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A Neuraminidase from *Streptococcus pneumoniae* Has the Features of a Surface Protein

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A gene from *Streptococcus pneumoniae* (*nanA*), with features entirely consistent with a neuraminidase gene, has been sequenced. High levels of neuraminidase activity were obtained after cloning of this gene, without flanking sequences, into a high-expression vector. RNA hybridization studies have shown that the gene is transcribed by a virulent pneumococcus strain. The predicted molecular weight of the protein and certain amino acid sequences are typical of other neuraminidases. NanA contains the four copies of the sequence SXDXGXTW that is present in all the bacterial neuraminidases previously described. Kyte and Doolittle analysis showed that NanA is a hydrophilic protein with hydrophobic domains at the N terminus and the C terminus. A putative signal peptide was found in the N terminus of this protein, indicating that the protein is exported from the pneumococcus. The C terminus has the features of the anchor motif found in other surface proteins from gram-positive bacteria. Electron microscopy studies showed the presence of neuraminidase associated with the cell surface of the pneumococcus.

Streptococcus pneumoniae is one of the major causes of lower respiratory tract infections, septicemia, and meningitis in humans (3). It produces several factors with properties that suggest that they could play a role in the virulence of this organism (6). One of these factors is a neuraminidase, an enzyme responsible for the cleavage of *N*-acetylneuraminic acid (NANA) from mucin, glycoproteins, and gangliosides (17, 37).

Although the precise role of the neuraminidase in pathogenesis has not been established, neuraminidase treatment of mucus could decrease its viscosity, thereby enhancing the colonization of the underlying tissues by the pneumococcus (37). Furthermore, removal of NANA by the neuraminidase could expose surface receptors for the pneumococcus (1).

There are some experimental data supporting the idea of a role in virulence for pneumococcal neuraminidase. All clinical isolates of the pneumococcus tested were positive for the production of this enzyme (19, 26). In patients with pneumococcal infection there was a direct relationship between the level of NANA in cerebrospinal fluid and the development of coma and an adverse outcome (26). Intracerebral inoculation of mice with purified neuraminidase caused neurological signs and death (18). Mice immunized with purified neuraminidase were partially protected against pneumococcal infection (21). However, intrathecal inoculation of the enzyme in dogs did not produce any symptoms (27).

Biochemical studies of pneumococcal neuraminidase have revealed a range of molecular weights and have led to controversy over the number and size of the neuraminidase(s) produced by the pneumococcus (22, 37, 40–42). Molecular studies have begun to resolve this controversy. The pneumococcus appears to have at least two neuraminidase genes which are unrelated at the DNA level. Cloning of both of these genes has been reported, and the two cloned sequences have been shown to be present in all pneumococcal strains tested (5, 7, 8). This paper describes the characterization of one of these cloned genes (8) and reveals that the gene product has a molecular weight consistent with it being a member of the "large" family (32) of bacterial neuraminidases.

There has been uncertainty as to the location of the pneumococcal neuraminidase (21). In this paper we provide evidence supportive of pneumococcal neuraminidase being a surface protein. Electron microscopy showed that the pneumococcal neuraminidase is a surface protein, and the sequence of NanA was consistent with this location. NanA has an N-terminal signal peptide, while the C terminus has a sequence with features of the anchor motif found in surface proteins from other gram-positive bacteria. We believe that this is the first time that this motif has been found in a pneumococcal protein.

MATERIALS AND METHODS

Bacterial strains and plasmids. The neuraminidase gene (*nanA*) was isolated previously from a gene bank of pneumococcal DNA from the nonencapsulated strain R36A (NCTC 10319) (8), derived from a type II capsular serotype strain.

RNA was purified from a virulent type III pneumococcal clinical isolate (GB05B). RNA from *Escherichia coli* LE392 (23) was used as a negative control in RNA dot blot hybridization experiments.

E. coli JM101 (47) SG13009::pREP4 and M15::pREP4 from a QIAexpressionist kit (QIAGEN, Hilden, Germany) were used as recipients for transformation. In these strains pREP4 carries the *lacI* gene encoding of the *lac* repressor. The vectors used for sequencing were pJDC9 (9), M13mp18 and M13mp19 (47), and pQE30 (QIAexpressionist; QIAGEN). Neuraminidase expression was studied by using pQE30.

DNA sequencing and analysis. Double-stranded plasmid DNA and single-stranded bacteriophage DNA templates were prepared as described before (23). M13 (single-stranded) and pJDC9 and pQE30 (double-stranded) DNA sequencing was done with the Sequenase Version 2.0 kit (U.S. Biochemical,

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Cleveland, Ohio) following the manufacturer's instructions. Both DNA strands were sequenced by using appropriate oligonucleotide primers.

DNA sequence analysis was performed with the University of Wisconsin Genetics Computer Group Sequence Analysis Package Version 6.2 (11).

