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Novel Lipoprotein Expressed by *Neisseria meningitidis* but Not by *Neisseria gonorrhoeae*

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The *ppk* gene, which codes for the enzyme polyphosphate kinase in *Neisseria meningitidis* strain BNCV, is preceded by an open reading frame coding for a protein with a predicted size of 19.2 kDa with a typical lipoprotein signal sequence of 21 amino acids. The protein has significant homology to the N-terminal portion of an outer membrane protein from *Haemophilus somnus* (J. Won and R. W. Griffith, Infect. Immun. 61:2813–2821, 1993). Sequencing of the same open reading frame from meningococcus strain M1080 predicted an almost identical protein. Antisera were raised against the lipoprotein, expressed in *Escherichia coli* as a fusion protein with glutathione *S*-transferase. The antisera reacted with meningococcal membrane fractions on a Western blot (immunoblot) but did not elicit complement-dependent bactericidal activity. Restriction enzyme digestion demonstrated conservation of this portion of the meningococcal and gonococcal chromosomes. However, antisera raised to the recombinant protein showed that the protein was absent from all strains of gonococcus tested. The sequences of the gene from several strains of *Neisseria gonorrhoeae* and *N. meningitidis* were compared and found to be almost identical, except that the coding sequences from all of the gonococcal strains were terminated prematurely as a result of a frameshift mutation. The significance of the remarkable conservation of these gonococcal genes is discussed.

The region of the chromosome of *Neisseria meningitidis* BNCV upstream from the characterized *ppk* gene (which codes for the enzyme polyphosphate kinase [PPK] [20a]) contains two additional open reading frames (ORFs). The more-upstream region, stretching from 1.1 to 0.6 kbp upstream of the *ppk* gene, could code for a protein with a predicted size of 19.2 kDa with a lipoprotein signal sequence (see Fig. 1). The positively charged/hydrophobic signal peptide is followed by a cleavage site for bacterial lipoprotein signal peptidase, Val LeuSerAlaCys (15), which would be processed between alanine and cysteine. The predicted neisserial protein shows significant homology to the N-terminal portion of an antigen from *Haemophilus somnus* (22), whose function remains unclear. The neisserial sequence information, however, was obtained from a library of fragmented chromosomal DNA in λgt11, and the sequenced fragment did not include an ATG initiation codon. On the supposition that the ORF did indeed code for a neisserial lipoprotein, and to evaluate its potential as an antigen for immunoprophylaxis, experiments were undertaken to characterize the gene and its product in the pathogenic neisseriae.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The origins of the neisserial strains used in this study have been described. The strains are *Neisseria gonorrhoeae* F62 (7, 8), MS11 (19, 20), R10 (16), and UU1 (a strain isolated by Z. A. McGee [University of Utah] from a case of disseminated gonococcal infection [12]) and *N. meningitidis* BNCV (a nonencapsulated variant of M986) and M1080 (3, 4). Neisseriae were grown in proteose peptone no. 3 (Difco) medium containing 1% IsoVitaleX (GC medium) (1) or on GC agar plates (18). Plates were incubated at 37°C in a moist atmosphere containing 5% carbon dioxide.

Fractionation of *N. meningitidis* BNCV. Meningococci were harvested from late-exponential-phase cultures in 200 ml of GC medium and fractionated into periplasm, cytoplasm, and membrane fractions by a method described previously (21).

Molecular genetic techniques. Unless otherwise noted, molecular genetic procedures were performed as described by Sambrook et al. (17). Cloning into the mp series of M13 vectors was performed as described by Messing (13). The PCR was carried out with the GeneAmp kit from Perkin-Elmer Cetus.

Restriction endonucleases and buffers were obtained from New England Biolabs. Other DNA-modifying enzymes were purchased from Pharmacia.

Gels for separation of DNA restriction fragments consisted of between 0.6 and 1.0% agarose (SeaKem; FMC Bioproducts) in taurine buffer (89 mM Tris, 28.5 mM taurine, 1 mM EDTA). Gels for purification of DNA fragments consisted of 0.7% low-melting-point agarose (SeaPlaque; FMC Bioproducts) in Tris-acetate-EDTA (TAE) buffer (17).

Southern transfer was performed by capillary blotting to Hybond-N^+ positively charged nylon membranes (Amersham). The membranes were placed for 10 min on filter paper soaked in 0.5 M NaOH, neutralized by three transfers to paper soaked in 1 M ammonium acetate containing 20 mM NaOH, and washed once in 50 mM NaOH. Hybridization of labelled probes and detection were performed with the Enhanced Chemiluminescence kit as described by the manufacturer (Amersham). Excess binding capacity of the membranes was blocked, and they were reacted with probes obtained by random-primed labelling of DNA with fluorescein-labelled deoxynucleoside triphosphate; bound probe was visualized with horseradish peroxidase-conjugated anti-fluorescein antibodies. Specifically, probes corresponding to the sequence of the lipoprotein gene were obtained by random-primed labelling of a PCR product which had been produced with chromosomal DNA as a template. (The design of the PCR product is described below, in "Production and purification of the cloned lipoprotein").

