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Conservation of Peptide Structure of Outer Membrane Protein-Macromolecular Complex from *Neisseria gonorrhoeae*

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The structural conservation of an outer membrane protein of *Neisseria gonorrhoeae* called OMP-MC (outer membrane protein-macromolecular complex) was investigated by determining the isoelectric point and amino-terminal amino acid sequence of the protein and by using high-performance liquid chromatography for comparative tryptic peptide mapping. The 76,000-dalton subunits generated by reduction and alkylation of the native 800,000-dalton complex from six test strains focused in ultrathin gels as bands of restricted heterogeneity at an approximate pI of 7.6. Dansyl chloride labeling indicated that all strains shared glycine as the amino-terminal amino acid. Sequence analysis of OMP-MC from two strains revealed no amino acid differences within the first 11 residues. Dual-label peptide maps revealed an extremely high degree of conservation of peptide structure. The results indicate that (i) OMP-MCs isolated from various strains of *N. gonorrhoeae* share structural homology and (ii) the 800,000-dalton complex is a homopolymer composed of 10 to 12 apparently identical 76,000-dalton subunits.

Proteins of the outer membrane (OM) of *Neisseria gonorrhoeae* have been studied extensively in the hope of gaining insight into the molecular interactions between the organism and its environment. Functionally, certain OM proteins have been associated with colony opacity (28), serum resistance (10, 18), leukocyte adherence (14, 18), and even binding of eucaryotic molecules such as calmodulin (Schleicher et al., Fed. Proc. **42**:861, 1983). Serologically, gonococcal OM proteins I, II, and III (PI, PII, and PIII) have been shown to be antigenic and to contribute to the variability evident in the serotyping of the species (11, 20). Structurally, the OM proteins of *N. gonorrhoeae* have been characterized to correlate antigenic diversity with differences in protein structure (9, 12, 13, 31).

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of solubilized OM from *N. gonorrhoeae* strains on 2 to 20% polyacrylamide gradient slab gels revealed a high-molecular-weight band of 800 kilodaltons (kd) in addition to previously described protein bands of apparent molecular weights (app. mol. wt.) of less than 110 kd (22). The complex, termed OMP-MC (outer membrane protein-macromolecular complex), appeared as a consistently uniform Coomassie blue staining band of identical app. mol. wt. in all strains and colonial variants of gonococci examined. Alkylation of the complex after reduction resulted in the generation of a single protein band of app. mol. wt. 76 kd, suggesting that the complex was composed of 10 to 12 76-kd subunits. Serological examination of the complex from a number of strains indicated a high degree of shared antigenic reactivity (M. V. Hansen and C. E. Wilde III, manuscript in preparation).

To evaluate the structural homology of OMP-MC, studies were initiated to determine the isoelectric point (pI) and the amino-terminal amino acid sequence of the 76-kd subunit of OMP-MC and to examine comparative tryptic peptide maps of these subunits isolated from six strains of *N. gonorrhoeae*. The results of these analyses indicate a high degree of structural conservation of OMP-MC from diverse strains of *N. gonorrhoeae*.

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MATERIALS AND METHODS

Materials. Dansyl chloride (DNS-Cl), phenylmethylsulfonyl fluoride (PMSF), Nonidet P-40, Trizma base, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), iodoacetamide, avidin, chicken egg white lysozyme, and all reference amino acids were from Sigma Chemical Co., St. Louis, Mo. Chymotrypsinogen A was supplied by Boehringer Mannheim Biochemicals, Indianapolis, Ind. Trypsin-tolylsulfonyl phenylalanyl chloromethyl ketone was obtained from Worthington Biochemical Corp., Freehold, N.J. Bovine gamma globulin fraction III was from Pentex, Inc., Kankakee, Ill. Electrophoresis and isoelectric focusing (IEF) reagents were purchased from Bio-Rad Laboratories, Richmond, Calif. Constant boiling HCl and trifluoroacetic acid (TFA) were purchased from Pierce Chemical Co., Rockford, Ill. High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and 2-propanol were purchased from Burdick and Jackson Laboratories, Inc., Muskeegon, Mich. Micropolyamide sheets were obtained from Schleicher and Schuell Co., Keene, N.H. Radiochemicals were supplied by ICN Pharmaceuticals, Inc., Irvine, Calif. (L-[4,5-^{3}H]lysine, 54 Ci/mmol) and New England Nuclear Corp., Boston, Mass. (L-[*U*-^{14}C]lysine, 350 mCi/mmol). NCS tissue solubilizer was obtained from Amersham Corp., Arlington Heights, Ill.

