INFECTION AND IMMUNITY, Mar. 1992, p. 798–803
0019-9567/92/030798-06$02.00/0

Vol. 60, No. 3

Conserved Outer Membrane Protein of *Neisseria meningitidis* Involved in Capsule Expression

MATTHIAS FROSCH,* DIRK MÜLLER, KRISTINE BOUSSET, AND ASTRID MÜLLER

Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, 3000 Hannover 61, Germany

Received 26 September 1991/Accepted 2 December 1991

In *Neisseria meningitidis*, translocation of capsular polysaccharides to the cell surface is mediated by a transport system that fits the characteristics of ABC (ATP-binding cassette) transporters. One protein of this transport system, termed CtrA, is located in the outer membrane. By use of a CtrA-specific monoclonal antibody, we could demonstrate that CtrA occurs exclusively in *N. meningitidis* and not in other pathogenic or nonpathogenic *Neisseria* species. Nucleotide sequence comparison of the *ctrA* gene from different meningococcal serogroups indicated that CtrA is strongly conserved in all meningococcal serogroups, independent of the chemical composition of the capsular polysaccharide. Secondary structure analysis revealed that CtrA is anchored in the outer membrane by eight membrane-spanning amphipathic beta strands, a structure of proteins that function as porins.

Capsular polysaccharides are important in the pathogenesis of *Neisseria meningitidis*, a major causative agent of septicemia and meningitis in infants. The capsules mediate resistance to complement-mediated bacteriolysis and phagocytosis (6). Additionally, the capsule of serogroup B meningococci is only weakly immunogenic, presumably because of immunological tolerance (24). Antibodies against the group B capsule, which is composed of α-2,8-linked polysialic acid, cross-react with the carbohydrate component of the neural cell adhesion molecule on embryonic neural and kidney tissues (2, 9).

Great efforts have been undertaken to develop a meningococcal vaccine. Because the capsular polysaccharide of group B meningococci, the predominate serogroup of sporadic meningococcal disease in North America and Europe (8), is nonimmunogenic, many vaccination studies have focused on subcapsular components, such as outer membrane proteins and lipooligosaccharides (10). However, major outer membrane proteins that induce protection after immunization exhibit serotype or subtype specificities, as has been shown for class 1, 2, and 3 outer membrane proteins (1, 11). Similarly, antibodies against meningococcal lipooligosaccharides are bactericidal, but a variety of different serotypes have also been described (26). Furthermore, there is some evidence that neisserial lipooligosaccharides undergo antigenic variation (17), thus limiting the use of these surface molecules in a meningococcal vaccine. Therefore, further attention has to be focused on more conserved surface proteins.

Recently, we have identified a gene within the cloned capsule gene complex (*cps*) of *N. meningitidis* (16) encoding an outer membrane protein designated CtrA (13). This protein is one component of a complex capsule transport system which fits the characteristics of ATP-binding cassette transporters (21). To investigate the antigenic variability of outer membrane protein CtrA, which might have implications in vaccine development, we determined the nucleotide sequence of *ctrA* genes from different meningococcal serogroups and deduced a membrane model from the primary structure of CtrA.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and plasmids. Plasmid vector pGEX-3X (29) was purchased from Pharmacia (Freiburg, Germany), and pEMBL8 (7) was described previously. *N. meningitidis* A1493 (serogroup A), B1936 and B1940 (both serogroup B), and C1701 (serogroup C) as well as nonpathogenic *Neisseria* species *N. lactamica* (116), *N. flava* (122), *N. perflava* (121), *N. subflava* (124), *N. mucosa* (112), and *N. sicca* (118) were obtained from U. Berger, Institute for Hygiene, Heidelberg, Germany. *N. meningitidis* serogroups W135 and Y were from the American Type Culture Collection (750020 and 55898), and serogroups 29E and Z were from the National Collections of Type Cultures, London (11202 and 10792). *N. gonorrhoeae* MS11 was a gift from E. Gotschlich, Rockefeller University, New York, N.Y. *N. gonorrhoeae* Ffn, GC5214, NG22584184, and NG877578186 as well as *N. cinerea* 493 were isolated in our own diagnostic laboratories. All *Neisseria* strains were grown on chocolate agar under 5% CO_2. *Escherichia coli* SURE^R was obtained from Stratagene (Heidelberg, Germany) and grown on LB agar. Outer membrane preparation of *N. meningitidis* B1940 was performed as described previously (30).

Recombinant DNA techniques. Restriction enzymes, T4 DNA ligase, and T7 DNA polymerase were purchased from Pharmacia LKB (Freiburg, Germany) and used under conditions recommended by the manufacturer. *Taq* DNA polymerase was obtained from Boehringer GmbH (Mannheim, Germany).