The procedures for isolation of chromosomal DNA from *Neisseria* species and for production of competent *E. coli* are described elsewhere (20a).

DNA sequencing techniques. DNA sequencing was performed with Sequenase II T7 DNA polymerase from United States Biochemical, by use of reagents from the 7-deaza-dGTP sequencing kit and α-^{35}S-dATP (New England Nuclear). Sequencing of double-stranded plasmid DNA was performed as described previously (17). Single-stranded DNA was produced for sequencing by isolating a single strand of a PCR product, produced from a primer labelled at its 5′ end with biotin. Biotin-labelled DNA was purified by the Dynabeads M-280 streptavidin system (Dynal).

DNA sequence information was processed with the computer program package from the Genetics Computer Group (version 7.3, 1993). Sequences obtained from *N. meningitidis* BNCV and *N. gonorrhoeae* F62 have been submitted to GenBank under accession numbers U16284 and U16285, respectively.

Cloning of *Eco*RI restriction endonuclease fragments of neisserial DNA containing the lipoprotein gene. DNA from *N. meningitidis* BNCV and from *N. gonorrhoeae* F62 was digested with restriction endonuclease *Eco*RI. The 6.5-kb fragment containing the lipoprotein gene sequence was identified by Southern blotting. The digested DNA was subjected to agarose gel electrophoresis, and the portion of the gel around the position of the reactive fragment was excised. DNA was purified by adsorption to glass in suspension (Geneclean; Bio 101) and ligated into the vector pBluescript IISK$^-$ (Stratagene). Transformants of *E. coli*

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XL1-Blue were grown on Luria-Bertani agar. Circles of Hybond-N^+ were placed onto the plates for 5 min. Upon removal of the membrane, the plates were stored at 4°C. The bacteria attached to the membrane were lysed, and the DNA bound to the membranes was probed as described for Southern blotting to identify recombinants containing the reactive *Eco*RI fragment.

Isolation of RNA from *N. meningitidis* and primer extension experiments. RNA was extracted from the mid-exponential phase of growth of *N. meningitidis* BNCV growing in liquid medium. Cells from 10 ml of culture were cooled rapidly on ice and harvested by centrifugation at 0°C. The pellet was resuspended at 0°C in 10 ml of 15 mM Tris-HCl (pH 8.0) containing 8 mM EDTA and 0.45 M sucrose, digested for 15 min with lysozyme (80 μl of a 50-mg/ml solution), and centrifuged at 0°C to pellet the spheroplasts. The spheroplasts were resuspended in 0.5 ml of lysis buffer (20 mM Tris-HCl [pH 8.0], 10 mM sodium chloride, 1 mM sodium citrate, 1.5% sodium dodecyl sulfate [SDS]) and, after addition of 30 μl of diethylpyrocarbonate, incubated at 37°C for 5 min. The suspension was mixed with 0.5 ml of saturated sodium chloride solution and incubated on ice for 10 min. After centrifugation for 45 min at 16,000 × *g*, at 4°C, the supernatant was extracted twice with an equal volume of phenol:chloroform:isopentanol (25:24:1; phenol was equilibrated to pH 6.8). The RNA was precipitated by the addition of 2 volumes of ethanol and stored in suspension at −70°C. The RNA was pelleted, washed with 80% ethanol, and dried under vacuum before use.

Mapping of the transcription start site was performed with a kit from Promega containing reverse transcriptase from avian myeloblastosis virus. Oligodeoxynucleotide primers corresponding to the DNA sequence of the noncoding strand of the gene were synthesized. Oligonucleotide primers were labelled at their 5′ ends with [γ-^{32}P]ATP, by use of T4 polynucleotide kinase, and hybridized to the RNA preparation. Copy DNA was produced by avian myeloblastosis virus reverse transcriptase, and the position of the 5′ ends of the RNA was determined by denaturing polyacrylamide gel electrophoresis (PAGE) by comparison of the chain lengths with those of DNA sequencing reactions produced with the same primers.

Production and purification of the cloned protein. The gene sequence from *N. meningitidis* BNCV corresponding to the mature protein after removal of its lipoprotein signal sequence was amplified by PCR. Oligonucleotide primers used were ctggatcCGCAACCAAAAGCAACGTCAAAGC (shown 5′ to 3′, where the last 24 bases correspond to the coding strand of the BNCV sequence, i.e., bases 65 to 88, and the first 7 bases provide a site for cleavage by restriction endonuclease *Bam*HI) and ttgaattcTGGCGGATACGTTTCGGTTTTGC (shown 5′ to 3′, where the last 23 bases correspond to the noncoding strand of the BNCV sequence, i.e., bases 511 to 489, and the first 8 bases provide an *Eco*RI cleavage site). The PCR product was inserted into the expression vector pGEX-1λT, and the protein was expressed in *E. coli* XL1-Blue MRF′ as a fusion protein with glutathione *S*-transferase (GST) at its N-terminal end, linked by a short amino acid sequence which is a cleavage site for thrombin.

