JOURNAL OF BACTERIOLOGY, Mar. 1998, p. 1323–1330 Vol. 180, No. 5
0021-9193/98/$04.00+0

Sequence Diversity, Predicted Two-Dimensional Protein Structure, and Epitope Mapping of Neisserial Opa Proteins

BURKHARD MALORNY,[1] GIOVANNA MORELLI,[1] BARICA KUSECEK,[1] JAN KOLBERG,[2] AND MARK ACHTMAN[1]*

Max-Planck Institut für molekulare Genetik, 14195 Berlin, Germany,[1] and Department of Vaccines, National Institute of Public Health, N-0462 Oslo, Norway[2]

Received 11 August 1997/Accepted 16 December 1997

The sequence diversity of 45 Opa outer membrane proteins from *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Neisseria sicca*, and *Neisseria flava* indicates that horizontal genetic exchange of *opa* alleles has been rare between these species. A two-dimensional structural model containing four surface-exposed loops was constructed based on rules derived from porin crystal structure and on conservation of sequence homology within transmembrane β-strands. The minimal continuous epitopes recognized by 23 monoclonal antibodies were mapped to loops 2 and 3. Some of these epitopes are localized on the bacterial cell surface, in support of the model.

The Opa (opacity) proteins are a family of antigenic- and phase-variable outer membrane proteins with a monomer molecular mass of approximately 28 kDa expressed by *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and commensal *Neisseria* species (33, 46). Purified Opa proteins are trimers or tetramers, as determined by gel filtration (2). The expression of certain Opa proteins promotes neisserial adherence to and invasion of epithelial and endothelial cells as well as professional phagocytes (20, 22, 28, 40, 44). Opa proteins can also mediate bacterial aggregation via interactions with lipopolysaccharide (6). Heparan sulfate proteoglycans on epithelial cell surfaces are a target for binding of some Opa proteins, and gonococcal invasion can be blocked by heparin or heparan sulfate (7, 37). The N-terminal domain of some members of the CD66 carcinoembryonic antigen family present on phagocytic cell surfaces is also a target for binding by Opa proteins (8, 16, 41, 42).

Multiple *opa* loci containing different *opa* alleles are scattered around the chromosomes of *N. gonorrhoeae* (11 to 12 *opa* loci) (4, 10, 20, 33) and *N. meningitidis* (3 to 4 loci) (3, 18, 25). Earlier comparisons of a limited number of alleles, primarily from two strains of *N. gonorrhoeae* and one strain of *N. meningitidis*, have indicated that sequence differences are concentrated in a semivariable region (SV) and two hypervariable regions (HV1 and HV2) within a conserved framework. Sequence differences between *opa* alleles arise by microevolution: translocations, deletions, point mutations, and import from unrelated neisseriae have been identified in *N. meningitidis* (17, 18, 25), and translocations have been documented for *N. gonorrhoeae* (5). The sequence variability of *opa* alleles is sufficiently large that it has been used for tracing of contacts among patients with gonorrhea (26).

Two slightly different two-dimensional Opa structure models were derived by using protein sequences from two gonococcal strains (4, 36). Both models predicted four surface-exposed loops, the first three of which corresponded to the SV, HV1, and HV2 regions. A few epitopes recognized by murine monoclonal antibodies (MAbs) which are predicted to be exposed on the cell surface have been mapped to the HV1 and HV2 regions (3, 9, 17, 18, 29). Numerous *opa* sequences from *N. meningitidis* (17, 25) and the commensal neisseriae *Neisseria sicca* and *Neisseria flava* (46) have since been described. We have compared these various sequences to determine whether they can be accommodated by the structural model(s) and whether they provide evidence for horizontal genetic exchange of *opa* genes between the different species. In addition, we have mapped the minimal binding sites of additional epitopes recognized by bactericidal MAbs in order to localize surface-exposed loops.

* Corresponding author. Mailing address: Max-Planck Institut für molekulare Genetik, Ihnestraße 73, D-14195 Berlin, Germany. Phone: (49 30) 8413 1262. Fax: (49 30) 8413 1387. E-mail: achtman@mpimg-berlin-dahlem.mpg.de.

MATERIALS AND METHODS

Nomenclature of *opa* alleles and proteins. Diverse nomenclatures have been used for *opa* sequences submitted to GenBank. In addition, the 106 alleles found in release 101 contained a number of incomplete sequences, duplicate sequences with different nomenclatures, and contradictory sequences for supposedly identical alleles. We have assigned arbitrary numbers to each unique allele and SV, HV1, and HV2 region (Table 1) (17, 25), derived from the original allelic or plasmid designations where possible. The complete data set is available upon request from M. Achtman.

DNA sequences. Duplicate sequences and sequences representing recombinational or translocation events (4, 17, 25) within the 106 alleles were excluded from analysis to ensure that only unique sequences were compared. Furthermore, only sequences encoding a mature Opa protein were used, thus excluding 15 partial sequences. Contradictory sequences were present for some *opa* alleles of *N. gonorrhoeae* MS11. In those cases, the sequences of Bhat et al. (4) were chosen because the PCR amplification and cloning method used by Kupsch et al. (20) has been shown to generate a high frequency of PCR-generated mistakes (25). The final data set consists of 45 sequences (Table 1) and includes 7 sequences from two serogroup B meningococci which have not been published elsewhere. Those sequences were obtained after PCR amplification of chromosomal DNA as described previously (25) and were sequenced by automated dye terminator cycle sequencing (ABI model 377 DNA sequencer) using primers O3510, O80, O82, O83, and O87 (17).