A genomic library of *N. meningitidis* A1493 was constructed by ligation of 1- to 2-kb *Sau*3A partially digested chromosomal DNA fragments into the *Bam*HI site of plasmid pEMBL8 and subsequent transformation into *E. coli* SURE^R. Colonies were tested by colony blotting (30) with monoclonal antibody 2619, which is directed against CtrA of all meningococcal serogroups (see below). One immunoreactive clone, termed pMF140, was used for sequencing the *ctrA* gene of serogroup A meningococci according to the nested deletion strategy described by Henikoff (20). Sequencing of double-stranded plasmid DNA was performed by using the T7 DNA polymerase sequencing kit obtained from Pharmacia.

For polymerase chain reaction (PCR), 50 ng of meningococcal chromosomal DNA was denatured at 94°C for 1.5

min. Oligonucleotide primers were annealed at 45°C for 2 min, and this was followed by the polymerase reaction at 72°C for 3 min. PCR was performed in an automatic Thermocycler constructed at the Medizinische Hochschule Hannover, using oligonucleotide primers 5′ GTGTTTAAAGTG AAATTTTA 3′ and 5′ TTAATTAGTTAAATTATTAATAC 3′, which encompass *ctrA* from the start to the stop codon. An additional oligonucleotide (5′ GCTCTGCGATTCCTT CATC 3′) was constructed homologous to the 5′ end of the coding sequence of the mature protein. Oligonucleotides were synthesized in a Gene Assembler Plus (Pharmacia LKB) by the phosphoamidite procedure.

For sequencing PCR products, DNA was purified by using PrimErase Quick columns (Stratagene). DNA (0.1 pmol) was mixed with a 100-fold molar excess of sequencing primers, denatured for 5 min at 95°C, and cooled for 5 min on ice-alcohol. Primer annealing was performed at 37°C for 5 min. After the addition of label mixes, 5 μCi of ^{35}S-dATP, and 2.5 U of *Taq* DNA polymerase, the mixture was incubated at 37°C for 5 min before the polymerase reaction was terminated by the addition of dideoxynucleotides. The termination reaction was performed at 60°C for 7.5 min. Annealing buffer, label, and termination mixes for *Taq* sequencing were obtained from United States Biochemical Corp. (Bad Homburg, Germany). Oligonucleotides designed for PCR were also used as sequencing primers. Additional oligonucleotides derived from the data of the following sequencing reactions were constructed to complete sequence determination.

Nucleotide sequence data were analyzed with the PC/GENE software package (IntelliGenetics, Mountain View, Calif.). Secondary structure prediction of membrane proteins was performed by using the AMPHI program described by Jähnig (23).

Monoclonal antibodies. For large-scale expression of CtrA, the complete *ctrA* gene of meningococcal strain B1940 was amplified by PCR using the same oligonucleotides described above, which encompass *ctrA* between the start and the stop codons. The PCR fragment was subcloned into the single *Sma*I site of expression vector pGEX-3X. Expression of the glutathione S-transferase fusion protein was induced by the addition of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). For immunization, a suspension of 10^8 IPTG-induced *E. coli* cells in phosphate-buffered saline was sonicated and injected intraperitoneally into BALB/c mice (Zentralinstitut für Tierversuchskunde, Hannover, Germany). Immunization was repeated four times at weekly intervals. Hybridoma cells were generated by standard protocols (3). CtrA-reactive monoclonal antibodies were detected by the enzyme-linked immunosorbent assay (ELISA) technique as described previously (14), using the same antigen that was used for immunization. ELISA results were compared with the ELISA reactivity with *E. coli* lysates expressing glutathione S-transferase without the CtrA moiety. The immunoglobulin class and subclass of the monoclonal antibodies were determined with a mouse hybridoma subtyping kit (Boehringer).

SDS-PAGE and immunoblot procedures. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 12.5% polyacrylamide gels by using the discontinuous buffer system of Laemmli (25). All cells were resuspended to an optical density at 550 nm of 1.2. Cell pellets from 1-ml cultures were resuspended in 100 μl of sample solution (20% glycerol, 3% SDS, 3% 2-mercaptoethanol, 1% bromphenol blue) and then denatured by heating at 100°C for 5 min. Western blotting (immunoblotting) to nitrocellulose was performed as described by Towbin et al. (31),