Bacterial strains and growth conditions. Gonococcal strains 2686 (K. H. Johnston, laboratory reference strain), 8035 (T. M. Buchanan, PI serogroup 6), 8038 (T. M. Buchanan, PI serogroup 8), 1342 (M. A. Apicella, lipopolysaccharide serogroup Gc_1), 1291 (M. A. Apicella, lipopolysaccharide serogroup Gc_2), and 7502 (T. M. Buchanan, disseminated gonococcal infection isolate) were used as the sources of OMP-MC in this study. Cells were grown on clear typing medium (27), and only nonpiliated, transparent (P^- O^-) organisms were utilized (27).

Intrinsic radiolabeling of all gonococcal strains was done in liquid culture in chemically defined WSJM medium (35). Either 2 μCi of L-[4,5-^{3}H]lysine per ml or 0.5 μCi of L-[*U*-^{14}C]lysine per ml was added to 0.5-liter cultures (starting optical density at 540 nm, 0.08) in WSJM medium containing 5% of the normal concentration of nonradioactive

L-lysine. Cultures were grown at 37°C with continuous shaking and harvested in late-exponential phase. Cell growth was monitored by optical density at 540 nm, and incorporation of radiolabel into macromolecules was followed by precipitation with 10% trichloroacetic acid (TCA).

Purification of OMP-MC. Cell envelopes and OMs were prepared and solubilized for chromatography by the method described by Newhall et al. (23), with the modification that all buffers contained 0.02% sodium azide and 0.001 M PMSF. OMP-MC was isolated from solubilized envelope or OM by gel filtration on a Sepharose CL-4B column (115 by 1.5 cm) equilibrated with 0.01 M Na-HEPES (pH 7.4)–0.1% SDS–0.02% NaN_3–0.001 M PMSF. Fractions were monitored by absorbance at 280 nm, by liquid scintillation counting (when applicable), and by electrophoresis over SDS–2 to 20% polyacrylamide gradient gels using the discontinuous buffer system of Laemmli (16). A portion of each pooled radiolabeled OMP-MC preparation was electrophoresed on an SDS–2.5% polyacrylamide/agarose composite tube gel, which was subsequently sliced into 1-mm segments; radioactivity associated with each fragment was determined by scintillation counting in a toluene-based cocktail after overnight digestion at 37°C in 0.5 ml of 90% NCS solubilizer. Comigration of radioactivity with the stained 800-kd band visible on duplicate gels indicated that the radiolabeled protein species was OMP-MC.

IEF. IEF procedures were patterned after the methods of O'Farrell (24) and Gorg et al. (6). Ultrathin (0.22 mm) gels were cast essentially as described by Gorg (6). The gel consisted of 5% acrylamide, 2.6% cross-linked with *N,N'*-methylene-bisacrylamide, 6 M urea, 0.1% Nonidet P-40, 5% ampholine (pH 3 to 10 or 7 to 9), and 0.05% ammonium persulfate. Polymerization of the gel mixture (8 ml, total volume) was catalyzed by the addition of 5 μl of *N,N,N',N'*-tetramethylethylenediamine (TEMED) just before casting the gel into the prepared form. If a narrow-range ampholine gel was run, pH 7 to 9 ampholines were mixed with the pH 3 to 10 ampholines at a 4:1 ratio. Gels were allowed to polymerize overnight before use.

Samples to be run on IEF gels were prepared as follows. One-tenth volume of 1.0 M Tris-hydrochloride (pH 8.0) was added to 100 μg of column-purified OMP-MC. Dithiothreitol was added to a final concentration of 0.040 M, and the sample was reduced by boiling for 2 min. After the sample had cooled to 22°C, iodoacetamide was added to a final concentration of 0.1 M, and the alkylation was allowed to proceed for 30 min at 22°C. TCA was added to a final concentration of 25%, and the OMP-MC was precipitated overnight at 4°C. Pelleted OMP-MC (40 krpm; 30 min) was washed twice with ice-cold absolute ethanol. The reduced and alkylated OMP-MC pellet was solubilized with 8 M urea and 1% Nonidet P-40 to a concentration of about 1 to 2 μg of protein per μl. SDS-solubilized OM samples were prepared for IEF in the same manner.

Focusing was done with a Bio-Rad model 1405 horizontal electrophoresis cell cooled to 10°C by a circulating water bath. Filter paper wicks were saturated with anolyte (0.06 N H_2SO_4) and catholyte [0.02 N $Ca(OH)_2$/0.04 N NaOH]. Samples were applied to the focusing gel surface by using either a plastic applicator mask with a 5-μl loading capacity or small Whatman no. 1 filter paper squares. Gels were focused at 5 W of constant power until a voltage limit of 2,000 V (wide-range ampholines) or 1,500 V (narrow-range ampholines) was reached. Peak voltage was maintained for 30 to 60 min. Applicator masks and filter paper squares were removed from the gel 1 h after the focusing run was initiated. A typical run was completed in about 3 h. The pH gradient was determined by elution in 1 ml of degassed distilled water of 1-cm^2 sections which were sliced from a portion of the gel not containing sample and measurement with a pH meter.