Production of the fusion protein was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG; 0.1 mM final concentration in 200 ml) to *E. coli* in Luria-Bertani medium. The cells were pelleted and then lysed by mild sonication at 0°C in 20 ml of a solution containing 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, and 0.5% (vol/vol) Nonidet P-40. Purification of the cloned protein from the cell lysate was achieved (as recommended by Pharmacia) by affinity chromatography on a glutathione-Sepharose 4B column, followed by cleaving of the linkage by thrombin. The GST was removed by another passage through a glutathione-Sepharose column. The yield of the neisserial protein was 8 mg.

Production of rabbit antisera. Antisera were prepared by Hazelton Laboratories, Denver, Pa., in two New Zealand White rabbits. The rabbits received one immunization of 250 μg of the purified protein in Freund's complete adjuvant on day 1 and then an additional three immunizations of antigen in incomplete adjuvant on days 24, 43, and 64 and were bled on day 90. The titers of the rabbit sera, measured against membranes from BNCV and against purified recombinant protein, increased by 50- and 5,000-fold, respectively, after the immunizations.

Immunological techniques. Western blotting was performed as described previously (2), after separation of proteins on a 12.5% polyacrylamide gel with the buffer system of Laemmli (10). The rabbit antiserum was used at a dilution of 1/10,000, and the alkaline phosphatase-conjugated goat anti-rabbit antiserum (TAGO Inc.) was used at a dilution of 1/3,000.

Complement-dependent bactericidal assays were performed by the following procedure, using rabbit antisera decomplemented by heating at 56°C for 30 min and serum from a volunteer as a complement source. Bacteria were grown in liquid medium to the mid-exponential phase, harvested by centrifugation, and resuspended to give an optical density at 600 nm of 1.0. The bacteria were then diluted 10^5-fold in Wong medium (23) containing 250 μM $CaCl_2$ but lacking glucose. Bactericidal reactions were carried out in tubes containing 100 μl of the bacterial suspensions, 600 μl of Wong medium-$CaCl_2$, 100 μl of rabbit antiserum, and 200 μl of the complement source or of decomplemented serum. Samples (100 μl) were taken in triplicate and spread onto GC agar plates immediately after addition of the complement source and again after 30 min of incubation at 37°C. Killing was expressed as 100 minus the percentage of surviving bacteria, relative to decomplemented controls.

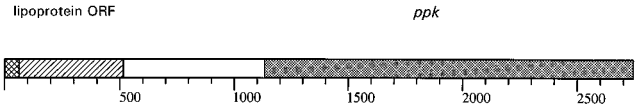

FIG. 1. Map of the λgt11 clone containing some of the *ppk* gene and an ORF which could code for a lipoprotein. Distances are shown in base pairs. The *ppk* gene is stippled. The ORF which could code for a lipoprotein (bp 1 to 516) is shaded, and the region coding for the putative signal sequence is indicated by additional cross-hatching.

RESULTS

Cloning of the regions of gonococcal and meningococcal chromosomes containing the gene for the lipoprotein. The sequence upstream of the *ppk* gene of *N. meningitidis* BNCV was obtained from a clone in λgt11 (20a). Immediately at the 5′ end of this clone, the sequence predicted a product with a signal sequence characteristic of a lipoprotein, but the clone did not include an ATG initiation codon or the promoter region (Fig. 1). Therefore, it was decided to clone this portion of the genome. Southern blots of *Eco*RI-digested chromosomal DNA from *N. gonorrhoeae* F62 and MS11 and from *N. meningitidis* BNCV and M1080, reacted with probes derived from the lipoprotein gene sequence, showed in each case a band with a size of 6.5 kb (data not shown). The fragments from strains F62 and BNCV were cloned into the vector pBluescript.

By using these clones, the areas of interest were sequenced on both strands. An initial sequence was obtained with primers based on the sequence of the λgt11 clone of BNCV. From this information, primers were designed to allow extension of the sequence determination further in the 5′ direction and then to sequence the other strand. The same area of the genome of M1080 was also sequenced, by use of the same primers and a PCR product as template. The sequences obtained are shown in Fig. 2. The sequences showed the presence of the ATG translational initiation codon and sequences characteristic of a canonical promoter region and ribosome binding site. Of interest is that the sequences are almost identical in two strains of meningococcus and one gonococcal strain. Thus, the DNA sequences clearly indicated the presence of a gene for a lipoprotein not previously reported in the pathogenic neisseriae.