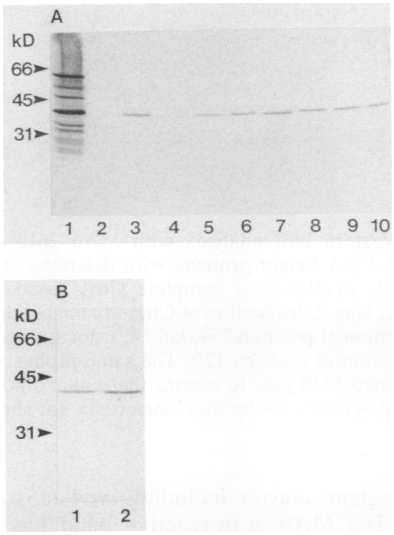

FIG. 1. (A) Western blot analysis with MAb 2619 of whole-cell lysates from recombinant *E. coli* clones expressing a 65-kDa glutathione S-transferase–CtrA fusion protein and from different *N. meningitidis* serogroups. Lane 1, *E. coli* clone expressing glutathione S-transferase–CtrA fusion protein. Expression of the fusion protein was unstable, and CtrA was degraded to the glutathione S-transferase moiety. Lane 2, *E. coli* clone expressing glutathione S-transferase without the CtrA moiety; lane 3, *N. meningitidis* B1940; lane 4, *N. meningitidis* B1940 with an 18-kb deletion within the *cps* locus including *ctrA* (15); lane 5, *N. meningitidis* A1493; lane 6, *N. meningitidis* C1701; lane 7, *N. meningitidis* 29E; lane 8, *N. meningitidis* W135; lane 9, *N. meningitidis* Y; lane 10, *N. meningitidis* Z. (B) Reactivity of MAb 2619 with outer membrane preparation of *N. meningitidis* B1940 (lane 2). Outer membrane preparation (10 μg) was loaded on the gel. Lane 1, *N. meningitidis* B1940 whole-cell lysate.

and blots were immunostained with 1:1,000-diluted ascites fluid of MAb 2619 and alkaline phosphatase-labeled goat anti-mouse immunoglobulin (Dianova, Hamburg, Germany). Antibodies were detected by the addition of BCIP-NBT substrate (0.05 mg of 5-bromo-4-chloro-3-indolyl phosphate per ml, 0.1 mg of nitroblue tetrazolium per ml in 100 mM Tris-HCl [pH 9.6], 4 mM $MgCl_2$).

Nucleotide accession number. The nucleotide sequence reported here has been deposited in the GenBank/EMBL data bank under accession number M80593.

RESULTS

Monoclonal antibody against CtrA. To investigate whether CtrA is common to all meningococcal serogroups, we raised a CtrA-specific monoclonal antibody. BALB/c mice were immunized with lysed *E. coli* cells that expressed CtrA fused to glutathione S-transferase. By this immunization strategy, one hybridoma cell clone was generated which secreted a CtrA-specific immunoglobulin G2a monoclonal antibody, designated MAb 2619.

Specificity of MAb 2619 for CtrA was confirmed in Western blots (Fig. 1) by its reactivity with *E. coli* cell lysates expressing a 65-kDa glutathione S-transferase–CtrA fusion protein and with meningococcal strain B1940, from which the capsule gene locus (*cps*) was cloned originally (16). In contrast, *E. coli* cells which synthesize only the glutathione S-transferase without the CtrA moiety and a mutant of meningococcal strain B1940 which has an 18-kb deletion in

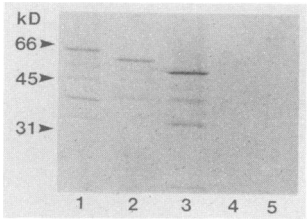

FIG. 2. Western blot analysis with MAb 2619 of glutathione S-transferase–CtrA fusion proteins with deletions at the 3′ end of CtrA. Lane 1, synthesis of complete CtrA fused to glutathione S-transferase; lane 2, truncation of CtrA at amino acid position 323; lane 3, truncation at position 259; lane 4, truncation at position 197; lane 5, truncation at position 120. The same probes were analyzed on silver-stained SDS gels to ensure that equal amounts of fusion proteins were synthesized by all clones (data not shown).

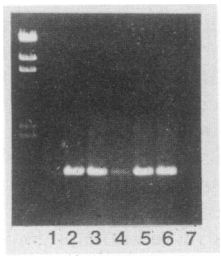

FIG. 3. PCR fragments generated with oligonucleotides limiting *ctrA* from the start to the stop codon (see also Fig. 4). Chromosomal DNA from *N. meningitidis* A1493 (lane 1), B1940 (lane 2), C1701 (lane 3), 29E (lane 4), W135 (lane 5), Y (lane 6), and Z (lane 7) was used as the template. *Hin*dIII-digested DNA from bacteriophage lambda was used as the marker and loaded in the left lane.

the capsule gene cluster including *ctrA* (15) exhibited no reactivity. The MAb 2619-reactive band has a molecular mass of about 37 kDa, which is in accordance with the predicted molecular mass for CtrA derived from recent sequencing data (13).

