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High-Molecular-Weight Antigenic Protein Complex in the Outer Membrane of *Neisseria gonorrhoeae*

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The outer membrane of *Neisseria gonorrhoeae* contains approximately 15 proteins, with 2 or 3 accounting for over 75% of the total protein mass. Samples of outer membrane from strain 2686 T4 analyzed by electrophoresis in 2% polyacrylamide gels revealed a band with an apparent molecular weight of 800,000. The band was protein material, as indicated by trypsin and pronase sensitivity and by L-[^{3}H]proline incorporation. Peptidoglycan, nucleic acids, and carbohydrate were not detected in the band. Dye binding, L-[^{3}H]proline incorporation, and labeling of solubilized outer-membrane proteins with ^{125}I-labeled Bolton-Hunter reagent indicated that the band made up 10 to 13% of the total protein mass of isolated outer membranes. The material in the band was purified by gel filtration and, after reduction and alkylation, quantitatively recovered as subunits with an apparent molecular weight of 76,000. The protein in complex form was exposed at the cell surface, as evidenced by labeling whole cells with ^{125}I by using a lactoperoxidase-catalyzed reaction and with CNBr-activated dextran. Rabbit serum raised against whole 2686 T4 gonococci contained antibody which reacted with the protein complex. The protein complex was detected in all gonococcal strains tested, but its presence could not be demonstrated in several other gram-negative species.

The outer membrane (OM) of *Neisseria gonorrhoeae* contains macromolecules which are likely to participate directly in the interactions between the organism and its host environment. One of the consequences of such interactions is the formation by the host of antibody directed against pili (3, 4), OM proteins (14, 33), and lipopolysaccharide (22, 33). Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) reveals a relatively simple OM protein profile for gonococci (7, 9, 11-14, 27, 34, 35). The most abundant OM protein accounts for about 60% of the total protein mass of the OM (7, 13). This protein, which is called the principal OM protein, is partially responsible for the serological diversity of gonococci (14). Thus, the value of the principal OM protein in the development of a broad-range, anti-gonococcal vaccine may be limited. Most of the other OM proteins are present in much smaller amounts, thereby hindering further studies of the antigenic makeup of the OM.

The several reports (7, 9, 11-14, 27, 34, 35) of the OM protein profile of gonococci do not describe OM proteins with molecular weights exceeding 110,000. In contrast, we consistently observed a sharp and intense band near the origin of 7.5% polyacrylamide gels during routine analysis of OM proteins by SDS-PAGE. This observation suggested the presence of high-molecular-weight proteins or a protein complex in the OM. The present studies used SDS-PAGE gels which include proteins with molecular weights up to 5 $\times 10^6$ to demonstrate the presence of a protein complex with an apparent molecular weight of 800,000. Further investigation revealed that this protein complex (i) comprised about 10% of the total OM protein mass, (ii) was exposed at the cell surface, (iii) possessed antigenic activity, (iv) could be quantitatively recovered after reduction and alkylation as subunits with a molecular weight of 76,000, and (v) could be purified in the complex form by gel filtration.

MATERIALS AND METHODS

Materials. Acrylamide, bisacrylamide, SDS, and Coomassie brilliant blue R-250 were from Bio-Rad Laboratories, Richmond, Calif. Sodium *N*-lauroyl sarcosinate (Sarkosyl) was from Pfaltz and Bauer, Flushing, N.Y. Agarose (type II, medium EEO) and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) were from Sigma Chemical Co., St. Louis, Mo. Brij-35, *N*-succinimidyl-3(4-hydroxyphenyl)-propionate (Bolton-Hunter reagent), and *o*-phthalaldehyde were from Pierce, Rockford, Ill. Enzymes used included trypsin and pronase B (Calbiochem, La Jolla, Calif.), lysozyme (Boehringer Mannheim Corp., Indianapolis, Ind.), deoxyribonuclease (Worthington Biochemicals Corp., Freehold, N.J.), and ribonuclease and

lactoperoxidase (Sigma). Radiochemicals were L-[^{3}H]proline and D-[^{3}H]glucosamine (ICN, Irvine, Calif.) and ^{125}I (carrier free, New England Nuclear Corp., Boston, Mass.). *Staphylococcus aureus* (Cowan I) cells bearing protein A were from Calbiochem. Molecular weight markers included: pentameric immunoglobulin M (IgM), dimeric IgA, and IgG, which were purified from human myeloma sera by standard techniques; ovalbumin and chymotrypsinogen (ISOLAB, Akron, Ohio); bovine serum albumin (Armour, Chicago, Ill.); and cytochrome *c* (Sigma).