Cloning and expression of the lipoprotein gene from *N. meningitidis* BNCV. To facilitate the characterization of the novel lipoprotein, it was decided to raise a specific antiserum to this antigen. PCR was used to copy a defined sequence from the lipoprotein gene to enable the production of large amounts of recombinant protein from an expression vector. Oligonucleotides, based on the known sequence of the region upstream of the *ppk* gene, were used to amplify a region of DNA, coding for the mature, predicted neisserial lipoprotein from chromosomal DNA of *N. meningitidis* BNCV, which was inserted in the expression vector pGEX-1λT. The neisserial protein was expressed in *E. coli* as a GST fusion protein and was purified by glutathione-Sepharose affinity chromatography. The lipoprotein was cleaved from the GST fusion protein by thrombin and purified again by glutathione-Sepharose chromatography (Fig. 3). The protein, predicted to consist of the mature lipoprotein with its N-terminal cysteine replaced by GlySer and an additional AsnSerSer at its C-terminal end, was used to prepare rabbit antisera.

The antisera were used to probe Western blots (immunoblots) of gonococci and meningococci (Fig. 4). Reactive bands with apparent molecular masses of 19 kDa were seen in the membrane fraction of the meningococci but were absent from all gonococci tested. This was surprising in view of the apparent conservation of the gene between the species.

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            -150                              -120
             *                                 *
F62          ATTCCGTATGCCGTACAAATACACCGCGTTTA**CTGATA**CGCACGTTTTTTATGC**TAATAT**
M1080        ATTCCGTATGCCGTACAAATACACCGCGTTTA**TTGATA**CGCACGTTTTTTATGC**TAATAT**
BNCV         ATTCCGTATGCCGTACAAATACACCGCGTTTA**CTGATA**CGCACGTTTTTTATGC**TAATAT**

            -90                               -60
             *                                 *
F62          TACGAAACAAAATCAAACGTTTAAAACTCTCCTGATGCGGCTCTTCCGAACAAAAGGCAG
M1080        TACAAACCAAAATCAAATGTTTAAAACTCTCCTGATGCGGCTCTTCCGAACAAAAGGCAG
BNCV         TACAAACCAAAATCAAATGTTTAAAACTCTCCTGATGCGGCTCTTCCGAACAAAAGGCAG

            -30                -10       +1
             *                  *         *
F62          ACGGGCATCGGGTAAA**AAAGG**ATTCTGCAT**ATG**AAAATCAAACAAATCGTCAAA
M1080        ACGGGCATCGGGTAAA**AGAGG**ATTCTGCAT**ATG**AAAATCAAACAAATCGTCAAA
BNCV         ACGGGCATCGGGTAAA**AGAGG**ATTCTGCAT**ATG**AAAATCAAACAAATCGTCAAA
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FIG. 2. Sequence upstream of neisserial lipoproteins, showing transcription start site for BNCV and putative recognition sequences. Sequences were determined from both strands of the cloned *Eco*RI fragments containing the gonococcal and meningococcal lipoprotein genes (F62 and BNCV). To obtain sequence information from M1080, primers were designed on the basis of sequence information from BNCV (extending further upstream and downstream than that shown in the figure). PCR was used to amplify this region from the chromosome of M1080, and the sequence was determined directly from the PCR product. The adenosine of the ATG translation initiation codon, shown in bold type, is numbered +1. It is preceded by a purine-rich sequence, the presumed ribosome binding site. Primer extension experiments located the start site (underlined) of the message from strain BNCV and allowed the assignment of putative −10 and −35 signals (bold type) for initiation of transcription. The positions of the presumed signals in the DNA of F62 and of M1080 are also shown. The assignation of transcription initiation signals and RNA start site is based on experimental evidence shown in Fig. 6.

The surface exposure and vaccine potential of this protein in meningococci were examined by determining whether it could serve as a target for antibody and complement-mediated bacteriolysis. The rabbit antisera did not have bactericidal activity at final dilutions of 1/10, 1/100, 1/500, or 1/2,500 in the presence of human complement (data not shown). Hence, it was concluded that the protein is not surface exposed and is unlikely to have potential as a vaccine component.

Comparison of the lipoprotein genes from various strains of *N. gonorrhoeae* and *N. meningitidis*. To explain the discrepancy between the apparent similarity of this region of the chromosome in the neisserial strains tested and the absence of the lipoprotein from the gonococci, sequencing of the gene from several strains of *Neisseria* was performed directly on DNA fragments produced by PCR from chromosomal DNA (6). The strains used, meningococcus strains BNCV and M1080 and gonococcus strains MS11, UU1, R10, and F62, were isolates taken at different times and from different places in the United States. The meningococcal sequences were very similar (Fig. 5), containing ORFs with lengths of 516 bp which code for proteins with a size of 19.2 kDa (17.1 kDa after removal of the 21-amino-acid signal sequence). The gonococcal sequences were similar to those of the meningococci. However, the insertion of 1 bp at position 74 of the gene results in a frame shift relative to the meningococcal reading frame, and the ORFs of all of the gonococcal sequences terminate at the same point approximately 100 bp later. Primer extension experiments demonstrated a site for initiation of transcription (in strain BNCV) at bp −85 relative to the ATG translational start site (Fig. 2 and 6).