PCR cloning of the predicted neuraminidase structural gene (ORF1) from pMC4170. The full-length predicted neuraminidase structural gene (bp 1284 to 4343) (see Fig. 2) encompassing the first putative ATG translation start codon, associated with a possible Shine-Dalgarno sequence, to the end of ORF1, was amplified by PCR from pMC4170 by using the following primers: primer A, 5'-AGAGGAACGGATCCAATCGGAG TGTTCAAGAAC-3' *Bam*HI site underlined); primer B, 5'-T TAGAATTGTCGACTTATTGTTCTCTCTTTTTCC-3' (*Sal*I site underlined).

The amplified DNA sequence was cloned into the expression vector pQE30 after restriction digestion with *Bam*HI and *Sal*I. The new recombinant, carrying ORF1, was transformed into *E. coli* M15::pREP4 and SG13009::pREP4. Preparation of crude protein extracts from these recombinants was done according to the manufacturer's instructions (QIAexpressionist; QIAGEN).

Assay of neuraminidase activity. Neuraminidase activity was assayed fluorometrically by mixing an equal volume of sample and 0.35% (wt/vol) of the fluorogenic substrate 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUAN) (Sigma) as previously described (22). The reaction mixture was incubated for 5 min at 37°C, and the reaction was stopped by the addition of 2 ml of 50 mM sodium carbonate buffer, pH 9.6. Fluorescence resulting from the release of 4-methylumbelliferone from MUAN was detected by using a Perkin-Elmer LS2B fluorimeter at an excitation wavelength of 366 nm and an emission wavelength of 446 nm.

Purification of RNA from the pneumococcus. RNA was purified from pneumococcal strain GB05B. A starter culture of this organism (1.26×10^8 bacteria) was inoculated into 400 ml of prewarmed brain heart Infusion (Oxoid) at 37°C. Samples (40 ml) were taken at 5 and 6 h of growth, centrifuged at 3,000 rpm for 5 min at 5°C in a Heraeus-Christ centrifuge, and resuspended in phenol–0.2 M sodium acetate, pH 5.2, at 60°C; 0.8 ml of a solution containing 0.02 M sodium acetate, pH 5.2, 0.5% (wt/vol) sodium dodecyl sulfate, and 1 mM EDTA was then added. After incubation with periodic shaking for 5 min at 60°C, samples were left on ice for a further 10 min and then centrifuged at 13,000 rpm for 5 min in an MSE MicroCentaur bench-top microcentrifuge. The top phase was transferred to a fresh Eppendorf tube, and 0.5 ml of phenol–0.2 M sodium acetate, pH 5.2, at 60°C was added. This was followed by incubation for 5 min at 60°C and a further 10 min on ice. Samples were centrifuged at 13,000 rpm for 5 min in the bench-top microcentrifuge, and top phases were subjected to chloroform extraction and precipitation with 2.5 volumes of ethanol. RNA pellets were resuspended in 40 μl of H_2O (treated with diethyl pyrocarbonate), and concentrations were determined as already described (23).

Dot blot hybridizations. RNA was blotted on to Hybond-N$^+$ nitrocellulose filters (Amersham), using a Bio-Dot microfiltration apparatus (Bio-Rad) according to the manufacturers' instructions. Blotted samples were hybridized with ^{32}P-labelled probes as already described (23).

Cell localization of pneumococcal neuraminidase. Cell localization of the neuraminidase was done by immunogold staining and electron microscopy with strain GB05B. Bacteria were grown for about 5 h before being harvested and fixed for 30 min in 0.5% (wt/vol) glutaraldehyde in 30 mM cacodylate buffer, pH 7.5, containing 5 mM $MgSO_4$. Fixed samples were washed in cacodylate buffer and then embedded in 10% (wt/vol) gelatin. Samples then were dehydrated through a series of alcohols and infiltrated within Lowicryl HM23 resin according to the manufacturer's instructions (Agar Scientific, Cambridge, United Kingdom); this was followed by UV polymerization. Sections, 70 nm thick, were cut on a Reicherf Ultracut OMU4 microtome. Cut sections were incubated for 10 min in phosphate-buffered saline, pH 7.0, containing 0.1% (vol/vol) Tween 20 (PBST) and 1% (wt/vol) bovine serum albumin (BSA) and then for 20 min in PBST containing 0.5% (wt/vol) gelatin. Sections then were incubated for 90 min at 4°C in rabbit antineuraminidase serum (5) diluted 1:100 in PBST containing 1% (wt/vol) BSA. Next, sections were washed five times with PBST and then incubated for 90 min in goat anti-rabbit immunoglobulin G–10 nm gold conjugate (1:100) (Amersham), as previously described (4). Following this procedure, the labelled sections were washed three times with PBST containing 1% (wt/vol) BSA and four times with PBST and then fixed with 1% (wt/vol) glutaraldehyde before electron microscopy on a Siemens 102 electron microscope.

The antineuraminidase serum was kindly provided by J. C. Paton, Adelaide Children's Hospital, Adelaide, Australia, and was raised against neuraminidase purified from the pneumococcus (22).