Bacteria and media. *N. gonorrhoeae* strains were provided as follows: 2686, types (T) 1, 2, 3, and 4 by D. S. Kellogg, Jr. (Center for Disease Control, Atlanta, Ga.); MS11 by J. Swanson (University of Utah, Salt Lake City); FA19, FA102, FA136, FA140, BR87, and BR54 by P. F. Sparling (University of North Carolina, Chapel Hill); CS7 and JW31 by S. A. Morse (University of Oregon, Portland); 7122 by R. B. Jones (Indiana University, Indianapolis); and RD_5 by F. E. Young (University of Rochester, Rochester, N.Y.). *Neisseria meningitidis* 13090 was obtained from the American Type Culture Collection, Rockville, Md. *Escherichia coli* B was obtained from R. C. Bockrath, Indiana University, Indianapolis. Cultures of *Branhamella catarrhalis*, *Bordetella pertussis*, *Moraxella lacunata*, *Neisseria sicca*, and *Neisseria perflava* were obtained from a departmental stock culture collection.

Gonococci were maintained by serial, selective subculture on clear typing medium (28). All T1 and T4 cultures were transparent, and all T2 and T3 cultures were opaque by the classification scheme of Swanson (28). Liquid cultures (LGCBIS [32]) containing 0.1 g of $NaHCO_3$ per liter were inoculated with bacteria from an overnight growth on clear typing medium. The initial density was about 10^7 bacteria/ml. Cultures were grown at 37°C and harvested in exponential phase at a density of about 2×10^8 bacteria/ml. On clear typing medium, these suspensions yielded predominantly the desired colonial type and color/opacity characteristics. No evidence of a gonococcal capsule was detected under these growth conditions. In some experiments, cells were grown at pH 6.0 by the method of Hebeler et al. (8). In some experiments, cells were grown in LGCBIS containing L-[^{3}H]proline (1 μCi/ml) to label protein or D-[^{3}H]glucosamine (0.017 μCi/ml) to label peptidoglycan (23). The distribution of these labels in different macromolecules has been described (23).

Protein content. Protein concentrations were determined by the method of Lowry et al. (18) by using bovine serum albumin as standard.

OM isolation. OM was isolated from cells ruptured in a French pressure cell by a modification of the method of Walstad et al. (34). The lysate from three passes through an ice-cold French pressure cell at 12,000 lb/in^2 was centrifuged at 10,000 × *g* for 10 min to remove nonruptured cells. The supernatant was centrifuged at 40,000 × *g* for 1 h at 4°C onto a 55% (wt/wt) sucrose cushion. The material banding on top of the cushion was suspended to about 5 mg of protein per ml in 10 mM HEPES (pH 7.4) containing 1% Sarkosyl to remove inner membrane. After incubation for 30 min at 23°C, the suspension was centrifuged for 1 h at 40,000 × *g*. The pellet was washed once in 10 mM HEPES (pH 7.4) by recentrifugation. The pellet of enriched OM was suspended in the same buffer and kept frozen at −70°C.

SDS-PAGE. Samples of OM were prepared for SDS-PAGE by solubilization in a solution of 3% SDS, 7% sucrose, and either 100 mM iodoacetamide or 40 mM dithiothreitol (DTT). Solubilization mixtures were incubated for 5 min at 100°C. In some cases samples reduced with DTT were alkylated with 100 mM iodoacetamide. OM protein profiles were obtained on 10% polyacrylamide gels by using the discontinuous buffer system of Laemmli (17). Electrophoresis in composite acrylamide-agarose gels was performed essentially as described by Peacock and Dingman (20). Composite gels contained 2% polyacrylamide that was 2.6% cross-linked with *N,N'*-methylene bisacrylamide, 0.5% agarose, and 0.2% SDS. Gels were buffered with 40 mM sodium acetate, 80 mM tris(hydroxymethyl)-aminomethane, and 4 mM ethylenediaminetetraacetic acid. The pH was adjusted to 8.3 with glacial acetic acid. The same buffer, also containing 0.2% SDS, was used in both electrode chambers. SDS-pore gradient electrophoresis was performed by using linear 2 to 20% polyacrylamide gradient slab gels (19) and the buffer system of Laemmli (17).

