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Purification of *Legionella pneumophila* Major Outer Membrane Protein and Demonstration that It Is a Porin

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We have purified the major outer membrane protein (MOMP) of *Legionella pneumophila*, determined that it is associated with peptidoglycan, and characterized it as a porin. To purify the MOMP, we used a simple, rapid, three-step procedure that gave us the protein in high yield. The first step of the purification procedure involved selectively extracting the MOMP from whole bacterial cells with calcium and zwitterionic detergent. The second and third steps achieved purification by ion-exchange and molecular-sieve chromatography. The dissociation of the MOMP into monomers was dependent upon the presence of a reducing agent and was enhanced by treatment at 100°C. To study the relationship of the MOMP to peptidoglycan, we extracted the protein by a modification of the Rosenbusch procedure. Like the *Escherichia coli* porins, the MOMP was peptidoglycan associated. The MOMP was at least partially dissociated from peptidoglycan in sodium dodecyl sulfate and a high salt concentration. To study the ion channel-forming properties of the MOMP, we reconstituted the MOMP in planar lipid membranes. The MOMP formed ion-permeable channels with a single-channel conductance size of 100 picoSiemens. The MOMP channels exhibited a fourfold selectivity for cations over anions and voltage-independent gating. These findings demonstrate that the MOMP is a porin with properties similar to those of *E. coli* porins.

Legionella pneumophila, the agent of Legionnaires disease, is a gram-negative bacterium that survives and multiplies within human mononuclear phagocytes (12, 19). A previous study demonstrated that the outer membrane of *L. pneumophila* contains a single major protein species with an apparent molecular weight of 28,000 (8a). This protein, the major outer membrane protein (MOMP), is also the major protein species of the bacterium (8a). The MOMP is exposed to the cell surface (8a).

To study the potential role of the MOMP in the physiology and pathogenicity of *L. pneumophila*, we sought to purify this protein. In this study, we describe its purification to apparent homogeneity by a simple and high-yield procedure. As a first step in the characterization of the MOMP, we examined its channel-forming properties. In this study, we demonstrate that the MOMP is a porin.

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MATERIALS AND METHODS

Media. Albumin-yeast extract broth and modified charcoal-yeast extract agar were prepared as described previously (13).

Bacteria. *L. pneumophila*, Philadelphia 1 strain, was grown in embryonated hen eggs, harvested, tested for viability and the presence of contaminating bacteria, and stored at −70°C, as described before (12). For experimental use, egg-yolk-grown *L. pneumophila* was cultured one time only on charcoal-yeast extract agar. Isolated colonies were inoculated into albumin-yeast extract broth and the bacteria were grown at 37°C to midlogarithmic phase (optical density of 0.7 to 0.8 at 540 nm measured in a Coleman 44 model spectrophotometer [Perkin-Elmer Corp., Norwalk, Conn.]). The doubling time of *L. pneumophila* at midlogarithmic phase was approximately 3 h.

Purification of the MOMP. The MOMP was purified by a modification of the method used by Blake and Gotschlich (5) to purify the MOMP of *Neisseria gonorrhoeae*. *L. pneumophila* cells were grown in albumin-yeast extract broth (1 liter) to an absorbance of 0.8 at 540 nm, pelleted by centrifugation at 11,000 × *g* for 10 min at 4°C, and washed twice with 0.15 M sodium chloride. The harvested bacteria were slowly suspended in 1 volume of 1.0 M sodium acetate, pH 4.0, containing 1 mM 2,3-dimercaptopropanol (Sigma Chemical Co., St. Louis, Mo.) and 10 volumes of 5% (wt/vol) *N*-tetradecyl-*N*,*N*-dimethyl-3-ammonia-1-propanesulfonate (Zwittergent-3,14; Calbiochem-Behring Corp., La Jolla, Calif.) in 0.5 M $CaCl_2$. The suspension was sonicated at room temperature for 30 s in a sonicating water bath (Heat System Ultrasonics, Farmingdale, N.Y.) and cooled at 0°C. Ice-cold absolute ethanol was added drop by drop to bring the final concentration of ethanol to 20% (vol/vol), and the mixture was stirred for 30 min at room temperature. The preparation was centrifuged at 17,000 × *g* for 10 min and the supernatant, containing mostly lipopolysaccharide (LPS), 18 mg of protein, and only low amounts of MOMP, was discarded. The pellet was suspended again in the sodium acetate–Zwittergent-3,14–$CaCl_2$ solution described above, and the mixture was sonicated, treated with ethanol, and centrifuged as above. The supernatant, which this time contained 1.7 mg of total protein and most of the MOMP, was collected, treated with ice-cold absolute ethanol to a final concentration of 75% (vol/vol) to precipitate proteins, incubated overnight at −20°C to allow the precipitate to collect, and centrifuged at 20,000 × *g* for 35 min. The pellet, containing the MOMP, was suspended in 50 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA disodium salt–0.5% Zwitt-