Analysis of MAb 2619 reactivity with meningococcal serogroups other than group B revealed an immunoreactive band with the same molecular weight as CtrA of group B meningococcal strain B1940 (Fig. 1A). Using purified outer membrane preparations of *N. meningitidis* B1940, an MAb 2619-reactive band of identical size was observed (Fig. 1B). In contrast, none of the *N. gonorrhoeae* strains or nonpathogenic *Neisseria* species tested expressed the MAb 2619-reactive epitope. These findings are in accordance with previous data which demonstrated that there is no DNA homology between the meningococcal capsule transport genes and *N. gonorrhoeae* or nonpathogenic *Neisseria* species, suggesting that these *Neisseria* species lack capsules comparable to that of meningococci (16). However, the occurrence of CtrA with an identical molecular weight in all meningococcal strains independent of the chemical composition of the capsular polysaccharide and reactivity with MAb 2619 indicate a close antigenic relationship of this outer membrane protein in all *N. meningitidis* serogroups.

Epitope mapping of MAb 2619. To map the epitope recognized by MAb 2619, we generated *ctrA* gene fragments by PCR using chromosomal DNA from strain B1940. These fragments, all beginning with the ATG start codon but with deletions at the 3′ end, were subcloned in frame into the *Sma*I site of expression vector pGEX-3X (29). The oligonucleotides used for this experiment are indicated in Fig. 4. By this strategy, glutathione S-transferase–CtrA fusion proteins were synthesized which terminated CtrA at amino acid positions 120, 197, 259, and 323, respectively. Binding of MAb 2619 to truncated CtrA was monitored by immunoblots. As shown in Fig. 2, clones 1 and 2, whose *ctrA* genes are truncated at nucleotides 976 and 778 (corresponding to amino acids 323 and 259), respectively, still bind MAb 2619, whereas clones 3 and 4, with truncation of *ctrA* at nucleotide positions 591 and 359 (corresponding to amino acids 197 and 120), respectively, are negative. From these data, we can conclude that the MAb 2619-reactive epitope is at least partially composed of the peptide fragment between amino acids 197 and 259 or that binding of MAb 2619 is influenced by secondary structures that are dependent on the presence of that region.

Nucleotide sequence analysis of *ctrA* genes from different meningococcal serogroups. To confirm the conserved nature of CtrA, we determined the nucleotide sequences of the *ctrA* genes of different meningococcal serogroups. For this purpose, we tried to amplify the *ctrA* gene of meningococcal serogroups A, B, C, 29E, W135, Y, and Z by PCR. Oligonucleotide primers were constructed which encompass the 1,160 bp of the *ctrA* gene from the start to the stop codon. By this strategy, a DNA fragment of the appropriate size was amplified from chromosomal DNA of serogroups B, C, W135, and Y. A faint band of identical size was also generated from chromosomal DNA of the serogroup 29E meningococcal strain. In contrast, no *ctrA* amplification from chromosomal DNA of serogroups A and Z could be achieved (Fig. 3). Because the 5′-end oligonucleotide was homologous to the signal sequence of *ctrA*, which may exhibit sequence variability from strain to strain, we used an additional oligonucleotide primer identical with the 5′ end of the coding sequence for the mature protein (Fig. 4). Again, the *ctrA* gene fragment could not be amplified by PCR from chromosomal DNA of serogroup A or Z meningococci, indicating sequence differences between the *ctrA* genes from group B, C, W135, and Y meningococci and those from serogroup A and Z strains.

We determined the nucleotide sequences of the *ctrA* genes from serogroup B (strain B1936), C (strain C1701), W135, and Y meningococci by direct sequencing of the PCR product and compared them with the already known nucleotide sequence of *ctrA* from group B meningococcal strain B1940. As shown in Fig. 4A, only one nucleotide difference was observed at position 1111, with cytidine-thymidine exchanges in meningococcal strains B1936 and W135. However, these base exchanges did not result in an altered amino acid sequence.

To obtain the nucleotide sequence of *ctrA* from serogroup A meningococci, we constructed a genomic library from *N. meningitidis* A1493. A total of 8,000 independent clones of this library were tested with MAb 2619 by colony blotting, and 5 immunoreactive clones could be isolated. One of these clones synthesized complete CtrA as confirmed by immunoblots (data not shown). This clone, termed pMF140, was used for sequence analysis of *ctrA*. The nucleotide sequence of *ctrA* from serogroup A meningococcal strain A1493 is shown in Fig. 4A. Comparison with the nucleotide sequence of serogroup B strain B1940 revealed an overall identity of 92.5%. However, the variability was highest in the signal sequence and the 5′ end of the coding sequence for the mature protein between nucleotides 1 and 53. This sequence variability might explain the failure of PCR-directed amplification of *ctrA* from serogroup A meningococci with oligo-

