Lutgenberg, B., R. Peters, H. Bernheimer, and W. Berendsen.** 1976. Influence of cultural conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. **147**:251–262.
36. **McAllister, T. J., J. W. Costerton, L. Thompson, J. Thompson, and J. M. Ingram.** 1972. Distribution of alkaline phosphatase within the periplasmic space of gram-negative bacteria. J. Bacteriol. **111**:827–832.
37. **McAllister, T. J., R. T. Irvin, and J. W. Costerton.** 1977. Cell surface-localized alkaline phosphatase of *Escherichia coli* as visualized by reaction product deposition and ferritin-labeled antibodies. J. Bacteriol. **130**:318–328.
38. **Malamy, H. M., and B. L. Horecker.** 1961. The localization of alkaline phosphatase in *Escherichia coli K-12*. Biochem. Biophys. Res. Commun. **5**:104–108.
39. **Mitchell, P.** 1961. Approaches to the analysis of specific membrane transport, **1**. 581–603. *In* T. W. Goodwin and O. Lindberg (ed.), Biological structure and function, vol. 2. Academic Press Inc., New York.
40. **Nakae, T.** 1975. Outer membrane of *Salmonella typhimurium*: reconstitution of sucrose-permeable membrane vesicles. Biochem. Biophys. Res. Commun. **64**:1224–1230.
41. **Nakae, T.** 1976. Identification of the outer membrane protein of *Escherichia coli* that produces transmembrane channels in reconstituted vesicle membranes. Biochem. Biophys. Res. Commun. **71**:877–889.
42. **Nakae, T.** 1976. Isolation of protein complex that produces transmembrane channels. J. Biol. Chem. **251**:2176–2178.
43. **Nakane, P. K., G. E. Nichoalds, and D. L. Oxender.** 1968. Cellular localization of leucine-binding protein from *Escherichia coli*. Science **161**:182–183.

44. **Neu, H. C., and L. A. Heppel.** 1965. The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. J. Biol. Chem. **240**:3685–3692.
45. **Nikaido, H.** 1973. Biosynthesis and assembly of lipopolysaccharide and the outer membrane layer of gram-negative cell wall, p. 131–208. *In* L. Leive (ed.), Bacterial membranes and walls. Marcel Dekker, New York.
46. **Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson.** 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*: isolation and characterization of cytoplasmic and outer membrane. J. Biol. Chem. **247**:3962–3972.
47. **Oxender, D. L., and S. C. Quay.** 1976. Isolation and characterization of membrane binding proteins, p. 183–234. *In* E. D. Korn (ed.), Methods in membrane biology, vol. 6. Plenum Press, New York.
48. **Palva, E. T., and L. L. Randall.** 1976. Nearest-neighbor analysis of *Escherichia coli* outer membrane proteins, using cleavable cross-links. J. Bacteriol. **127**:1558–1560.
49. **Palva, E. T., and L. L. Randall.** 1978. Arrangement of protein I in *Escherichia coli* outer membrane: cross-linking study. J. Bacteriol. **133**:279–286.
50. **Pardee, A. B., and K. Watanabe.** 1968. Location of sulfate-binding protein in *Salmonella typhimurium*. J. Bacteriol. **96**:1049–1054.
51. **Randall, L. L., and I. S. Hardy.** 1977. Synthesis of exported proteins by membrane bound polysomes from *Escherichia coli*. Eur. J. Biochem. **72**:43–53.
52. **Randall-Hazelbauer, L. L., and M. Schwartz.** 1973. Isolation of the bacteriophage lambda receptor from *Escherichia coli*. J. Bacteriol. **116**:1436–1446.
53. **Reitmeier, R. A. F., and P. D. Bragg.** 1977. Crosslinking of the proteins in the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **466**:245–256.
54. **Rosen, B. P., and L. A. Heppel.** 1973. Present status of binding proteins that are released from gram negative bacteria by osmotic shock, p. 209–230. *In* L. Leive (ed.), Bacterial membranes and walls. Marcel Dekker, New York.
55. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*, regular arrangement on the peptidoglycan and unusual dodecyl sulfate binding. J. Biol. Chem. **249**:8019–8029.
56. **Salton, M. R. J., and P. Owen.** 1976. Bacterial membrane structure. Annu. Rev. Microbiol. **30**:451–482.
57. **Schmitges, C. J., and U. Henning.** 1976. The major proteins of *Escherichia coli* outer cell envelope membrane; heterogeneity of protein I. Eur. J. Biochem. **63**:47–52.
58. **Schnaitman, C.** 1971. Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. J. Bacteriol. **108**:545–552.
59. **Schnaitman, C.** 1974. Outer membrane proteins of *Escherichia coli*. III. Evidence that the major protein of *Escherichia coli* O111 outer membrane consists of four distinct polypeptide species. J. Bacteriol. **118**:442–453.
60. **Schumacher, G., and K. Bussmann.** 1978. Cell-free synthesis of proteins related to *sn*-glycerol-3-phosphate transport in *Escherichia coli*. J. Bacteriol. **135**:239–250.
61. **Silhavy, T. J., M. J. Casadaban, H. A. Shuman, and J. R. Beckwith.** 1976. Conversion of β-galactosidase to a membrane-bound state by gene fusion. Proc. Natl. Acad. Sci. U.S.A. **73**:3422–3427.
62. **Smith, W. P., P. C. Tai, B. C. Thompson, and B. D. Davis.** 1977. Extracellular labeling of nascent polypeptide traversing the membrane of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:2830–2834.
63. **Steven, A. C., B. ten Heggeler, J. Kistler, and J. P. Rosenbusch.** 1977. Ultrastructure of a periodic protein layer in the outer membrane of *Escherichia coli*. J. Cell Biol. **72**:292–301.
64. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124**:112–118.
65. **Szmelcman, S., M. Schwartz, T. J. Silhavy, and W. Boos.** 1976. Maltose transport in *Escherichia coli K-12*. A comparison of transport kinetics in wild-type and λ resistant mutants with the dissociation constants of the maltose-binding protein as measured by fluorescence quenching. Eur. J. Biochem. **65**:13–19.
66. **Tien, H. T., S. Carbone, and E. A. Dawidowicz.** 1966. Formation of "black" lipid membranes by oxidation products of cholesterol. Nature (London) **212**:718–719.
67. **Uemura, J., and S. Mizushima.** 1975. Isolation of outer membrane proteins of *Escherichia coli* and their characterization on polyacrylamide gel. Biochim. Biophys. Acta **413**:163–175.
68. **von Meyenburg, K., and N. Nikaido.** 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.
69. **Yu, F., and S. Mizushima.** 1977. Stimulation by lipopolysaccharide of the binding of outer membrane proteins O-8 and O-9 to the peptidoglycan layer of *Escherichia coli K-12*. Biochem. Biophys. Res. Commun. **74**:1397–1402.