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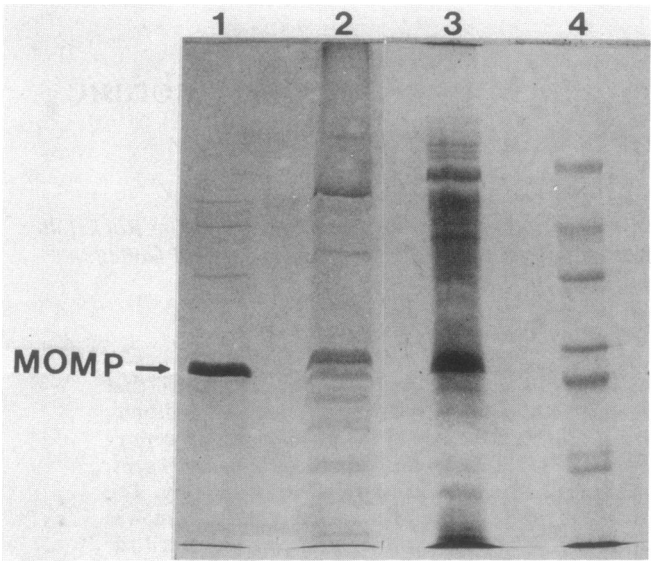

FIG. 1. Protein profiles of first and second extractions of *L. pneumophila* cells by treatment with calcium and zwitterionic detergent. *L. pneumophila* cells were treated twice with 5% Zwittergent-3,14 and 0.5 M $CaCl_2$ as described in the text. After each treatment, the supernatant proteins were collected, ethanol precipitated, and subjected to SDS-PAGE. Lane 3 shows the total cell proteins. Lane 2 shows the protein profile of the supernatant obtained after the first treatment (36 μg of protein loaded on the gel). It contains little MOMP. Lane 1 shows the protein profile of the supernatant obtained after the second treatment (4 μg of protein loaded on the gel). The MOMP constitutes the great majority of the total protein. Lane 4 contains molecular weight standards: trypsin inhibitor, 20,100; trypsinogen, 24,000; carbonic anhydrase, 29,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; ovalbumin, 45,000; and bovine albumin, 66,000.

ergent-3,14 and applied to a column (1.5 by 20 cm) of DEAE-Sepharose CL-6B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) equilibrated in the same buffer. The column was washed with the 50 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA–0.5% Zwittergent-3,14 buffer until the 280-nm absorbance fell to base line. Then a 200-ml salt gradient of 0.05 to 0.8 M NaCl in the same buffer was applied to the column at a flowrate of 20 ml/h. The fractions containing the MOMP were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and pooled, and the proteins were precipitated by the addition of ethanol (4°C) to a final concentration of 75% (vol/vol). The precipitate was allowed to collect overnight at −20°C and recovered by centrifugation at 20,000 × *g* for 35 min. The pellet was suspended in a minimum volume of 10 mM Tris-hydrochloride–200 mM NaCl–10 mM EDTA–0.5% Zwittergent-3,14 (pH 8.0) and applied to a column (2.5 by 50 cm) of Sephacryl S-200 (Pharmacia Fine Chemicals). The column was eluted in the same buffer at a flow rate of 10 ml/h. Fractions containing the purified MOMP were identified by SDS-PAGE, pooled, and precipitated with ethanol (75%, vol/vol; 4°C) as described above. The final pellet was solubilized in 50 mM Tris-hydrochloride (pH 7.5)–5 mM EDTA–0.5% Zwittergent-3,14, aliquoted, and stored at −70°C.

SDS-PAGE. PAGE in the presence of SDS (BDH Chemicals Ltd., Poole, England) was performed according to Laemmli (14) as modified by Ames (1). For protein analysis, the separating gel contained 12.5% acrylamide (BDH) and 0.33% *N,N'*-methylene bisacrylamide (BDH). Samples were solubilized by heating them at 100°C for 5 min in an equal volume of SDS-PAGE sample buffer consisting of 125 mM Tris-hydrochloride, 20% glycerol, 4% SDS, 4 mM EDTA, 10% 2-mercaptoethanol (Sigma), and 0.01% bromophenol blue (Sigma), pH 6.8. Molecular weight standards (α-lactalbumin, 14,200; trypsin inhibitor, 20,100; trypsinogen, 24,000; carbonic anhydrase, 29,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; ovalbumin, 45,000; and bovine albumin, 66,000 were obtained from Sigma Chemical Co. After electrophoresis, the gels were fixed in 10% acetic acid–25% isopropanol and stained for proteins with 0.2% Coomassie brilliant blue R-250 (Sigma) in fixing solution.

Assays for protein and LPS in purified MOMP preparations. The protein concentration of ethanol-precipitated MOMP preparations was assayed by the Lowry method (15). Since Zwittergent-3,14 interferes with the Lowry method, the protein concentration of MOMP preparations in Zwittergent-3,14 was estimated spectrophotometrically, using 20 as an approximate value for the extinction coefficient at 280 nm of a 1% solution of bacterial outer membrane porins (6, 20). Protein concentration was also approximated by evaluating the intensity of the protein band obtained on stained gels after SDS-PAGE. LPS contamination was estimated by determining the amount of 2-keto-3-deoxyoctonate (KDO) present in MOMP preparations. KDO was released from the protein preparation (500 μg to 1 mg of protein) by hydrolysis with 0.02 N H_2SO_4 at 100°C for 20 min and directly assayed by the thiobarbituric acid method of Weissbach and Hurwitz (30) as modified by Osborn et al. (23).

