

# Cloning and DNA Sequence of the *omc* Gene Encoding the Outer Membrane Protein-Macromolecular Complex from *Neisseria gonorrhoeae*

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The *omc* gene, encoding the outer membrane protein-macromolecular complex (OMP-MC), was cloned in two pieces from *Neisseria gonorrhoeae* 2686. The 5' fragment of the *omc* gene included a promoter sequence, as indicated by its unregulated expression in *Escherichia coli*. Attempts to reconstruct an intact *omc* gene were unsuccessful, suggesting that expression of the complete OMP-MC protein was toxic to *E. coli*. Complete sequence determination revealed a coding sequence of 2,133 nucleotides; the deduced amino acid sequence indicated a mature protein of 687 amino acids with an NH<sub>2</sub>-terminal signal peptide of 24 amino acids. Analysis of the deduced amino acid sequence revealed that the NH<sub>2</sub>-terminal half of OMP-MC is generally hydrophilic, while the COOH-terminal portion contains alternating hydrophobic and hydrophilic regions. Serological analyses demonstrated that the NH<sub>2</sub>-terminal portion of OMP-MC is exposed on the gonococcal surface and the COOH-terminal portion is membrane associated.

The surface components of gonococci have been under vigorous investigation in attempts to expand our knowledge about the biology of this organism and to find potential vaccine antigens for gonorrhea. Most antigens studied to date exhibit extensive structural and antigenic variations, e.g., pili (10, 19, 22), protein I, the major outer membrane protein with porin function (15, 31, 35), the heat-modifiable proteins II, which are associated with colony opacity (5, 13, 40), and lipopolysaccharide (1). In contrast, protein III exhibits a conserved structure (16) yet induces blocking antibodies that interfere with the bactericidal effects of other specific antibodies (26). The H.8 antigen is antigenically conserved, yet anti-H.8 monoclonal antibodies fail to be bactericidal in vitro and protective in vivo (36).

The surface-exposed outer membrane protein-macromolecular complex (OMP-MC) is structurally and antigenically conserved among diverse gonococcal strains (11, 34). OMP-MC is a major component that accounts for about 10% of the gonococcal outer membrane protein mass (11, 25). In the complex form, OMP-MC has a molecular mass of 800 kilodaltons (kDa) and is composed of 10 to 12 identical subunits of 76 kDa. The OMP-MC subunits of diverse gonococcal strains were shown to be structurally conserved by both tryptic and chymotryptic peptide mapping (10; W.-M. Tsai, M. J. Corbett, J. R. Black, and C. E. Wilde III, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, D15, p 74). Antigenic cross-reactivities of OMP-MC among heterologous gonococcal strains were also demonstrated by immunoelectrophoretic transfer with anti-OMP-MC polyclonal antibodies (34). OMP-MC is immunogenic in humans (J. R. Black, M. J. Corbett, M. Thompson, B. Ellis, and C. E. Wilde III, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 75, 1985), and anti-OMP-MC antibodies are capable of promoting complement-mediated bacteriolysis of homologous and heterologous strains of gonococci (6). These properties of OMP-MC make it a potential antigen for immunoprophylaxis.

We describe in this paper the identification and character-

ization of the gene encoding OMP-MC of *Neisseria gonorrhoeae* 2686. We propose the utilization of *omc* (outer membrane complex) to designate the genetic locus encoding the OMP-MC subunit polypeptide, in accordance with the recommendations arising from the sponsored roundtable discussion on neisserial genetics at the Sixth International Pathogenic Neisseria Meeting, October 1989, Callaway Gardens, Ga. (33).

(Portions of this work have been presented at the Fifth (Noordwijkerhout, The Netherlands, September 1987) and Sixth (Callaway Gardens, Ga., October 1989) International Pathogenic Neisseria Meetings.)

## MATERIALS AND METHODS

**Bacterial strains, bacteriophages, and plasmids.** The *N. gonorrhoeae* strains used in this study were 2686 (K. H. Johnston, laboratory reference strain) and FA19 (20). *Escherichia coli* Y1090 (39), JM103 (23), NM538 (9), and RR1ΔM15 (2) have been previously described. λgt11 phage (38), λEMBL3 phage (9), and pUC18 and pUC19 plasmids (37) were used for molecular cloning.

**Media.** *N. gonorrhoeae* was grown on clear typing medium or in peptone broth supplemented with NaHCO<sub>3</sub>, iron, glucose, and L-glucosamine (25). *E. coli* strains were grown in ZY medium (0.5% NaCl, 0.5% yeast extract, 1.0% NZ amine; Humko Sheffield Chemicals, Lynnhurst, N.J.) with the following additions when appropriate: ampicillin (50 μg/ml), isopropylthio-β-galactoside (IPTG; 100 μg/ml), and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal; 0.005%).