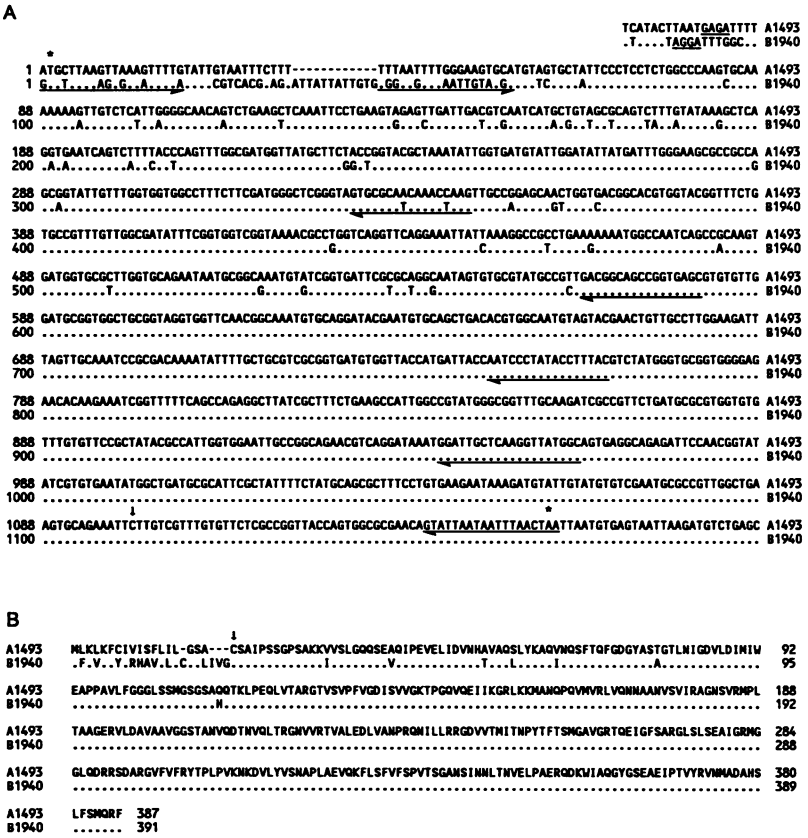

FIG. 4. (A) Nucleotide sequence of *ctrA* of *N. meningitidis* A1493 compared with the sequence of *ctrA* from strain B1940 (13). Nucleotide positions are given on the left and refer to the ATG start codon. Start and stop codons are indicated with asterisks. The putative Shine-Dalgarno sequences are underlined (18). Sequences of oligonucleotides used for amplification of complete *ctrA* or gene fragments by PCR are indicated with arrows. The sequences of primers indicated with arrows from the 3′ to the 5′ end must be read complementary. A cytidine residue at nucleotide position 1111 (indicated with a small arrow above) was found to be replaced by a thymidine residue in *N. meningitidis* B1936 and W135. Complete sequence determination of the PCR products of meningococcal strains B1936, C1701, W135, and Y also included the nucleotide sequence of the annealing sites of the PCR primers. Therefore, single-nucleotide variations in the corresponding chromosomal fragments cannot be excluded. (B) Alignment of the amino acid sequences of CtrA from *N. meningitidis* A1493 and B1940. Amino acid positions are given on the right. Signal sequence cleavage site is indicated with an arrow.

nucleotides whose sequences are identical with the 5′ end of *ctrA* from serogroup B meningococci.

Compared with the *ctrA* sequence of strain B1940, a modest nucleotide sequence variability was observed between nucleotides 54 and 509, with 49 nucleotide exchanges resulting in 89% identity in this segment. The following 417 nucleotides are completely conserved between the *ctrA* genes of group A and B meningococci.

Comparison of the deduced amino acid sequences of CtrA from serogroup A and B meningococci (Fig. 4B) without signal sequence revealed a 98.1% identity, with seven amino acid exchanges. Considering conserved amino acid substitutions (isoleucine versus valine at amino acid positions 36 and 68 and threonine versus alanine at positions 58 and 82), a similarity of 99.2% between both polypeptides was observed. The amino acid sequence of the leader peptide is completely different in the CtrA molecules of both serogroups. However, both signal sequences exhibit properties of lipoprotein precursors. The leader peptides may be cleaved off by the signal peptidase II. The mature protein has an N-terminal cysteine residue to which glyceride-fatty acids may be attached (22).

Secondary structure prediction for CtrA. The hydrophobicity and secondary structure of CtrA from strain B1940 were analyzed by using the algorithm of Jähnig (23). Eight membrane-spanning amphipathic beta strands were predicted in the N-terminal part around amino acids 38, 62, 88, 122, 140, 153, 166, and 173 (Fig. 5). These beta strands could form a membrane-spanning beta barrel. The predicted structure for CtrA is very similar to that found for the *E. coli* OmpA protein, an outer membrane protein whose N-terminal part is incorporated in the membrane by eight membrane-spanning amphipathic beta strands followed by a large C-terminal periplasmic domain (33).

From the secondary structure data, we deduced a simplified model for the arrangment of CtrA in the outer membrane (Fig. 6). The predicted periplasmic loops shown in this model were confirmed by determination of insertion sites of transposon Tn*phoA* (27) within *ctrA*, which we recently used to localize CtrA in the outer membrane (13). Two alkaline phosphatase-positive insertions were located before amino acids 74 and 136 in the first and second periplasmic loops. Additional Tn*phoA* insertions were found in the predicted periplasmic domain between amino acids 188 and 391 at positions 257, 261, 363, and 378.