Extraction of *L. pneumophila* peptidoglycan-associated proteins. Peptidoglycan-associated proteins were extracted from

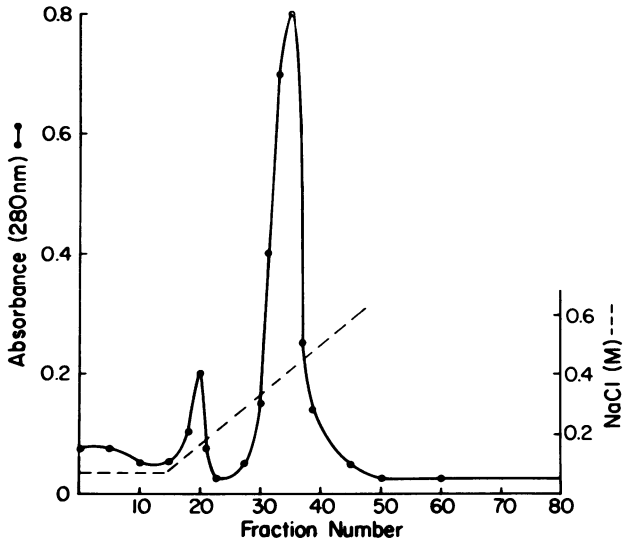

FIG. 2. Elution profile of the *L. pneumophila* MOMP on a DEAE-Sepharose column. The supernatant proteins obtained after the second extraction of *L. pneumophila* with $CaCl_2$-Zwittergent-3,14 were dissolved in a buffer consisting of 50 mM Tris-hydrochloride (pH 8.0), 10 mM EDTA, and 0.5% Zwittergent-3,14 and applied to a DEAE-Sepharose CL6B column as described in the text. The column was washed with the same buffer and the bound proteins were eluted with a salt gradient (0.05 to 0.8 M NaCl). Fractions were assayed for protein content by measuring absorbance at 280 nm (solid line) and for salt concentration by measuring conductivity (dashed line). SDS-PAGE analysis revealed that the MOMP was in the major peak, eluting at 0.4 M.

L. pneumophila by a modification of the Rosenbusch procedure used to purify *E. coli* porins (25). Briefly, 100 ml of *L. pneumophila* was harvested at an absorbance of 0.8 at 540 nm, washed with 10 mM Tris-hydrochloride (pH 7.4), and solubilized by heating in 2% (wt/vol) SDS (Sigma)–10% glycerol–2 mM $MgCl_2$–10 mM Tris-hydrochloride (pH 7.4) at 60°C for 30 min. The insoluble fraction, containing peptidoglycan and associated proteins, was pelleted by centrifugation at 100,000 × *g* for 1 h and washed twice with 2 mM $MgCl_2$–10 mM Tris-hydrochloride, pH 7.4. The MOMP was dissociated from the peptidoglycan by suspending the pellet in 2% SDS (wt/vol)–0.5 M NaCl–10 mM Tris-hydrochloride (pH 7.4) and incubating the suspension at 37°C for 30 min. The solubilized material was separated from the insoluble peptidoglycan by centrifugation at 100,000 × *g* for 1 h. The supernatant proteins were precipitated by adding 9 volumes of acetone and incubating at −20°C.

Membrane conductance measurements. Since Zwittergent-3,14 interferes with membrane conductance measurements, MOMP preparations free of this detergent were prepared. The protein solution was precipitated twice with 2 volumes of ethanol (−20°C). The second precipitate was dissolved in water and precipitated once again with ethanol (−20°C). Finally, the third precipitate was dissolved in water and lyophilized.

Electrical characteristics of the MOMP were studied by reconstituting the protein in planar black lipid membranes (BLM). The BLM was formed at room temperature by brushing a solution of 5% diphytanoylphosphatidylcholine (Avanti Polar Lipids, Birmingham, Ala.) in *n*-decane (Aldrich Chemical Co., Milwaukee, Wis.) across a 1-mm-diameter hole in a Teflon partition. The membrane separated symmetrical solutions containing 100 mM NaCl, 10 mM 2-(*N*-morpholino) ethanesulfonic acid, 3 mM $MgCl_2$, and 1 mM EDTA (pH 6.5). Membrane formation was monitored by microscope observation, and only completely black films were used. An Ag/AgCl electrode was submerged in each compartment and connected to a voltage clamp current-monitoring amplifier. This enabled the potential difference across the BLM to be clamped at selected values.

