N-Terminal Amino Acid Sequences of the Major Outer Membrane Proteins from a Neisseria meningitidis Group B Strain Isolated in Brazil

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The four dominant outer membrane proteins (46, 38, 33 and 28 kDa) were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a semi-purified preparation of vesicle membranes of a Neisseria meningitidis (N44/89, B:4:P1.15:P5.5,7) strain isolated in Brazil. The N-terminal amino acid sequence for the 46 kDa and 28 kDa proteins matched that reported by others for class 1 and 5 proteins respectively, whereas the sequence (25 amino acids) for the 38 kDa (class 3) protein was similar to class 1 meningococcal proteins. The sequence for the 33 kDa (class 4) was unique and not homologous to any known protein.

Key words: Neisseria meningitidis group B - outer membrane proteins - class specific proteins - N-terminal amino acid sequence

Within the past decade, groups of investigators have begun to characterize on a molecular level the structural components of Neisseria meningitidis, the ethiologic agent of meningococcal disease. Many of these investigators (Tsai et al. 1981, Achtman et al. 1983, Morse et al. 1983, Poolman et al. 1983, Frasch et al. 1986, Barnejee-Bhatnagar & Frasch 1990) have sought to identify and to analyze several meningococcal surface components in an attempt to define important immunogens or potential vaccinant compounds. Unfortunately, for the human disease caused by the meningococci serogroup B, the capsular polysaccharide (CP) has shown poor immunogenicity (Wyle et al. 1972) and in some cases induced immunological tolerance (Frasch 1990, Poolman 1995). A likely explanation for this last property of CP is its structural identity, a homopolymer of α2→8 sialic acid, with polysialyated host glycoproteins (Finne et al. 1987).

The N. meningitidis serogroup B is the predominant etiologic agent of the infection in many countries (Whiattle & Greenwood 1976, Bower et al. 1977, Peltona 1985, Cougant et al. 1986), and specifically in Brazil it has been reported that the number of cases is increasing since 1976 (Sacchi et al. 1992). Current experimental vaccines are therefore based on the outer membranes compounds (Poolman 1995) which have been treated to remove the potentially toxic lipopolysaccharide (LPS) (Poolman et al. 1983, Cowe et al. 1989, Banerjee-Bhatnagar & Frash 1990). Such preparations contain variable amounts of five classes of major outer membrane proteins (OMP), namely C1 through C5, which have apparent molecular weights of 46.000, 41.000, 38.000, 33.000 and 28.000, respectively (Tsai et al. 1981, Van der Ley et al. 1991, 1993 a,b). C1 (class 1 OMP) is a porin-like molecule and is generally present in most meningococcal strains. The antigenic variations of this protein define the subtype of a meningococcal strain (Poolman 1995) and to date, monoclonal antibodies (MAbs) have been made against 15 different subtypes (Abdillahi & Poolman 1988). As C1, the C2 (class 2 OMP) and C3 (class 3 OMP) are porin-like proteins (anion-specific) and are mutually exclusive since meningococcal strains have either the class 2 or class 3 proteins. These antigens represent the predominant proteins on the outer membrane (Tommassen et al. 1990, Van der Ley et al. 1991, Van der Ley & Poolman 1992) and, because of their limited antigenic variation, they have been used to define at least 20 serotypes (Griffiss et al. 1977). C4 (class 4 OMP) appears to be the highly conserved between meningococcal...
strains. Although its cellular function is unknown, it shares sequence homology with *Escherichia coli* Omp A (Klugman et al. 1989). Antibodies directed against this protein are non-bactericidal and have the additional ability to block the lytic effect of antibodies directed against other outer surface antigens (Munkley et al. 1991). Finally, C5 (class 5 OMP, ope or opacity-associated protein), in contrast to the other major OMP, is quite variable both in its qualitative and quantitative expression (Achtman et al. 1991, Aho et al. 1991, Poolman 1995) but may induce bactericidal antibodies (Danelli et al. 1995). This group of proteins also elicits strong but strain-specific antibody responses and confers important interstrain antigenic differences which may be detected by MAbs (Zollinger et al. 1984).

As some of these proteins are targets for bactericidal antibodies and show characteristic differences it was decided to determine the primary sequence of the class 1-5 proteins. Advances in the structural studies of OMPs not only contributed significantly to the understanding the structure-function relationships, but made it possible to characterize conformational changes which accompany the reajustments of amino acid residues involved in the immune response of the host.

In this work we describe the N-terminal amino acid sequence of the class 1-5 *N. meningitidis* OMPs from a strain recently isolated in São Paulo, Brazil. The data can contribute to the identification of important antigenic determinants and the elucidation of the structural and functional properties of the molecules.

**MATERIALS AND METHODS**

*Bacterial strain and obtention of the outer membrane proteins* - *N. meningitidis* N44/89 (B:4:P1:15:P5:5,7) was isolated from a patient with meningococcal septicemia by the Bacteriological Division of the Adolfo Lutz Institute, São Paulo, Brazil. The outer membrane proteins were prepared as described previously (Frasch 1990) with some modifications. Briefly, the bacterium was cultured in Catlin’s medium and the outer membrane vesicles (OMVs) were obtained by centrifugation (3.000g, 1hr, 25°C). The pellet was resuspended in 0.1M Tris-HCl buffer, pH 8.5, containing 0.2 mM EDTA (10% w/v, Tris-EDTA buffer) and the suspension sheared in an ice cooled, omnimixer (Sorvall) for 10 min. The cells were removed by two successive centrifugation steps at 10.000g and 12.000g for 20 min each. The OMVs in the supernatant were then pelleted at 37.000g for 45 min, resuspended in Tris-EDTA buffer and stored at -20°C until use.