**DNA isolation and manipulation.** Gonococcal chromosomal DNA was prepared by the method described by Evans and Clark (8). Restriction endonucleases, mung bean nuclease, T4 DNA ligase, and Sequenase were obtained from New England BioLabs, Inc., Bethesda Research Laboratories, Inc., Boehringer Mannheim Biochemicals, and United States Biochemical Corp., respectively. Plasmid DNA extraction and transformation into *E. coli* were performed by the methods described by Maniatis et al. (21).

**Library construction.** A λgt11 genomic library was made from *N. gonorrhoeae* 2686. Chromosomal DNA of gonococ-

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cal strain 2686 was mechanically sheared and treated with *E. coli* methylase to block internal *EcoRI* restriction sites. *EcoRI* linkers were ligated to the ends of the sheared and size-fractionated (10 to 20 kilobases [kb]) DNA fragments. The DNA fragments were cut with *EcoRI* and inserted into the unique *EcoRI* site of  $\lambda$ gt11. A second gonococcal genomic library was constructed by using phage  $\lambda$ EMBL3. Gonococcal strain 2686 chromosomal DNA was partially digested with *Sau3AI* and 10- to 20-kb fragments were isolated by preparative agarose gel electrophoresis. These fragments were mixed with the left and right arms of  $\lambda$ EMBL3 prepared by *Bam*HI digestion, ligated, and packaged in vitro (14).

**Plaque screening.** For  $\lambda$ gt11 genomic library screening, recombinant phages were plated on a lawn of *E. coli* Y1090 and incubated at 42°C for 3 to 4 h. IPTG-saturated nitrocellulose membranes (NCM) were overlaid and incubated at 37°C for an additional 2 to 8 h. The membranes were then processed and probed as for immunoelectrophoretic transfer. For  $\lambda$ EMBL3 genomic library screening, plaques were transferred onto NCM and probed with DNA fragments which had been labeled by nick translation.

**DNA hybridization.** Probes for hybridization were isolated from preparative agarose gels by electroelution and were nick translated using  $\alpha$ -<sup>32</sup>P-deoxyribonucleotides (27). Chromosomal digests were separated on horizontal agarose gels and transferred to NCM (30). DNA-containing NCM were hybridized with nick-translated probes at 68°C overnight. Subsequently, the NCM were washed with 2 $\times$  SSC (1 $\times$  SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.5% sodium dodecyl sulfate (SDS) and 2 $\times$  SSC-0.1% SDS at room temperature for 5 and 15 min, respectively, followed by

soaking the membranes in 0.1 $\times$  SSC-0.5% SDS at 68°C for 2 h. Hybridizing bands were detected by autoradiography.

**Progressive deletion.** To obtain progressive deletions for DNA sequencing, the method described by Yanisch-Perron et al. (37) was used with some modifications. The 4.5-kb *SalI* fragment was subcloned into pUC18 between the *Bam*HI and *SalI* sites in the multiple cloning site. Two restriction sites in the multiple cloning site were chosen to provide an insert-proximal exonuclease III (Exo III)-sensitive 5' end and an insert-distal Exo III-resistant 3' end. For this study, either *Bam*HI and *Sph*I or *Sst*I and *Xba*I were used to generate 5' or 3' unidirectional progressive deletions, respectively. Two micrograms of plasmid DNA was digested by appropriate restriction endonucleases, the DNA fragments thus generated were treated with 8 U of Exo III at 37°C, and samples were removed at 60-s intervals. Since Exo III requires a 3'-OH and prefers double-stranded DNA, unidirectional progressive deletions were generated. The mixtures were then treated with mung bean nuclease to remove single-stranded DNA, and the plasmids were reformed by blunt-end ligation, using T4 DNA ligase.

**DNA sequencing.** DNA sequences were determined by the dideoxy chain termination method of Sanger et al. (28) as modified in the instructions of the Sequenase kit (United States Biochemical). Synthetic reaction mixtures were labeled with [ $\alpha$ -<sup>35</sup>S]dATP. All templates were double stranded, and sequences were confirmed by sequencing both strands.