Nucleotide sequence accession number. The nucleotide sequence of pMC4150 has been deposited with EMBL, and the sequence accession number is X72967.

RESULTS

Neuraminidase gene nucleotide sequence determination and analysis. We have previously reported that plasmid pMC2150 (Fig. 1) (8) codes for a product with neuraminidase activity. Three fragments obtained from the digestion of pMC2150 with *Hin*dIII were subcloned into M13mp18 and M13mp19 for nucleotide sequencing. Contiguity of the three subcloned *Hin*dIII fragments was confirmed by determination of the nucleotide sequence encompassing the *Hin*dIII sites in pMC2150, using the appropriate oligonucleotides to prime sequencing reactions. Three open reading frames (ORFs) were predicted (Fig. 1), but the 3' end of the largest one (ORF1) was not present in pMC2150. To determine the entire sequence of ORF1, a DNA fragment was subcloned from the recombinant phage lambda EMBL301-Neu1 (8). A new recombinant, pMC4170, containing a 7.0-kb insert, was selected on the basis of neuraminidase production and its DNA restriction map (Fig. 1). A 2.35-kb *Sph*I-*Xba*I fragment within pMC4170, which overlapped the 3' end of pMC2150, was used to complete the sequencing of ORF1. All DNA was sequenced on both strands (Fig. 1). Features of the sequence of ORF1 and the complete sequences of ORF1, -2 and -3 are shown in Fig. 2.

ORF1 is 3.1 kb and, after analysis of the deduced amino acid sequence, is proposed to represent the neuraminidase gene (*nanA*) within the neuraminidase-producing clone pMC4170. It contains the amino acid sequence SXDXGXTW, where X represents any amino acid. This sequence, known as the "aspartic box", is found in all other reported microbial neuraminidase amino acid sequences (15, 16, 29, 31, 36). This motif is present in NanA in four copies, as in other neuraminidases. It was absent from ORF2 and -3 (Fig. 2 and 3). The distances between some of the aspartic boxes within the bacterial neuraminidases are well conserved. The spacing of the sequences within NanA fell within the expected pattern, as is shown in Fig. 3.

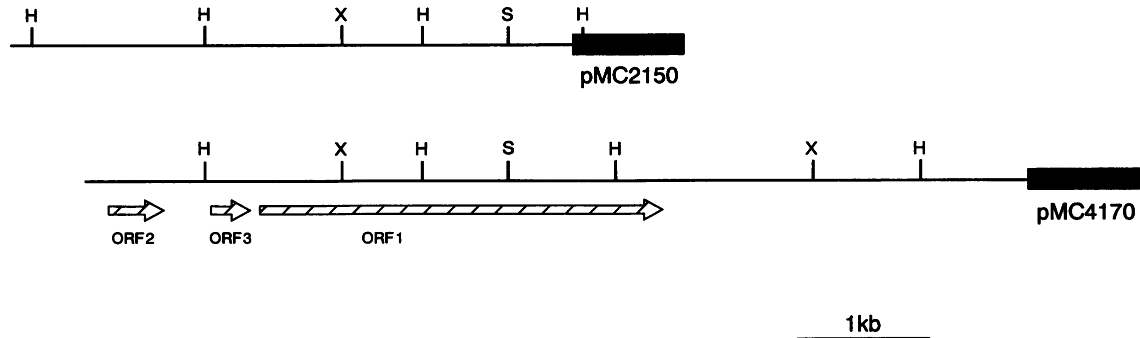

FIG. 1. DNA restriction maps of the neuraminidase clones. Restriction map of cloned pneumococcal DNA in pMC2150 and pMC4170. Restriction endonuclease sites are as follows: H, *Hin*dIII; S, *Sph*I; X, *Xba*I. The solid portions represent vector sequence. Arrows indicate the orientations and locations of ORF1, ORF2, and ORF3.

nanA has three potential translation start codons (ATG). Two of them, at positions 1284 and 1353 of the DNA sequence shown in Fig. 2, are associated with putative Shine-Dalgarno sequences. These start points would give proteins with maximum predicted sizes of 112 and 110 kDa, respectively. The other ATG is located at position 1239. This start point would result in a protein with a maximum predicted size of 114 kDa, but this ATG lacks an obvious Shine-Dalgarno sequence. We have no evidence to indicate which ATG is used. Nevertheless, all these predicted sizes are close to the reported size of a neuraminidase purified from the pneumococcus (22). The predicted molecular weight is at the upper end of the range of reported molecular weights of pneumococcal neuraminidases (22, 37, 40–42).

The DNA sequence shows two putative −10 and −35 promoter regions present at −172 and −218 from the first ATG. Downstream of *nanA* there is a potential rho-independent transcription terminator (Fig. 2).

The largest predicted peptide sequence from *nanA* was used as the basis for subsequent analysis. Kyte and Doolittle analysis (20) of the amino acid sequence showed a hydrophilic protein with two main hydrophobic regions, one at the N terminus, from amino acids 32 to 49, and the other at the C terminus, from amino acids 1013 to 1030 (Fig. 4).

