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Respiratory syncytial virus fusion glycoprotein: nucleotide sequence of mRNA, identification of cleavage activation site and amino acid sequence of N-terminus of F_1 subunit

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ABSTRACT

The amino acid sequence of respiratory syncytial virus fusion protein (Fo) was deduced from the sequence of a partial cDNA clone of mRNA and from the 5' mRNA sequence obtained by primer extension and dideoxysequencing. The encoded protein of 574 amino acids is extremely hydrophobic and has a molecular weight of 63371 daltons. The site of proteolytic cleavage within this protein was accurately mapped by determining a partial amino acid sequence of the N-terminus of the larger subunit (F_1) purified by radioimmunoprecipitation using monoclonal antibodies. Alignment of the N-terminus of the F_1 subunit within the deduced amino acid sequence of Fo permitted us to identify a sequence of lys-lys-arg-lys-arg-arg at the C-terminus of the smaller N-terminal F_2 subunit that appears to represent the cleavage/activation domain. Five potential sites of glycosylation, four within the F_2 subunit, were also identified. Three extremely hydrophobic domains are present in the protein; a) the N-terminal signal sequence, b) the N-terminus of the F_1 subunit that is analogous to the N-terminus of the paramyxovirus F_1 subunit and the HA_2 subunit of influenza virus hemagglutinin, and c) the putative membrane anchorage domain near the C-terminus of F_1.

INTRODUCTION

Human respiratory syncytial virus (RS virus), a pleomorphic enveloped negative strand RNA virus, is an important etiological agent of severe lower respiratory disease among infants and young children (1,2). The viral envelope acquired from the host cell during viral morphogenesis has two integral virus-encoded membrane glycoproteins (ca 84 kdal and 68 kdal respectively) and is bound to the viral core nucleocapsid through an abundant viral matrix protein (M) that lines the inner aspect of the envelope (3-7). In this respect and in other features, RS virus resembles the paramyxoviruses but it differs from the latter group of viruses in morphology of the nucleocapsid and lack of hemagglutinin and neuraminidase activities (8).

Paramyxoviruses have two surface glycoproteins that are involved in the induction of immunity against infection (9-11). The paramyxovirus HN

glycoprotein has hemagglutinating and neuraminidase activities and is responsible for adsorption to viral receptors on the host cell (12). Antibodies against the HN glycoprotein inhibit hemagglutination and neuraminidase activities as well as neutralize viral infectivity. The other glycoprotein, F, mediates cell fusion and is required for virus penetration and cell to cell spread (13,14). Monospecific antibodies to paramyxovirus F glycoprotein neutralize infectivity and and inhibit cell to cell spread following infection (15,16). The biological activities of the F glycoprotein are dependent upon specific cleavage by a host protease of a precursor protein, Fo, into two subunits, F_1 and F_2, that are held together by disulfide bonds (17). In this respect the fusion glycoprotein is analogous to the influenza virus hemagglutinin (HA), but unlike the latter, the paramyxovirus fusion glycoprotein causes cell fusion at neutral pH (18). Proteolytic cleavage of the precusor Fo exposes a markedly hydrophobic region at the newly generated N-terminus of the F_1 subunit (19). Among the paramyxovirus F_1 subunits that have been sequenced there is significant homology within the first 18 amino acids of the N-terminal region(20-23). The biological importance of this portion of the molecule is also underscored by the fact that chemically synthesized oligopeptides homologous to this region competitively inhibit penetration and cell fusion by SV5 and Sendai paramyxoviruses (24).

Infection of cell monolayers with RS virus causes syncytium formation. Because the F glycoproteins of a variety of paramyxoviruses have been shown to mediate this type of cell damage it has been assumed that the F glycoprotein of RS virus is responsible for its ability to induce syncytia. Metabolic labelling experiments with precursor amino acids and sugars have shown that the 68 kdal envelope glycoprotein is converted to two subunits of 48 kdal and 20 kdal, respectively, following reduction and denaturation (6,7,25). Genetic analysis of conditional lethal ts mutants has identified eight nonoverlapping complementary groups (26,27). Although the different ts lesions have not been localized in the genome nor the affected gene product(s) identified, one of these mutants, namely, ts_2, is defective in syncytium formation and in viral penetration at nonpermissive temperature (28,29). In addition, certain monoclonal antibodies against purified RS virus neutralize the virus and immunoprecipitate a 68 kdal glycoprotein that is converted to two polypeptides of ca 49 kdal and 20 kdal upon reduction and denaturation (30,31).