Multiple alignment of protein sequences. After translation, the amino acid sequences of mature Opa proteins were aligned by using PILEUP (version 9.0; Genetics Computer Group, University of Wisconsin). The alignment was then edited manually, especially in the variable regions, by placing alignment gaps such that they increased the protein sequence similarities.

Sequence analysis. Alignments stored as an MSF file were analyzed by using a self-written program, PsFind (ftp://novell-del-valle.rz-berlin.mpg.de), which can calculate percent uniformity, defined as the percentage of the most frequent amino acid at each position (excluding gaps introduced by the alignment). PsFind was also used to calculate the properties of the most common amino acid at each position. Phylogenetic trees were calculated in ARB (http://www.mikro.biologie.tu-muenchen.de), using PAM distances and the neighbor-joining method (30). Bootstrap analysis was performed in ARB, using 200 repetitions.

MAbs. The murine MAbs used (Table 2) include antibodies secreted by five new hybridomas which were generated as previously described (1) after immunization of BALB/c mice with meningococci of serogroup A, subgroup IV-1 (O623) or subgroup III (L614, U506), or of serogroup B, ET-5 complex (192/B8, 210/G9).

TABLE 1. Unique designations of *opa* alleles and their variable regions

opa allele[a]	Species[b]	Cluster[c]	GenBank accession no.	Designation[d]		
				SV	HV1	HV2
41	Nm C C1938	Nm-1	X06445	109	35	37
46	Ng JS3	Ng-3	X12625	113	82	83
47	Ng MS11	Ng-3	X60709	130	77	78
49	Ng MS11	Ng-1	X60711	127	52	53
65	Ng VP1	Ng-2	Z18940	116	94	95
66	Ng VP1	Ng-1	Z18941	126	55	56
67	Ng VP1	Ng-1	Z18942	119	88	89
68	Ng VP1	Ng-1	Z18936	126	55	57
69	Ng F62-SF	Ng-3	X15780	122	80	81
70	Ng MS11	Ng-1	X52373	117	73	74
71	Ng MS11	Ng-1	X52370	127	58	59
74	Ng MS11	Ng-1	X52368	123	73	74
75	Ng MS11	Ng-2	X52367	124	75	76
77	Ng MS11	Ng-3	X52365	123	84	85
78	Ng MS11	Ng-1	X52371	128	68	69
79	Ng MS11	Ng-1	X52364	120	71	96
87	Ns	Outgroup	U12287	132	90	91
88	Nf	Outgroup	U12288	131	92	93
92	Nm A	Nm-1	AF001199	101	5	6
93	Nm A	Nm-1	AF001200	102	5	6
94	Nm A	Nm-1	AF001201	100	7	8
99	Nm A	Nm-2	AF001181	100	1	2
100	Nm A	Nm-1	AF001195	100	9	10
101	Nm A	Nm-2	AF001179	109	3	4
102	Nm A	Nm-1	AF001204	103	24	25
118	Nm A	Nm-1	AF001186	103	13	14
123	Nm B 188/87	Nm-1	AF016290	100	38	19
124	Nm B 188/87	Nm-1	AF016288	111	39	40
126	Nm B 188/87	Nm-2	AF016292	106	41	42
127	Nm B 190/87	Nm-1	AF016286	100	43	44
128	Nm B190/87	Nm-1	AF016289	106	45	46
129	Nm B190/87	Nm-1	AF016285	100	43	48
130	Nm B190/87	Nm-1	AF016291	107	49	50
131	Nm A	Nm-1	AF001194	100	9	10
132	Nm A	Nm-2	AF001180	100	1	2
133	Nm A	Nm-1	AF001185	103	13	14
134	Nm A	Nm-1	AF001193	100	15	51
540	Nm C FAM18	Nm-1	X63110	103	30	31
709	Ng FA1090	Ng-2	X06436	118	63	67
900	Nm C FAM18	Nm-1	X63111	103	32	97
1100	Ng FA1090	Ng-2	X06435	115	63	64
1800	Nm C FAM18	Nm-1	X63109	133	33	98
5100	Nm C Z4197	Nm-1	U37256	109	28	540
5200	Nm C Z4197	Nm-2	U37257	103	22	23
15063	Ng MS11A	Ng-1	U13708	121	61	62

[a] Unique designation for each allele. Opa proteins implicated in invasion: Opa68, Opa71 (formerly called Opa50) (20), and Opa15063 (43).

[b] Nm, *N. meningitidis*; Ng, *N. gonorrhoeae*; Ns, *N. sicca*; Nf, *N. flava*. Strain designations are included for *N. gonorrhoeae* and *N. meningitidis* serogroup B and C (17) strains. The serogroup B strains 188/87 and 190/87 were isolated in Norway and belong to the ET-5 complex; the serogroup C strains FAM18 and Z4197 are ET-37 complex strains isolated in the USA and Mali, respectively. The serogroup A strains were of subgroups III and IV-1 (25).

