JOURNAL OF VIROLOGY, July 1988, p. 2228–2233
0022-538X/88/072228-06$02.00/0

Vol. 62, No. 7

Further Characterization of the Soluble Form of the G Glycoprotein of Respiratory Syncytial Virus

DAVID A. HENDRICKS,[1]* KENNETH McINTOSH,[2] AND JEAN L. PATTERSON[2,3]

Gene Trak Systems, 31 New York Avenue, Framingham, Massachusetts 01701,[1] and Division of Infectious Diseases, Department of Pediatrics, the Children's Hospital,[2] and Department of Microbiology and Molecular Genetics, Harvard Medical School,[3] Boston, Massachusetts 02115

Received 28 December 1987/Accepted 21 March 1988

A soluble form of the G glycoprotein, the attachment protein, of respiratory syncytial virus is shed from infected HEp-2 cells. The G_s proteins of the Long and 18537 strains have apparent molecular sizes of 82 and 71 kilodaltons, respectively, 6 to 9 kilodaltons smaller than the virion-associated forms (G_v). The G_s protein of the Long strain was further characterized. Approximately one in six of all of the radiolabeled G molecules in these cultures at 24 h postinfection was present as the G_s protein. The G_s protein was clearly evident in culture fluids at 6 h postinfection, but the G_v protein could not be discerned until 12 h after infection, an observation that is consistent with the 12-h eclipse period for respiratory syncytial virus. Therefore, the G_s protein is shed, in part at least, from intact, infected cells and before the appearance of progeny virus. The appearance of a smaller G_s protein (74 kilodaltons) in fluids of infected calls which were incubated with tunicamycin shows that addition of N-linked oligosaccharides is not required for the genesis and shedding of the G_s protein. Sequencing of the amino terminus of purified G_s protein revealed two different termini, whose generations are consistent with cleavages of the full-length G protein between amino acids 65 and 66 and between residues 74 and 75. This result suggests that the G_s protein is present in two different forms which lack the proposed intracytoplasmic and transmembrane domains of the full-length G protein.

Although respiratory syncytial (RS) virus, a pneumovirus in the family *Paramyxoviridae* (19), causes considerable morbidity in children and repeatedly infects individuals (17), many aspects of the replication of this virus have not been elucidated. Recently there has been considerable interest in the molecular biology of RS virus, perhaps in response to the acknowledgement that conventional vaccines were unsuccessful because they produced inadequate protection or more severe disease after the next natural infection (18, 38). A major advance which resulted from this new focus has been a comprehensive description of the genetic organization of RS virus (summarized by Collins and Wertz [5]).

Some aspects of viral replication have become clearer from studies of the appearance and processing of viral glycoproteins in infected cells. The large glycoprotein (G; 90 kilodaltons [kDa]), which functions in viral attachment of cells (21, 36), has a protein backbone of about 32.6 kDa (predicted from its nucleotide sequence [28, 37]) and contains both N- and O-linked oligosaccharides (7, 11, 28, 37). A 45-kDa glycoprotein which is endoglycosidase H sensitive (contains high mannose oligosaccharides) appears to be chased, after at least 30 min, into an 84- to 90-kDa mature, endoglycosidase H-resistant (contains complex oligosaccharides) form of the G protein (7, 12). The 45-kDa protein is present but not processed to the mature G protein in monensin-treated cells, and smaller forms of both the precursor to the G protein and the G protein are produced in tunicamycin (TM)-treated cells (7, 27, 33). The fusion (F) protein, which produces syncytia in infected cells (35) and probably fusion of absorbed virions to infected cells, is a disulfide-linked heterodimer composed of a small, distal polypeptide (F_2; 20 kDa) and a larger, proximal protein (F_1; 48 kDa) (8, 10, 20, 34).