To detect the focused proteins, the IEF gel was covered with a sheet of dry nitrocellulose membrane (Millipore Corp., Bedford, Mass.) and gently peeled from the glass plate. The proteins were transferred cathodally at 2 A for 1 h in a Bio-Rad Trans-Blot electrophoretic chamber filled with degassed 1% acetic acid (5). The nitrocellulose membrane containing the transferred, focused proteins was washed with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20 for 30 min at 22°C, incubated with an appropriate dilution of rabbit anti-2686 OMP-MC serum for 1 h at 22°C, rewashed, and then incubated with radioiodinated protein A for 1 h at 22°C. The processed nitrocellulose membrane was stained with amido black (25) and autoradiographed using Kodak X-Omat AR film along with a DuPont Cronex Lighting Plus ZG intensifying screen in a cardboard holder. Films were exposed at −70°C for 8 to 16 h.

Tryptic digestion. Five milligrams of bovine gamma globulin was added as carrier to the pooled column fractions containing radiolabeled OMP-MC of each strain, and the individual pools were adjusted to 20% TCA; precipitation was allowed to proceed overnight at 4°C. The TCA-precipitable material was pelleted by centrifugation at 15 krpm for 30 min and washed twice with ethanol. Precipitates were solubilized and reduced by boiling for 3 min in 3.0 ml of 0.1 M Tris-hydrochloride (pH 8.0)–10 M urea–0.04 M dithiothreitol. After cooling for 30 min at room temperature, L-[*U*-^{14}C]lysine-labeled OMP-MC from strain 2686 was added at a 1:10 (^{14}C to ^{3}H) ratio to L-[4,5-^{3}H]lysine-labeled OMP-MC from each of the five other test strains. The mixtures were alkylated by the addition of iodoacetamide to a final concentration of 0.1 M for 1 h. The reduced and alkylated pools were dialyzed overnight at 4°C against two changes of 0.1 M $NaHCO_3$ (pH 8.5). The dialyzed mixtures were made 1 mM in $CaCl_2$, and trypsin was added at a ratio of 1 part trypsin to 10 parts carrier protein. After incubation at 37°C for 1 h, additional trypsin was added at an enzyme to carrier ratio of 1:20, and the digestion was continued for 12 to 16 h at 37°C. The pH of each sample was lowered to approximately 3 with glacial acetic acid, and the samples were lyophilized. The samples were rehydrated with 0.6 ml of water and relyophilized. Before HPLC analysis, the samples were dissolved in 0.3 to 0.5 ml of 0.05% TFA and centrifuged to remove any insoluble material.

HPLC. Peptides were resolved on an Ultrasphere-octadecyl-silane column using a Beckman model 332 gradient liquid chromatograph. Approximately 10^5 cpm of ^{3}H and 10^4 cpm of ^{14}C were applied to the column per run. The aqueous phase was 0.05% trifluoroacetic acid, and the organic phase was a 1:1:1 (vol/vol/vol) mixture of acetonitrile, methanol, and 2-propanol. An initial flow rate of 0.5 ml/min was maintained over two linear gradient changes. The first gradient was from 0 to 30% organic phase over 60 min, and the second gradient increased the percentage of the organic phase to 60% over an additional 40 min. After an increase in flow rate to 1.0 ml/min, the organic phase was increased to 100% over 10 min and maintained for an additional 5 min to elute any residual material before reequilibration of the column to starting conditions. Fractions were collected at 0.5-min intervals. The content of each fraction was transferred to a scintillation vial, the tube was rinsed with 0.5 ml water, and the rinse was added to the vial. The vials were dried overnight at 70°C and counted under dual-label condi-

tions in 10 ml of a toluene-based cocktail containing 0.5 ml of 90% NCS solubilizer.