Analysis of the N terminus of the predicted sequence of NanA showed amino acids characteristic of signal sequences (2) (Fig. 2). The features of the sequence are a hydrophobic domain from amino acids 32 to 49 followed by a region rich in basic and neutral amino acids, a proline at amino acid 50, and the signal peptidase cleaving site, VLA, at positions 51 to 53. This signal peptide would be present if transcription started at the ATG at position 1284. The presence of this putative signal sequence predicts that this neuraminidase is exported across the pneumococcal membrane. However, if transcription was from the ATG at 1353, the signal peptide would be truncated.

If the neuraminidase is exported it may become anchored on the cell surface. Analysis of the C terminus identified a region, between amino acids 898 and 998, containing a very high proportion of proline or glycine (27%) and threonine or serine (19%) residues. This region has a group of 20 amino acids contiguously repeated three times (Fig. 2). Closer to the C terminus there is the sequence LPETGN followed by a hydrophobic domain of 18 amino acids, ending with five charged amino acids at the C terminus (Fig. 5). This arrangement closely resembles those in the C-terminal regions of many other gram-positive surface proteins (12).

Upstream of the neuraminidase gene is found a region of DNA with repeated nucleotide sequences. These sequences correspond to the previously described intergenic repeated DNA sequences, named BOX (24). Located 752 bp upstream of *nanA* there are three different BOX sequences, boxA, boxB, and boxC (Fig. 2). boxB, composed of 45 nucleotides, is tandemly repeated four times, whereas boxA and boxC, with 59 and 50 nucleotides, respectively, are present only once. Interestingly, ORF2 spans these BOX elements, starting 4 bp before boxA and finishing 7 bp after boxC, implying that the BOX sequences may not be restricted to intergenic regions of the chromosome of *S. pneumoniae*, as reported previously (24). However, no Shine-Dalgarno or putative promoter consensus sequences have been found for ORF2. The translation start codon for this ORF is GTG (Fig. 2).

Confirmation of neuraminidase gene expression from ORF1. To prove that the neuraminidase activity from pMC4170 was associated with ORF1, we cloned ORF1, without any flanking DNA sequences, into the expression vector pQE30. The cloned sequence contained the two ATGs at positions 1284 and 1353 (Fig. 2), as they are the only ones associated with putative Shine-Dalgarno sequences. The new recombinant, named pQMC1, was transformed into *E. coli* M15::pREP4 and SG13009::pREP4. The correct cloning of ORF1 was confirmed by nucleotide sequence determination of both ends of the inserted pneumococcal DNA in pQMC1. Sonicates of cultures of bacteria harboring pQMC1 were assayed for neuraminidase activity. Both recombinant strains had neuraminidase activity (295,750 fluorimeter units per ml of culture for *E. coli* M15::pREP4::pQMC1 and 190,700 fluorimeter units per ml of culture for *E. coli* SG13009::pREP4::pQMC1). These activities are between four- and sixfold higher than the ones detected in the pneumococcal clinical isolate GB05B (data not shown). These results confirm the prediction of ORF1 as the sequence coding for the neuraminidase activity in pMC4170.

Determination of ORF1-specific mRNA expression and neuraminidase activity in a virulent pneumococcal strain. Although DNA hybridization studies showed that this neuraminidase gene was present in the chromosomes of several pathogenic pneumococcal strains (8), it was not known whether it was transcribed in the pneumococcus. Furthermore, because of the evidence for two neuraminidase genes, the presence of neuraminidase activity from pneumococci was not sufficient evidence that *nanA* was transcribed. Therefore, we investigated ORF1 transcription and production of neuraminidase activity during pneumococcal growth in batch culture. Neuraminidase activity was not seen after 2 h of growth but