There is a paucity of information concerning the release of glycoproteins from RS virus-infected cells. Recently, we described a non-virion-associated (soluble) form of the G protein of RS virus which was recovered from culture fluids of infected HEp-2 cells (13). We establish here that the G_s protein appears in culture fluids before the appearance of cytopathology and the end of viral eclipse. We show that the addition of N-linked oligosaccharides to the G protein is not required for the appearance of the G_s protein. Finally, we determine, by amino acid sequencing, that the G_s protein is a mixture of two proteins which lack the proposed intracytoplasmic and transmembrane domains of the full-length G protein.

MATERIALS AND METHODS

Virus and cells. The Long and 18537 strains of RS virus were twice purified by limiting dilution.

Radiolabeling of viral glycoproteins. Viral glycoproteins were radiolabeled as previously described (13). In some experiments, TM was added to culture fluid to achieve a final concentration of 2 μg/ml, and the addition of [^{3}H]glucosamine and processing of culture fluids were carried out as described previously (13).

Discontinuous sucrose gradients. A discontinuous sucrose gradient was used to separate virions and G_s proteins in culture fluids. A sample of 0.40 to 0.55 ml of clarified culture fluid containing 100 mM $MgSO_4$ and 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.5, was layered onto 2.4 ml of a 1:2 dilution of MHN buffer (1 M $MgSO_4$, 50 mM HEPES [pH 7.5], 150 mM NaCl [7], which rested on a 2.5-ml cushion of 1.667 M sucrose in MHN buffer (density, 1.33 g/cm^3). Centrifugation was for 90 min at 100,000 × *g* in an SW50.1 rotor, during which virions would be expected to sediment through the middle layer (density equivalent to about 13% sucrose) and to concentrate on the cushion. Then 0.5-ml fractions were collected, and acid-precipitable counts were determined.

* Corresponding author.

Radioimmunoprecipitation assay. A radioimmunoprecipitation assay which employed a hyperimmune guinea pig serum to the Long strain or a hyperimmune rabbit serum to purified G protein of the 18537 strain was described previously (13).

Purification of G_s protein and determination of its amino acid sequence. G_s protein was purified from culture fluids of flasks (150 cm^2) of HEp-2 cells infected with the Long strain. Subconfluent monolayers of HEp-2 cells were infected at a multiplicity of infection of 1; after viral adsorption, monolayers were covered with 10 ml of minimal essential medium (MEM) plus 0.1% bovine albumin. At 27 h postinfection, culture fluids were clarified by centrifugation at 10,500 × *g* for 20 min, and virus was removed from clarified fluids by centrifugation at 65,000 × *g* for 2 h. Buffer was added to virus-free fluids to achieve final concentrations of 0.5 M NaCl, 0.02 M Tris hydrochloride (pH 7.4), 0.1% Triton X-100, and 0.1% sodium deoxycholate (ConA buffer), and glycoproteins were absorbed to a Concanavalin A-Sepharose 4B (Sigma Chemical Co.) column, a procedure found to enrich for the G protein of RS virus (unpublished data). After extensive washing with ConA buffer, the column was incubated overnight at 4°C with ConA buffer supplemented with 0.5 M α-methyl-D-mannoside (Sigma), and the eluate was passed over a cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals) immunoaffinity column containing anti-G protein monoclonal antibodies 91-4, 104-2, 402-5, 18-1, and 131-2G, which were generously supplied by B. Fernie and L. Anderson. G protein was removed from the column by elution with 0.1 M glycine buffer (pH 2.5), and 0.5-ml fractions were collected in tubes containing 0.5 ml of 0.2 M Tris hydrochloride (pH 7.4) buffer. The G_s preparation was simultaneously concentrated and dialyzed in a collodion bag concentrator (Schleicher & Schuell Co.). Automated Edman degradation was performed at the Harvard Microsequencing Facility, Harvard Biological Laboratories, Cambridge, Mass., on an Applied Biosystem 470A Protein sequencer equipped with an on-line 120APTH analyzer.