Gels were stained and destained in 4-liter circulating solutions of (i) 0.05% Coomassie brilliant blue R-250, 25% isopropanol, and 10% acetic acid (24 h), (ii) 0.005% Coomassie blue, 10% isopropanol, and 10% acetic acid (24 h), and (iii) 20% methanol and 10% acetic acid (48 h in the presence of activated charcoal). Quantitation of protein bands was performed by scanning stained gels at 580 nm with an ISCO model 659 gel scanner (ISCO, Lincoln, Nebr.) and determining peak areas with a planimeter. Apparent molecular weights were estimated by comparison with a plot of migration distance versus log molecular weight using pentameric IgM, dimeric IgA, IgG, bovine serum albumin, ovalbumin, chymotrypsinogen, and chytochrome *c* as standards.

Analysis of whole-cell lysates on 2% composite gels was used as a method of screening bacteria (other than gonococci) for the presence of high-molecular-weight proteins or protein complexes. Bacteria were suspended at an optical density of 0.4 at 540 nm in 0.1 M sodium phosphate (pH 7.4) containing 0.85% NaCl. The cells from 1 ml of this suspension were centrifuged at 10,000 × *g* for 10 min. The pellet was solubilized in a final volume of 300 μl as described above, except 0.4 M 2-mercaptoethanol was used in place of DTT. Each lysate (100 μl) was analyzed by SDS-PAGE.

Enzymatic treatments. Enriched OM suspended in 10 mM HEPES (pH 7.4) was incubated with trypsin (100 μg/ml), pronase (100 μg/ml), lysozyme (100 μg/ml), or deoxyribonuclease (40 μg/ml) and ribonuclease (40 μg/ml) for 3 h at 37°C. Samples were then solubilized for electrophoresis in the presence of 2-mercaptoethanol and analyzed on 2% composite gels.

Periodic acid-Shiff (PAS) staining. OM proteins were resolved on a 3% polyacrylamide gel which was similar to the 2% gels except for the absence of agarose. After electrophoresis, the gel was stained for carbohydrate by the methods of Fairbanks et al. (5) and Zacharius et al. (36). Ovalbumin was used as a PAS-positive marker, and bovine serum albumin was used as a PAS-negative marker.

Gel filtration. Samples of OM were prepared for

gel filtration by solubilization for 5 min at 100°C in 10 mM HEPES (pH 7.4) containing 2% SDS, 0.4 M 2-mercaptoethanol, and 8 M urea. A 1-ml portion containing about 6 mg of OM protein was applied to a Sepharose CL-4B column (110 by 1 cm; Pharmacia Fine Chemicals, Piscataway, N.J.) equilibrated with 10 mM HEPES (pH 7.4) containing 0.1% SDS. The column eluate was collected in 1.5-ml fractions and continuously monitored for optical density at 280 nm. The column was calibrated with pentameric IgM, IgG, and bovine serum albumin as molecular weight markers. Fluorescent monitoring of amino-containing material in column effluents was performed by a modification of the technique of Benson and Hare (1). A portion (50 μl) of each fraction was mixed with 0.5 ml of a solution containing 0.2 mg of *o*-phthalaldehyde per ml, 14 mM 2-mercaptoethanol, and 0.1% Brij-35 in 0.2 M sodium borate buffer (pH 10.5). After a 5-min reaction time at 22°C, relative fluorescence was determined in an Aminco filter fluorocolorimeter (American Instrument Co., Silver Spring, Md.) by using a 6-by-50-mm cuvette, a 360-nm excitation filter, and a 436-nm emission filter (Wratten 47B).

^{125}I surface labeling. Cells harvested from liquid culture were surface labeled with ^{125}I by the lactoperoxidase-catalyzed iodination method of Swanson et al. (31). OM was isolated by the method of Johnston (12). Samples of OM were analyzed by SDS-PAGE, and the radioactivity associated with each OM protein was determined. In some cases, the surface-labeled OM proteins were reiodinated after isolation either with the procedure as described above or with an alternative method as described by Fraker and Speck (6).

CNBr-activated dextran surface labeling. CNBr-activated dextran (Pharmacia) was prepared and used to identify proteins exposed on the cell surface by the methods of Kamio and Nikaido (15). Cells harvested from liquid culture containing L-[^{3}H]proline were washed and suspended in 0.1 M $NaHCO_3$ buffer (pH 8.5). After incubation with CNBr-activated dextran at 23°C for 60 min, the cells were washed, and OMs were isolated essentially as described above. A 70% (wt/wt) sucrose cushion was necessary for collection of the labeled OM. A control sample, untreated with CNBr-activated dextran, was prepared simultaneously. The samples were analyzed on 2% composite gels by SDS-PAGE and the migration profile was determined by the distribution of radioactivity.