Dansylation procedures. The procedures used for dansylation were derived from established protocols (7, 21, 36). Proteins prepared for dansylation were suspended in buffer (0.01 M HEPES [pH 7.4], 0.1% SDS, 0.02% NaN_3, 0.001 M PMSF), reduced, and alkylated. The samples were precipitated overnight at 4°C after the addition of TCA to a final concentration of 25%. The TCA precipitates were pelleted by centrifugation, washed twice with ethanol, and redissolved in 0.05 M $NaHCO_3$ (pH 10.0)–0.2% SDS. DNS-Cl (20 mg/ml in acetone) was mixed with the samples at a 6:1 (wt/wt) ratio of DNS-Cl to protein and allowed to react for 1 h at 37°C. The dansylated proteins were precipitated with 10 volumes of acetone overnight at −70°C and pelleted at 40 krpm for 30 min. The acetone pellet was dried under vacuum, dissolved in 500 to 750 μl of 6.1 N constant boiling HCl and transferred to Pyrex ignition tubes (16 by 125 mm; Corning Glass Works, Corning, N.Y.). The tubes were sealed under vacuum, and the proteins were hydrolyzed for 22 h at 110°C. After hydrolysis, the samples were subjected to two cycles of lyophilization and dissolved in 20 to 40 μl of a 3:2 acetone to acetic acid solution. Glass micropipets (1 to 5 μl; Clay Adams Co., Parsippiny, N.J.) were used to apply 1 μl of sample to one corner (0.5 cm from each edge) of a micropolyamide sheet (4.5 by 4.5 cm). Chromatography in the first dimension was done with a 1.2:50 (vol/vol) ratio of formic acid to water, and the second vector solvent was an 8:2 (vol/vol) ratio of benzene to glacial acetic acid. The polyamide sheets were dried thoroughly after both the first- and second-dimension separations. Individual amino acids were detected as brightly colored, fluorescent spots upon illumination with a hand-held UV lamp. One hundred micrograms of each OMP-MC strain was dansylated. Ten nanomoles each of avidin, egg white lysozyme, and chymotrypsinogen A, included as standards with known amino termini, were treated in the same manner. A non-protein-containing control consisting of buffer alone was processed at the same time as the protein samples. Fluorescent spots on polyamide sheets loaded with protein samples (OMP-MC or standards) were identified by comparing their positions to those of spots produced by known, labeled amino acids migrating on the opposite side of the same sheet.

Amino acid sequencing. Approximately 20 nmol of column-purified OMP-MC from strains 2686 and 1342 was precipitated with 20% TCA, washed with ethanol, and sent for sequence analysis to J. D. Capra, Department of Microbiology, University of Texas Health Science Center, Dallas. Automated sequence analysis was performed with a Beckman model 890C Sequenator modified with a cold trap (19). Polybrene was included with the samples (15), and the resultant phenylthiohydantoin (PTH) amino acids were identified by HPLC.

RESULTS

IEF. Samples prepared for IEF included reduced and alkylated purified OMP-MC and OM of each of the six test strains of *N. gonorrhoeae*. The reduced and alkylated samples were checked by electrophoresis on an SDS-2 to 20% polyacrylamide gradient slab gel to ensure conversion of the 800-kd complex to the 76-kd subunits. Complete conversion was necessary since the 800-kd complex would not be able to migrate through the 5% acrylamide gel used for focusing.

Figure 1 shows an autoradiogram of an IEF gel (pH 7 to 9 ampholines) on which various OMs and OMP-MCs from the test strains were run. The 76-kd subunit protein bands from all strains tested, as detected by anti-2686 OMP-MC serum, migrated uniformly and focused with an apparently identical pI of 7.6. The migration of the subunit polypeptides as apparently homogeneous single bands indicated a common subunit structure; i.e., OMP-MC is a homopolymer of 10 to 12 apparently identical 76-kd subunits. The IEF migration patterns of isolated OMP-MC and OMP-MC that were focused as part of an OM preparation were identical. The utility of monospecific antisera for detecting a specific focused band is apparent in that the restricted banding pattern visible in lanes containing focused OMs had the same mobility as bands visible in lanes containing purified, focused OMP-MC. In contrast, when focused OMs were treated with anti-whole gonococcal cell serum, multiple bands were resolved, but only a simple, restricted pattern appeared when anti-OMP-MC serum was used as a probe for focused OMs (data not shown). These findings, as well as other data (Hansen and Wilde, in preparation), indicate that the anti-OMP-MC serum used possessed restricted specificity and reacted equivalently with OMP-MC of all strains.

Peptide mapping. Preliminary experiments had shown that macromolecules of *N. gonorrhoeae* could be intrinsically labeled by inclusion of L-[4,5-^{3}H]lysine in WSJM medium. The greatest degree of incorporation occurred in cultures containing less than 10% of the nominal L-lysine concentration. The cultures exhibited exponential growth throughout the 180 min of labeling. Based on these observations, WSJM containing 5% of the nominal L-lysine concentration was utilized to label cells through approximately two generations for the isolation of radiolabeled OMP-MC.

Determination of the release of radioactivity from TCA-precipitated material treated with lysozyme (100 μg/ml) or proteinase K (100 μg/ml) showed that 95% of the counts remained TCA precipitable after lysozyme treatment, whereas an average of 90% of the counts were converted to a TCA-soluble form by proteinase K. These data indicated that the L-lysine radiolabel was predominantly incorporated into cellular proteins rather than converted to diaminopimelic acid and incorporated into peptidoglycan.