[c] Cluster within tree in Fig. 3.

[d] Unique designation based on nucleotide sequence. The amino acid sequences of SV103 and SV106 are identical, whereas the nucleotide sequences differ by 1 bp. These sequences are shown in Fig. 2 as SV-103. The amino acid sequences of HV1-13 and HV1-22 are identical, whereas the nucleotide sequences differ by 1 bp. These sequences are shown in Fig. 2 as HV1-13.

Epitope mapping. Multiple synthetic N-terminally acetylated peptides containing 12 or 10 amino acids were synthesized on pins as previously described (15), using an epitope scanning kit (Cambridge Research Biochemicals) with a dilution aid (Epiguide; Labsystems) and the modifications described elsewhere (23). The pins were screened by enzyme-linked immunosorbent assay (ELISA) for reactivity as previously described (17).

Exposure of epitopes. Bactericidal activity was tested in microtiter wells as described previously (12), using various concentrations of MAbs in the presence of 20% human complement and the serogroup A, subgroup IV-1 strain C623, which expresses Opa132, Opa136, and Opa137. MAbs were scored as bactericidal when at least 50% of the bacteria were killed by low concentrations compared to control tests lacking antibodies. Immunofluorescence microscopy with live bacteria was performed as described previously (24).

RESULTS

Sequence variation among 45 Opa protein sequences. Sequence variation has recently been examined among *opa* alleles from hundreds of *N. meningitidis* serogroup A, subgroup III strains isolated globally since the 1960s and from representative subgroup IV-1 and IV-2 strains (25). Those sequence variants which represent *opa* alleles inherited from a common ancestor of these subgroups, or which were subsequently imported by horizontal genetic exchange, were chosen for a comparison of diverse neisserial Opa proteins. The unique *opa* sequences from two serogroup C strains of the ET-37 complex (17) and from two serogroup B strains of the ET-5 complex, as well as one sequence from strain C1938 of unknown clonal assignment, were included (Table 1). Sequence variants reflecting translocation or recombinational events between *opa* alleles were excluded from the comparison because they do not represent unique alleles. These 25 sequences were supplemented by 18 unique sequences available in GenBank from *N. gonorrhoeae* MS11, FA1090, VP1, and JS3 as well as by two sequences from the commensal species *N. sicca* and *N. flava*. The resulting list (Table 1) contains those 45 unique *opa* alleles for which complete sequences encoding the entire mature Opa protein were available. After translation, the amino acid sequences were aligned, with manual addition of gaps within the variable regions to ensure maximal homology, and the amino acid variability at each position was calculated (Fig. 1).

The former limits of the variable SV, HV1, and HV2 regions (3, 4, 18, 32) were based on only a few sequences, primarily

TABLE 2. MAbs which react with Opa proteins

MAb[a]	Immunoglobulin subclass	Minimal epitope	Specificity
O623	G2a	NKS	HV1-1
U506	G1	NKS	HV1-1
O521[b]	G3	NRQD	HV1-1
T116	ND[c]	TWKEL	HV1-7
L614	G1	KESNYS	HV1-9
P219[b]	G1	IY	HV2-2
P322[b,d]	G3	TIYNQ	HV2-2
P414[b]	G2a	TPTIYNQ	HV2-2
W320/15	G2a	NIP	HV2-2
W124	ND	NIP	HV2-2
W104	ND	PTNIPGGT	HV2-2
AB419	G2b	TTFL	HV2-6
W320/16	ND	PSGSTT	HV2-8
U205	G2b	PVPQGPTPK	HV2-10
O516/2[b]	G2b	KGATQ	HV2-14
P110[b,d]	G2b	KPTKGAT	HV2-14, HV2-19
P112[b,d]	G2a	QP	HV2-14
N312	G1	QP	HV2-14
P515[b,d]	G3	KGAT	HV2-14
P416[b,d]	G2a	PTKGATQP	HV2-14
P514[b,d]	G2a	PTKGATQP	HV2-14
210/G9	G2a	QPGKL	HV2-14, HV2-19
192/B8	G1	TQPGKLV	HV2-14, HV2-46

[a] All have been previously described by Achtman et al. (1) except for D309 (not shown) (2), O521 (18), and 192/B8, 210/G9, L614, O623, and U506 (described for the first time in this report).

[b] Strong immunofluorescence with bacteria expressing Opa proteins recognized by the MAb.

[c] ND, not determined.

[d] Bactericidal at concentration of <8 μg/ml.