In addition to the questions that must be answered concerning the basic

properties of RS virus as an unusual paramyxovirus, there is an urgent need to develop effective immunoprophylaxis against RS virus disease in infancy and early childhood. Previous approaches using either attenuated conditional lethal mutant live vaccines or formalin inactivated vaccine were unsuccessful, the former due to the genetic instability and the latter due to a failure to induce a balanced immune response (1). As an alternate approach, it may be possible to immunize with purified viral glycoproteins amplified by expression of recombinant cDNAs encoding these proteins in a eukaryotic expression system. As a first step towards this goal, we have sequenced a cDNA clone of mRNA encoding RS virus fusion glycoprotein. The identity of the cDNA clone encoding this protein was confirmed by sequencing the N-terminal amino acids of purified F_1 subunit. The salient features of the primary structure of the protein relevant to its biologic function are discussed.

MATERIALS AND METHODS

Cells and viruses

RS virus (strain A_2) was propagated in HEp-2 cell monolayers. Procedures for isolation of mRNA and virus purification have been described (7).

Nucleotide sequencing

Nucleotide sequencing of cloned RS viral DNA was accomplished by the method of Maxam and Gilbert (32). DNA primer extension on a mRNA template and dideoxysequencing were performed as described previously (33). The DNA sequence was analyzed by the computer program of Queen and Korn (34). Hydropathicity profile of the protein was determined according to Kyte and Doolittle (35).

Isolation of viral proteins

Subconfluent Hep2 cell monolayers were infected with RS virus at a multiplicity of 5 plaque forming units per cell. At 24 hr. postinfection, cells were fed with fresh medium deficient in the amino acid(s) used for labeling. [^{3}H] amino acid mixture or [^{35}S]-methionine was then added (20-25 µci/ml) and incubation continued for 24 hr. The cells were then harvested, rinsed in phospate buffered saline (PBS) and suspended in 3 volumes of buffer A [10 mM Tris HCl, pH 7.5, 0.6 M KCl, 0.15 M NaCl, 2% Triton X 100, 5 mM EDTA, 0.1% aprotinin and 2 mM phenyl methyl sufonyl fluoride (PMSF)]. The cell suspension was frozen and thawed twice, centrifuged at 2000 X g for 10 min to remove nuclei and debris. The post nuclear supernatant was centrifuged in a SW 50.1 rotor at 40,000 rpm for 1 hr and the resulting

supernatant used for immunoprecipitation. The viral fusion protein was immunoprecipitated with monoclonal antibodies directed against RS virus fusion glycoprotein. Procedures for preparation and characterization of RS virus monoclonal antibodies will be described in detail elsewhere (E. Norrby unpublished data). Immunoprecipitates were electrophoresed on SDS-polyacrylamide (12.5%) gels under reducing conditions, protein bands visualized by autoradiography and extracted essentially as described (36).

Amino acid sequencing

Automated sequence analysis was performed using a Beckman 890C sequencer and the Beckman 0.1 M Quadrol Program 121078 as described (37).

RESULTS

Identification of putative RS virus cDNA plasmid encoding the fusion glycoprotein.

Previously we described construction of a RS cDNA recombinant plasmid library and characterization of cDNA clones representing four viral genes (7). The library was subsequently screened by colony hybridization to identify viral recombinants that did not react with the previously identified genes. The plasmid DNA contained in these clones was labeled by nick translation and hybridized to mRNA from infected cells. Single clones that belonged to three nonoverlapping classes and that reacted with virus specific mRNAs of ca. 2000, 1050, and 1000 bases, respectively, were identified (38). One of these ($pRSA_{14}$) containing 1755 bp of cloned viral sequence had the largest insert among the recombinants reacting with the mRNA of about 2000 bases. It was used to select specific mRNA from RS infected cells and the mRNA was translated *in vitro* to yield a 59 kdal protein as its major product (Fig. 1). Huang and Wertz (39) have also shown that a viral mRNA of a similar size translates *in vitro* to yield 59 kdal protein as its sole product. Although a protein of this size was not detected among purified viral proteins or in infected cell lysates, its size suggested that it might be the unglycosylated precursor of the 68 kdal fusion glycoprotein (39,40). This situation is analogous to the uncleaved, unglycosylated precursor of the paramyxovirus fusion glycoprotein that is detected among *in vitro* translation products of viral mRNA (41).