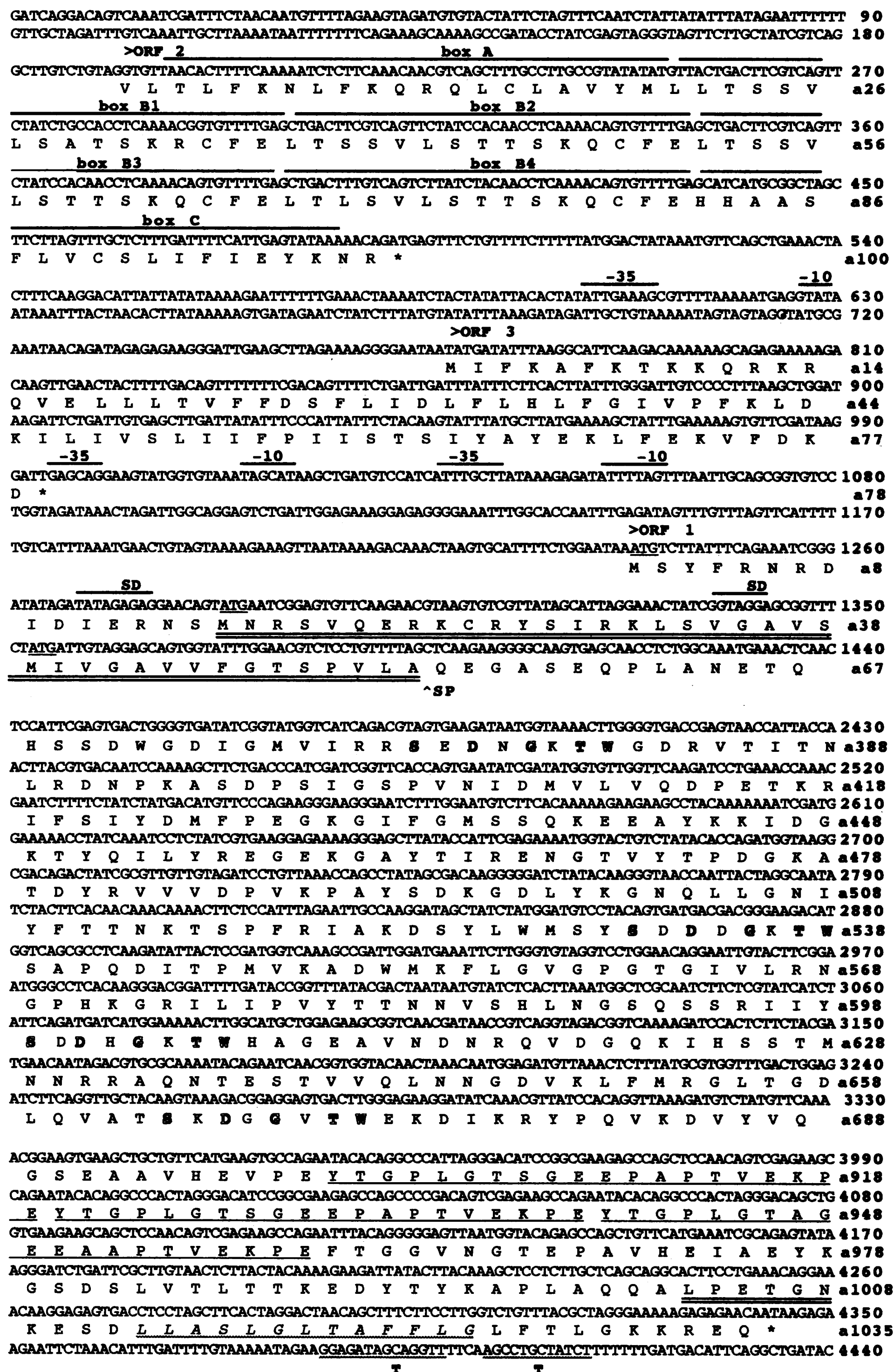

FIG. 2. Features of the nucleotide and deduced amino acid sequences of NanA. Nucleotides and amino acids are numbered on the right of the sequences. The DNA strand is shown 5′ to 3′. The deduced amino acid sequences are written below the nucleotide sequence: ORF1, corresponding to *nanA*, starting at the ATG codon in position 1239; ORF2, starting at the GTG codon in position 193; and ORF3, starting at the ATG codon in position 769. Two putative −35 and −10 promoter sequences and a ribosome binding site (SD) are indicated upstream of ORF1. The wavy line shows the presence of a putative rho-independent transcription terminator for ORF1. A putative signal peptide for ORF1 is double underlined, with a putative signal peptidase site (SP) indicated by an arrowhead. C-terminal repeated amino acid sequences in ORF1 are underlined. The surface anchor motif near the C terminus is double underlined. This is followed by a hydrophobic domain indicated by italics and a wavy line. Neuraminidase aspartic box motifs are shown in outline. DNA BOX elements present near the 5′ end of ORF1 are indicated.