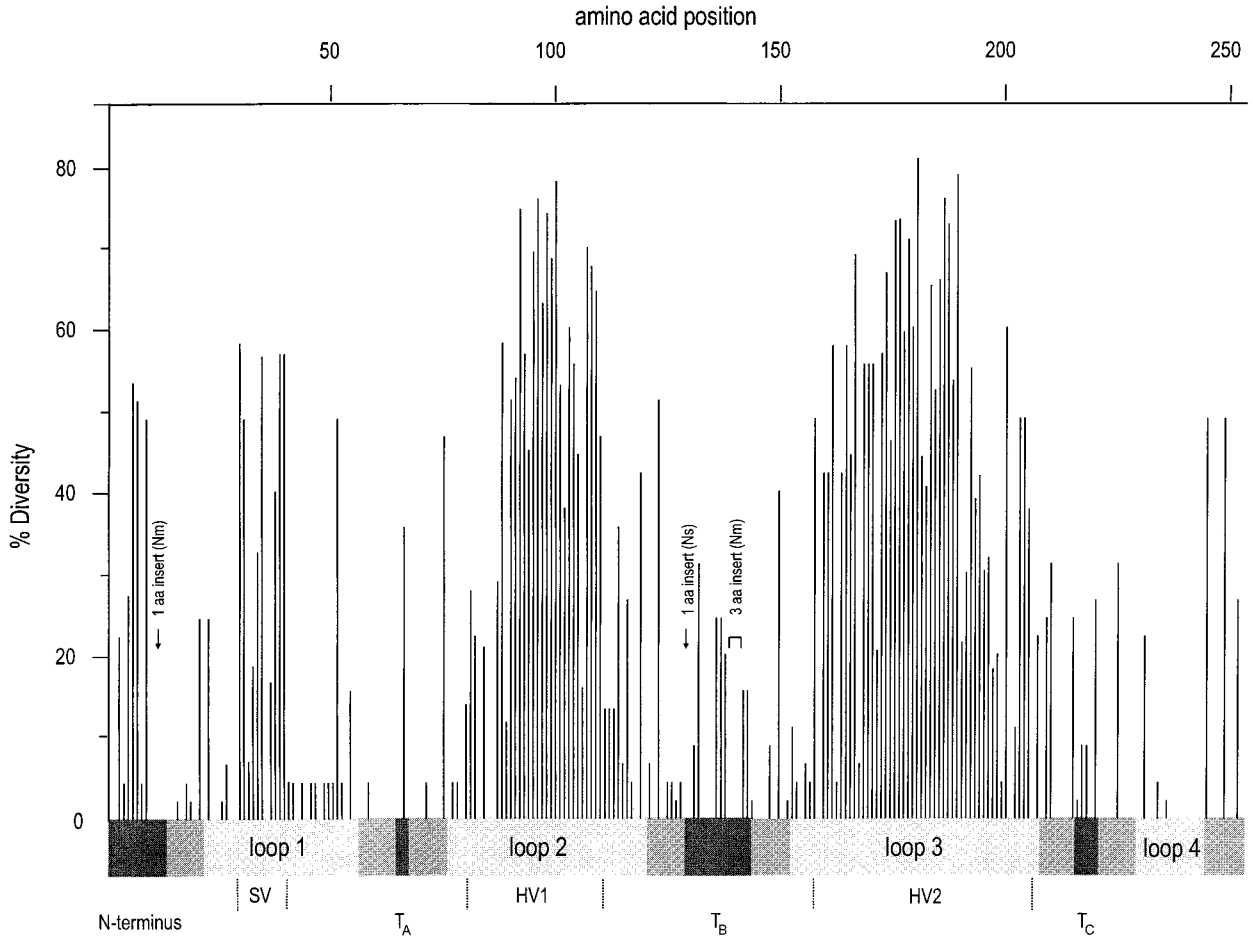

FIG. 1. Sequence diversity of 45 Opa proteins. The percentage occurrence of the most common amino acid (percent uniformity) was calculated at each position within an alignment of the 45 Opa proteins, using the PsFind program. The percentage diversity consists of percent uniformity subtracted from 100. The regions predicted to correspond to exposed loops on the cell surface are shaded light gray, regions putatively exposed to the periplasm are shaded dark gray, and predicted transmembrane strands are indicated by intermediate shading. The SV, HV1, and HV2 regions were redefined to include the maximal sequence diversity, as indicated aa, amino acid.

from *N. gonorrhoeae*, and need to be redefined as indicated in Fig. 1 and 2 to account for the variability in this data set. Additional sequence variability was concentrated at the N terminus and in the regions assigned to periplasmic turns T_B and T_C (Fig. 1; see below).

Species-specific differences. A rooted neighbor-joining (30) phylogenetic tree using *N. sicca* and *N. flava* as an outgroup contained two main clusters of sequences from *N. meningitidis* (Nm-1 and Nm-2) and three clusters of sequences from *N. gonorrhoeae* (Ng-1, Ng-2, and Ng-3) (Fig. 3). Clusters Nm-1, Ng-1, and Ng-2 were obtained in over 50% of 200 bootstrap repetitions, whereas the other clusters were less robust (Fig. 3). Opa proteins from *N. gonorrhoeae* MS11 were found in all three Ng clusters, and Opa proteins from *N. meningitidis* subgroups III/IV-1 or the ET-5 complex were found in both Nm clusters. Similar results were obtained whether the distance algorithm was based on percentage identity or the PAM matrix and when only sequences from the conserved regions were compared (data not shown). The observation that sequences from *N. gonorrhoeae* and *N. meningitidis* never clustered together indicates that horizontal genetic transfer of *opa* alleles between the two pathogenic species is rare. In agreement, the SV, HV1, and HV2 regions from *N. meningitidis* were also all distinct from those of *N. gonorrhoeae* (Table 1). The N-terminal portions of all meningococcal Opa proteins contain an additional amino acid (Y) (Fig. 1). Some meningococcal Opa proteins contain a duplication of three amino acids (DKF) at positions 140 to 142.