Nucleotide Sequence of mRNA encoding the RS virus fusion protein.

Recombinant cDNA clone $pRSA_{14}$ was selected for DNA sequence analysis with the expectation that translation of cloned RS viral sequence might reveal structural domains similar to those of other well defined viral

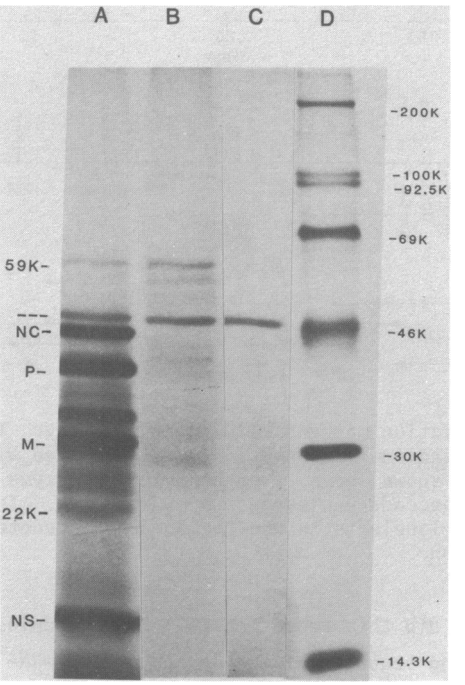

FIGURE 1. In vitro translation of mRNA hybrid selected by recombinant plasmid $pRSA_{14}$: plasmid DNA was partially digested with HpaII (1 unit enzyme per 10 µg DNA for 30' at 37°C). The digest was deproteinized by phenol/chloroform extraction and the DNA denatured by brief boiling in 0.1 N NaOH. Following neutralization, the solution was adjusted to 6 X SSC and the DNA immobilized to nitrocellulose filters. Filters containing 40 µg DNA were hybridzed with 100 µg of total cytoplasmic RNA. Conditions of hybridization and elution of specific RNA were described before (33). RNA was translated in a messenger dependent rabbit reticulocyte system and the ^{35}S-methionine labeled translation products analyzed by SDS/polyacrylamide gel electrophoresis. Translation products of total RNA from infected cells, infected cell RNA hybrid selected with $pRSA_{14}$ and in uninfected cell RNA mock selected with $pRSA_{14}$ are electrophoresed in lanes A, B, and C. Commercial ^{14}C labeled protein markers were run alongside (lane D).

surface glycoproteins such as influenza hemagglutinin or the F_1 subunit of paramyxovirus fusion glycoprotein. The restriction map and sequencing strategy are illustrated in Fig. 2. The RS viral cDNA sequnce of 1755 nucleotides had a single long open reading frame in one orientation starting at the first nucleotide and ending 85 nucleotides short of the end of cloned viral sequence. This end codon was followed by another in-frame end codon 78 nucleotides downstream. The cDNA insert within $pRSA_{14}$ as well as those contained within other clones belonging to this category, did not have a 3'

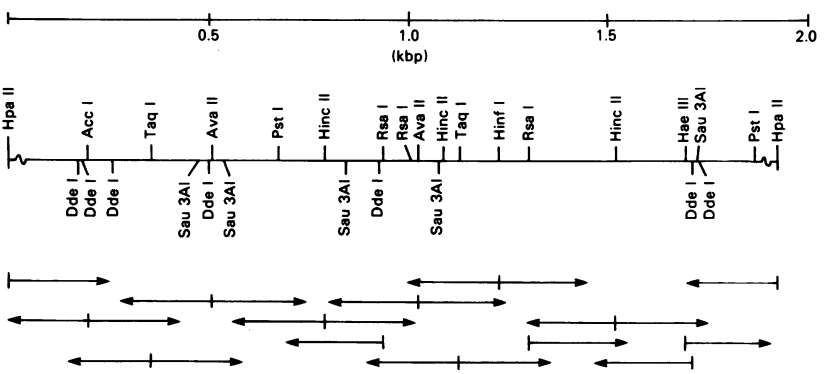

FIGURE 2. DNA restriction map and sequencing strategy: The HpaII insert of $pRSA_{14}$ used for sequencing is schematically illustrated with the length in kilobase pairs (kbp) shown above. The restriction enzyme sites used for 5' end labeling and/or secondary cleavages are indicated. The bar of each arrow represents the labeled site and the arrowhead denotes the extent of sequence determination.