**Immunoelectrophoretic transfer.** After SDS-polyacrylamide gel electrophoresis using a Laemmli buffer system (18), separated proteins were electrophoretically transferred to NCM in 0.05 M sodium phosphate buffer at pH 7.5. The

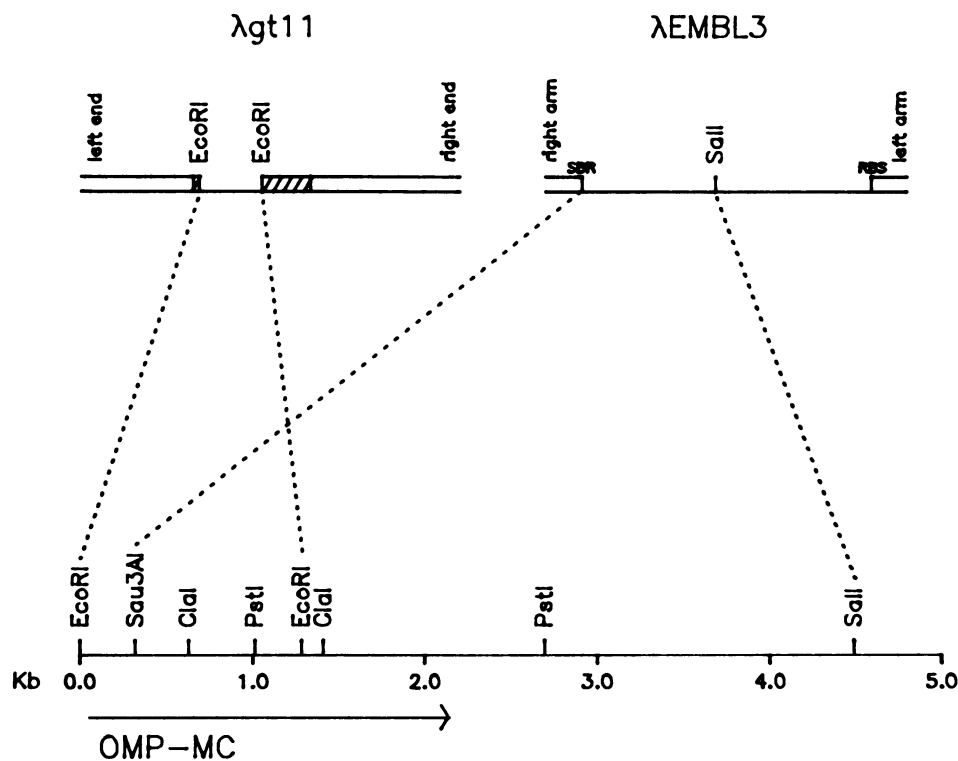


FIG. 1. Restriction map of the *omc* gene. The *omc* gene was cloned in two overlapping fragments: (i) the 1.3-kb *EcoRI*-*EcoRI* fragment was identified by screening a  $\lambda$ gt11 genomic library made from gonococcal strain 2686 with rabbit anti-OMP-MC polyclonal antibody; (ii) the 4.5-kb *SalI*-*SalI* fragment was subcloned from a  $\lambda$ EMBL3 construct isolated from a genomic library made by partial *Sau3AI* digestion of strain 2686 DNA and identified by using the 1.3-kb *EcoRI* fragment as a probe.

NCM were washed for 1 h in 0.01 M sodium phosphate (pH 7.4)–0.15 M NaCl–0.05% Tween 20 (PBST) to block unoccupied protein-binding sites. Rabbit anti-*N. gonorrhoeae* 2686 OMP-MC serum, diluted to 1:5,000 with PBST, and radioiodinated staphylococcal protein A ( $2 \times 10^6$  cpm for 9-by 14-cm NCM) were incubated sequentially with the NCM, each for 1 h with two intervening PBST washes. Following two additional washes with PBST, the NCM were air dried and the positions of reactive protein bands were detected by autoradiography.

**Papain digestion of OMP-MC.** Isolated OMP-MC (11) in 10 mM sodium HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.4)–0.1% SDS–0.02% Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> was treated with papain at an enzyme/substrate ratio of 1:20 for 30 min at 37°C. Samples were electrophoresed on SDS, 2 to 20% gradient polyacrylamide gels (25) and either stained with Coomassie blue or subjected to immunoelectrophoretic transfer analysis using rabbit anti-OMP-MC antiserum and <sup>125</sup>I-protein A.

## RESULTS

**Identification of clones containing the *omp* gene.** Screening of the  $\lambda$ gt11 library identified more than 15 clones that reacted with anti-OMP-MC polyclonal antibody. However, the inserted DNA fragments in these clones were all of the same size, about 1.3 kb, without internal *Eco*RI restriction sites (Fig. 1). Two oligonucleotides, one 17-mer and one 20-mer, with their sequences corresponding to two separate portions of the NH<sub>2</sub>-terminal amino acid sequence of OMP-MC, hybridized with the 1.3-kb *Eco*RI-*Eco*RI fragment recovered from  $\lambda$ gt11 clones. This confirmed that the 1.3-kb *Eco*RI fragment contained the 5' end of the *omp* gene.