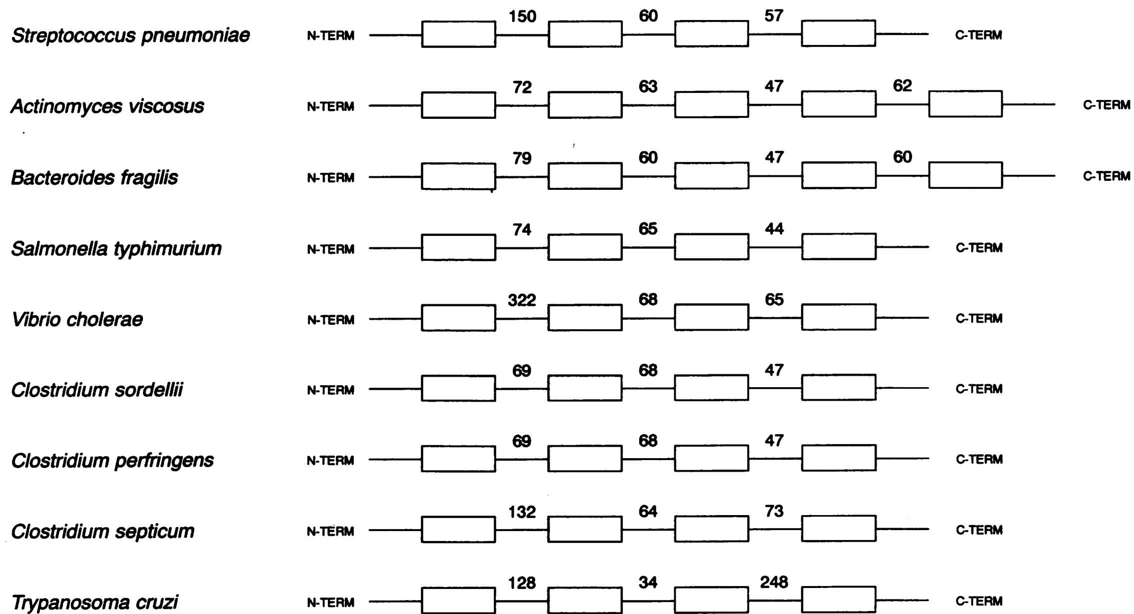

FIG. 3. Presence of aspartic boxes in microbial neuraminidases. The aspartic box consensus motif -Ser-X-Asp-X-Gly-X-Thr-Trp- is represented by a box. The numbers between boxes are the numbers of amino acids between motifs. References are as follows: *A. viscosus* (15); *B. fragilis* (36); *S. typhimurium* (16); *C. septicum* (35); *C. cholerae*, *C. sordellii*, and *C. perfringens* (31); *T. cruzi* (29).

could be detected after 3 h of growth (1,750 fluorimeter units per 10^8 bacteria), and maximum levels of activity were reached after 5 to 6 h (3,710 and 4,602 fluorimeter units per 10^8 bacteria, respectively). Therefore, bacteria were harvested for RNA purification at 5 and 6 h of growth, when the enzyme activity was maximum, just before autolysis occurred.

Purified RNA from these bacteria were subjected to dot blot hybridization. As a negative control, RNA purified from *E. coli* LE392 was used. This blot was hybridized with the PCR product used to construct pQMC1, which corresponds to *nanA* and was also used as a positive control. As shown in Fig. 6, *nanA* DNA hybridized to RNA purified from *S. pneumoniae* grown for 5 and 6 h in batch culture. *nanA* DNA did not hybridize to *E. coli* RNA. These results show that the neuraminidase gene described here is expressed in vitro during the growth of a virulent pneumococcal strain.

Localization of neuraminidase in the pneumococcus. To investigate the location of the neuraminidase, immunogold staining using a polyclonal antineuraminidase serum was done. The antiserum was raised against neuraminidase purified from the pneumococcus. The antiserum reacted with extracts of *E. coli*::pQMC1 in an enzyme-linked immunosorbent assay and immunodot blots but not with the control *E. coli*::pQE30. As shown in Fig. 7, the antineuraminidase serum bound only to the surface of the pneumococcus. No evidence for a cytoplasmic location was seen. These findings support the idea that NanA is located on the surface of the pneumococcus.

DISCUSSION

Three ORFs were found by sequencing of pMC2150 and the *Sph*I-*Xba*I fragment of pMC4170. ORF1 was first considered to encode the neuraminidase in these clones since the predicted amino acid sequence from this ORF contains the aspartic box SXDXGXTW present in other microbial neuraminidases (15, 16, 29, 31, 35, 36). This prediction was corrob-

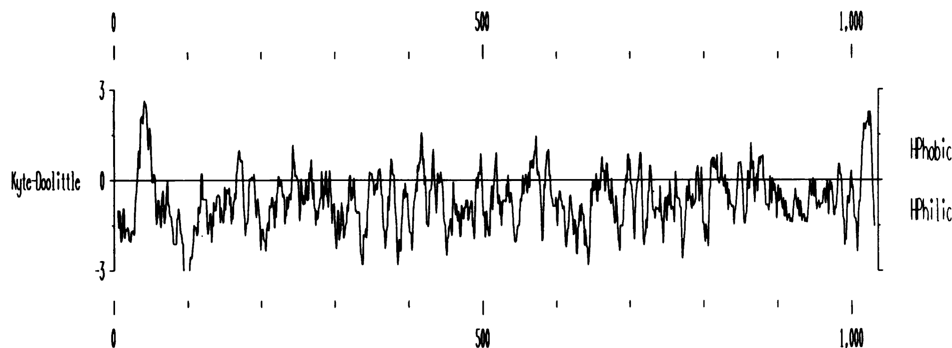

FIG. 4. Hydropathy plot of the NanA protein. The graph was generated by using the standard parameters of Kyte and Doolittle (20) with a window size of 9. The *x* axis represent the position of amino residues starting from the first initiation codon. The *y* axis indicates hydrophilicity or hydrophobicity.