poly(A) sequence nor did they represent full length copies of the their mRNA template. The missing sequence at the 5' end of the mRNA was then determined by DNA primer extension on RS mRNA and dideoxysequencing of the extended product. A 69 bp MboII/AccI fragment with its MboII site 34 nucleotides downstream of the 5' end of the cloned insert was asymmetrically 5' end labeled at the AccI site and hybridized to mRNA from RS virus infected cells. The DNA primer was then extended using reverse transcriptase. A single extension product approximately 100 nucleotides longer than the primer was obtained. This established the orientation of the messenger strand within $pRSA_{14}$. The missing sequence of the 5' end of the RS mRNA was then determined by sequencing the extended product by the dideoxynucleoside triphosphate (ddNTP) technique.

The general strategy and the sequence of the 5' end of mRNA in the antimessenger sense are illustrated in Fig. 3. The 67 nucleotides corresponding to the 5' end of mRNA together with the downstream sequences present in pRSA14 represent a continuous sequence of 1821 nucleotides starting at the 5' end of the mRNA and ending approximately 70 nucleotides upstream of the putative 3' end of the mRNA.

The sequence of RS virus Fo is presented in Fig. 4 in the messenger sense. The initial NGGGCAAAT sequence is conserved among all RS viral transcripts sequenced thus far (S.Venkatesan, et al., unpublished data).

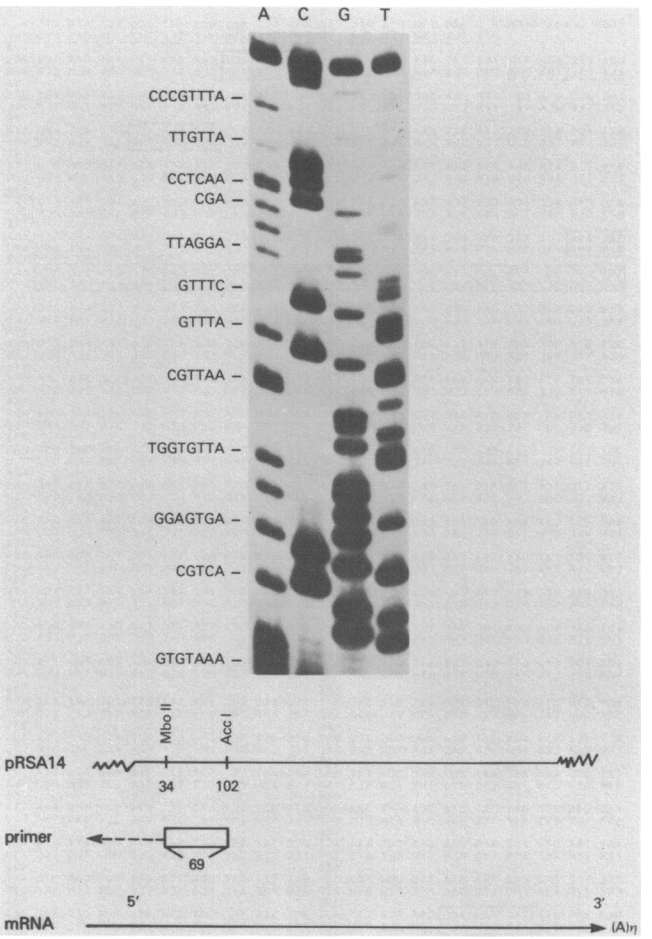

FIGURE 3. Dideoxysequencing of 5' end of the fusion protein mRNA: The schematic diagram denotes the strategy. Two pmol of a 69 base pair AccI/MboII fragment asymmetrically 5' end labeled at the AccI site was annealed to poly(A) containing RNA (10 µg RNA/pmol DNA) from infected cells. The DNA/RNA hybrid was recovered and the DNA partially extended on the template using dideoxynucleoside triphosphate inhibitors. Conditions for hybridization and dideoxysequencing were as described (33). The sequencing products were electrophoresed on the acrylamide gels (8%) containing 8M urea. The base specific reactions are shown in lanes A, C, G, and T with the deduced sequence written on the left side.