Labeling with ^{125}I Bolton-Hunter reagent. ^{125}I-labeled Bolton-Hunter reagent was prepared by the method of Bolton and Hunter (2). OM proteins were solubilized by incubation for 1 h at 37°C in 10 mM HEPES (pH 7.4) containing 0.1% SDS. The pH of the solubilized OM suspension was raised to 9.0 by the addition of 0.2 M sodium borate (pH 10.5). A portion containing about 100 μg of protein was labeled with about 0.5 μCi of ^{125}I-labeled Bolton Hunter reagent (2). The unreacted reagent was removed by passing the sample over a Sephadex G-25 desalting column which was equilibrated with solubilization buffer.

Immunoprecipitation. A fraction of ^{125}I-labeled Bolton Hunter-labeled OM proteins containing about 100 μg of protein was diluted to 1 ml with 0.1 M sodium phosphate (pH 7.4) containing 0.1% SDS. To remove material that would bind nonspecifically, 10 μl of preimmune rabbit serum was incubated with the sample for 1 h, followed by incubation with 100 μl of 10% (wt/vol) *S. aureus* bearing protein A for 30 min at 37°C. The staphylococci were recovered by centrifugation at 7,000 × *g* for 15 min. The supernatant was mixed with 10 μl of rabbit hyperimmune serum raised against whole 2686 T4 gonococci as described previously (21) and incubated for 1 h at 37°C. To precipitate immune complexes, 100 μl of staphylococci was then added, and the mixture was incubated for 30 min at 37°C. Staphylococci were recovered as before. The staphylococcal pellet from this treatment was washed three times with 0.1 M sodium phosphate buffer containing 0.1% SDS by centrifugation as before. Immunoprecipitated protein was released by boiling the cell pellets for 10 min in a solution of 2% SDS, 10 M urea, and 0.4 M 2-mercaptoethanol. Solubilized protein was separated from the staphylococci by centrifugation. ^{125}I disintegrations per minute were monitored throughout the procedure. The total amount of 800,000 molecular weight protein in the starting material and in the immunoprecipitate was determined by SDS-PAGE by using 2% composite gels.

Radioactivity. Gels of ^{125}I-labeled OM protein were cut into 1-mm slices; ^{125}I disintegrations per minute were determined for each slice in a Beckman model 4000 gamma counter (Beckman, Palo Alto, Calif.). Slices of gels containing L-[^{3}H]proline or D-[^{3}H]glucosamine were incubated with NCS tissue solubilizer (Amersham, Arlington Heights, Ill.) for 1 h at 55°C before addition of a toluene-based scintillation cocktail and determination of radioactivity as described (24).

RESULTS

Biochemical characterization. To visualize proteins that have high apparent molecular weights, samples of OM from strain 2686 T4 (used throughout except as noted) were subjected to electrophoresis in 2% polyacrylamide gels that contained 0.5% agarose for mechanical support. This composite gel system revealed a band with an apparent molecular weight of 800,000 (Fig. 1). This OM protein macromolecular complex is hereafter referred to as OMP-MC. To obtain an alternate measure of the molecular weight of OMP-MC, solubilized OM proteins were subjected to gel filtration on a Sepharose 4B column in the presence of SDS. The column eluate contained four distinct peaks of optical density at 280 nm (Fig. 2). Most of the OM proteins eluted in peak C. Material eluting in the void and total volumes (peaks A and D, respectively) contained no detectable protein when analyzed by SDS-PAGE and no amino groups when relative fluorescence was monitored after treatment with *o*-phthalaldehyde. The high ultraviolet absorbance of peak D was due to 2-mercaptoethanol and that of peak A was due to nucleic acid. The smallest peak, B, contained no detectable protein other than OMP-MC. In comparison with molecular weight markers, OMP-MC eluted from the column with

an apparent molecular weight of about 900,000. Thus, two methods of different principle demonstrated the high apparent molecular weight of OMP-MC. The discrepancy of molecular weight estimates between the two, i.e., 800,000 by gel electrophoresis and 900,000 by gel filtration, may reflect differential SDS binding or conformational differences, or both.