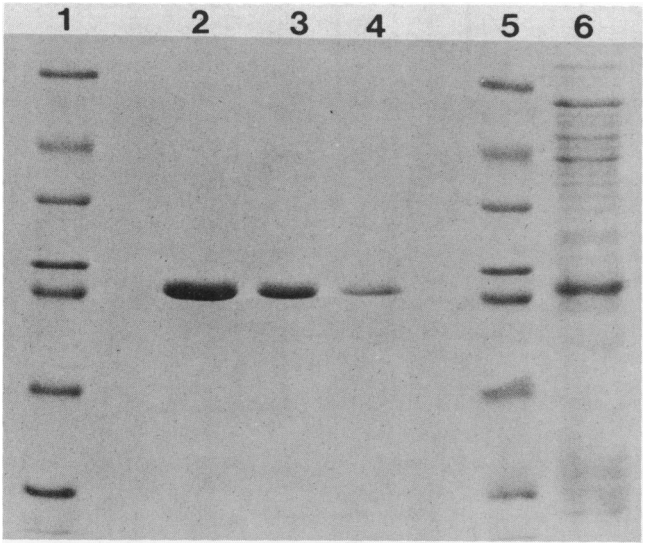

FIG. 3. SDS-PAGE analysis of the purified *L. pneumophila* MOMP. The MOMP preparation obtained after molecular-sieve chromatography on a Sephacryl S-200 column was analyzed by SDS-PAGE. Lanes 1 and 5, Molecular weight standards (α-lactalbumin, 14,200K; trypsin inhibitor, 20,100; trypsinogen, 24,000; carbonic anhydrase, 29,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; ovalbumin, 45,000; and bovine albumin, 66,000. Lanes 2, 3, and 4, Purified MOMP (20, 10, and 5 μg, respectively). Lane 6, Whole *L. pneumophila* extract.

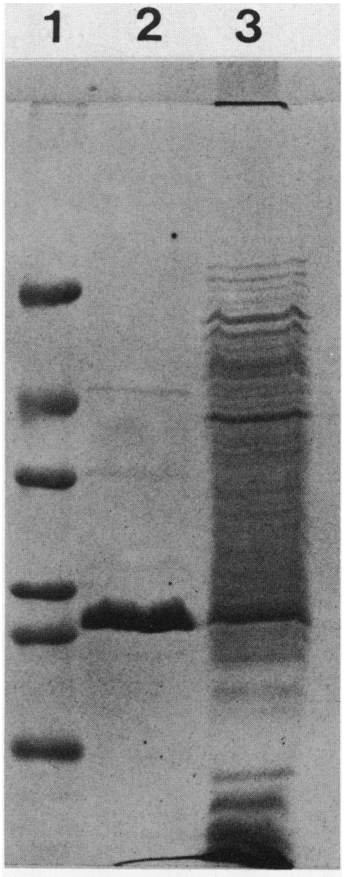

FIG. 4. SDS-PAGE of peptidoglycan-associated protein(s). *L. pneumophila* cells were solubilized in 2% SDS–2 mM $MgCl_2$ and incubated at 60°C as described in the text. The insoluble peptidoglycan-containing fraction (lane 2), recovered by centrifugation at 100,000 × *g* for 1 h, was solubilized in sample buffer and analyzed by SDS-PAGE; approximately 10 μg of protein was loaded onto the gel. The supernatant fraction (lane 3) was precipitated with acetone (9 volumes), and the precipitate was dissolved in the same volume of sample buffer as the fraction in lane 2. Molecular weight standards (lane 1) were the same as in the legend to Fig. 1.

MOMP-containing vesicles were prepared by the sonication–freeze-thaw technique (7). A 5-mg portion of lipid (4 mg of diphytanoylphosphatidylcholine and 1 mg of egg phosphatidylethanolamine) and 50 μg of MOMP were hydrated with 0.5 ml of a solution of the same composition as that used to bathe the BLM. This mixture was sonicated to clarity at 4°C for about 10 min. The vesicles were frozen in a slurry of dry ice and ethanol and then thawed at room temperature three times.

MOMP was reconstituted in the BLM by osmotically promoting fusion of the MOMP-containing vesicles with the BLM (7). A 50-μl amount of the vesicle mixture was added to one compartment, termed the *cis* compartment. While this compartment was stirred with a small magnetic stir bar, urea was added to the same compartment to a concentration of 200 mM to establish an osmotic gradient across the BLM. Evidence for fusion was sought by examining the voltage

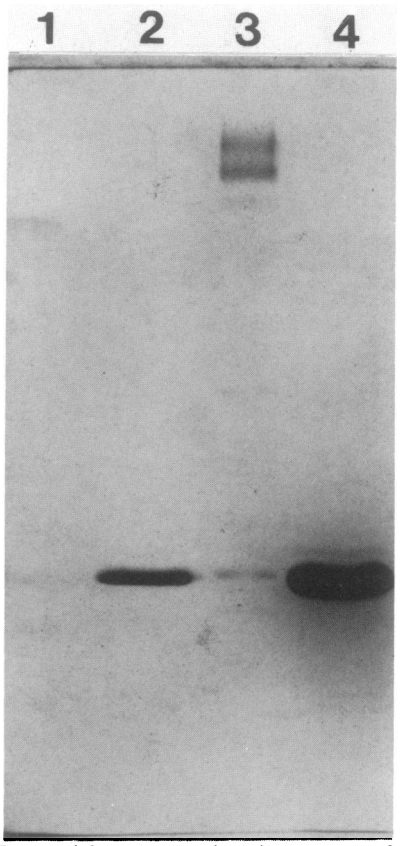

FIG. 5. Heat and 2-mercaptoethanol treatment of MOMP. The MOMP (10 μg) was ethanol precipitated, suspended in sample buffer either with or without 2-mercaptoethanol, and incubated for 5 min at either 100°C or room temperature. The samples were analyzed by SDS-PAGE. Lane 1, MOMP in absence of 2-mercaptoethanol at room temperature; lane 2, MOMP in presence of 2-mercaptoethanol at room temperature; lane 3, MOMP in absence of 2-mercaptoethanol at 100°C; lane 4, MOMP in presence of 2-mercaptoethanol at 100°C.

clamp current record for jumps, corresponding to the insertion of one or more ion channels into the BLM. Such current jumps were observed, and after 100 to 200 jumps fusion was terminated by adding urea to the *trans* compartment to abolish the osmotic gradient.