There is one long open reading frame starting at position 14 and ending at position 1738 from the 5' end of the transcript. The other two reading frames are extensively blocked throughout. The AUG initiator codon is

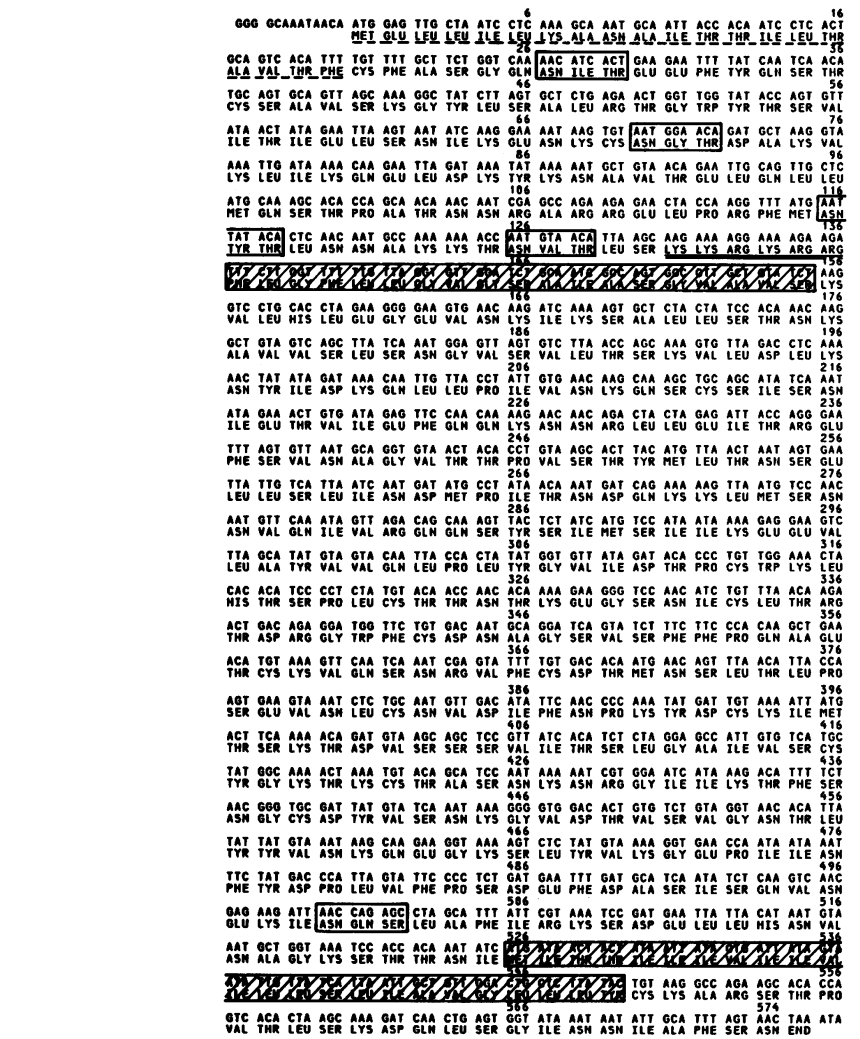

FIGURE 4. Nucleotide sequence of the fusion protein mRNA and the deduced amino acid sequence of the protein: The DNA sequence is presented in the messenger sense. The putative signal sequnce at the N-terminal is underlined by an interrupted line. The potential glycosylation sites of the type Asn-X-Ser or Asn-X-Thr are boxed in. The amino acid sequence lys-lys-arg-lys-arg-arg at the cleavage site is denoted by a double line. The hydrophobic domain of 19 amino acids (residues 137-155) at the N-terminus of F_1 subunit is hatched and boxed in as is the putative C-terminal membrane anchorage domain between residues 526 and 549.

embedded in a ACAATGG sequence that conforms to the PXXAUGG sequence that surrounds most functional eukaryotic translational initator codons (42). The translated protein of 574 amino acids has a molecular weight of 63371 daltons. The protein has 34% hydrophobic amino acids and 18.6% charged residues. The N-terminal 20 amino acids are highly hydrophobic and except for the occurrence of glutamic acid at position 2 and lysine at position 7, this region resembles other signal sequences required for translocation across the microsomal membranes (43). There is a prominent cluster of hydrophobic amino acids near the C-terminus between positions 525 and 549. This region resembles the hydrophobic transmembrane anchorage domain(s) found at the C termini of the HA_2 subunit of influenza virus hemagglutinin (44), VSV G protein (45) and F_1 subunit of SV5 viral fusion protein (46). The remaining 25 residues at the C-terminus are relatively hydrophilic and probably represent the cytoplasmic tail of the glycoprotein.

Identification of the cleavage site within the fusion glycoprotein.