Comparison of the radioactivity incorporated into isolated cell envelopes (inner plus outer membranes) compared to that recovered as OMP-MC showed that OMP-MC comprised an average of 2.9% of the total protein mass of the envelopes for all strains (Table 1). Determination of the radioactivity in the isolated OM confirmed previous estimates of approximately 10% of the total protein mass of the OM consisting of OMP-MC (23). Purified, radiolabeled, reduced, and alkylated OMP-MC of each strain was electrophoresed on SDS-10% polyacrylamide tube gels. The radioactivity profiles obtained by slicing the gels into 1-mm segments indicated that, in all cases, the purified, radiolabeled OMP-MC subunits migrated as apparently homogeneous peaks that comigrated with the 76-kd band on stained gels.

Profiles of radiolabeled tryptic peptides resolved by HPLC are shown in Fig. 2. The striking similarity of profiles among these chromatograms suggests that similar tryptic cleavage sites existed for all samples examined. However, such a conclusion depends on run-to-run consistency. In the present study, the use of a dual-label analysis allowed more accurate comparisons to be made. Each ^{3}H-labeled OMP-MC was codigested and cochromatographed with the same standard, ^{14}C-labeled OMP-MC from strain 2686. This design provides that any variations in tryptic cleavage sites should be readily apparent as peaks containing only one of the two radiolabels. However, all peptide peaks observed

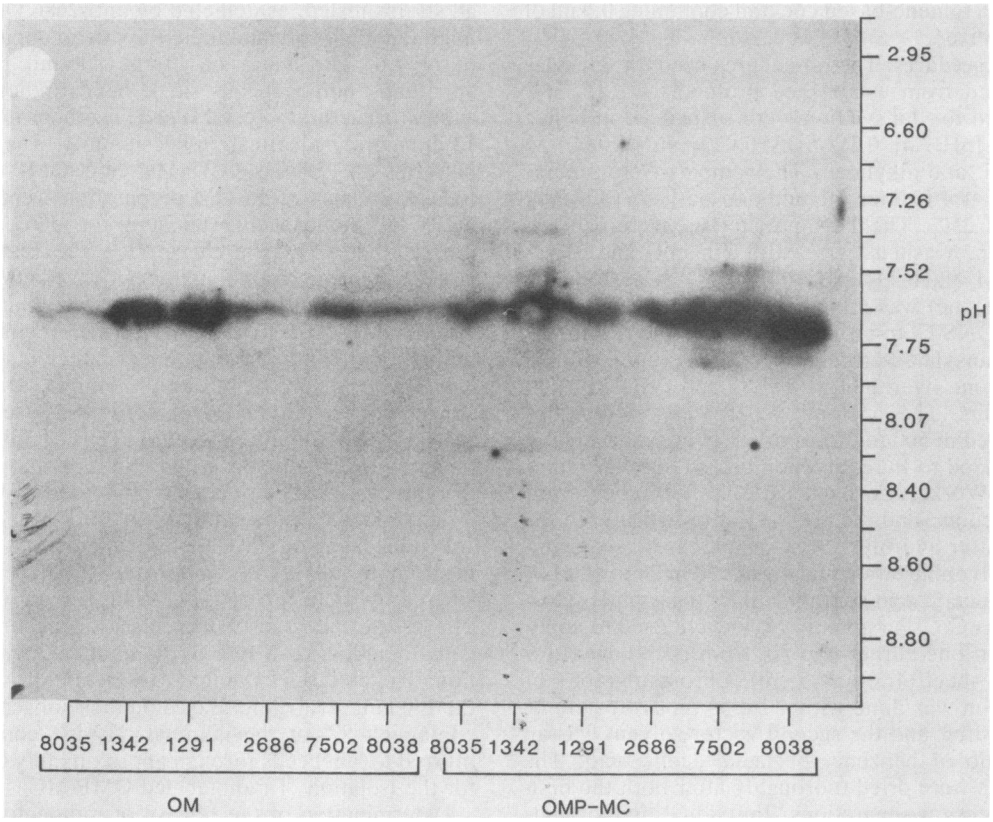

FIG. 1. Autoradiogram of an immunoelectrophoretic transfer of reduced and alkylated gonococcal OMs and OMP-MCs focused using narrow-range ampholines (pH 7 to 9). All lanes were developed with a 1:20,000 dilution of anti-2686 OMP-MC serum. The pH gradient was determined after elution of slices of the focused gel not containing sample.

contained both ^{3}H and ^{14}C labels. Single-label controls counted under dual-label conditions showed that this coincidence was not due to spillover during counting.

Within each run, the ratio of ^{3}H to ^{14}C was essentially constant (Table 2); however, variation from this ratio was observed for occasional peaks (e.g., the peak around fraction 80). This variation may have resulted from the gain or loss of a peptide component from the test strain relative to strain 2686. In addition, subtle variations within peaks were observed in which the migration of the ^{3}H component was slightly offset from that of the ^{14}C component. Such occasional lack of absolute comigration of peptides could result from minor differences in peptide amino acid composition, differences that were insufficient to allow separation into discrete peaks under the chromatographic conditions used. However, in no instance was a discrete peak observed that contained only one radiolabel; this observation reinforces the existence of a high degree of homology of peptide structure among OMP-MCs from diverse strains.