RESULTS

Purification of the MOMP. We purified the MOMP of *L. pneumophila* to apparent homogeneity in high yield by a three-step procedure detailed in Materials and Methods. The first step in the purification procedure was based on the finding that the outer membrane proteins of *N. gonorrhoeae* are selectively solubilized in the presence of a zwitterionic detergent and Ca^{2+} (5). When we treated the *L. pneumophila* with 5% Zwittergent-3,14 and 0.5 M $CaCl_2$, we found that the *L. pneumophila* MOMP was also selectively solubilized, but only with the second extraction. The first extraction released mostly LPS (168 μg of KDO per mg of protein) and low amounts of MOMP (Fig. 1). The second extraction yielded large amounts of MOMP (Fig. 1) and smaller amounts of LPS (25 μg of KDO per mg of protein); this was most of the MOMP of the bacteria. The second step in the purification procedure was the application of the Zwittergent-extracted material to an ion-exchange chromatography column, using a 0.05 to 0.8 M sodium chloride gradient (Fig. 2). The MOMP elutes at 0.4 M and thus can be separated from a major contaminant which elutes earlier in the gradient (Fig. 2). The third and final step of the purification procedure was the application of the ion-exchange-purified material to a molecular-sieve chromatography column. This step achieves purification by decreasing LPS contamination below the level of detection by the KDO assay. Before this step, the pooled material from ion-exchange chromatography contained 12 μg of KDO per mg of protein. After this step, the pooled material contained <1 μg of KDO per mg of protein. The purified MOMP preparation was highly homogeneous; 10 μg yielded only one band on SDS-PAGE, when the gel was stained with either Coomassie brilliant blue (Fig. 3) or Bio-Rad silver stain kit. Silver staining for LPS (27) detected approximately 0.2 μg of LPS in the 10-μg MOMP sample (data not shown). In typical preparations, we obtained 1 mg of MOMP per liter of culture at an optical density of 0.8, which contains approximately 0.7 g (dry weight) of bacteria (24).

The MOMP, like *E. coli* K-12 porins, is noncovalently associated to peptidoglycan. We also extracted the MOMP by a modification of the Rosenbusch procedure used to purify *E. coli* porins (25). We first incubated *L. pneumophila* in 2% SDS–2 mM Mg^{2+} at 60°C for 30 min, conditions under which most of the cellular proteins are solubilized but peptidoglycan and associated proteins remain insoluble. We then

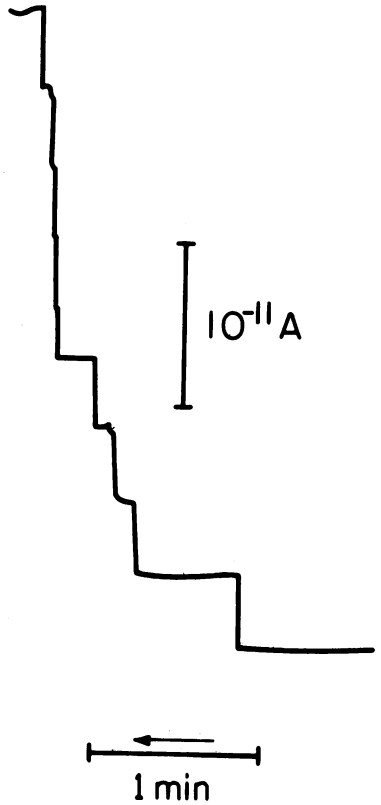

FIG. 6. Reconstitution of MOMP in a planar lipid bilayer. MOMP-containing vesicles were prepared and allowed to fuse with the *cis* compartment of a BLM as described in the text. Fusion was driven by an osmotic gradient with the *cis* compartment hyperosmotic (200 mM urea). The potential across the BLM was clamped at 20 mV, *cis* compartment positive. Individual fusion events are manifested by discrete jumps in the current record. Each jump represents the insertion of one or more MOMP into the BLM.