Southern blot analysis of chromosomal DNA digested with *Eco*RI, using the 1.3-kb *Eco*RI fragment as a probe, yielded a single 1.3-kb hybridizing band. This indicated that during construction of this  $\lambda$ gt11 library, methylation was incomplete and thus the *Eco*RI sites on both ends of the 1.3-kb fragment were inherent to the *omp* gene rather than imported from the linkers. A second gonococcal genomic library, made in *Bam*HI-digested  $\lambda$ EMBL3 by replacement of the central fragment ("stuffer") with partially *Sau*3AI-digested chromosomal DNA fragments, was screened using the 1.3-kb *Eco*RI fragment as a probe. A clone was identified with an insert of 15 kb (Fig. 1). A 4.5-kb *Sal*I-*Sal*I fragment from this clone was then subcloned into pUC18 and mapped by restriction endonuclease digestion. By combining mapping data obtained with both the 1.3-kb *Eco*RI and 4.5-kb *Sal*I fragments, a restriction map of the genomic *omp* gene from *N. gonorrhoeae* 2686 was deduced (Fig. 1).

**Expression of OMP-MC gene fragments in *E. coli*.** When whole-cell lysates of the  $\lambda$ gt11 clone were examined by immunoelectrophoretic transfer, using anti-OMP-MC antibodies, a 45-kDa immunoreactive band was observed instead of a fusion protein normally expected from  $\lambda$ gt11 recombinant clones. The 1.3-kb *Eco*RI fragment was subsequently cloned into pUC19 in two different orientations; representative subclones expressed anti-OMP-MC-reactive proteins of slightly different apparent molecular masses (Fig. 2). Immunoreactive proteins were expressed from both orientations of the 1.3-kb *Eco*RI fragment, even in the absence of IPTG induction. These findings indicated that the 1.3-kb *Eco*RI fragment contained not only the 5' end of the *omp* structural gene but also sequences capable of expressing promoter function in *E. coli*. In contrast, pUC18 clones containing the 4.5-kb *Sal*I fragment expressed a 70-kDa

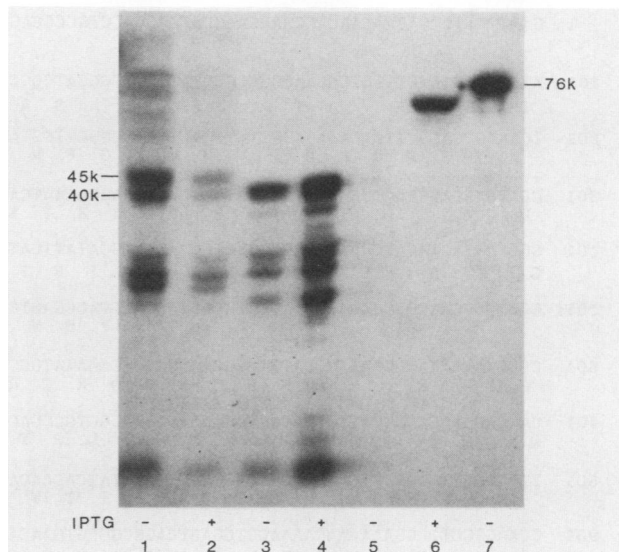


FIG. 2. Immunoelectrophoretic transfer analysis of whole-cell lysates from *E. coli* clones containing fragments of the *omp* gene. The DNA fragments identified from  $\lambda$ gt11 and  $\lambda$ EMBL3 genomic libraries were subcloned into either pUC18 or pUC19. The clones thus obtained were grown in the presence (+) or absence (–) of IPTG, and lysates were analyzed by immunoelectrophoretic transfer, using rabbit anti-*N. gonorrhoeae* 2686 OMP-MC serum and <sup>125</sup>I-protein A. Lanes 1 and 2, pUC19 clone containing the 1.3-kb *Eco*RI fragment in the opposite orientation to the *lacZ* promoter; lanes 3 and 4, pUC19 clone containing the 1.3-kb *Eco*RI fragment in the same orientation as the *lacZ* promoter; lanes 5 and 6, pUC18 clone containing the 4.5-kb *Sal*I fragment in the same orientation as the *lacZ* promoter; lane 7, the 76-kDa subunit of OMP-MC isolated from gonococcal strain FA19.

anti-OMP-MC-reactive protein only when correctly oriented in relation to the *lacZ* promoter of the vector and when induced in the presence of IPTG (Fig. 2). The sizes of both the expressed protein and the inserted DNA fragment suggested that the 4.5-kb *Sal*I fragment contained most of the structural *omp* gene but lacked the promoter region and some of the coding sequence for the NH<sub>2</sub>-terminal portion of OMP-MC.