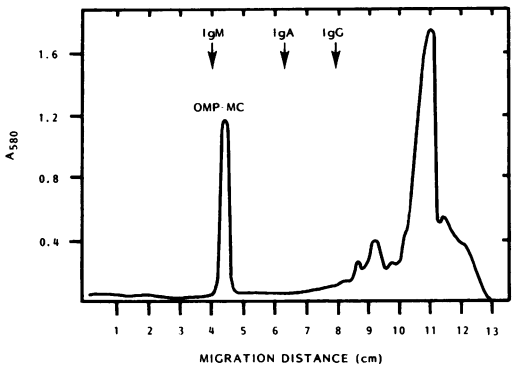

FIG. 1. *Absorbance scan of Coomassie blue-stained gonococcal outer-membrane proteins after migration in the 2% composite gel during SDS-PAGE. In relation to the migration distances of molecular weight markers (indicated by arrows), OMP-MC had an apparent molecular weight of 800,000. Other individual OM proteins with lower molecular weights were not resolved in this gel system.*

The relative amount of OMP-MC in the OM was quantitated on the basis of (i) Coomassie blue dye-binding, (ii) biosynthetically incorporated L-[^{3}H]proline, and (iii) labeling of solubilized OM proteins with ^{125}I-labeled Bolton-Hunter reagent. All methods gave essentially the same results and indicated that OMP-MC made up 10 to 13% of the total protein mass of isolated OM.

To characterize the composition of OMP-MC, samples of OM were treated with either trypsin, pronase, lysozyme, or deoxyribonuclease and ribonuclease and analyzed for the continued presence of OMP-MC by SDS-PAGE. Trypsin and pronase eliminated OMP-MC, which indicated the presence of protein. In contrast, peptidoglycan and nucleic acids were apparently absent since neither lysozyme nor deoxyribonuclease and ribonuclease had an effect. An independent measure of peptidoglycan content in OMP-MC was obtained by labeling cells with D-[^{3}H]glucosamine and determining the ^{3}H activity associated with the OMP-MC band. When an OM sample (15,000 ^{3}H dpm, 150 μg of protein) was analyzed on a 2% gel, no ^{3}H disintegrations per minute were recovered in OMP-MC. Peptidogly-

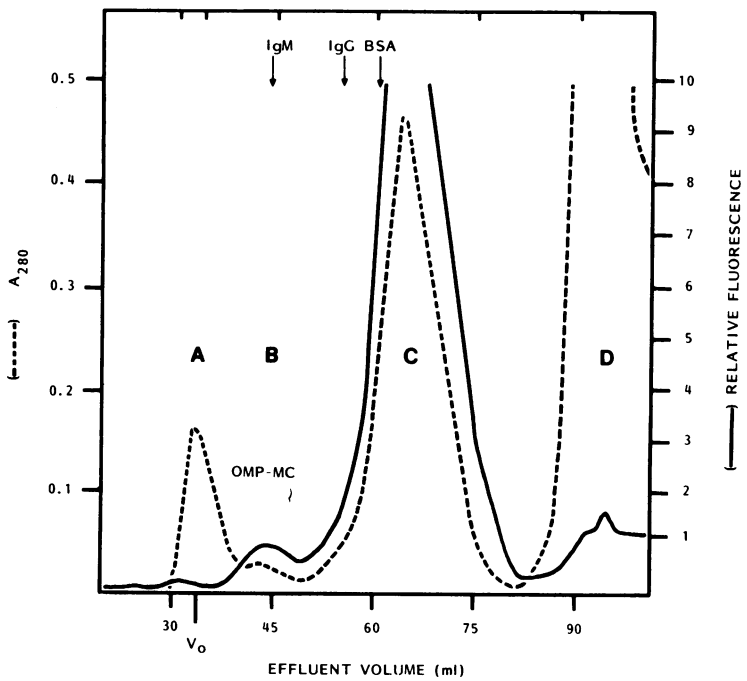

FIG. 2. *Elution profile of SDS-solubilized 2686 T4 outer membrane proteins applied to a Sepharose CL-4B column as measured by ultraviolet absorbance and fluorescence of OPA. Peak B contained only OMP-MC when analyzed by SDS-PAGE. The compositions of other peaks are discussed in the text. Molecular weight markers (indicated by arrows) were pentameric IgM, IgG, and bovine serum albumin (BSA).*

can isolated from the same cells had a specific activity of 4.7×10^5 dpm/mg of peptidoglycan. Since it was possible to measure reliably 50 dpm above background, and the OMP-MC content in the sample was approximately 15 μg, OMP-MC contained less than 1% peptidoglycan by mass. To test for the presence of carbohydrate, OM proteins were resolved on 3% polyacrylamide gels and stained by two different PAS procedures. Carbohydrate was not detected with either procedure.

Johnston and Gotschlich reported that about 7% of radiolabeled OM protein did not penetrate a 10% polyacrylamide gel (13). It is possible that the nonpenetrating material was OMP-MC, as described herein. Alternatively, OMP-MC might have been lost during membrane fractionation and purification. To test the latter possibility, OM was isolated by the methods of other investigators (9, 12–14). Each procedure tested gave OM fractions that contained OMP-MC in similar amounts. These results suggested that OMP-MC was present but not resolved in other investigations of the OM protein profile.