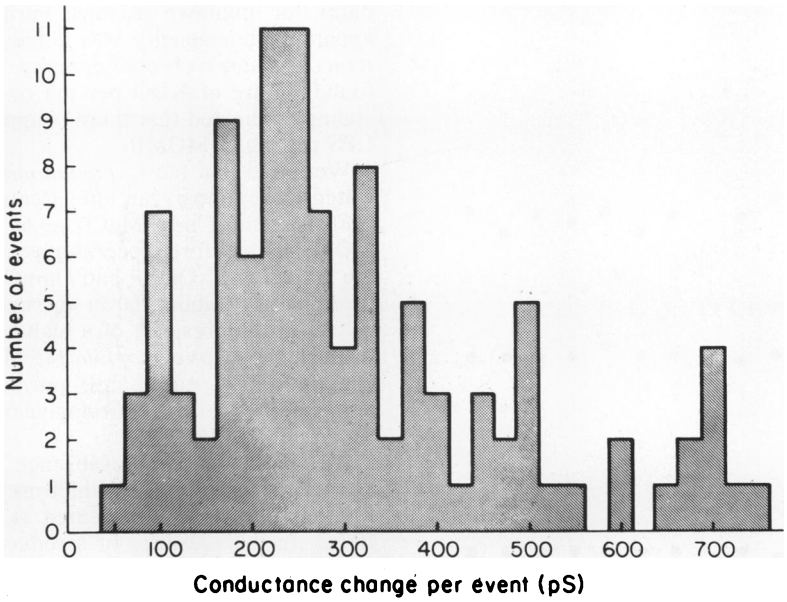

FIG. 7. Histogram of jump sizes in BLM conductance with fusion of MOMP-containing vesicles with the planar membrane. Individual current jumps in the records from three independent experiments (105 events) were converted to the corresponding increase in BLM conductance by dividing the current jump by the membrane voltage (20 mV). The resolution of the conductance jumps was 25 picoSiemens (pS). The mean size of the smallest mode is 100 pS, which corresponds to the size of a single ion channel formed in the BLM by MOMP.

recovered the insoluble fraction by centrifugation and examined it by SDS-PAGE (Fig. 4). This revealed that the MOMP was associated with the peptidoglycan-containing fraction. We dissociated the MOMP from the peptidoglycan by incubating the complex in SDS–0.5 M NaCl, a procedure which also dissociates OmpF and OmpC *E. coli* porins from peptidoglycan (10). However, only about 50% of the MOMP was dissociated from the peptidoglycan by this procedure.

Sensitivity of MOMP to heat and reducing conditions. Since the structures of MOMP of several bacteria are altered by heat and reducing agents (21, 28, 29), we examined the effect of heat and 2-mercaptoethanol treatment on the migration of MOMP in SDS-PAGE (Fig. 5). In the presence of 2-mercaptoethanol, treatment of the MOMP at 100°C resulted in approximately twice as much protein migrating to the position of the monomer as treatment of the MOMP at room temperature. In the absence of 2-mercaptoethanol, only a small proportion of the MOMP enters the gel and migrates to the position of the monomer, whether the MOMP is treated at 100°C or at room temperature.

The MOMP is a porin. In several gram-negative bacteria that have been investigated, the outer membrane contains major proteins called porins which form channels in planar lipid bilayers (2, 3, 8, 16, 17, 22, 26; F. Greco, M. S. Blake, E. C. Gotschlich, and A. Mauro, Fed. Proc. **39**:1813, 1980). In *E. coli*, these porins are peptidoglycan associated, under the conditions noted above. In view of this, we examined the MOMP of *L. pneumophila* to determine if it is a porin.

We first sought evidence that the MOMP formed ion channels in a BLM. We reconstituted the MOMP in lipid vesicles and promoted fusion of these vesicles with the BLM by establishing an osmotic gradient across the membrane. We then monitored the voltage clamp current record for jumps corresponding to the insertion of one or more ion channels into the BLM. Many such jumps were recorded, indicating that the MOMP forms ion channels in a BLM (Fig. 6).

To determine the conductance of a single ion channel created by the MOMP, we measured current jumps from a number of records similar to those in Fig. 6 and converted each jump to the corresponding change in BLM conductance by dividing the jump size by the membrane voltage (20 mV). The conductance changes for 105 current jumps are plotted as a histogram in Fig. 7. The modal value for the smallest jump size was 100 picoSiemens, which is somewhat smaller than the conductance of single ion channels formed in BLM by *E. coli* porin (3).

The channel formed by *L. pneumophila* porin is cationic selective. To determine if MOMP channels exhibit ion selectivity, we measured the membrane potential at which the net membrane current was zero in the presence of a salt gradient. With 1.0 M NaCl in the *cis* compartment and 0.1 M NaCl plus 1.8 M glucose in the *trans* compartment, this reversal potential was −20 mV (*cis* compartment negative with respect to *trans*). From the Goldman-Hodgkin-Katz equation (4), this value for the reversal potential indicates that the ion channel formed by the MOMP exhibits a fourfold selectivity for cations over anions. This selectivity is identical to that measured for porin from *E. coli* (4).

The channel formed by *L. pneumophila* porin exhibits voltage-independent gating. To determine if the conductance produced in BLM by insertion of MOMP channels exhibits voltage dependence, we allowed a large number of MOMP-containing vesicles to fuse with the BLM, terminated further fusion, and measured the steady-state conductance over a wide range of membrane potentials (Fig. 8). The conductance was independent of membrane potential over the range measured (−140 to +140 mV) in each of three independent experiments (Fig. 8). This indicates that the functional channels produced by MOMP in planar bilayers are predominantly in the open or conducting state for membrane potentials in this range. This type of voltage-independent conductance behavior has also been observed with porin from *E. coli* (3, 26).