We sought to confirm the identity of the 63 kdal RS viral protein as the Fo precusor by direct amino acid sequence analysis of purified fusion glycoprotein. Infected cell monolayers were labeled with [^{3}H]-leucine, [^{3}H]-isoleucine, or [^{35}S]-methionine and the cell lysates were immunoprecipitated with monoclonal antibodies directed against the fusion protein. The monoclonal antibodies specficially immunoprecipitate a 68 kdal virus fusion glycoprotein that is converted to two polypeptides (F_1 and F_2) of 49 kdal and 19 kdal upon reduction (data not shown).

The N-terminal amino acid sequencing profile of the purified F_1 subunit labeled either with isoleucine or leucine is shown in Fig. 5. For the F_1 subunit labeled with [^{3}H]-leucine, radioactivity above background levels was observed at cycles 2,5,6,22,24,35, and 36 and at cycles 12 and 31 when [^{3}H]-isoleucine was used to label the protein. Occurrence of leucine and isoleucine at the designated positions locates the N-terminus of F_1 at the phenylalanine residue at position 137 of the precursor protein (Fig. 4).

The N-terminus of the F_1 subunit is preceeded by a lys-lys-arg-lys-arg-arg sequence. There is a string of 19 uncharged and hydrophobic residues at the N-terminus of F_1 resembling the N-terminal hydrophobic domains of the HA_2 subunit of influenza hemagglutinin (44) and the F_1 subunit of paramyxovirus fusion protein (23,46). The RS viral F_1 subunit has 438 amino acids and a molecular weight of 47955 daltons. The observed molecular weight of 49 kdal agrees well with the deduced molecular weight by gel electrophoresis when glycosylation at the single N-glycosylation site

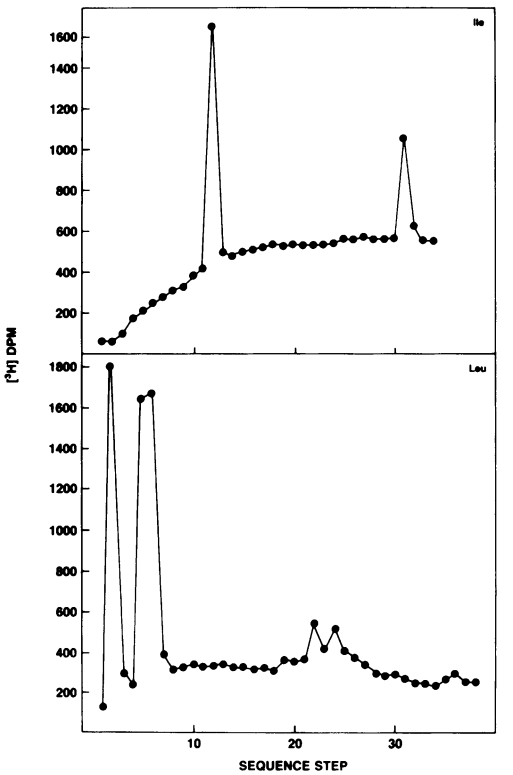

FIGURE 5. N (terminal) radiosequence analysis of the F_1 subunit of fusion protein: Conditions for protein labeling of infected cells are described in Materials and Methods. Amino acid radiosequencing of the immunoaffinity-purified protein was according to Colligan, et al. (37). Total cpm in the thiazolinone derivative extracted by butyl chloride at each sequencing step is plotted. The amino aicd sequence at the bottom represents residues 137-155 of the deduced sequence for the fusion protein (Fig. 4). The numbers above the sequence assign positions for leucine and isoleucine in the sequencing cycle.

(Asn-Gln-Ser) between residues 500-502 is taken into account. These are 4 potential N-glycosylation sites in the F_2 subunit (Asn-X-Thr) at residues 27-29, 70-72, 116-119, and 126-128 (Fig. 4). Although is is not known whether all the N-glycosylation sites are used, the above finding is

consistent with the observed molar excess incorporation of sugar residues into the F_2 subunit over the F_1 subunit both in infected cells and in purified virus. The F_2 subunit of the mature fusion glycoprotein of RS virus contains 110 amino acids (MW = 12,421) if the first 20 amino acids representing the signal sequence are processed during the vectorial translocation of the protein through the microsomes and the six basic residues at the junction between F_2 and F_1 are removed during cleavage.