Subunit composition. By analogy with other equally large proteins, e.g., IgM, it seemed likely that OMP-MC was a complex composed of lower-molecular-weight subunits. Solubilization of the OM proteins in the presence of DTT or 2-mercaptoethanol did not affect the amount or the migration behavior of OMP-MC. The failure to demonstrate subunits by these measures suggested that OMP-MC either (i) was a single polypeptide chain of unprecedented size, (ii) possessed disulfide or other bonds between subunits which were inaccessible or unusually resistant to these treatments, (iii) gave an anomolous molecular weight in the systems used, or (iv) had disulfide bonds between subunits that were reduced by DTT and were reformed during electrophoresis or gel filtration as the subunits moved away from the reducing agent. This latter hypothesis was tested by first reducing a solubilized OM sample with DTT and then alkylating free sulfhydryl groups with iodoacetamide before electrophoresis. Such reduction and alkylation resulted in the disappearance of OMP-MC with the concomitant appearance of subunits (Fig. 3, lane 7). The conversion of OMP-MC into subunits was complete. OMP-MC purified by gel filtration was labeled with ^{125}I-labeled Bolton-Hunter reagent, mixed with a solubilized OM sample, and reduced and alkylated. When analyzed on a 10% polyacrylamide gel, all disintegrations per minute comigrated with a Coomassie blue staining band of approximately 76,000 molecular weight. These results suggest that OMP-MC is a protein complex composed of 10 to 12 76,000 subunits linked by disulfide bonds that have a high propensity to reoxidize after removal of reducing agent.

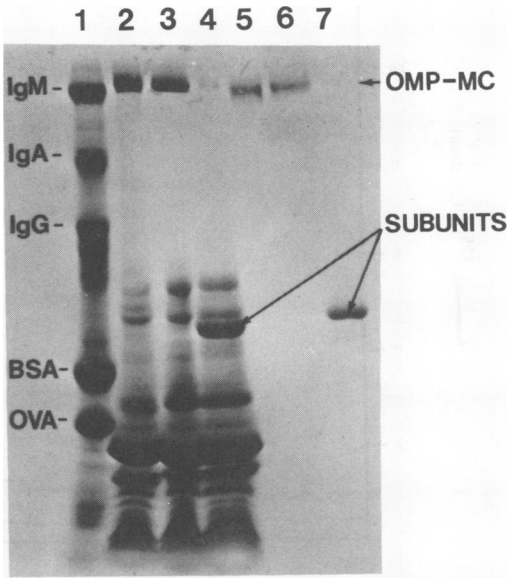

FIG. 3. *Coomassie blue-stained 2 to 20% polyacrylamide gradient slab gel of 2686 T4 outer-membrane proteins and purified OMP-MC illustrating the conversion of OMP-MC into subunits. (Lane 1) Molecular weight markers: pentameric IgM, dimeric IgA, IgG, bovine serum albumin (BSA) and ovalbumin (OVA). (Lanes 2–4) Isolated outer-membrane proteins. (Lanes 5–7) OMP-MC purified by gel filtration. Samples 2 and 5 were alkylated with iodoacetamide; 3 and 6 were reduced with dithiothreitol; and 4 and 7 were reduced and alkylated. The positions of OMP-MC and its subunits are indicated by arrows.*

Surface labeling. The location of OMP-MC in the OM is suggested by its enrichment in samples of partially purified OM compared with samples of inner membranes (data not shown). To confirm the OM location and determine whether OMP-MC is exposed at the cell surface, intact 2686 T4 gonococci were surface-labeled with ^{125}I by using a lactoperoxidase-catalyzed reaction. The enzyme will not penetrate lipid bilayers and is too large to penetrate known membrane pores. Analysis of labeled OM proteins on a 2% composite gel indicated that OMP-MC was labeled (Fig. 4) and thus exposed at the cell surface. The relative degree of labeling was less than with other OM proteins previously demonstrated to be exposed at the surface (10, 31). Reiodination of isolated OM (i) with lactoperoxidase before SDS solubilization or (ii) by the method of Fraker and Speck (6) after solubilization resulted in similar low extents of iodination of OMP-MC. We conclude that the low yield of surface-labeled OMP-MC was due to its intrinsic low iodination potential by these oxi-