TABLE 1. Incorporation of L-[4,5-^{3}H]lysine into OMP-MC

Strain	dpm[a] incorporated into:			OMP-MC as % of:	
	Envelopes[b]	OM	OMP-MC	Envelopes	OM
2686	31.34	7.97	0.81	2.6	10.1
8038	40.98	7.51	0.79	1.9	10.4
8035	42.94	ND[c]	1.59	3.7	
7502	77.40	ND	1.80	2.3	
1342	65.47	ND	1.93	2.9	
1291	52.62	ND	2.03	3.8	

[a] Values are dpm × 10^{6} incorporated into material precipitable in 10% TCA.
[b] Envelopes are inner plus outer membranes.
[c] ND, Not determined.

Dansylation. Preliminary experiments done with a set of 20 stock amino acids and with proteins of known amino termini verified the utility of the dansylation procedure and established the best chromatographic conditions. Less than 0.5 nmol of an individual DNS-amino acid spotted on a polyamide sheet was easily detectable. The expected DNS-amino acid was observed for each of the test proteins: chymotrypsinogen A (cysteine), avidin (alanine), and lysozyme (lysine).

Identical dansylation and chromatographic procedures were performed on freshly isolated, reduced, and alkylated OMP-MC of the six test strains. Each analysis of the purified OMP-MC samples yielded DNS-glycine as the sole amino-terminal amino acid; ε-DNS-lysine was also observed due to labeling of internal lysine residues. A non-protein-containing sample processed in the same manner yielded no labeled amino acids, even when 10 times more sample volume was loaded onto the polyamide sheet than for the protein-containing samples.

Amino acid sequencing. The amino-terminal amino acid sequences of OMP-MC from strains 2686 and 1342 are presented in Fig. 3. Determination of the entire first 20 amino acids from strain 1342 was hindered for technical