Epitope mapping revealed that binding of MAb 2619 is dependent on the peptide fragment between amino acids 197 and 259, which is located in the predicted C-terminal peri-

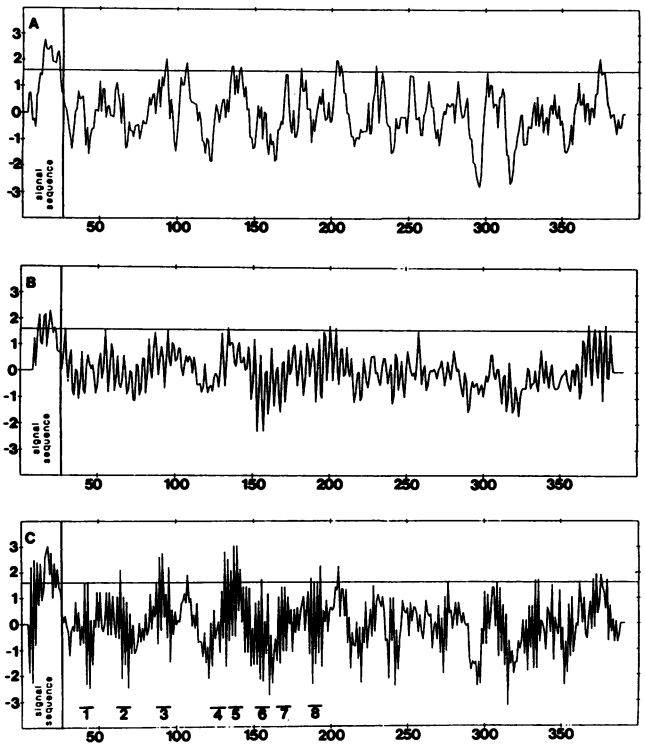

FIG. 5. Secondary structure predictions for CtrA according to the algorithm of Jähnig (23). (A) Hydrophobicity blot with a window of seven consecutive residues; (B) α-helix potential; (C) beta-strand potential. Predicted membrane-spanning beta strands are indicated with bars in panel C. According to the rules of Jähnig (23), the beta strands were not disrupted by beta-turn formations. Beta turns were predicted by the method of Chou and Fasman (5; data not shown).

plasmic domain. In immunofluorescence studies, no binding of MAb 2619 to the surface of meningococci could be observed (data not shown), thus also confirming the proposed topology of CtrA in the outer membrane.

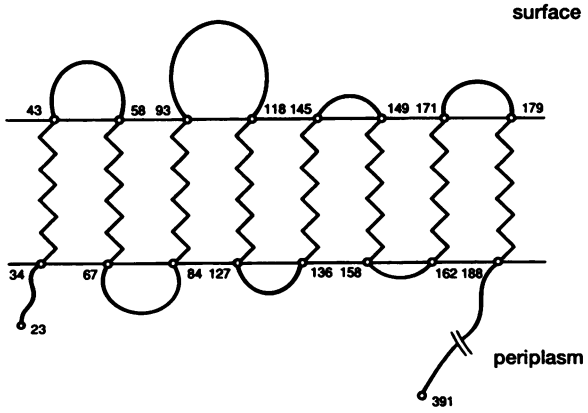

FIG. 6. Simplified model for the arrangement of CtrA in the outer membrane. The bars symbolize the lipid bilayer, and the zig-zag lines symbolize the membrane-spanning beta strands. Amino acid positions are given.

DISCUSSION

Recent studies on the molecular mechanisms of capsule expression in *N. meningitidis* and translocation of capsular polysaccharide to the cell surface led to the identification of a complex transport system (13) which has striking similarities with other transporters of the ABC (ATP-binding cassette) transporter type (21). One protein component of this system was located in the outer membrane and designated CtrA (Ctr = capsule transport). CtrA has properties of a lipoprotein. Because the capsular polysaccharide is attached to the outer membrane by a phospholipid to which the polysaccharide is linked at its reducing end (19), one can speculate that CtrA functions as a membrane anchor for capsular polysaccharides. However, we were not able to coprecipitate CtrA and capsular polysaccharides of group B meningococci with either MAb 2619 or MAb 735 (14), which is directed against the capsular polysaccharide of group B meningococci (11a).