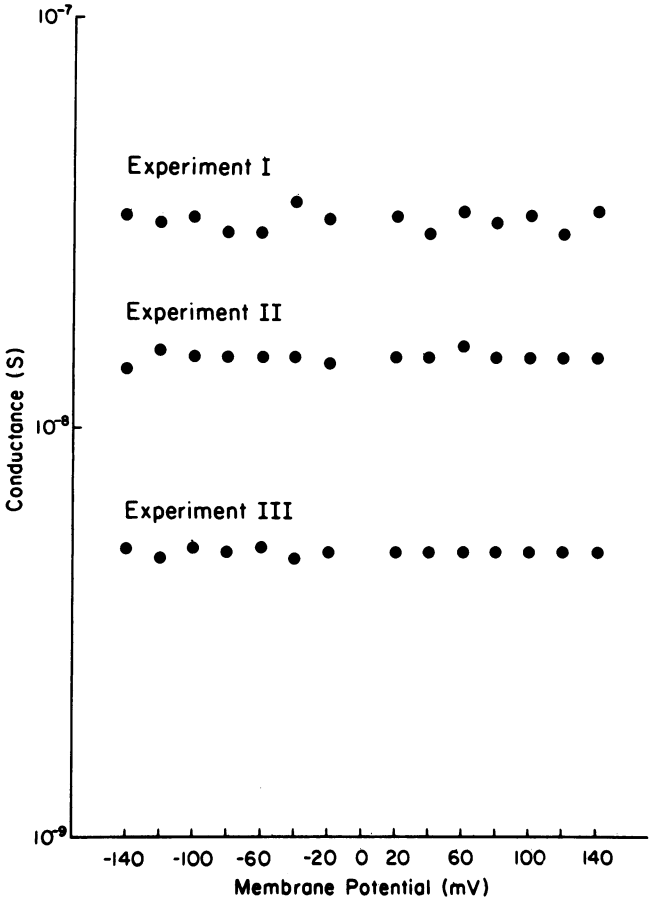

FIG. 8. Conductance of BLM reconstituted with MOMP as a function of voltage. An osmotic gradient was established across a BLM, and MOMP-containing vesicles were allowed to fuse with the BLM as described in the text. After 100 to 200 fusion events were detected, fusion was arrested by abolishing the osmotic gradient. The membrane voltage was then clamped at the indicated membrane potentials for several minutes each, and the net conductance of the BLM was measured at the end of each period. Net conductances for three independent experiments are shown plotted against membrane potential. In each experiment, the next conductance depends on the number of MOMP reconstituted, which varied over a large range. The net conductances of each untreated BLM was <10 pico-Siemens. The net conductance of MOMP-containing BLM is independent of membrane voltage over the range of −140 to +140 mV. S, Siemens.

DISCUSSION

In this paper, we describe a simple and efficient method for the purification of the *L. pneumophila* MOMP. The MOMP is selectively extracted in large quantity by treating *L. pneumophila* with calcium and Zwittergent-3,14, a treatment which selectively extracts outer membrane proteins of *N. gonorrhoeae* (5). Blake and Gotschlich have hypothesized that, in the presence of a high concentration of calcium, LPS aggregates and decreases its association with membrane proteins, allowing the Zwitterionic detergent to interact with exposed hydrophobic sites of the proteins and solubilize them. In our study, two treatments with calcium and zwitterionic detergent were required to remove the *L. pneumophila* MOMP. The first treatment did not effectively release MOMP from the *L. pneumophila* outer membrane, even with increased detergent concentration (unpublished data), for unknown reasons. Purification of the MOMP to apparent homogeneity was achieved by ion-exchange and molecular-sieve chromatography. In this preparation, we found <1 μg of KDO per mg of MOMP; however, silver staining indicated that there is approximately 20 μg of total LPS per mg of MOMP.

We found that the *L. pneumophila* MOMP remains associated to peptidoglycan after treatment of the bacteria with 2% SDS and 2 mM $MgCl_2$ at 60°C. The *L. pneumophila* MOMP is therefore a peptidoglycan-associated protein, like the *E. coli* K-12 OmpF and OmpC porins. The MOMP was incompletely (about 50%) dissociated from the peptidoglycan in the presence of a high salt concentration. In this respect, the *L. pneumophila* MOMP differs from the *E. coli* OmpF, OmpC, and LamB porins, which are completely dissociated from the peptidoglycan in the presence of high salt (9, 10).

We found that in the absence of a reducing agent the MOMP forms aggregates which are too large to enter the gel, whether the MOMP is treated at room temperature or at 100°C. In the presence of a reducing agent, the MOMP is dissociated into monomers, and treatment at 100°C seems to increase the amount dissociated. The sensitivity of the MOMP aggregates to 2-mercaptoethanol, also reported by others (11), may reflect the presence of interchain disulfide linkages as in the case of *Chlamydia trachomatis* and *Chlamydia psittaci* (21). That disulfide bridges may be present in the porins of both *L. pneumophila* and *Chamydia* sp., two obligate intracellular pathogens, but not in *E. coli* (18) suggests the possibility that disulfide bridges may be important in intracellular parasitism.

We have also demonstrated that the *L. pneumophila* MOMP is a porin. The MOMP is readily reconstituted in BLM by promoting fusion of MOMP-containing vesicles with the membrane. The ion channels formed in planar bilayers by the reconstitution of MOMP are similar to those formed by porin from *E. coli*. Although the conductance of a single channel formed by *L. pneumophila* porin is smaller than that formed by *E. coli* porin, both porin channels exhibit smaller selectivity for cations over anions and voltage-independent gating.