DISCUSSION

Meningococci contain a gene encoding a lipoprotein, situated approximately 600 bp upstream of the *ppk* gene, which has

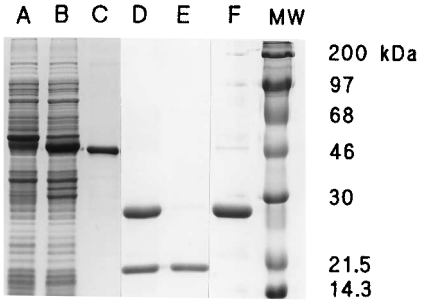


FIG. 3. Purification of the lipoprotein-GST fusion protein and cleavage to release the recombinant lipoprotein. Portions of the fractions obtained during purification of the recombinant neisserial lipoprotein were subjected to SDS-PAGE and staining with Coomassie blue. Lanes: A, whole-cell lysate of *E. coli* XL1-Blue containing recombinant plasmid in the absence of inducer; B, *E. coli* containing recombinant plasmid induced with IPTG; C, lipoprotein-GST fusion purified by glutathione affinity chromatography; D, purified fusion protein after digestion with thrombin; E, purified lipoprotein after removal of GST by a second passage through the glutathione-Sepharose column; F, GST eluted from the affinity column; MW, molecular mass marker proteins (Rainbow markers; Amersham).

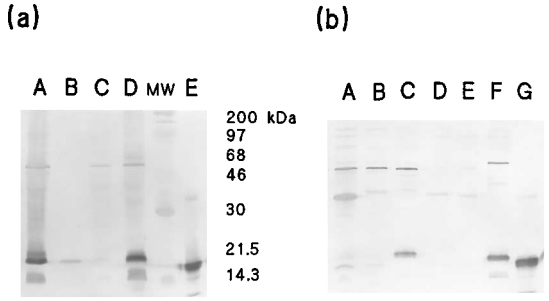
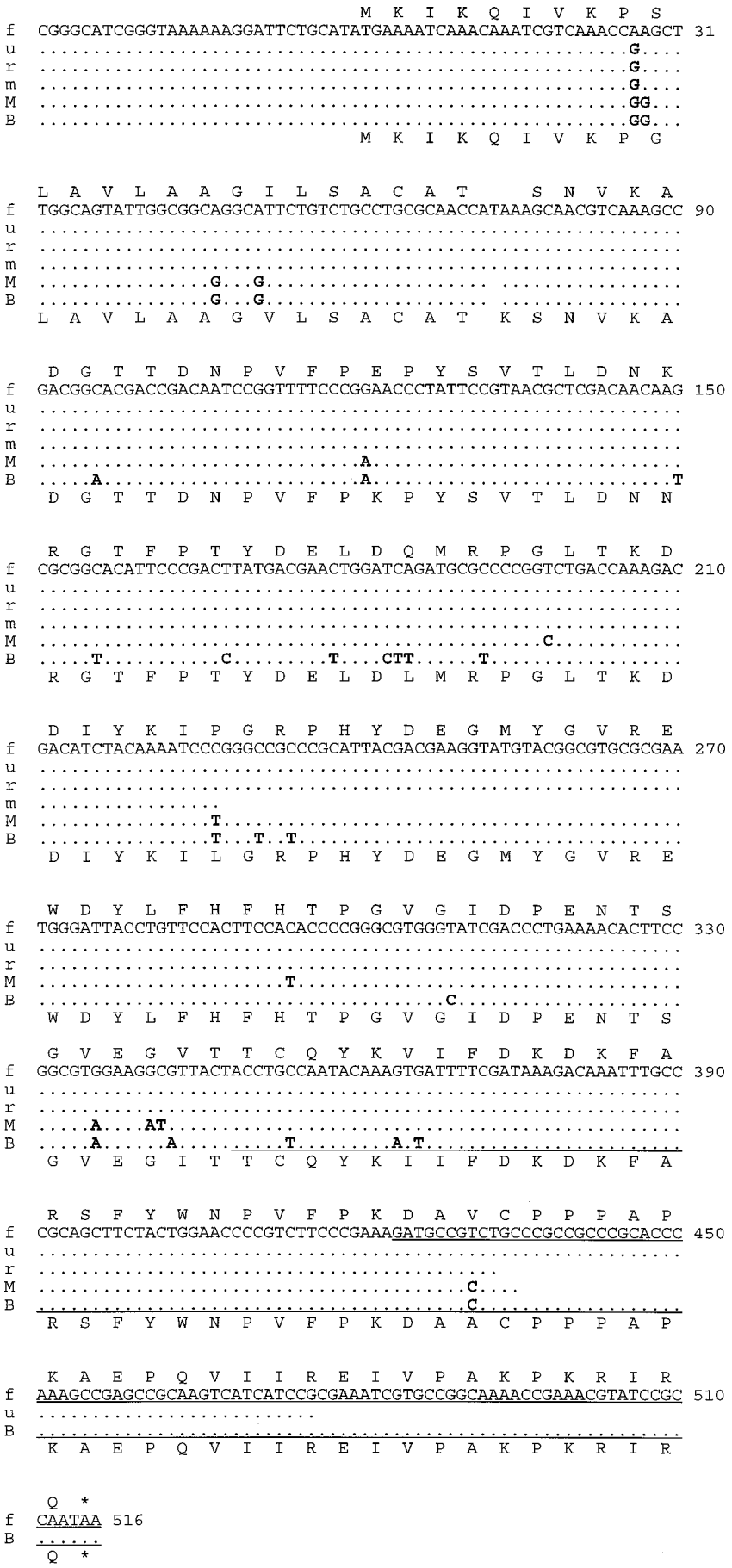