The Opa proteins from *N. sicca* (*opa87*) and *N. flava* (*opa88*) resembled each other but differed at numerous sites from those of the pathogenic neisseriae, even within otherwise conserved regions (Fig. 4). Pairwise comparisons between Opa proteins from the commensal species and the Opa proteins from the pathogenic neisseriae yielded 50 to 59% identities, whereas Opa proteins from *N. meningitidis* were 60 to 84% identical to those of *N. gonorrhoeae*. The SV, HV1, and HV2 regions of the Opa proteins from the commensals were also shorter than those from the pathogenic neisseriae.

Despite the species specificity, certain variable regions were similar between gonococcal and meningococcal proteins, with stretches of identity of up to 13 amino acids. The most similar of these regions were SV-123 (gonococcal *opa* allele 74 or 77) versus SV-111 (meningococcal Opa124), where 8 of 11 amino acids were identical, HV1-94 (gonococcal Opa65) versus HV1-30 (meningococcal Opa540), where 23 of 25 amino acids were identical, and HV2-78 (gonococcal Opa47) versus HV2-10 (meningococcal Opa100 and Opa131), where 33 of 41 amino acids were identical. These observations are consistent with occasional recombination between the two species. Simi-

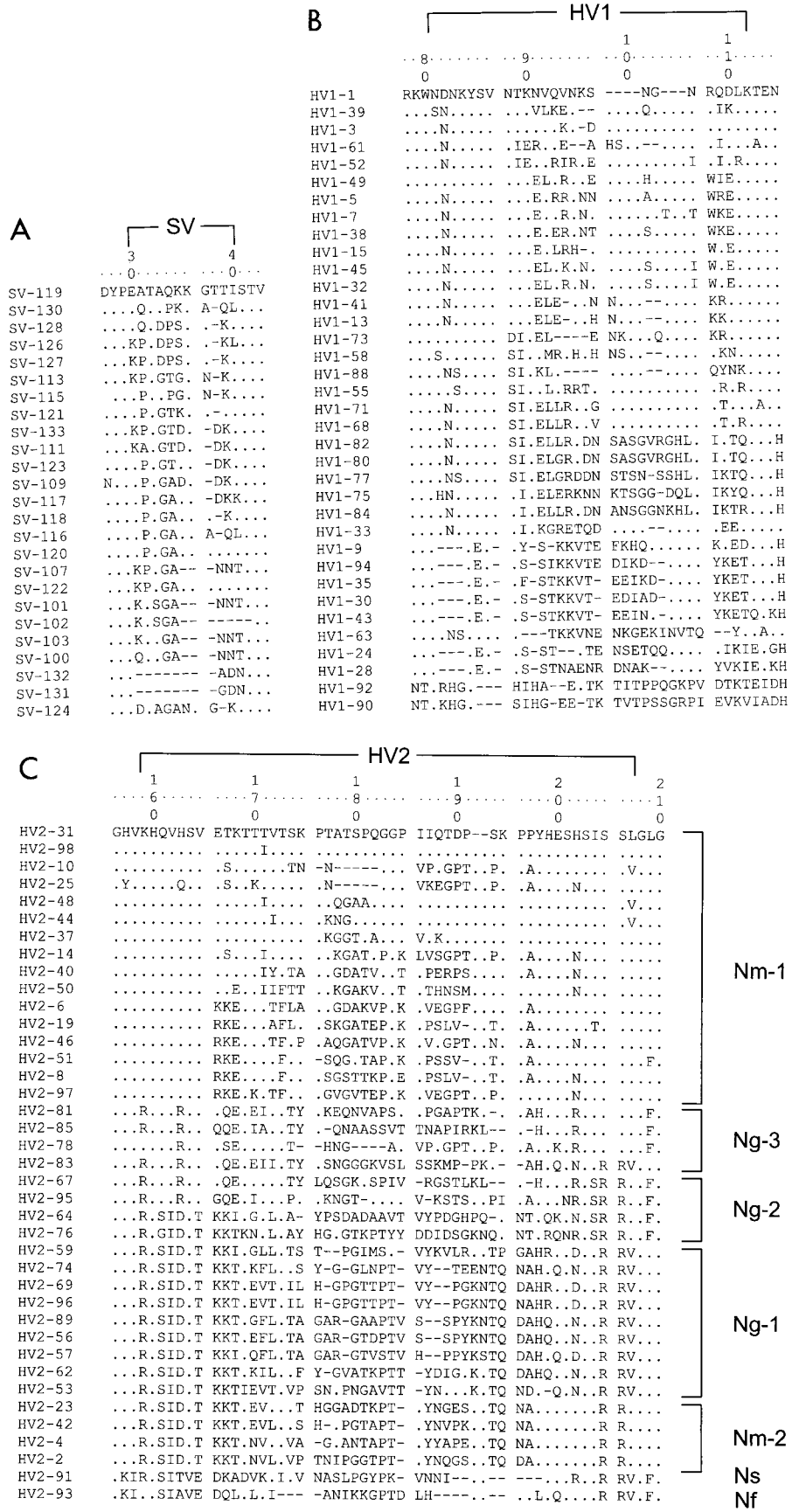

FIG. 2. Multiple alignments of the SV, HV1, and HV2 regions. Dots indicate identity to the top sequence shown, and gaps introduced to maximize homology are indicated by hyphens. The numbers at the top designate positions in a consensus sequence. (A) SV positions 30 to 40; (B) HV1 positions 81 to 112; (C) HV2 positions 159 to 207. The ordering of the sequences was by maximal visual similarity except for panel C, where the sequences were grouped by phylogenetic protein cluster.