RESULTS

Quantitation of G_s protein in culture fluids. To determine the amount of G_s protein relative to the total G protein in infected cultures, it was first necessary to separate G_s protein from virions (and their associated G_v protein), both of which were present in culture fluids. Previously, this separation was accomplished by rate zonal centrifugation in a continuous sucrose gradient. For these experiments, we resolved [^{3}H]glucosamine-labeled G_s proteins from virions in a discontinuous sucrose gradient (Fig. 1). The two top fractions (9 and 10) from gradients overlaid with culture fluids from either mock- or virus-infected materials contained most (82 and 74%, respectively) of the acid-precipitable counts in the gradients (Fig. 1A), a result that would be expected if most of the proteins were soluble or associated with aggregates that have low sedimentation coefficients. Fraction 6, the fraction atop the sucrose cushion, contained a peak of radioactivity in the virus-infected preparation but not in the mock-infected preparation. This peak of radioactivity was always found in either fraction 5 or 6.

Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1B) of the top five fractions from these gradients revealed an abundant or heavily labeled, putative G_s protein in virus-infected preparations (lanes 4 and 5) but not in mock-infected preparations (lanes 9 and 10). Longer exposures of dried gels revealed a possible G_v protein in fraction 6 and a dramatic decrease in the density of the putative G_s protein band from fractions 10 through 7 (lanes 5 through 2), an observation which suggested that some of the G_s protein was associated with aggregates. One glycoprotein (97 kDa) in mock-infected preparations (lanes 9 and 10) had an electrophoretic mobility which was similar to but slower than those of the G_v and G_s proteins. Figure 1B was used for quantitation of G_s protein by densitometry.

G proteins in gradient fractions, culture fluids, and cell extracts were quantitated from densitometric scans of autoradiograms; comparison of these data showed that approximately 16% of all of the G protein in these cell cultures (24 h postinfection) was present as the G_s protein (i.e., in fraction 10 of the discontinuous sucrose gradient). About 16% of the G protein in culture fluids was detected in the fraction atop the sucrose cushion; thus, at most 16% of the G protein in culture fluid (5% of the total G protein in these infected cells) was virion associated. Since fraction 9 may contain some G_s protein and it was not included in these calculations, it is likely that as much as 20% of the total G protein in this culture was released as the G_s protein. Fractions 10 and 9 contained 62 and 17%, respectively, of all of the G protein in culture fluids; therefore, most of the G protein in these culture fluids was present as the G_s protein.

Apparent molecular weights of G_s and G_v proteins. To identify more clearly viral glycoproteins in sucrose gradients and to determine their molecular weights, samples of fractions from a discontinuous gradient were incubated with an anti-RS virus hyperimmune guinea pig serum, and immunoprecipitates were analyzed by SDS-PAGE (Fig. 1C). These results clearly revealed a heterogenerously migrating G_s protein (82 kDa), which was most abundant in the two top fractions of the gradient (lanes 5 and 6). A slower-migrating G_v protein (88 kDa) and putative F_1 and F_2 glycoproteins were observed only in the fraction atop the sucrose cushion (lane 1). These results suggested that virions were found only on the sucrose cushion and that the F protein was not present in a soluble form. A 43- to 45-kDa glycoprotein which was found only in the top fraction (lane 6) of the gradient overlaid with the viral preparation may be a protein which has been shown to be antigenically related to the G protein (13). The presence of small amounts of G_s protein in the intermediate layer of the discontinuous sucrose gradient (lanes 2 through 4) suggests a possible association of G_s proteins with aggregates. A 97-kDa glycoprotein was immunoprecipitated from mock-infected materials and a priori it may be assumed to be present and immunoprecipitated from viral preparations. However, it should not be confused with the G_v or G_s proteins for the following reasons. (i) It has a distinct and slower electrophoretic mobility from both forms of the G protein. (ii) It has never been observed in the fraction atop the sucrose cushion and thus should not be confused with the G_v protein. (iii) It appears to be present only in small quantities in the top fraction of the gradient; if it were present in viral preparations, it should not significantly supplement the amount of G_s protein which is precipitated. This 97-kDa glycoprotein did not appear to be present in immunoprecipitates of viral preparations in which its resolution and visualization should be possible (lanes 2 through 4).