**Sequence of the OMP-MC gene.** The DNA sequence shown in Fig. 3 was determined by the dideoxy chain termination method of Sanger et al. (28) in three ways: (i) subclones of smaller restriction fragments in either pUC18 or pUC19 were sequenced in both orientations by using universal and reverse primers; (ii) since there are few useful restriction sites in the sequence (Fig. 1), progressive deletions were generated in the 4.5-kb *Sal*I fragment and deletion clones were sequenced; (iii) remaining gaps were then filled in by sequencing with synthetic oligonucleotide primers. The nucleotide sequence will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under the accession number M22564.

Analysis of the sequence showed only one large open reading frame, which lies between bases 77 and 2209. Based on the amino acid sequence published previously (11), the first residue of the mature protein is glycine at base 149, giving a mature protein of 687 amino acids and a signal peptide of 24 amino acids (Fig. 3). The molecular weight predicted from the deduced amino acid sequence is 74,980, which is close to the apparent molecular mass of 76 kDa for the OMP-MC subunit determined by gel electrophoresis (25). The predicted 24-residue signal peptide has character-

1	GAATTCTCCGACAAAAACCCGAACAAGCGGCAGCACCTGCCGACAGAAAATTAAGAAGAGGATTACTCCATTATGAATACCAAACTGACAAAAATC	100
		M N T K L T K I
101	ATTTCGGTCTCTTTGTCGCAACCGCCGCTTTCAGACGGCATCGGCAGGAAACATTACAGACATCAAAGTTTCTCCCTGCCCAACAAACAGAAAAATCG	200
	I S G L F V A T A A F Q T A S A <span style="border: 1px solid black; padding: 2px;">G N I T D I K V S</span> S <span style="border: 1px solid black; padding: 2px;">L P N K Q K I V</span>	
201	TCAAAGTCAGCTTTGACAAAGAGATTGTCAACCGACCGGCTTCGTAACCTCCTCACCAGCCGCGCATCGCCTTGGACTTTGAACAAACCGGCATTCCAT	300
	K V S F D K E I V N P T G F V T S S P A R I A L D F E Q T G I S M	
301	CGATCAACAGGTAATCGAATATGCCGATCCTCTGTTGAGCAAAATCAGTGCCGCACAAAACAGCAGCCGTCGCGCTGCGTTCTGAATTTGAACAAACCG	400
	D L Q V L E Y A D P L L S K I S A A Q V N S S R A R L V L N L N K P	
401	GGCCAATACAATACCGAAGTACGCGGGAACAAAGTTTGGATATTCATTAACGAATCGGACGATACCGTGTCCGCCCCGCGCCAGCCGTAAGGCCG	500
	G Q Y N T E V R G N K V W I F I N E S D D T V S A P A R P A V K A A	
501	CGATGCGCACCGGCAAAACAACAGGCTGCCGACCGTTTACCGAGTCCGTAGTATCCGATTCGCGACCGTTAGCCCGGCAAAACAACAGGCGAGCGCAT	600
	L R T G K T T G C R T V Y R V R S I R I R T V Q P G K T T G S G I	
601	CGGCAAAACAACCAATATCGATTTCCGCAAGACGGCAAAAATGCCGGCATTATCGAATGGCGGCATTGGGCTTTGCGGGCAGCCGACATCAGCCAA	700
	G K T N Q Y R F P Q R R Q K C R H Y R I G G I G L C G Q P D I S Q	
701	CAGCAGCACCATCATCGTTACGCTGAAAACCATACCTGCCGACCGCGCTCCAACGCGAGTTGGATGTGGCAGACTTCAAACACCGGTTCAAAGG	800
	Q H D H I I V T L K N H T L P T A L Q R S L D V A D F K T P V Q K V	
801	TTACGCTGAAACGCTCAATAACGACACCCAGCTGATTATCACAACACCGGCAACTGGGAACCTCGTCAACAAATCCGCCGCGCCGGATCTTTACCTT	900
	T L K R L E N N D T Q L I I T T T G N W E L V N K S A A P G Y F T F	
901	CCAAGTCTGCCGAAAAACAACCTCGAGTACGGCGCGTGAACAATGCGCCAAAACCTTACAGGCGGAAAAATCTCCCTTGACTTCAAAGATGTC	1000