FIG. 4. Western blots of gonococci and meningococci with rabbit anti-lipoprotein antiserum. Rabbit antiserum to the recombinant neisserial lipoprotein was used to probe Western blots of cellular fractions of meningococcus BNCV (a) and of whole-cell lysates of meningococci M1080 and BNCV and gonococci F62, MS11, UU1, and R10 (b). Lanes in panel a: A to D; BNCV whole cells (lane A), periplasm (lane B), cytoplasm (lane C), and membrane fraction (lane D); E, purified recombinant lipoprotein from *E. coli*; MW, molecular mass marker proteins (Rainbow markers; Amersham) whose positions are indicated in kilodaltons. Lanes in panel b: A to F; whole-cell lysates of strains F62 (lane A), MS11 (lane B), M1080 (lane C), UU1 (lane D), R10 (lane E), and BNCV (lane F); G, purified recombinant lipoprotein from *E. coli*.


M K I K Q I V K P S
f CGGGCATCGGGTAAAAAAGGATTCTGCATATGAAAATCAAACAAATCGTCAAACCAAGCT 31
u .....G....
r .....G....
m .....G....
M .....GG...
B .....GG...
M K I K Q I V K P G
L A V L A A G I L S A C A T S N V K A
f TGGCAGTATTGGCGGCAGGCATTCTGTCTGCCTGCGCAACCATAAAGCAACGTCAAAGCC 90
u .....
r .....
m .....
M .....G...G..... .....
B .....G...G..... .....
L A V L A A G V L S A C A T K S N V K A
D G T T D N P V F P E P Y S V T L D N K
f GACGGCACGACCGACAATCCGGTTTTCCCGGAACCCTATTCCGTAACGCTCGACAACAAG 150
u .....
r .....
m .....
M .....A.....
B .....A.....T
D G T T D N P V F P K P Y S V T L D N N
R G T F P T Y D E L D Q M R P G L T K D
f CGCGGCACATTCCCGACTTATGACGAACTGGATCAGATGCGCCCCGGTCTGACCAAAGAC 210
u .....
r .....
m .....
M .....C.....
B .....T.....C.....T....CTT.....T.....
R G T F P T Y D E L D L M R P G L T K D
D I Y K I P G R P H Y D E G M Y G V R E
f GACATCTACAAAATCCCGGGCCGCCCGCATTACGACGAAGGTATGTACGGCGTGCGCGAA 270
u .....
r .....
m .....
M .....T.....
B .....T...T..T.....
D I Y K I L G R P H Y D E G M Y G V R E
W D Y L F H F H T P G V G I D P E N T S
f TGGGATTACCTGTTCCACTTCCACACCCCGGGCGTGGGTATCGACCCTGAAAACACTTCC 330
u .....
r .....
M .....T.....
B .....C.....
W D Y L F H F H T P G V G I D P E N T S
G V E G V T T C Q Y K V I F D K D K F A
f GGCGTGGAAGGCGTTACTACCTGCCAATACAAAGTGATTTTCGATAAAGACAAATTTGCC 390
u .....
r .....
M .....A....AT.....
B .....A.....A.....T.....A.T.....
G V E G I T T C Q Y K I I F D K D K F A
R S F Y W N P V F P K D A V C P P P A P
f CGCAGCTTCTACTGGAACCCCGTCTTCCCGAAAGATGCCGTCTGCCCGCCGCCCGCACCC 450
u .....
r .....
M .....C....
B .....C.....
R S F Y W N P V F P K D A A C P P P A P
K A E P Q V I I R E I V P A K P K R I R
f AAAGCCGAGCCGCAAGTCATCATCCGCGAAATCGTGCCGGCAAAACCGAAACGTATCCGC 510
u .....
B .....
K A E P Q V I I R E I V P A K P K R I R
Q *
f CAATAA 516
B .....
Q *