Culture fluids of HEp-2 cells that were infected with the 18537 strain of RS virus also contained a G_s protein (71 kDa) and a G_v protein (80 kDa), which were somewhat smaller than the analogous proteins of the Long strain, an observation that is consistent with strain-specific variations in the

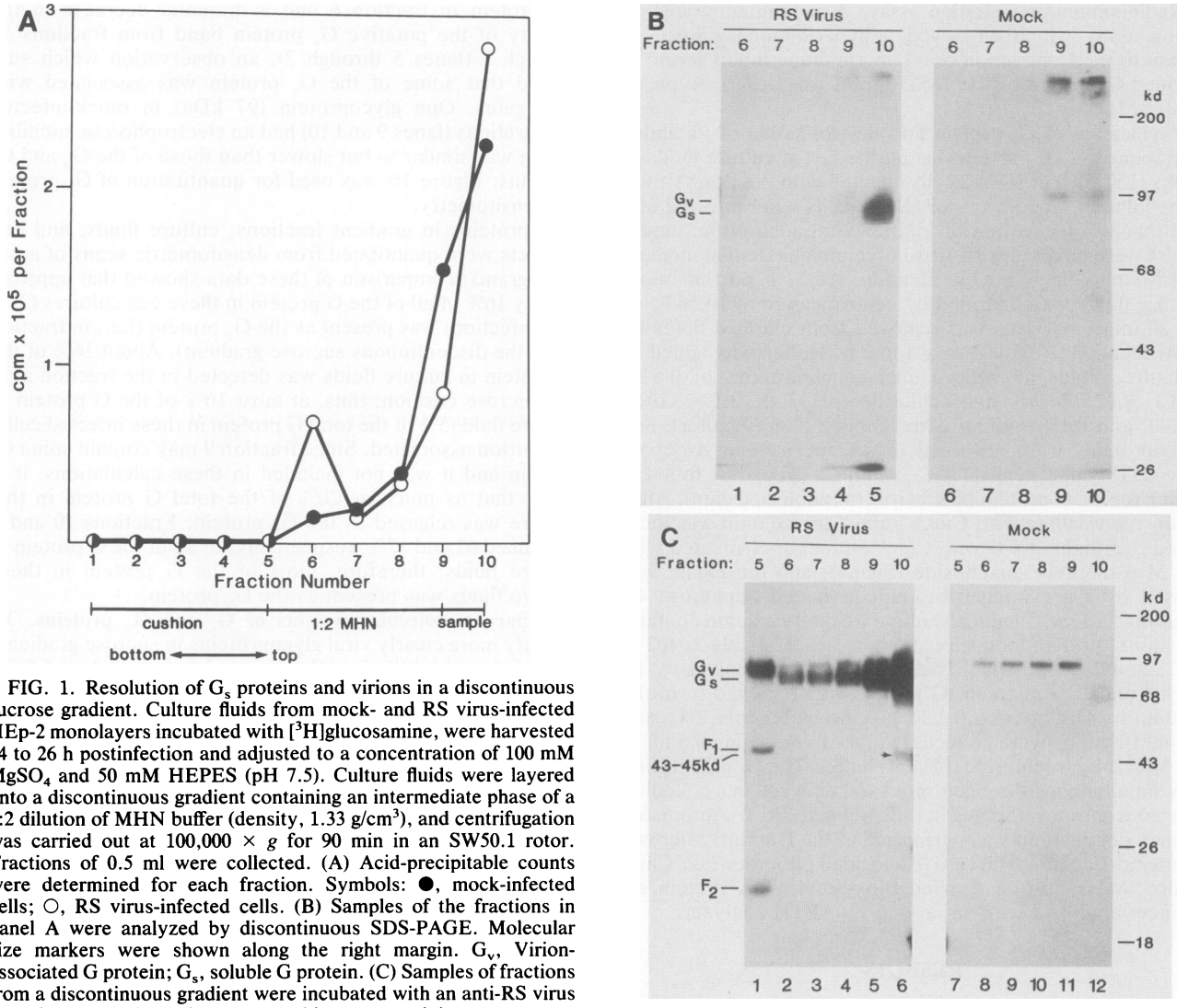

FIG. 1. Resolution of G_s proteins and virions in a discontinuous sucrose gradient. Culture fluids from mock- and RS virus-infected HEp-2 monolayers incubated with [^{3}H]glucosamine, were harvested 24 to 26 h postinfection and adjusted to a concentration of 100 mM $MgSO_4$ and 50 mM HEPES (pH 7.5). Culture fluids were layered onto a discontinuous gradient containing an intermediate phase of a 1:2 dilution of MHN buffer (density, 1.33 g/cm^3), and centrifugation was carried out at 100,000 × *g* for 90 min in an SW50.1 rotor. Fractions of 0.5 ml were collected. (A) Acid-precipitable counts were determined for each fraction. Symbols: ●, mock-infected cells; ○, RS virus-infected cells. (B) Samples of the fractions in panel A were analyzed by discontinuous SDS-PAGE. Molecular size markers were shown along the right margin. G_v, Virion-associated G protein; G_s, soluble G protein. (C) Samples of fractions from a discontinuous gradient were incubated with an anti-RS virus hyperimmune guinea pig serum, and immunoprecipitates were analyzed by discontinuous SDS-PAGE. F_1 and F_2, Membrane-proximal and -distal portions, respectively, of fusion glycoproteins; 43-45kd, a glycoprotein believed to be a precursor of G protein.

masses of G proteins reported by Norrby et al. (26). The two forms of the G protein of the 18537 strain were immunoprecipitated by a hyperimmune serum (kindly provided by E. Walsh) that was prepared in a rabbit immunized with purified G protein of the 18537 strain. That a G_s protein was released from cells infected with either the Long or 18537 strains of RS virus suggests that this is an RS virus-specific phenomenon and not a strain-specific characteristic.