**SDS-PAGE and protein determination** - The OMVs were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE, Laemmli 1970) under reducing conditions and the protein concentration was measured according to Lowry’s method (Lowry et al. 1951), using bovine serum albumine as a standard.

**N-terminal amino acid sequence analysis** - For sequencing experiments, the isolated proteins were run on a 15%-acylamide SDS-PAGE (Laemmli 1970) and the proteins blotted on to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories Ltd, UK) using a semi-dry blotting apparatus (Bio-Rad).

The partial amino N-terminal sequences were performed by automatic Edman degradation using a gas-phase protein microsequencer (model PSQ-1, Shimadzu) coupled to an on-line high-performance liquid chromatography (model 6A; Shimadzu, Kyoto, Japan), as described previously (Giovanni De Simone et al. 1994). The initial yield was 55-65% and the repetitive yield 90-92%. A Digital Deccp computer was used to store the GenBank, EMBL and PIR databases. The nucleotide and deduced amino acid sequences were used to query these databases using Wisconsin GCG FASTA algorithm.

**RESULTS AND DISCUSSION**

The SDS-PAGE analysis of the isolated OMPs (*N. meningitidis* serogroup B) is shown in Fig. The Mr of the identified proteins are in good accordance with those from the meningococcal described in the literature.

Amino acid analysis of C1, C3, C4 and C5 proteins showed that none of them presented identical N-terminal sequences with the possible exception of the C1 and C3. These two proteins were homologous (40% identity in 25 amino acids) but clearly different in the positions 3,7,13-25. The common sequence $D^1 V^2 S^3 L^4 Y^5 G^6 F^7 K^9 A^{10} G^{11} V^{12}$ was identical for the first 12 amino acids to the sequence predicted from DNA sequencing of different genes (position 20-39) for meningococcal serogroup B and C class 1 (Table I) and class 3 precursor proteins (Table II). A high similarity with PIA and PIB gonococcal proteins was also observed.

The N-terminal amino acid sequence of the class 4 protein begins with the glycine residue and is distinct from the class 1 and 3 proteins but similar (50% in 10 amino acids) to a class 4 serotype 2a deduced meningococcal sequence (Table III). The amino acid sequence similarity of gonococcal protein III with the OmpA protein of *E. coli* and other enterobacteria has been described elsewhere (Gotschlich et al. 1987, Klugman et al.)
form) isolated protein was observed with a translated DNA sequence (pFLOB540) from FAM18 cells (Kawula et al. 1988) but no identity was seen with the PII gonococcal protein (Table IV).

Although a very high homology between the class 1 (20 amino acids) and class 5 proteins (10 amino acids) of the *N. meningitidis* serogroup B was observed with similar proteins from serogroup A described in the literature, previous immunological characterization of the strains showed that they present different antigenic properties. Moreover, the sequence of amino acids 1 through 20, of the class 1 and 3 proteins, has been suggested to comprise a highly conserved transmembrane protein segment of *Neisseria* sp, while the variable segment seem to be at the outside of the membrane forming an important antigenic site (McGuiness et al. 1990, Bash et al. 1995). Thus it is important go on in the primary structure of these proteins in order to define their variable regions.

The class 1 protein is unique to meningococci, it is immunogenic and antibodies directed against it are bactericidal in vitro (Frasch et al. 1986, Poolman et al. 1987) and protect against infection in an experimental animal model (Saukkonen et al. 1987).

The antigenic differences in the class 2/3 outer membrane proteins are important and accountable for serotype specificity, while antigenic determinants on the class 1 protein generate subtype specificity. A recent study, using synthetic peptides from three strains of *N. meningitidis* (MC50, MC51, H44/76) defined the regions VR1 (24-36) and VR2 (179-187) from the class 1 proteins as responsible for the serosubtype specificity (McGuiness et al. 1990). The determination of the structure-function relationship can provide important clues for the understanding of the multiple biological effects of these outer membrane proteins on different strains of *N. meningitidis*. In addition, the DNA sequence or selected constituent oligonucleotide portions, including synthetic oligonucleotides, may be used as molecular gene probes for the detection

**TABLE I**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Gen bank accession no./Reference</th>
<th>N-terminal sequence</th>
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<tbody>
<tr>
<td>N44/89</td>
<td>B:4P1.15</td>
<td>This work</td>
<td>DVSLY⁵ GEIKA¹⁰ GVQG¹³ NFQLE²⁰ IEPP²⁵</td>
</tr>
<tr>
<td>H44/76</td>
<td>B:15P1.7.1</td>
<td>McGuinness et al. 1990</td>
<td>DVSLY GEIKA GVEGR NYQLQ LTEAQ</td>
</tr>
<tr>
<td>MC50</td>
<td>C:NT:P1.16</td>
<td>Barlow et al. 1990</td>
<td>DVSLY GEIKA GVEGR NIQAQ LTEQP</td>
</tr>
<tr>
<td>MC51</td>
<td>C:NT:P1.15</td>
<td>McGuinness et al. 1990</td>
<td>DVSLY GEIKA GVEGR NFQLE LTEPP</td>
</tr>
<tr>
<td>2996</td>
<td>B:2b:1.2</td>
<td>X60105</td>
<td>DVSLY GEIKA GVEGR NIQLQ LTEPL</td>
</tr>
</tbody>
</table>

NT: not typed
of the organism in various body fluids. Knowledge of the amino acid sequence also allows the testing of strategic synthetic peptides to identify and confirm immunodominant B-cell or T-cell epitopes. Synthetic peptides also may be used as the basis for improve meningococcal and gonococcal serologic tests and/or meningococcal synthetic peptide vaccines.

## REFERENCES


Barnejee-Bhatnagar N, Frasch CE 1990. Expression of *Neisseria meningitidis* iron-regulated outer membrane proteins, including a 70 Kilodalton transfer-