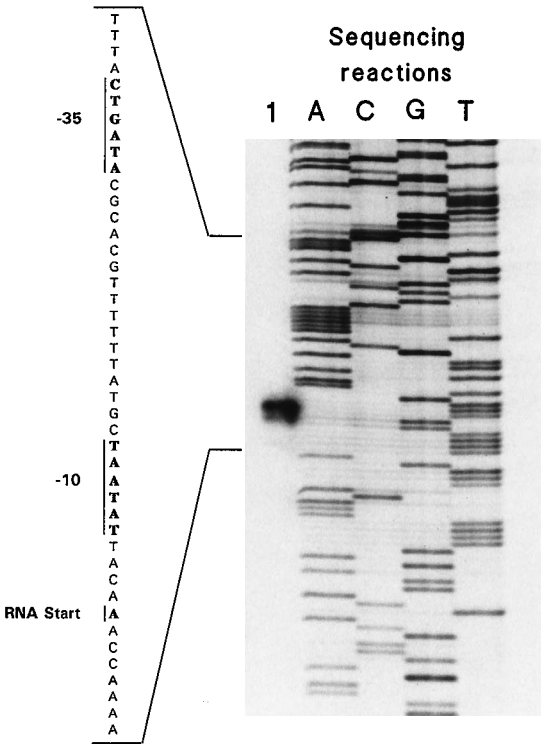


FIG. 6. Location of the start of the transcript coding for the lipoprotein in BNCV. A ^{32}P-end-labelled oligonucleotide corresponding in sequence to the noncoding strand of the lipoprotein gene (from position 45 to position 29) was hybridized to RNA extracted from BNCV, and the DNA chain was extended to the 5′ end of the RNA transcript by reverse transcriptase. The labelled DNA (primer extension product) was electrophoresed on a sequencing gel (lane 1) together with sequencing reactions (lanes A, C, G, and T) with the same primer. The sequence shown to the left of the autoradiograph is (reading from top to bottom) the reverse complement of the sequenced DNA, which is, therefore, the sequence of the coding strand of the gene. The positions of the putative −35 and −10 regions and of the initiation of transcription are marked by vertical lines.

not been described previously. The composition of the mature protein is 45% hydrophobic amino acids, consistent with its location in the membrane fraction of meningococci. The N-terminal portion of the protein in meningococci shows homology to a 31-kDa antigen from *H. somnus* (Fig. 7). The antigen from *H. somnus* also shows homology, in its C-terminal region, to the Rmp (PIII and class 4) proteins from the pathogenic *Neisseria* species (5, 9) and to the homologous portion of OmpA proteins from enterobacteria. In addition to the obvious homologies in Fig. 7, two other features are of interest. The lipoprotein sequence contains a proline-rich region PPP APKAEPQVII (amino acids 146 to 158) which has homology to the PIII sequence (AVPEPEPAPVAVV; amino acids 68 to 80) but is absent in the *H. somnus* antigen. Also, a potential disulfide loop is conserved between the lipoprotein (defined by cysteines 118 and 145) and the *H. somnus* antigen (cysteines 106 and 128).

The difference between the gene sequence in gonococci and that in meningococci is striking. The gonococcal sequences were very similar to those of the meningococci, except for the insertion of 1 bp at position 74, causing premature termination of the gonococcal ORFs with a TGA at position 173. The strains used, meningococcus strains BNCV and M1080 and gonococcus strains MS11, UU1, R10, and F62, were isolates taken at different times and from different places in the United States, supporting the idea that the inactivation of this gene by this particular frameshift mutation is a characteristic of all gonococci. This is particularly remarkable in view of the similarity of the rest of the gene, both translated and (presumed) untranslated portions, to the meningococcal gene. Changes are seen in 28 of 516 bp, mostly in strain BNCV, resulting in the alteration of 25 of 172 codons. Of these, 15 do not change the coded amino acid and 3 change valine for isoleucine. Within the species, the conservation is even more striking. The predicted meningococcal proteins differed by only one amino acid in 171. Of the gonococci, only one strain (F62) differed by 1 bp in its gene sequence, and were it not for the frame shift, the gonococci would code for identical proteins. The similarity in DNA sequence between gonococci and meningococci extends to the upstream region where the promoter for strain BNCV was identified, suggesting that gonococci also produce the transcript.

The possible function of the neisserial lipoprotein is open to investigation. The results of this study suggest that the frameshift mutation is a characteristic of the gonococcal species in which it differs from the closely related meningococcus. If ceasing to produce this protein was a gonococcal adaptation to growth in the laboratory, it is improbable that each strain would have the identical mutation. If the lack of this protein was an evolutionary change, the extreme conservation of the mostly untranslated gonococcal gene is surprising, since conservation of genetic information might be expected to reflect utility of the gene product. However, cases in which premature termination of translation is avoided by genetically programmed ribosomal frame shifting have been described previously (discussed by Levin et al. [11]). It would be interesting to determine whether conditions exist under which gonococci might turn on production of a protein by such a mechanism.