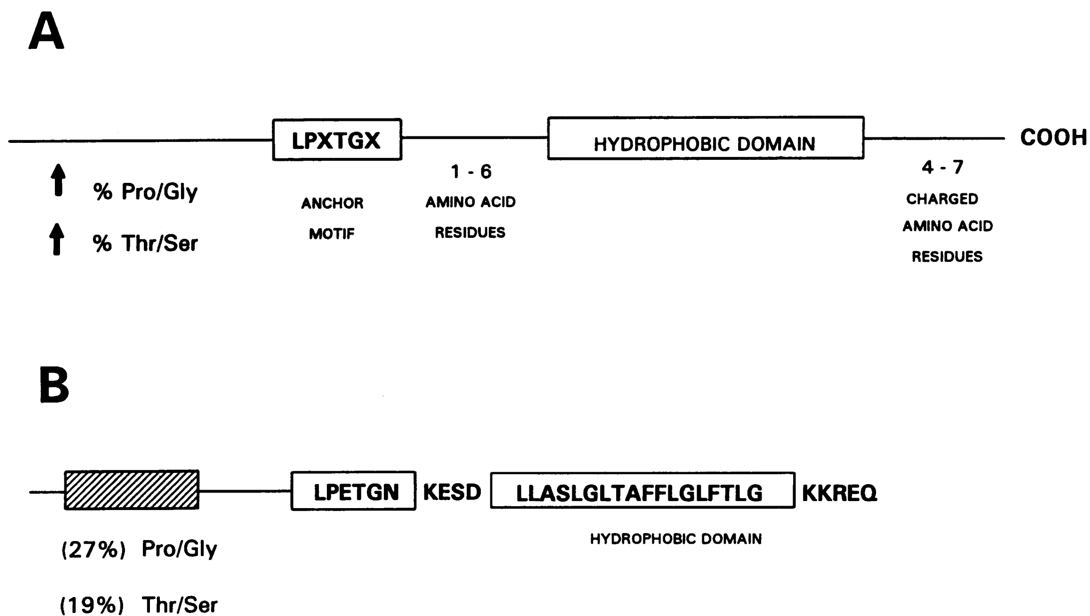

FIG. 5. Presence of a surface protein anchor motif in the NanA predicted amino acid sequence. (A) Consensus structure of a gram-positive bacterial surface protein anchor motif (12). (B) Structure of the C terminus of NanA, resembling a surface anchor motif in gram-positive bacteria. The hatched box indicates the presence of the three 20-amino-acid repeats, within the 100-amino-acid P/G- and T/S-rich region, found near the C terminus of NanA.

orated by the cloning of this ORF via PCR into a high-expression vector and detection of high levels of neuraminidase after this construct was cloned in *E. coli*.

The bacterial neuraminidases have been classified into two groups on the basis of molecular size (32). The family of small enzymes have molecular sizes of around 42 kDa, whereas the large neuraminidases have molecular sizes greater than 60 kDa. The predicted molecular size of NanA of between 110 and 140 kDa suggests that this enzyme is a member of the large family of bacterial neuraminidases.

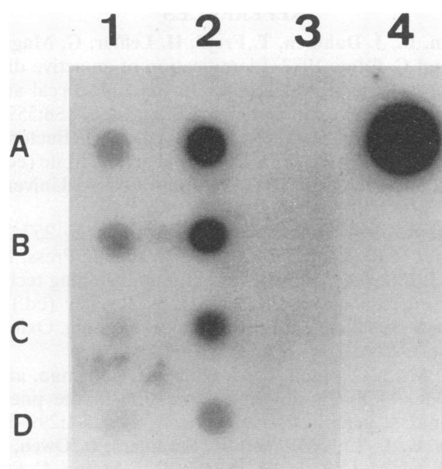

FIG. 6. Dot blot hybridization of RNA from the pneumococcal strain GB05B. Lanes: 1 and 2, RNA purified from *S. pneumoniae* GB05B at 5 and 6 h of growth, respectively; 3, 10 μg of purified RNA from *E. coli*; 4, ORF1 DNA. Rows A to D correspond to the following concentrations of RNA from lanes 1 and 2: A, 10 μg; B, 5 μg; C, 2.5 μg; D, 1.75 μg.

The number of copies of the aspartic box consensus sequence (four) and the distance between some of them were conserved when compared with the other neuraminidases. Such conservation suggests a role for these sequences in the enzymatic activity of the neuraminidase. Analysis of the crystal structure of a neuraminidase from *Salmonella typhimurium* showed that the aspartic boxes lie on the outside of the protein, away from the active site, in this enzyme (10), but it was suggested (10) that these sequences are involved in secretion or protein folding. However, other sequences outside these regions also appear to play a role in activity (33, 44).

An interesting feature of pMC2150 was that an active neuraminidase was produced even though it lacked 233 amino acids from the C terminus. This shows that these amino acids are not required for activity. The observation also supports the notion (22) that the pneumococcal neuraminidases with lower reported sizes are enzymatically active degradation products of a larger enzyme.