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LITERATURE CITED

1. **Ames, G. F. L.** 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. **249**:634–644.
2. **Benz, R., and R. E. W. Hancock.** 1981. Properties of the large ion-permeable pores formed from protein F of *Pseudomonas aeruginosa* in lipid bilayer membranes. Biochim. Biophys. Acta **646**:298–308.
3. **Benz, R., K. Janko, W. Boos, and P. Lauger.** 1978. Formation of large, ion-permeable membrane channels by the matrix protein (porin) of *Escherichia coli*. Biochim. Biophys. Acta **511**:305–319.
4. **Benz, R., K. Janko, and P. Lauger.** 1979. Ionic selectivity of pores formed by the matrix protein (porin) of *Escherichia coli*. Biochim. Biophys. Acta **551**:238–247.
5. **Blake, M. S., and E. C. Gotschlich.** 1984. Purification and partial

characterization of the opacity-associated proteins of *Neisseria gonorrhoeae*. J. Exp. Med. **159**:452–462.
6. **Chen, R., C. Kramer, W. Schmidmayr, and U. Henning.** 1979. Primary structure of major outer membrane protein I of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:5014–5017.
7. **Cohen, F. S., M. H. Akabas, J. Zimmerberg, and A. Finkelstein.** 1984. Parameters affecting the fusion of unilamellar phospholipid vesicles with planar bilayer membranes. J. Cell. Biol. **98**:1054–1062.
8. **Douglas, J. T., M. D. Lee, and H. Nikaido.** 1981. Protein I of *Neisseria gonorrhoeae* outer membrane is a porin. FEMS Microbiol. Lett. **12**:305–309.
8a. **Gabay, J. E., and M. A. Horwitz.** 1985. Isolation and characterization of the cytoplasmic and outer membrames of Legionnaires' disease bacterium (*Legionella pneumophila*). J. Exp. Med. **161**:409–422.
9. **Gabay, J. E., and K. Yasunaka.** 1980. Interaction of the LamB protein with the peptidoglycan layer in *Escherichia coli* K-12. Eur. J. Biochem. **104**:13–18.
10. **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.
11. **Hindahl, M. S., and B. H. Iglewski.** 1984. Isolation and characterization of the *Legionella pneumophila* outer membrane. J. Bacteriol. **159**:107–113.
12. **Horwitz, M. A., and S. C. Silverstein.** 1980. The Legionnaires' disease bacterium (*Legionella pneumophila*) multiplies intracellularly in human monocytes. J. Clin. Invest. **66**:441–450.
13. **Horwitz, M. A., and S. C. Silverstein.** 1983. Intracellular multiplication of Legionnaires' disease bacteria (*Legionella pneumophila*) in human monocytes is reversibly inhibited by erythromycin and rifampin. J. Clin. Invest. **71**:15–26.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Lugtenberg, B., and L. Van Alphen.** 1983. Molecular architecture and functioning of the outer membrane of *Escherichia coli* and other gram-negative bacteria. Biochim. Biophys. Acta **737**:51–115.
17. **Nakae, T.** 1976. Outer membrane protein of *Salmonella*. Isolation of protein complex that produces transmembrane channels. J. Biol. Chem. **251**:2176–2178.
18. **Nakae, T., J. Ishii, and M. Tokunaga.** 1979. Subunit structure of functional porin oligomers that form permeability channels in the outer membrane of *Escherichia coli*. J. Biol. Chem. **254**:1457–1461.
19. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1984. Interaction between the Legionnaires' disease bacterium (*Legionella pneumophila*) and human alveolar macrophages. Influence of antibody, lymphokines, and hydrocortisone. J. Clin. Invest. **74**:771–782.
20. **Neuhaus, J. M.** 1982. The receptor protein of phage lambda: purification, characterization and preliminary electrical studies in planar lipid bilayers. Ann. Microbiol. (Inst. Pasteur) **133A**:27–32.
21. **Newhall, W. J. V., and R. B. Jones.** 1983. Disulfide-linked oligomers of the major outer membrane protein of chlamydiae. J. Bacteriol. **154**:998–1001.
22. **Nikaido, H., and E. Y. Rosenberg.** 1983. Porin channels in *Escherichia coli*: studies with liposomes reconstituted from purified proteins. J. Bacteriol. **153**:241–252.
23. **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.
24. **Pine, L., R. George, M. W. Reeves, and W. K. Harrell.** 1979. Development of a chemically defined liquid medium for growth of *Legionella pneumophila*. J. Clin. Microbiol. **9**:615–626.
25. **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.
26. **Schindler, H., and J. P. Rosenbusch.** 1978. Matrix protein from *Escherichia coli* outer membrane forms voltage-controlled channels in lipid bilayers. Proc. Natl. Acad. Sci. U.S.A. **75**: 3751–3755.
27. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
28. **Van Alphen, L., T. Riemens, J. Poolman, and H. C. Zanen.** 1983. Characteristics of major outer membrane proteins of *Haemophilus influenzae*. J. Bacteriol. **155**:878–885.
29. **Verstreate, D. R., M. T. Creasy, N. T. Caveney, C. L. Baldwin, M. W. Blab, and A. J. Winter.** 1982. Outer membrane proteins of *Brucella abortus*: isolation and characterization. Infect. Immun. **35**:979–989.
30. **Weissbach, A., and J. Hurwitz.** 1959. The formation of 2-Keto-3 deoxy-heptonic acid in extracts of *Escherichia coli* B. J. Biol. Chem. **234**:705–709.