	Q V L P K K Q N L E S G G V N N A P K T F T G R K I S L D F Q D V	
1001	GAAATCCGACCATCTGCAGATTTGGCAAAAGAAATCCGGGATGAACATTGTTGCCAGCGACTCCGTCAGCGGCAAAATGACCTCTCCCTCAAAGACG	1100
	E I R T I L Q I L A K E S G M N I V A S D S V S G K M T L S L K D V	
1101	TACCTTGGGATCAGGCTTTGGATTGGTTATGACAGGCGGCAACCTCGATATGCGCCAGCAAGGGAACATCGTCAACATCGCGCCGCGACGAGCTGCTTG	1200
	P W D Q A L D L V M Q A R N L D M R Q Q G N I V N I A P R R A A C	
1201	CCAAGACAAAGCCTTCTTACAAGCGGAAAAAGACATTGCCGATGTGGCGCGCTGTATTCCCAAACTTCCAATTGAAATACAAAATGTGGAAGAATT	1300
	T S G K R L T S G K R H C R S T G V P K L P I E I Q K C G R I	
1301	CCGCGCATCTGCTTTGGACAATGCGGACACGACCCGGAACCGCAACACGCTTGTGACGCGCAGGGGCGAGCTGCTGATCGATCCCGCCCAACACCC	1400
	P Q H P A L D N A D T T G N R N T L V S G R G S V L I D P A T N T L	
1401	TGATTGTTACCGATACCCGACGCGTCATCGAAAAATTCGCAAACTGATTGACGAATGGACGTACCCGCGCAACAAGTGATGATTGAGGCGGATCGT	1500
	I V T D T R S V I E K F R K L I D E L D V P A Q Q V M I E A R I V	
1501	CGAAGCGGACAGCGCTTCTCGCGGATTTGGCGTTAAGTTCGCGCGACAGGCGGAAAAACTGAAAAATGAGACGAGCGCATTCGCTGGGGCGTG	1600
	E A A D G F S R D L G V K F G A T G R K K L K N E T S A F G W G V	
1601	AACTCCGGCTTCGGGGCGGCGATAAATGGGAGGCCAAACCAAATCAACCTGCCGTTGCCGTCGCGCAAAACAGCATTTGCTGGTGCAGCGGATTTCT	1700
	N S G F G G G D K W E A K P K S T C R R L P C R K Q H F A G A A A R D F S	
1701	CCGCGCGTTGAATTTGGAAATGTCCGATCCGAGTCGCTTTCAAASACCAACCGCTTGCCAAATCCGCGGCTGACCCAAAGCCGAAGAGGCCAA	1800
	G A L N L E L S A S E S L S K T K T L A N P R V L T Q N R K E A K	
1801	AATCGAATCCGGTACGAAATTCCTTTTACCGTAACTACAGCTCGGGCGGCGCAACTCTACCAACAGGAACTCAAAAAGCGCTTTGGGGGTGACC	1900
	I E S G Y E I P F T V T T R S G G G N S T N T E L K K A V L G L T	
1901	GTTACGGCGAATCAGCCCGACGGACAAATCATGACCGTCAAAATCAACAAAGACTCGCCTCGACAATGTGCTTACGGCAACAACAATCCTAT	2000
	V T A N I T P D G Q I I M T V K I N K D S P R Q C A S G N N T I L C	
2001	GTATTTGACCAAAAGCCTGAATACGCGAGCTATGGTTGAAAACCGGCGCACITTTGATTGTCCGCGGATTTATGAAGAAAACACGGCAATACGCTGAC	2100
	I S T K S L N T Q A M V E N G G T L I V G G I Y E E N N G N T L T	
2101	CAAAGTCCCCTGTTGGCTACATCCCGTTATCGGCAACCTCTTAAACACTCGGGAAAAAACCGACCGCGGAACTGCTGATTTTCAATTACCCCGG	2200
	K V P L L A T S P L S A T S L K H S G K N R P P R T A D F Q L P P	
2201	AGGGAATTATAGATACGGCGCAACAGCCTGCGCTATTGATGCGTCAAAATAAGGGCATATGTTTTACAGCATA 2273	
	R E L	

FIG. 3. Nucleotide sequence of the *omc* gene, with the corresponding deduced amino acid sequence of the OMP-MC protein. Boxed amino acid residues indicate homology with the previously determined amino acid sequence (11). The AGGA ribosome-binding site is underlined.

istics typical of bacterial signal peptides (32), with a positively charged NH<sub>2</sub>-terminal region, a central hydrophobic region, a more polar COOH-terminal region, and an Ala-X-Ala cleavage site for processing. Examination of the sequence of the OMP-MC gene disclosed a typical AGGA ribosome-binding site (29) 10 nucleotides upstream from the ATG initiation codon. However, no sequences in the -10 and -35 regions could be found which conformed to the consensus sequences for bacterial promoters (12).