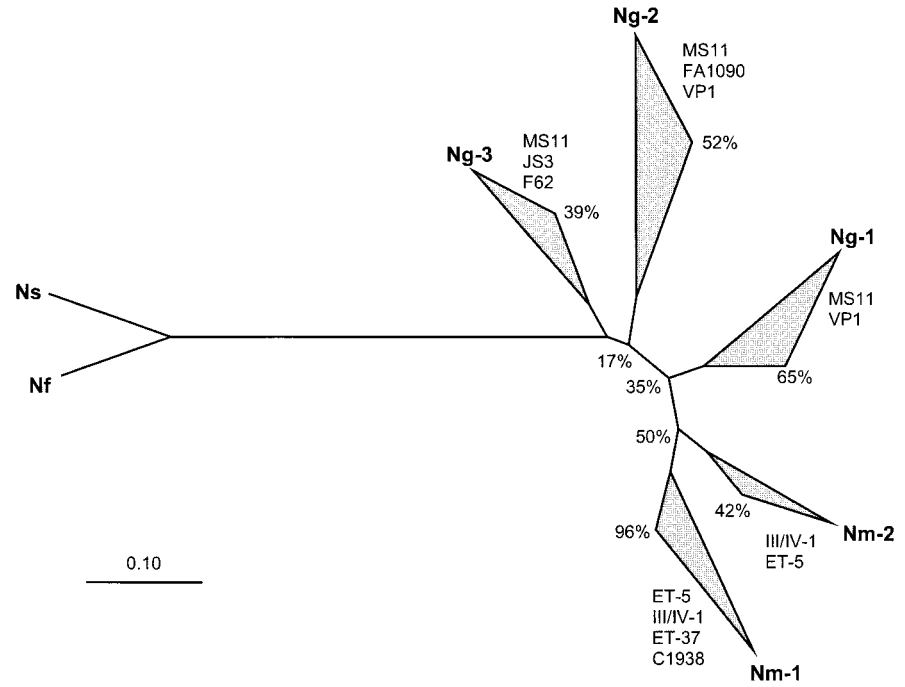

FIG. 3. Phylogenetic neighbor-joining tree of 45 Opa proteins, using sequences from commensal neisseriae (Nf, *N. flava*; Ns, *N. sicca*) as an outgroup. Clockwise of each cluster are indicated the strains (*N. gonorrhoeae*, clusters Ng-1 through Ng-3) or subgroups (*N. meningitidis*, clusters Nm-1 and Nm-2) which they encompassed, as well as confidence levels (percentages) calculated from bootstrap tests with 200 repetitions. The values at the nodes are also bootstrap confidence levels. The genetic distance scale is indicated by a horizontal line at the bottom.

larly, after exclusion of the hypervariable regions and two short insertions of one and three amino acids, 145 of 157 amino acids were identical between gonococcal Opa70 and meningococcal Opa900.

Two-dimensional model for the structure of Opa proteins. Antiparallel amphipathic β strands span the outer membrane within porins (11, 45), and strand prediction has been used to devise two slightly different two-dimensional models of gonococcal Opa proteins (4, 36). Transmembrane strands correspond to the most conserved sequences within outer membrane protein families (19). The C-terminal amino acid of outer membrane proteins is usually a phenylalanine and is preceded by a transmembrane strand (34). Porins characteristically possess short periplasmic turns, containing turn-promoting amino acids and lacking turn-inhibiting amino acids (27), and their transmembrane strands are flanked by aromatic residues (11, 45).

We devised a two-dimensional β-barrel model containing eight transmembrane strands which maximized all of the criteria cited above (Fig. 4). Minor discrepancies to the criteria were the slightly hydrophilic amino acid S_{125} and turn T_B, of 14 amino acids, which is exceptionally long. The model predicts that four hydrophilic loops (L1 to L4) are exposed on the cell surface. The SV region is part of loop L1, HV1 corresponds to L2, and HV2 corresponds to L3. Loop L4 is short and is also strongly conserved. Of the domains predicted to face the periplasm, the N terminus and turns T_B and T_C show considerable sequence variability. The model (Fig. 4) differs slightly from former models (4, 36) in that the transmembrane strands are of uniform length and the ends of the external loops are translocated by up to three amino acids (marked with asterisks in Fig. 4).