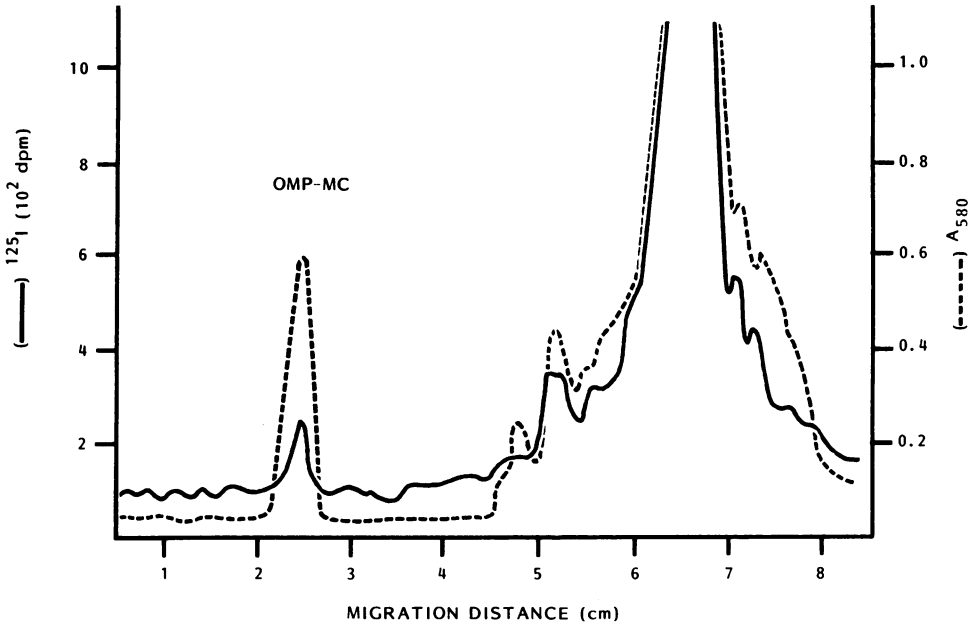

FIG. 4. *2% composite gel of 2686 T4 outer-membrane proteins surface-labeled with ^{125}I. The absorbance scan was obtained from a duplicate sample stained with Coomassie blue.*

dative methods rather than to a low degree of exposure at the cell surface.

Further confirmation of the surface exposure of OMP-MC was obtained with CNBr-activated dextran. This agent forms covalent linkages with free amino groups and in its polymerized activated form does not penetrate cells (15). Thus, proteins exposed on the cell surface and labeled with CNBr-activated dextran are greatly increased in molecular weight. Analysis of the OM proteins of treated cells showed a marked loss of OMP-MC, indicating surface exposure (Fig. 5). Compared with an untreated control, some protein was shifted to high molecular weights as indicated by counts per minute at the origin (Fig. 5) and at the top of the stacking gel. A decrease in counts per minute was also noted at the migration distance for low-molecular-weight proteins. The surface exposure of some of these proteins has been demonstrated previously (10, 16, 31).

Immunoprecipitation. Exposure at the cell surface suggested that OMP-MC may play a role in the immunological interaction of the host with gonococci. The antigenic activity of this protein was tested by determining whether rabbit serum raised against whole 2686 T4 gonococci contained anti-OMP-MC antibody. Approximately 30% of the original OMP-MC present was recovered in the immune complex staphylococcal precipitate. In contrast, only 6% could be shown to bind nonspecifically to the staphylococci after the initial incubation with preimmune serum. We conclude that OMP-MC is a significant surface antigen.

Presence of OMP-MC in other strains and species. The previous data were obtained with strain 2686 T4. OMP-MC was also found in the OM preparations of all other gonococcal strains tested. In all cases both the apparent molecular weights and the relative amounts of OMP-MC were similar to 2686. OMP-MC was identified in each of the colonial variants of 2686 (T1, T2, T3, and T4); in other wild-type laboratory strains including RD_5, CS7, and JW31; in each strain of an isogenic set of mutants having altered antibiotic sensitivities (FA19, FA102, FA136, FA140, BR87, BR54 [25, 26]); in cells grown at pH 6.0 (RD_5 and 2686); in the LA^+ and LA^- variants of strain MS11 (16, 29, 30); and in strain 7122 which has properties associated with disseminated gonococcal infection isolates. The presence of OMP-MC was also found to be independent of colony color or colony opacity.

The presence of OMP-MC in other species was also investigated by SDS-PAGE analysis of whole cell lysates. OMP-MC was detected in *N. gonorrhoeae* by this technique but was not found in *N. meningitidis*, *N. sicca*, *N. perflava*, *B. catarrhalis*, *M. lacunata*, *E. coli*, or *B. pertussis*. *N. meningitidis* and *N. sicca* had small amounts of Coomassie blue staining material which migrated more slowly than OMP-MC.