Hydrophilicity calculations (17) indicated that the NH<sub>2</sub>-terminal half of the OMP-MC protein is generally hydrophilic, with a hydrophobic signal peptide and the COOH-terminal portion exhibiting the alternating hydrophilic-

hydrophobic character associated with membrane proteins (24) (Fig. 4). The orientation of OMP-MC in the gonococcal membrane was deduced by immunoelectrophoretic transfer analysis following papain digestion. Papain treatment of isolated OMP-MC converted the 800-kDa complex to a lower-molecular-mass form of ca. 400 kDa, with the concomitant loss of antigenic reactivity with polyclonal anti-OMP-MC antiserum (Fig. 5). Similar results were obtained following papain treatment of isolated outer membranes and whole gonococci (data not shown). This antiserum, as well as a panel of monoclonal antibodies recognizing six distinguishable epitopes, bound to intact gonococci (6) and recognized the 45-kDa peptide expressed from the 1.3-kb *EcoRI*

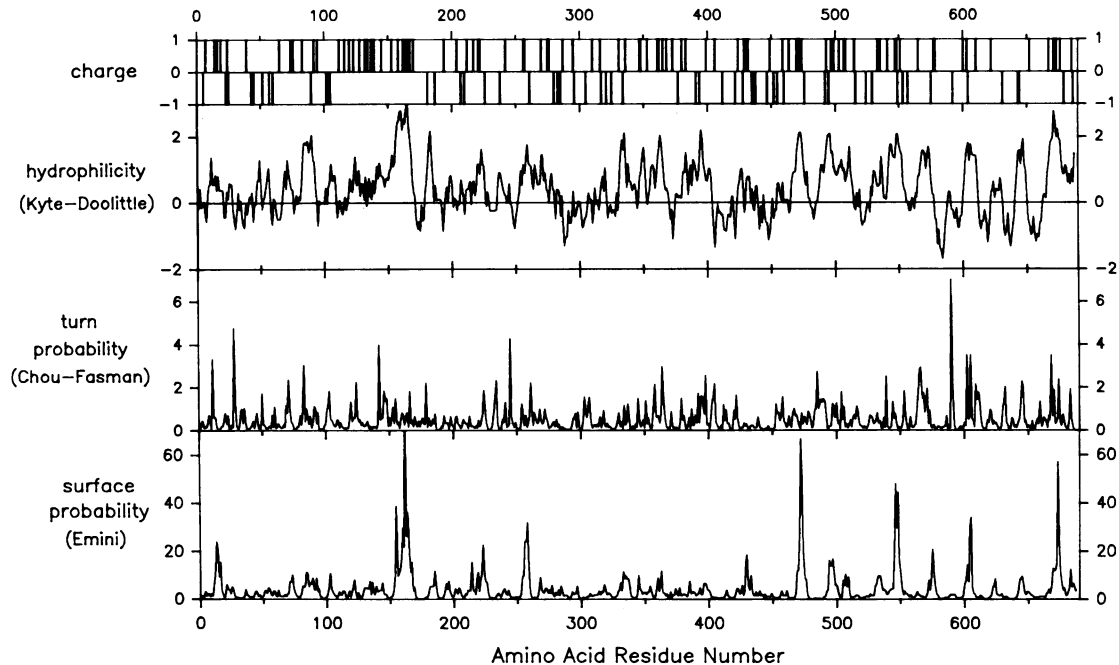


FIG. 4. Structural analysis of the deduced amino acid sequence of the mature OMP-MC protein. Charge distribution is plotted by using values of +1 for Arg and Lys and -1 for Asp and Glu. Hydrophilicity was calculated by the method of Kyte and Doolittle (17), using an averaging window of 10 residues, the probability of turn formation was calculated by the method of Chou and Fasman (4), and surface probability was calculated by the method of Emini et al. (7).

fragment derived from the 5' end of the *omp* gene. Thus, we conclude that the NH<sub>2</sub>-terminal portion of OMP-MC is exposed on the gonococcal outer membrane and the COOH-terminal portion may contain the membrane insertion site(s).

#### DISCUSSION

Gonorrhea continues to be a prominent disease within the sexually active population. The increasing incidence of isolation of gonococcal strains exhibiting multiple drug resistance reinforces the need for the development of immunoprophylactic measures to control this infection. Among the major outer membrane components of gonococci, OMP-MC exhibits many characteristics that are desirable for a vaccine antigen. This protein complex is exposed on the

outer membrane, is immunogenic during infection, serves as a target for potentially protective immune responses, and shows little, if any, structural variation among diverse gonococcal strains.