Properties of the gene and the predicted amino acid sequence of this neuraminidase allowed us to make a number of predictions about the cellular location of the enzyme. A putative signal peptide has been found in the N terminus of the predicted protein sequence. The structure of this signal peptide is comparable to the consensus structures for signal peptides in proteins from gram-positive bacteria, described previously (13, 30, 44, 45). The presence of such a sequence suggested that the enzyme is exported.

The immunostaining experiments showed that the neuraminidase is a surface protein in the pneumococcus. Unfortunately, the possibility that more than one neuraminidase gene is expressed in the pneumococcus (8) means that the immunostaining data does not provide a definitive answer to the location. This is because the antiserum recognizes the neuraminidase product of the clone of Berry and colleagues (5), in addition to recognizing the neuraminidase described here. Nevertheless, because we have shown that the gene described

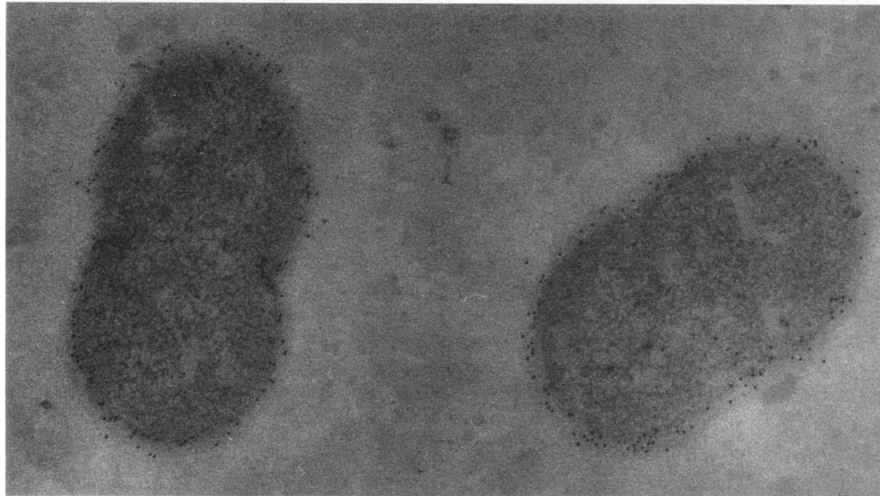

FIG. 7. Electron micrograph of pneumococcal strain GB05B. Bacteria were treated with antineuraminidase serum and then an anti-immunoglobulin G gold probe.

in this paper is expressed in the pneumococcus, the immunostaining data are completely consistent with the conclusion that this neuraminidase has a surface location.

Once exported, the enzyme probably becomes fixed as a surface protein via the C-terminal amino acids. Analysis of the C terminus of NanA revealed the presence of an amino acid sequence typical of membrane anchor motifs found in many other proteins from gram-positive bacteria (12, 25). To our knowledge this is the first time that these sequences have been found in a pneumococcal protein. Such regions have been shown to play a role in the anchoring of proteins in the cell wall of *Staphylococcus aureus* (38, 39).

Although it seems likely that the neuraminidase is a surface protein, some features allow us to propose additional locations. A further possibility is that the exported protein is released into the extracellular environment. This speculation is based on the report that group A streptococcal M protein, which has the anchor sequences, was released from spheroplasts into the medium by the action of a surface protease cleaving in the LPXTGX sequence (28). Thus, a common mechanism of secretion of certain proteins from gram-positive bacteria may exist. In the first stage, the protein is exported and anchored to the cell surface through the anchor motif. In the second stage, the protein would be cleaved by a surface protease and released into the culture medium.

The presence of two possible start codons may allow synthesis of two forms of neuraminidase from a single gene. One of these would have a signal sequence and would be exported, while the other would lack the signal peptide and be retained within the cell. However, no evidence of an intracellular location was provided from the immunostaining experiments. If the production of two neuraminidases occurs in this fashion, the physiological reason is unclear.

Within the bacterial kingdom, neuraminidase appears to be an enzyme of variable location. The presence of a signal peptide is not a universal feature of bacterial neuraminidases. They have been found in the neuraminidases from *Clostridium septicum* (35), *Clostridium sordellii* G12 (34), and *Vibrio cholerae* (43). The neuraminidase from *Actinomyces viscosus* has been reported to have a leader peptide (48). Presumably these enzymes are exported. In at least one of these species, *V. cholerae* (43), the enzyme accumulates extracellularly. The neuraminidase of *Bacteroides fragilis* also is reported to be surface exposed (14). In contrast, a signal peptide has not been found in the enzyme from *Clostridium perfringens* (31) or *S. typhimurium* (16). Further analysis of the *A. viscosus* neuraminidase indicates that it also has a C-terminal membrane anchor. However, in this case the six-amino-acid sequence LSRTGT does not fit the consensus sequence exactly. As before, the reason for different locations in different species is unknown but presumably reflects functional differences.

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