Nucleotide and amino acid sequence comparison of the *ctrA* genes from different meningococcal serogroup strains demonstrated that CtrA is a strongly conserved protein of the outer membrane. No amino acid sequence differences were found in CtrA proteins from serogroup B, C, W135, and Y meningococcal strains, whose capsules are all composed of neuraminic acid, either as a homopolymer or as a heteropolymer (8). In contrast, compared with CtrA from group B meningococci, seven amino acid substitutions were found in CtrA of serogroup A *N. meningitidis*, whose capsule is composed of *N*-acetylmannosamine (8). The almost complete conservation at the nucleotide sequence level of the *ctrA* gene from the neuraminic acid capsule group contrasts with variations in the nucleotide sequence of *ctrA* in serogroup A meningococcus A1493, resulting in only a 92.5% identity to the nucleotide sequence of *ctrA* in serogroup B, C, W135, and Y strains. Although most of these nucleotide sequence variations are silent substitutions that do not lead to an altered amino acid sequence, the nucleotide variability between *ctrA* of group A meningococci and the neuraminic acid-containing capsular serogroups may reflect phylogenetic distance between meningococcal serogroups whose capsule is composed of neuraminic acid and serogroups with other chemical compositions.

The conserved protein sequence of CtrA in different serogroups indicates a common structure and function for capsular polysaccharide transport. Secondary structure analysis revealed that CtrA is anchored in the outer membrane by eight membrane-spanning amphipathic beta strands, which may form a beta barrel. This structure is comparable to that for proteins with porin functions (32, 33). Assuming that CtrA has porin properties, similarities to protein K of *E. coli* are evident. This protein is also a porin (34), and its expression in *E. coli* correlates with capsulation (28). However, further biophysical and structural analyses of CtrA are necessary to verify the predicted porin nature of CtrA and to obtain insight into its role in surface expression of capsular polysaccharides.

CtrA is reminiscent of other conserved proteins of pathogenic *Neisseriae* species, such as the H.8 antigen (4) and the recently described P.833 (12). However, these proteins are common to the pathogenic *Neisseria* species *N. meningitidis* and *N. gonorrhoeae*. Furthermore, expression of these surface proteins was also described in the nonpathogenic *Neisseria* species *N. lactamica* and *N. cinerea*. In contrast, CtrA is the first outer membrane protein described that is restricted to a single neisserial species, namely, *N. menin-*

gitidis. This makes CtrA an ideal candidate for a rapid diagnosis of meningococci based on serological methods, and the monoclonal antibody described here may be a valuable tool for such purposes. Furthermore, because of its conserved antigenic features, CtrA is a candidate for development of a vaccine against meningococcal disease.

ACKNOWLEDGMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft to M.F. (689/2-2).

We thank B. D. Robertson for critically reviewing the manuscript and R. Haas for help with the computer work necessary for secondary structure analysis.

REFERENCES

1. **Abdillahi, H., and J. T. Poolman.** 1988. Definition of meningococcal class 1 OMP subtyping antigens by monoclonal antibodies. FEMS Microbiol. Lett. **47:**139–144.
2. **Bitter-Suermann, D., and J. Roth.** 1987. Monoclonal antibodies to polysialic acid reveal epitope sharing between invasive pathogenic bacteria, differentiating cells and tumor cells. Immunol. Res. **6:**225–237.
3. **Burger, R., U. Deubel, U. Hadding, and D. Bitter-Suermann.** 1982. Identification of the functionally relevant determinants on the complement component C3 with monoclonal antibodies. J. Immunol. **129:**2042–2050.
4. **Cannon, J. G., W. J. Black, I. Nachamkin, and P. W. Stewart.** 1984. Monoclonal antibody that recognizes an outer membrane antigen common to the pathogenic *Neisseria* species but not to most nonpathogenic *Neisseria* species. Infect. Immun. **43:**994–999.
5. **Chou, P. Y., and G. D. Fasman.** 1978. Empirical predictions of protein conformations. Annu. Rev. Biochem. **47:**251–276.
6. **Cross, A. S.** 1990. The biological significance of bacterial encapsulation. Curr. Top. Microbiol. Immunol. **150:**87–96.
7. **Dente, L., G. Cesareni, and R. Cortese.** 1983. pEMBL: a new family of single stranded plasmids. Nucleic Acids Res. **11:**1645–1655.
8. **Devoe, I. W.** 1982. The meningococcus and mechanisms of pathogenicity. Microbiol. Rev. **46:**162–190.
9. **Finne, J., D. Bitter-Suermann, C. Goridis, and U. Finne.** 1987. An IgG monoclonal antibody to group B meningococci crossreacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. J. Immunol. **138:**4402–4407.
10. **Frasch, C. E.** 1989. Vaccines for prevention of meningococcal disease. Clin. Microbiol. Rev. **2:**S134–S138.
11. **Frasch, C. E., W. D. Zollinger, and J. T. Poolman.** 1985. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev. Infect. Dis. **7:**504–510.

11a. **Frosch, M.** Unpublished data.