DISCUSSION

A recombinant plasmid (pRSA14) containing a partial copy of RS virus fusion protein gene was used to derive the cDNA sequence of this gene. The plasmid lacked 67 nucleotides corresponding to the 5' end and about 70 nucleotides corresponding to the 3' end of the mRNA. The latter value was calculated based upon the estimated size of fusion glycoprotein mRNA detected by Northern blot analysis. The missing 5' end sequence was obtained by primer extension and dideoxysequencing of the extended product that represents the 5' end of RS viral mRNA. The 5' end of the mRNA has a nine nucleotide 5' NGGGCAAAT- sequence that is conserved in all RS viral genes sequenced thus far. Although the identity of the 5' nucleotide N of the Fo gene was not determined directly, it is likely to be G based on the complete analogous sequence of a bicistronic clone containing the entire 5' end of another RS viral gene (Elango,N., *et al.*, unpublished data). Alternatively, the 5' terminal nucleotide may represent the methylated guanosine residue of the cap structure that was fortuitously copied by reverse transcriptase (47). Our sequence data predict a protein of 574 amino acids (MW = 63371) that is rich in hydrophobic residues. The calculated molecular weight is somewhat larger than the 59 kdal unglycosylated translation product of mRNA selected by the cDNA plasmid. This may be due to an artifact of SDS-polyacrylamide gel electrophoresis. Hydropathicity profile of the protein indicates three domains of extreme hydrophobicity (Fig. 6). These include a N-terminal region of 20 residues presumambly representing the signal sequence, a second domain of 20 residues 136 residues downstream from the N-terminus and 24 residues near the C-terminus. Amino acid sequence analysis of the F_1 subunit confirmed the identity of the cDNA sequence and located the N-terminus for the F_1 subunit at residue 136, which is phenylalanine.

Purified fusion glycoprotein of paramyxoviruses incorporated into artifical membranes causes cell fusion at neutral pH mimicking the

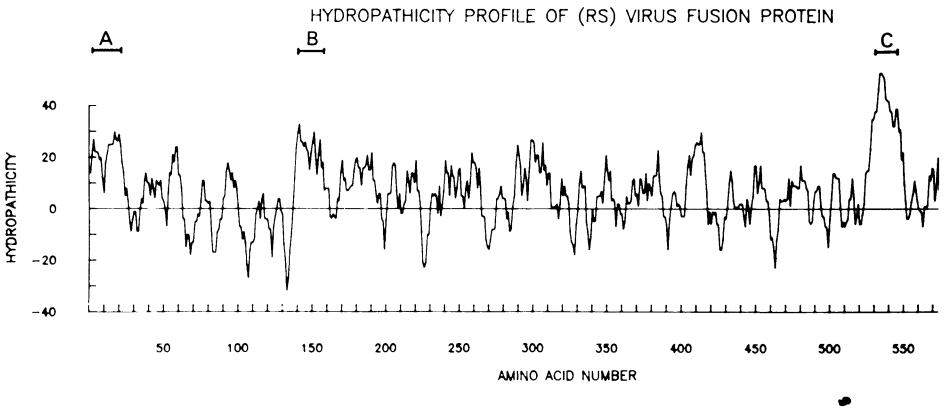

FIGURE 6. Hydropathicity profile of RS virus fusion protein: The relative hydropathicity of the protein was calculated according to Kyte and Doolittle (35). Regions denoted by A, B, and C represent the hydrophobic signal sequence, the NH_2-terminus of F_1 subunit and the putative C-terminal membrane anchorage domain of F_1 subunit.

biological role of this protein in viral penetration and cell fusion (48). These activites require cleavage of the precursor Fo that generates a new N_2-terminus for the F_1 subunit. In each of the paramyxoviruses examined so far, the first 18 amino acids at the N-terminus are hydrophobic and conserved among the different viruses with mismatches at only five positions, namely, the 2nd, 4th, 5th, 8th, and 9th residues (23). In this respect these domains resemble the N_2-terminus of the HA_2 subunit of influenza A virus. The F_1 subunit of RS virus has a similar structure but lacks overall homology with the influenza HA or SV5 virus fusion protein implying that RS virus is evolutionarily distinct. The Phe-Leu-Gly sequence at the N_2-terminus of F_1 is, however, reminiscent of the conserved Phe-X-Gly sequence found in paramyxoviruses. Overall, RS virus F protein is considerably more hydrophobic throughout than influenza A HA. In this respect it resembles the paramyxovirus SV5 F protein (46). Possibly, hydrophobicity may be required for fusion activity at neutral pH. At the C-terminus (residues 525-549), the F_1 subunit of RS virus has a region of marked hydrophobicity similar to the C-terminal anchorage domains of HA_2 of influenza virus (44) and F_1 subunit of SV5 fusion glycoprotein (46). In the case of influenza A virus hemagglutinin it has been shown that deletion of the sequence encoding the C-terminal 178 amino acids (49) or alteration of the C-terminal hydrophobic sequence (50) prevents insertion of the HA into the cell surface thereby causing it to be secreted or to accumulate