Mapping of cell surface-exposed epitopes. MAbs that react with intact bacteria must recognize epitopes on the cell surface. We have formerly mapped five such epitopes to the regions corresponding to L2 and L3 of Opa proteins from serogroup C meningococci (17) and have now analyzed 26 other MAbs which react specifically with serogroup A meningococci in whole-cell ELISAs and with native Opa proteins purified from those meningococci. Six of these MAbs were bactericidal, and 10 bound to live bacteria, as determined by immunofluorescent light microscopy (Table 2). The specificity of the 26 MAbs correlated with the sequences of individual HV1 or HV2 regions (Table 2). The minimal epitopes recognized by these MAbs were mapped by Pepscan analysis of L2 and L3 of the Opa proteins with which they react. We synthesized 12-mers overlapping by nine amino acids on pins and tested them for reactivity with the 26 MAbs. For 23 MAbs, the results defined epitopes whose limits were further refined by Pepscan with 10-mers overlapping by nine amino acids (data not shown). The locations of the minimal continuous epitopes that these MAbs recognize are summarized in Table 2 and Fig. 5. Only weak reactivity was obtained with two other MAbs (U106 and U214), and a third (D309) reacted with so many peptides that the results were uninterpretable.

Two of the MAbs whose epitopes had been mapped, 192/B8 and 210/G9, reacted with Opa proteins containing HV2-14 from serogroup A meningococci but had been isolated after immunization with serogroup B meningococci expressing Opa128 (HV2-46) and Opa123 (HV2-19), respectively. MAb 192/B8 recognizes TQPGKLV in HV2-14, whereas HV2-46 contains the sequence TVPGKIV (differences are underlined). MAb 210/G9 recognizes the sequence QPGKL in HV2-14, whereas HV2-19 contains the sequence EPGKI. Similarly, P110, raised against serogroup A Opa protein Opa137, also reacted with Opa123 from serogroup B meningococci. The minimal binding site was KPTKGAT in HV2-14 (Opa137), whereas HV2-19 contains KPSKGAT. These results presumably reflect the ex-

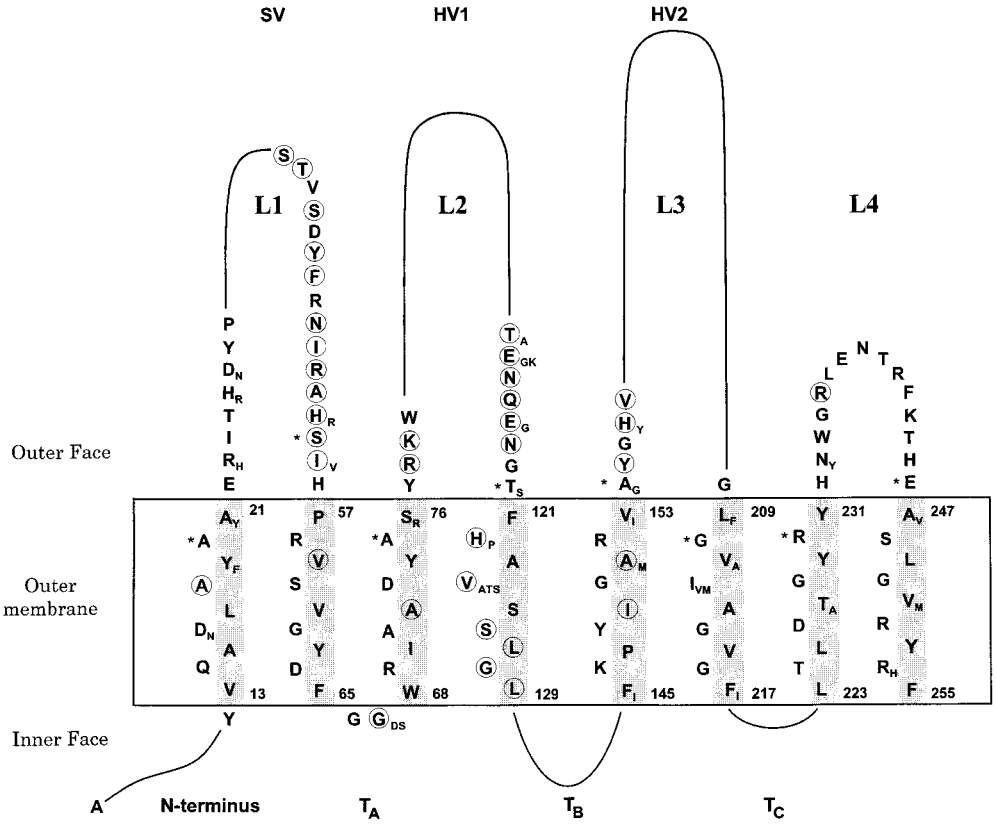

FIG. 4. Predicted two-dimensional structure of Opa proteins. Variable stretches are indicated by a continuous line, and only details of conserved amino acids are shown. Minor sequence variation is indicated by additional subscript letters next to the most frequent amino acids. Circled amino acids were variable only in the Opa proteins from *N. flava* or *N. sicca*. Asterisks indicate the first amino acids predicted to lie outside the outer membrane in the model of Bhat et al. (4). The inner and outer faces of the outer membrane are indicated by horizontal lines. The gray shading indicates the nonpolar side of the eight transmembrane β strands.

changeability of certain amino acids within minimal epitopes and were not further investigated.

DISCUSSION

The sequences of 45 informative Opa protein sequences from four neisserial species were analyzed in order to test for frequent interspecies recombination and to construct an improved two-dimensional structural model.