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FIG. 5. Comparison of the sequences of lipoprotein genes from various *Neisseria* strains. Abbreviations: f, u, r, and m, *N. gonorrhoeae* F62, UU1, R10, and MS11, respectively; M and B, *N. meningitidis* M1080 and BNCV, respectively. Sequences of strains UU1, R10, and M1080 were determined directly from both strands of the PCR products to the extent shown in the figure. The sequence of strain MS11 was determined directly from one strand of a PCR product, in one direction only. Sequences from F62 and from BNCV were determined by direct sequencing of the PCR product and were also obtained by sequencing both strands of the cloned *Eco*RI fragment encompassing this region. Where these data overlapped, the sequences determined by PCR were identical to those determined by plasmid sequencing. The 3′ ends of the sequences from plasmid sequencing only are underlined. The translation above the sequences is of F62, and that below is of BNCV. Numbering of bases starts from the A of the ATG translation start codon in BNCV. Regions in which sequences are identical to that of F62 are shown by periods. Bases which differ from those of F62 are shown in bold type. Note also the insertion of a thymidine residue at position 74 in the gonococcal sequences which is absent from the meningococcal sequences. To emphasize the similarity between the gonococcal and meningococcal sequences, translation of the gonococcal sequence is shown as if this T were not present. In actuality, the gonococcal ORFs terminate with TGA at position 173. The amino acid sequence of the predicted protein is MKIKQIVKPSLAVLAAGIL SACAT**IKQRQSRRHDRQSGFPGTLFRNARQQARHIPDL**, where residues in bold type differ from those shown in the figure as a result of the frame shift at position 74. The two meningococcal sequences predict identical proteins, with the single exception being the changes at bases 341 and 342 (in M1080), which results in the coding for aspartate in place of glycine.

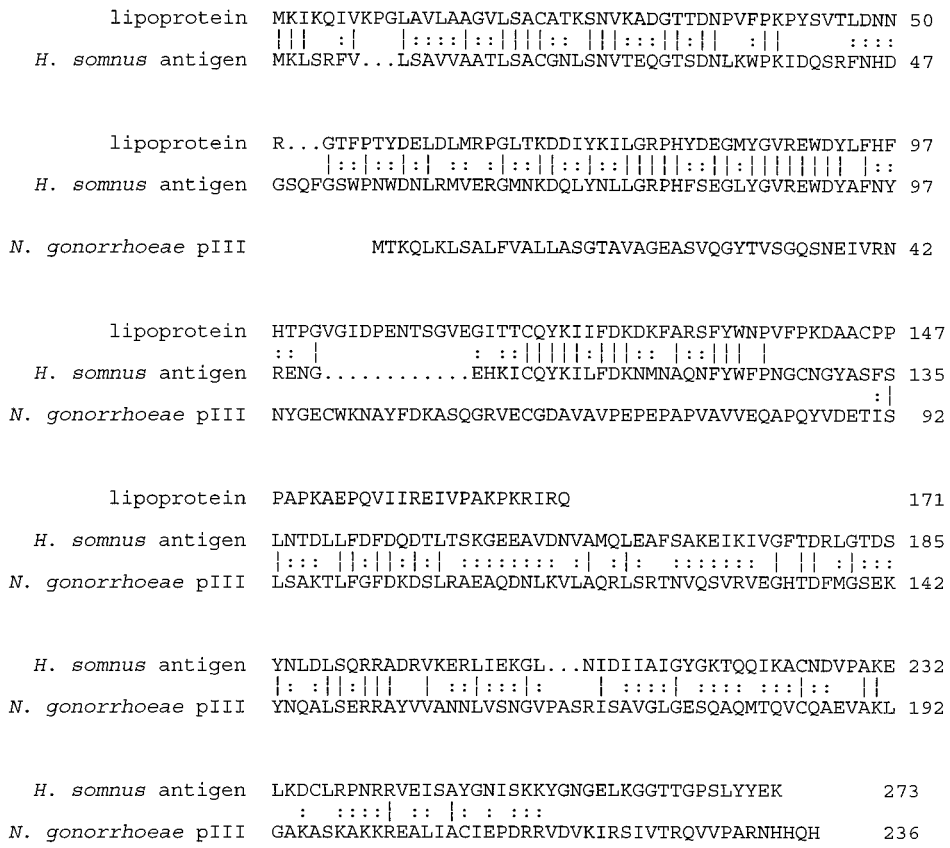

FIG. 7. Comparison of the sequences of the neisserial (BNCV) lipoprotein, the 31-kDa antigen from *H. somnus*, and protein III from *N. gonorrhoeae*. Sequence comparisons were performed by the program fasta as described by Pearson and Lipman (14). Lines indicate identical amino acids; colons show conservative changes.

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