intracellularly. A similar role can be envisioned for the C terminal hydrophobic domain of RS virus fusion glycoprotein. The remaining sequence at the C-terminus is relatively polar and resembles the cytoplasmic domains of influenza A virus HA_2 and SV5 virus F_1 subunit.

Growth and virulence of both influenza and paramyxoviruses require cleavage of a viral glycoprotein (HA and F, respectively) by a trypsin-like host protease. This occurs at a precise location upstream of a hydrophobic region that becomes the N-terminus of the C-terminal cleavage product (HA_2 and F_1 respectively). In the case of H1, H2, and H3 hemagglutinins of influenza A virus, a single arginine residue at the cleavage site is removed but in the case of H7 hemagglutinin a pentapeptide is lost at the cleavage site. This suggests a requirement for two peptidases in the processing of the hemagglutinin of the H7 serotype (51). SV5 virus fusion protein is readily cleaved in tissue culture and has five arginine residues preceding the N-terminus of F_1 (46). The ease with which SV5 virus fusion glycoprotein and influenza A virus H7 hemagglutinin are cleaved suggests that trypsin-like cell protease(s) preferentially cleave after a stretch of basic amino acids rather than a single basic amino acid such as the arginine that is present just upstream of the N-terminus of the HA_1 subunit of influenza A H1, H2 or H3 (46). RS virus conforms to the former pattern because it has a lys-lys-arg-lys-arg-arg sequence at the cleavage site preceding the N-terminus of F_1 and is readily cleaved by host cell proteases as indicated by the observation that exogenous trypsin is not required for plaque formation in heteroploid cell cultures. Nonetheless, RS virus grows poorly *in vitro* and has a fairly narrow host range.

Genetic studies of conditional lethal *ts* mutants generated by nitrosoguanidine mutagenesis indicate that the ts_2 mutant of RS virus is defective in syncytium formation and penetration of its host cell at nonpermissive temperature (29). These observations suggest that the *ts* mutation of this mutant is located in the F glycoprotein gene.

Although the cDNA clone sequenced in this study lacks 67 nucleotides at the 5' end of the mRNA, it should be possible to clone a primer extension product containing these bases. Subsequently they can be ligated to the remaining structural sequence in pRSA14. The resulting full length cDNA copy can then be amplified by cloning and used for expression studies in eukaryotic cells. Availability of the full coding sequence of this gene should allow us to construct strategically located in-frame deletions or point mutations within the cleavage activation site, the NH_2-terminal domain

of F_1 or the C-terminal anchorage domain and study their biological effects. Although the functional role of the N-terminus of HA_2 subunit of influenza A virus has been established, it is by no means clear how this domain that lies 100 Å from the distal end and 35 Å from the membrane initiates cell fusion. It has been suggested that under low pH conditions normally encountered within intracellular organelles such as lysosomes, the molecule undergoes a second conformational change bringing the active site into proximity with the membranes (44). RS virus casues fusion at neutral pH and it will be interesting to determine whether deletions or alterations of the sequence at N-terminus of F_1 produced by site directed mutagenesis will shift the pH optimum for fusion and/or alter the normal route of intra- and intercellular transport.

Monoclonal antibodies directed against RS virus fusion glycoprotein are effective in neutralizing virus in vitro. Hence, active immunization using purified fusion glycoprotein expressed in eukaryotic systems is a possible alternative to immunization using live attenuated virus. Transfer of a full length clone coding for fusion protein to a selectable vaccinia virus vector would yield, in effect, a replicating antigen that can be evaluated for antigenicity and immunoprophylaxis in animal model systems. Such an approach has been tested and found to be feasible both in the case of infleunza virus and hepatitis B virus (52,53).

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The editorial assistance of Marianne Guiler and Linda Jordan is greatly appreciated.

Abbreviations: RS, respiratory syncytial; F, fusion protein; HA, influenza virus hemagglutinin; HN, hemagglutinin neuraminidase; N-terminal, amino-terminal; C-terminal, carboxy-terminal

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