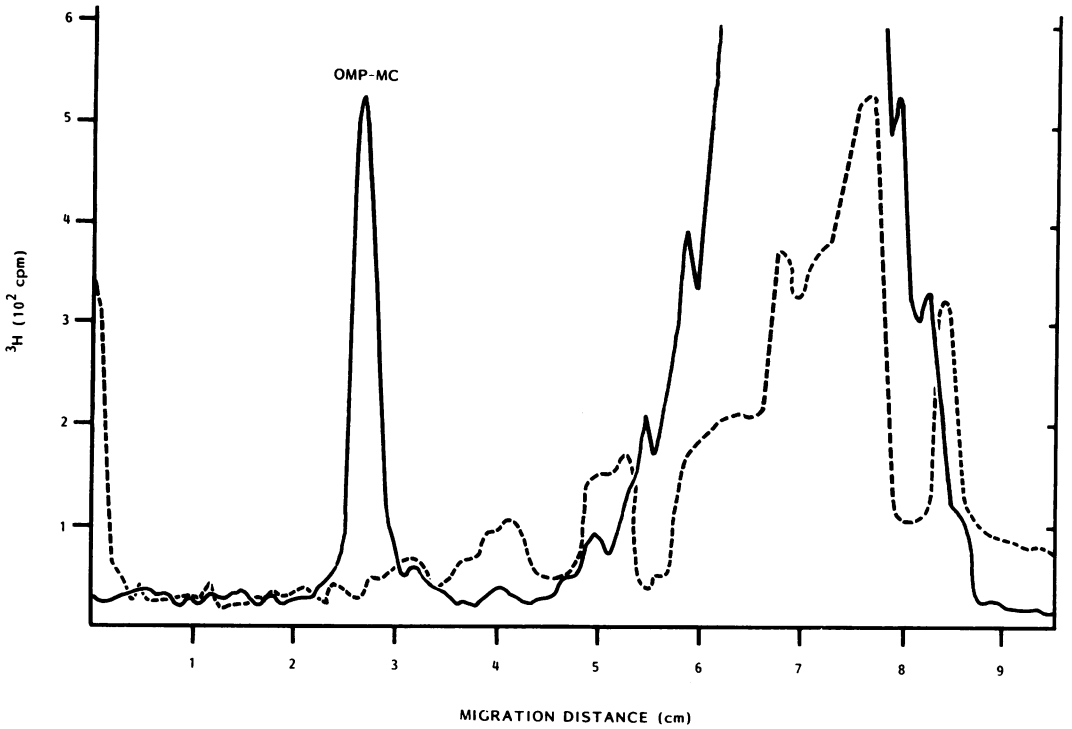

FIG. 5. *Radioactivity profile determined from 2% composite gels of* L-*[^{3}H]proline-labeled 2686 T4 outer-membrane proteins. Samples were surface labeled with CNBr-activated dextran* (-----) *or left untreated* (——).

DISCUSSION

The OM of gram-negative bacteria represents the interface between the organism and its external environment. This interface functions both as a permeability barrier and as the site for biochemical and biophysical interactions with host cells and soluble components, e.g., antibody and complement. In many cases, these functions have been related directly to the OM proteins. Investigations of the OM proteins of *N. gonorrhoeae* have demonstrated the presence of about 15 proteins with 2 or 3 accounting for over 75% of the total protein mass.

The present study describes the presence of a previously unresolved protein, herein called OMP-MC, which accounts for approximately 10% of the OM protein mass. SDS-PAGE and gel filtration indicated that OMP-MC had an apparent molecular weight of about 800,000 to 900,000. The high molecular weight of OMP-MC suggested that the protein had a subunit structure. Attempts to demonstrate subunits by conventional techniques were unsuccessful. However, when samples were both reduced and alkylated, OMP-MC was converted into subunits with a molecular weight of 76,000.

Johnston et al. (14) have developed a serotyping scheme based on the antigenic behavior of an OM complex consisting of lipopolysaccharide and two OM proteins. Both of these proteins are exposed at the surface and together they comprise a unique antigenic set which is responsible for differences in serotype. Similarly, the OMP-MC was exposed at the cell surface, had antigenic activity, and, in addition, was found in all *N. gonorrhoeae* strains tested. These findings indicate that OMP-MC deserves further consideration in studies aimed at (i) describing shared OM antigens, (ii) developing vaccines, (iii) developing serotype schemes, and (iv) interrelating the structure and function of the gonococcal OM.

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