In this report, we present the complete nucleotide sequence of the *omp* gene from *N. gonorrhoeae* 2686. This gene was cloned in two overlapping fragments. Several attempts to reconstruct an intact *omp* gene were unsuccessful (data not shown); these efforts involved the ligation of fragments of the 1.3-kb *Eco*RI insert with appropriate fragments of the 4.5-kb *Sal*I fragment. In all recovered clones, the constituent fragments had been ligated in opposite orientations and the expressed protein had a molecular weight consistent with its expression solely from the *Eco*RI-derived fragment. Since all attempts at reconstruction have included the promoter sequence on the *Eco*RI fragment, these findings are consistent with the conclusion that the unregulated expression of complete OMP-MC is toxic to the *E. coli* host. Similar findings have been reported for the gonococcal protein I gene (3).

Complex mechanisms exist in gonococci for the expression of pilin and proteins II. Multiple genetic loci lead to variations in pilin expression produced by gene conversion involving recombination events between expressed and silent loci (10). Similar mechanisms may exist for controlling the expression and antigenic variabilities of proteins II (5). In contrast, a single *omp* locus probably exists in *N. gonorrhoeae*, since Southern blot analysis of *Eco*RI-digested chromosomal DNA, using the 1.3-kb *Eco*RI fragment as a probe, revealed a single hybridizing band of 1.3 kb in both strains 2686 and FA19 (data not shown).

The NH<sub>2</sub>-terminal portion of OMP-MC is exposed on the surface of intact gonococci, and the COOH-terminal portion may be membrane bound. This conclusion is based on the reactivities of anti-OMP-MC antibodies with the 45-kDa

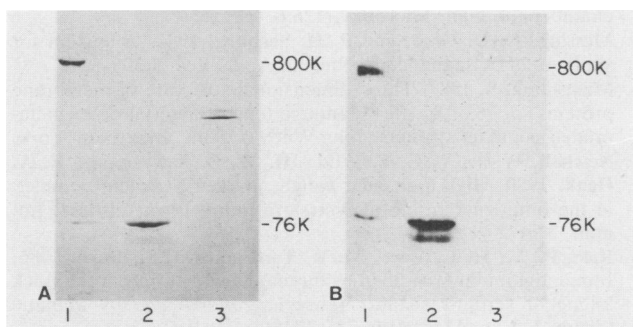


FIG. 5. Papain digestion of intact OMP-MC. Intact polymeric OMP-MC (lanes 1), the 76-kDa subunit of OMP-MC (lanes 2), and papain-digested polymeric OMP-MC (lanes 3) were electrophoresed on SDS, 2 to 20% polyacrylamide gradient gels (25). The gel in panel A was stained with Coomassie blue, while that in panel B was subjected to immunoelectrophoretic transfer analysis using anti-OMP-MC antiserum and <sup>125</sup>I-protein A.

peptide expressed by the 5' *EcoRI* fragment of the *omc* gene and with intact gonococci and the loss of these reactivities following treatment of gonococci and isolated OMP-MC with papain.

Several regions of potential antigenicity have been identified as those with simultaneously high hydrophilicity (17), surface probability (calculated by the method of Emini et al. [7]), and turn probability (calculated by the method of Chou and Fasman, [4]); these areas include approximate amino acid residue positions 155 to 165, 252 to 262, 466 to 476, 542 to 552, and 667 to 677. Analysis of the distribution of charged residues indicated an overall basic nature for OMP-MC and a relatively even distribution of positive and negative charges, with the exception of one potentially interesting region between residues 113 and 171; within this region are clustered 17 basic residues and no acidic residues. This region may be important for the function of OMP-MC, perhaps by promoting electrostatic interactions with acidic ligands such as eucaryotic cell surface molecules or DNA.

Computerized searches of the GenBank and EMBL data bases failed to identify other gene sequences with significant DNA homology to the *omc* gene. Similarly, amino acid sequence comparisons of OMP-MC with other bacterial outer membrane proteins have not revealed significant similarities, using either stringent or simplified matching criteria. Since sequence comparisons at both the nucleotide and protein levels have failed to identify a homologous sequence, the function of OMP-MC remains undefined. However, the successful cloning of the *omc* gene will allow construction of *N. gonorrhoeae* mutants that have defined alterations in the gene. These mutants, in turn, will help to elucidate the biological functions of OMP-MC and its potential role in the pathogenesis of gonorrhea.

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#### LITERATURE CITED

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