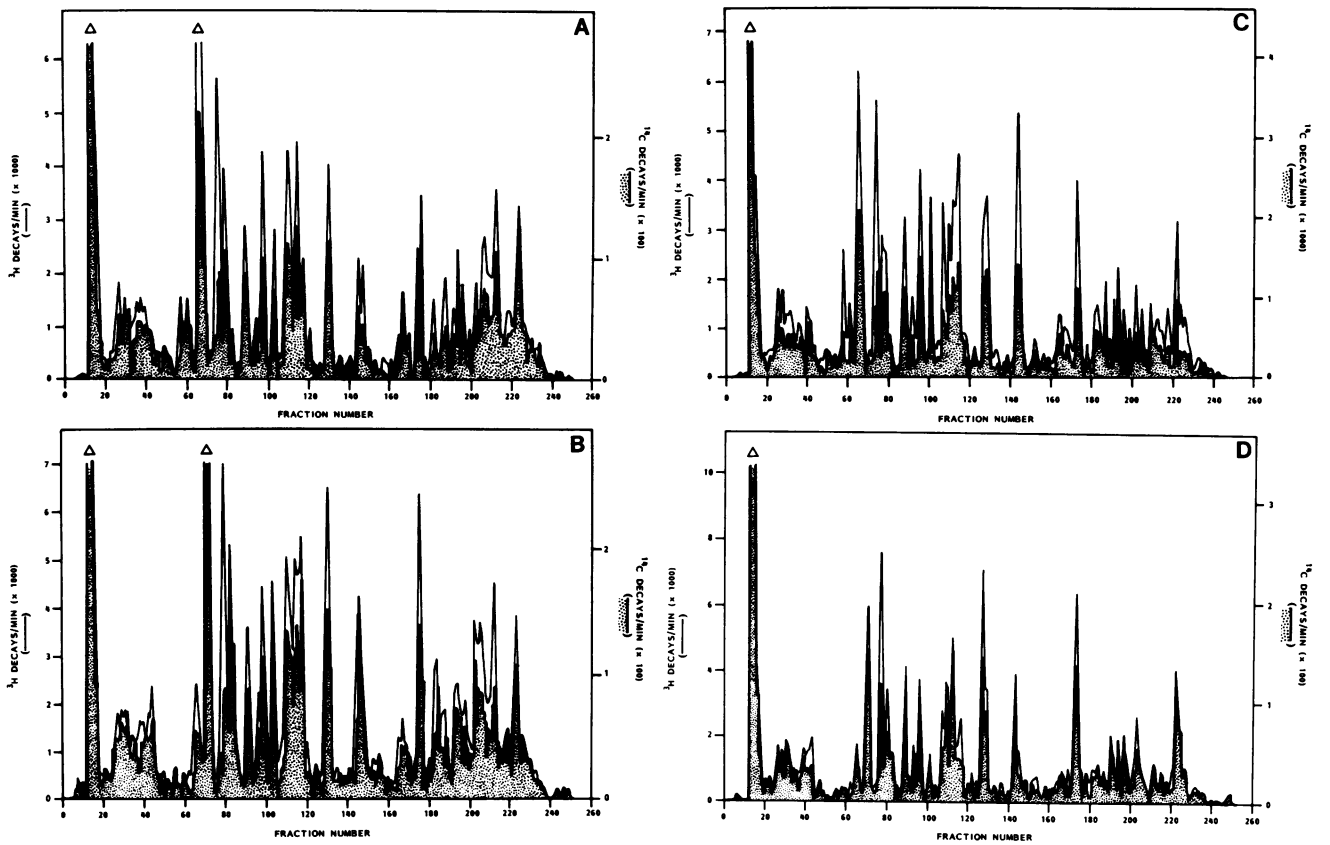

FIG. 2. Tryptic peptide maps of L-[4,5-^{3}H]lysine-labeled OMP-MC from strains (A) 7502, (B) 1291, (C) 1342, and (D) 8035; each ^{3}H-labeled protein was codigested and cochromatographed with L-[*U*-^{14}C]lysine-labeled OMP-MC from strain 2686. Chromatography conditions are described in the text.

reasons. Several points can be made from these data. First, the identity of the amino-terminal residue was confirmed as glycine. Second, the presence of a single sequence indicates that (i) all 76-kd subunits comprising the native complex are identical or (ii) if nonidentical subunits are present, only one molecular form possesses a free (unblocked) amino terminus, making it available for sequencing. Third, no differences in amino acid sequence among the nine comparable positions were observed between the proteins isolated from strains 2686 and 1342; this reinforces the homology of peptide structure observed by tryptic peptide mapping. Computer-assisted analysis of the sequence of the first 18 amino acids of OMP-MC from strain 2686 was performed by P. Davidson, Department of Biology, Indiana University, Bloomington. Comparison of this sequence with 1,993 sequences (containing 330,893 residues) in the Protein Sequence Database from the Atlas of Protein Sequence and Structure indicated that no protein exhibited homology at more than 7 of the 18 positions (less than 39% homology). Of the nine proteins that shared seven homologous residues, none was a bacterial membrane protein and none of the homologous regions were at or near the amino termini. These data suggest that OMP-MC is unrelated to previously sequenced proteins.

DISCUSSION

The outer membrane of *N. gonorrhoeae* contains relatively few protein species, with two or three accounting for greater than 75% of the total protein mass (23). These proteins, whose location in the OM favors their interaction with host cells and soluble components, have been shown to be immunoreactive. Serological examinations have demonstrated that degrees of heterogeneity exist among these proteins isolated from diverse strains. Similarly, biochemical analyses have shown marked degrees of diversity of peptide profiles of these various proteins.

As the most abundant OM protein species, PI of the gonococcus is a non-heat-modifiable, deoxycholate-insoluble protein (8, 26) that varies in app. mol. wt. among strains (17, 32). At least three distinct species of PI have been demonstrated based on protease sensitivity, SDS-polyacrylamide gel electrophoresis migration, and two-dimensional peptide mapping (1, 3, 29). PII is a family of heat-modifiable proteins of varying app. mol. wt. associated with the opacity

TABLE 2. Ratio of ^{3}H to ^{14}C dpm for representative major tryptic peptide peaks resolved by HPLC

Strain	Ratio[a] for peak at approx. fraction no.:							
	15	66	72	80	90	130	146	176
7502	34	30	37	57	28	35	40	38
1291	41	43	39	69	49	43	38	42
1342	25	34	29	53	26	28	32	28
8035	41	32	42	86	42	46	44	46

[a] Ratio =

$$\frac{\text{sum of } ^3\text{H dpm within peak for OMP-MC of the indicated strain}}{\text{sum of } ^{14}\text{C dpm within peak for OMP-MC of strain 2686}}$$

Position	1	5	10	15	20
2686 OMP-MC	GLY ASN ILE THR ASP ILE LYS VAL SER ASP LEU PRO ASN LYS GLN LYS ILE VAL				LYS
1342 OMP-MC	GLY ASN ILE	ASP ILE LYS VAL SER	LEU		

FIG. 3. Amino-terminal amino acid sequence of OMP-MC from strains 2686 and 1342. A blank indicates that no PTH amino acid was identified at that position.

properties of gonococci (9, 26, 28); multiple species of PII may exist in a particular gonococcal strain (17, 18, 32). Despite the appearance of some apparently common peptides, considerable differences in peptide profiles exist among PIIs of different strains and among multiple PIIs present in a single strain (9, 30). PIII is an apparently conserved protein that exists in the OM as a heteropolymer with PI (20, 22). PIII is a non-heat-modifiable protein found in all strains that exhibits an increase in app. mol. wt. after treatment with reducing agents (3, 20, 31). Strikingly similar peptide maps are observed upon examination of PIII from diverse strains (2, 12, 13). The identification of OMP-MC as a major protein component of the gonococcal OM (23) prompted investigations into the extent of structural conservation and subunit composition of this protein complex.