The two Opa sequences available from the commensal species, *N. sicca* and *N. flava*, differed strongly from sequences from the pathogenic species, *N. gonorrhoeae* and *N. meningitidis* (Fig. 3). The commensal sequences possessed shorter SV, HV1, and HV2 regions, corresponding to shorter L1, L2, and L3 (Fig. 2), as well as a large number of different amino acids at otherwise conserved positions (Fig. 4). Additional sequences are necessary to determine whether these differences are typical of the commensal neisseriae.

opa alleles can translocate from one locus to another in *N. gonorrhoeae* (5) and *N. meningitidis* (17, 18, 25), leading to formation of mosaic genes, and import of *opa* alleles from unrelated neisseriae during epidemic spread has also been documented for *N. meningitidis* (17, 18, 25). Simple cocultivation in the laboratory of different neisserial species suffices to allow DNA transformation of *opa* alleles (14). Interspecies transfer has been demonstrated for several (non-*opa*) genes within the neisseriae (citations in reference 21).

In contrast, the results presented here indicate that transfer of *opa* alleles between neisserial species is rare in nature. In addition to the size differences between Opa proteins from commensal and pathogenic species, all 25 meningococcal Opa proteins contain a unique insertion of one amino acid in the N-terminal region (Fig. 1). Furthermore, Opa proteins from the different species clustered separately within a phylogenetic tree (Fig. 3). These results resemble those found for housekeeping genes (39), for which the lack of genetic overlap between *N. gonorrhoeae* and *N. meningitidis* has been interpreted as indicating ecological isolation (31). We note, however, that one example of horizontal genetic exchange between the pathogenic neisseriae has already been described (38) and that the genetic diversity of Opa proteins is likely to be so large that the current sample of 45 sequences is too small to have detected rare genetic exchange.

Two-dimensional structural models based on a limited number of Opa sequences (4, 36) were refined by combining rules based on the properties of amino acids (13, 27) with those derived from porin structures (11, 35, 45) and by minimizing the sequence variability within the nonpolar face of transmembrane β strands. The resulting model (Fig. 4) predicts that the protein traverses the outer membrane eight times, resulting in four hydrophilic loops on the cell surface and terminating at the inner face of the outer membrane. L1, L2, and L3 are highly variable in sequence and correspond to the variable regions called SV, HV1, and HV2. Considerable sequence variability was also found in the N terminus and within turns T_B and T_C, all predicted to face the periplasmic side of the outer membrane. In light of this degree of variability, the structure

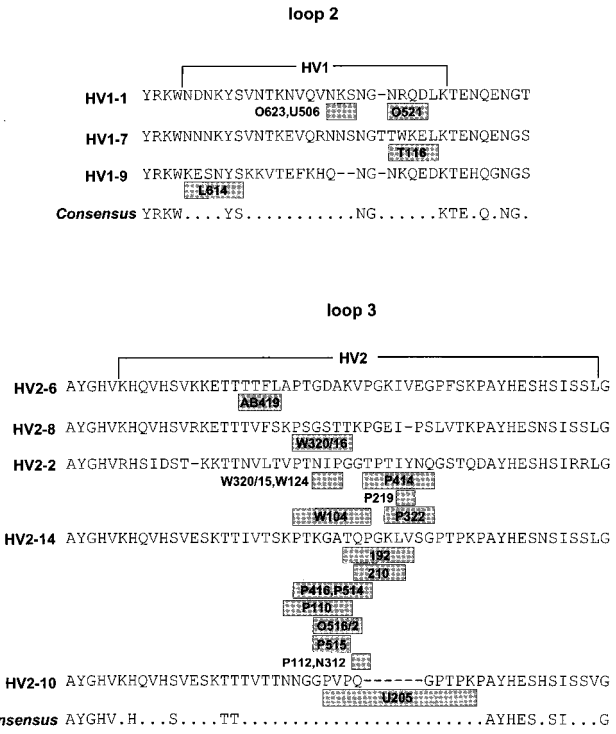

FIG. 5. Minimal continuous epitopes in L2 and L3 recognized by 23 MAbs. The minimal epitopes are shown in gray. The epitopes were defined by Pepscan with 10-mers overlapping by nine amino acids, except that the epitopes recognized by MAbs U205 and L614 were mapped by using 12-mers overlapping by nine amino acids.

of L4 is strikingly constant, suggesting that it might have an important role in protein structure.

The exposure of L2 and L3 to the cell surface was confirmed by mapping continuous epitopes recognized by 23 MAbs to those loops and by showing that some of these MAbs are bactericidal and/or bind to live bacteria (immunofluorescence). No MAbs that bind to L1 and L4 have been described, suggesting that L2 and L3 are immunodominant.

It seems likely that the structures of most Opa proteins will resemble that of the model presented here, especially because the two Opa proteins from *N. sicca* and *N. flava* possessed the same structure despite being only 50 to 60% homologous to other Opa proteins. The model also provides the possibility to test the significance of apparent homologies between Opa proteins and proteins from other genera whose genomes are being sequenced.

ACKNOWLEDGMENT

Burkhard Malorny was supported by grant Ac36/6 from the Deutsche Forschungsgemeinschaft.

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