12. **Frosch, M., R. Droste, H. Fiedler, and T. F. Meyer.** 1991. Identification and expression in *E. coli* of a conserved surface epitope of pathogenic *Neisseriae*, p. 219–224. *In* M. Achtman, P. Kohl, C. Marchal, G. Morelli, A. Seiler, and B. Thiesen (ed.), Neisseriae 1990. Walter de Gruyter & Co., Berlin.
13. **Frosch, M., U. Edwards, K. Bousset, B. Krauße, and C. Weisgerber.** 1991. Evidence for a common molecular origin of the capsule gene loci in Gram-negative bacteria expressing group II capsular polysaccharides. Mol. Microbiol. **5:**1251–1263.
14. **Frosch, M., I. Görgen, G. J. Boulnois, K. N. Timmis, and D. Bitter-Suermann.** 1985. NZB mouse system for production of monoclonal antibodies to weak bacterial antigens: isolation of an IgG antibody to the polysaccharide capsules of *Escherichia coli* K1 and group B meningococci. Proc. Natl. Acad. Sci. USA **82:**1194–1198.
15. **Frosch, M., E. Schultz, E. Glenn-Calvo, and T. F. Meyer.** 1990. Generation of capsule-deficient *Neisseria meningitidis* strains by homologous recombination. Mol. Microbiol. **4:**1215–1218.
16. **Frosch, M., C. Weisgerber, and T. F. Meyer.** 1989. Molecular characterization and expression in *Escherichia coli* of the gene complex encoding the polysaccharide capsule of *Neisseria meningitidis* group B. Proc. Natl. Acad. Sci. USA **86:**1669–1673.
17. **Gibson, B. W., J. W. Webb, R. Yamasaki, S. J. Fisher, A. L. Burlingame, R. E. Mandrell, H. Schneider, and J. M. Griffiss.** 1989. Structure and heterogeneity of the oligosaccharides from the lipopolysaccharides of a pyocin-resistant *Neisseria gonorrhoeae*. Proc. Natl. Acad. Sci. USA **86:**17–21.
18. **Gold, L., and G. Stormo.** 1987. Transcriptional initiation, p. 1302–1306. *In* F. C. Neidhard, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
19. **Gotschlich, E. C., B. A. Fraser, O. Nishimura, J. B. Robbins, and T. Liu.** 1981. Lipid on capsular polysaccharides of Gram-negative bacteria. J. Biol. Chem. **256:**8915–8921.
20. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates target breakpoints for DNA sequencing. Gene **28:**351–359.
21. **Higgins, C. F., S. C. Hyde, M. M. Mimmack, U. Gileadi, D. R. Gill, and M. P. Gallagher.** 1990. Binding protein-dependent transport systems. J. Bioenerg. Biomembr. **22:**571–592.
22. **Hussain, M., S. Ichihara, and S. Mizushima.** 1982. Mechanism of signal peptide cleavage in the biosynthesis of the major lipoprotein of the *Escherichia coli* outer membrane. J. Biol. Chem. **257:**5177–5182.
23. **Jähnig, F.** 1989. Structure prediction for membrane proteins, p. 707–717. *In* G. D. Fasman (ed.), Prediction of protein structures and the principles of protein conformation. Plenum Press, New York.
24. **Jann, B., and K. Jann.** 1990. Structure and biosynthesis of the capsular antigens of *Escherichia coli*. Curr. Top. Microbiol. Immunol. **150:**19–42.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
26. **Mandrell, R. E., and W. D. Zollinger.** 1977. Lipopolysaccharide serotyping of *Neisseria meningitidis* by hemagglutination inhibition. Infect. Immun. **16:**471–475.
27. **Manoil, C., and J. Beckwith.** 1985. Tn*phoA*: a transposon probe for protein export signal. Proc. Natl. Acad. Sci. USA **82:**8129–8133.
28. **Paakkanen, J., E. C. Gotschlich, and P. H. Mäkelä.** 1979. Protein K: a new major outer membrane protein found in encapsulated *Escherichia coli*. J. Bacteriol. **139:**835–841.
29. **Smith, D. B., and K. S. Johnson.** 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione-S-transferase. Gene **67:**31–40.
30. **Stern, A., P. Nickel, T. F. Meyer, and M. So.** 1984. Opacity variants of *Neisseria gonorrhoeae*: gene expression and chromosomal linkage to the pilus genes. Cell **37:**447–456.
31. **Towbin, H., T. Stähelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
32. **Van der Ley, P., J. E. Heckels, M. Virji, P. Hoogerhout, and J. T. Poolman.** 1991. Topology of outer membrane porins in pathogenic *Neisseria* spp. Infect. Immun. **59:**2963–2971.
33. **Vogel, H., and F. Jähnig.** 1986. Models for the structure of outer-membrane proteins of *Escherichia coli* derived from Raman spectroscopy and prediction methods. J. Mol. Biol. **190:**191–199.
34. **Whitfield, C., R. E. W. Hancock, and J. W. Costerton.** 1983. Outer membrane protein K of *Escherichia coli*: purification and pore-forming properties in lipid bilayer membranes. J. Bacteriol. **156:**873–879.