IEF is a technique that provides a sensitive means for comparing proteins based on structural and compositional differences. For example, Terhorst et al. (33) were able to differentiate two ribosomal proteins that differed solely on the basis of the presence or absence of an acetyl moiety on the alpha amino group of the amino terminal serine. Serological detection of the 76-kd subunit of OMP-MC after focusing revealed a pattern of restricted heterogeneity at a pI of 7.6; this isoelectric point was common to OMP-MC from all strains examined and independent of the degree of purity of the applied samples. The restricted pattern indicates that all of the subunits of OMP-MC share the same pI; thus, if OMP-MC exists as a heteropolymer of two (or more) different forms of the 76-kd subunit, each form must be similar enough in composition to share pI. Furthermore, the consistency of focusing behavior of OMP-MC from each of the test strains indicates that strain-to-strain differences in composition (if any) must be minimal.

Analyses of peptides generated by tryptic digestion of OMP-MC indicated that all strains tested displayed remarkably similar peptide profiles. The use of a dual intrinsic radiolabel design allowed OMP-MC from each of the test strains to be compared to a common internal standard. Such a design overcomes problems associated with differences in degrees of digestion and variations in chromatographic conditions that may occur when proteins are examined individually. For example, extrinsic labeling with oxidative iodination methods could result in different proteins being labeled at different positions and to different degrees (e.g., mono- versus diiodotyrosine), leading to variations in peptide chromatographic behavior. Biosynthetic labeling with L-lysine ensured that each lysine-containing peptide would be labeled to the same degree and, since trypsin cleaves on the carboxyl side of lysine residues, each tryptic peptide should contain only one labeled residue. Despite the high resolving power of reverse-phase HPLC, all OMP-MC peptide peaks contained both ^{3}H and ^{14}C radiolabels in approximately constant ratios within each run. This finding indicates that tryptic cleavage sites are conserved in OMP-MC and that any differences in tryptic peptide compositions are of insufficient magnitude to preclude coelution of homologous peptides.

Amino-terminal analyses, both by DNS-Cl and amino acid sequencing, indicated that OMP-MC from all strains tested shared common features. Glycine was determined to be the terminal amino acid of OMP-MC from each strain. Despite the ubiquity of glycine as a potential environmental contaminant, the absence of DNS-glycine upon analyses of proteins with known amino termini and sham controls validated the results for OMP-MC. The identity of the amino terminus of OMP-MC was confirmed for two strains by amino acid sequencing. The presence of a single amino-terminal amino acid and a single amino acid sequence complements the conclusion drawn from IEF that the 800-kd complex is a homopolymer formed of 10 to 12 identical 76-kd subunits. Furthermore, these data strengthen the conclusion that OMP-MC is structurally conserved among diverse strains of gonococci. Where comparisons could be made, no difference in amino acid sequence was observed for OMP-MC from strains 2686 and 1342. Although these results are indicative of conservation of structure, extension of sequence analyses at the protein and/or nucleic acid level is necessary to determine the precise degree of homology.

Of the gonococcal OM proteins examined to date, only PIII exhibits structural conservation comparable to that observed for OMP-MC. Peptide maps generated from PIII labeled either in solution or on the surface of intact cells demonstrated a high degree of structural conservation (12, 13). In contrast, both PI and PII demonstrated marked variability of peptide maps in strain-to-strain comparisons (9, 12, 13, 29, 32, 34); this variability was most evident when the proteins were labeled on the surface of intact organisms, suggesting that the exposed portions of these proteins are the repositories of the unique structural moieties (9, 13). This conclusion is further supported by serological studies. PI can be segregated into nonoverlapping serogroups (1, 4, 11, 20); a similar pattern may hold true for PII (W. J. Newhall V, L. B. Mail, C. E. Wilde III, and R. B. Jones, manuscript in preparation). Although PII may be antigenically conserved (20), its serological independence may be overshadowed by its close physical association with PI (20, 22).

The structural characterization of OMP-MC reveals marked conservation of peptide structure; this result complements recent serological investigations which demonstrated a high degree of cross-reactivity among OMP-MC from diverse strains of gonococci (Hansen and Wilde, in preparation). OMP-MC is a major, surface-exposed component of the outer membrane of *N. gonorrhoeae*; the degree of structural and serological conservation shown by this complex is uncommon among gonococcal OM antigens and suggests a possible role for OMP-MC in immunoprophylaxis.

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