Times of appearance of the G_s and G_v proteins in culture fluids after infection. To elucidate the genesis of the G_s protein it was important to determine whether it was released from degraded virions or lysed cells or whether it was shed from infected, intact cells. Therefore, appearances of G_s protein and virions, detected by the presence of G_v proteins, in culture fluids were determined by sampling culture fluids at various times after infection, separating G_s proteins from virions in discontinuous sucrose gradients, and immunoprecipitating [^{3}H]glucosamine-labeled proteins from the top or cushion fractions. Immunoprecipitates were analyzed by SDS-PAGE analysis (Fig. 2). The G_s protein was evident in culture fluids as early as 6 h postinfection (lane 1) and increased in abundance and apparent heterogeneity until 18 h postinfection (lanes 3, 5, 7, 9, and 11). In contrast, the G_v protein, which was slightly larger than the G_s protein, could not be seen in immunoprecipitates until 12 h after infection (lane 6) and increased in intensity until the end of the experiment (lanes 8, 10, and 12). Clearly, the G_s protein was much more abundant than the G_v protein. The appearance and production of the G_v protein are consistent with the 12-h eclipse period of RS virus and the continuous release of virus until destruction of the cell monolayer. Other soluble, viral structural proteins could not be detected by the radioimmunoprecipitation assay until 12 to 15 h after infection (data not shown). Cytopathic effects were first discernable at 15 h after infection. The 43- to 45-kDa protein, believed to be a precursor to the G protein, was found only in a soluble form in culture fluids and was first apparent at 12 h postinfection (lane 5) (13). Because the G_s protein was present in culture fluids during viral eclipse and before the appearance of cytopathic effects, it is likely that the G_s protein is shed, in part at least, from infected, intact cells.

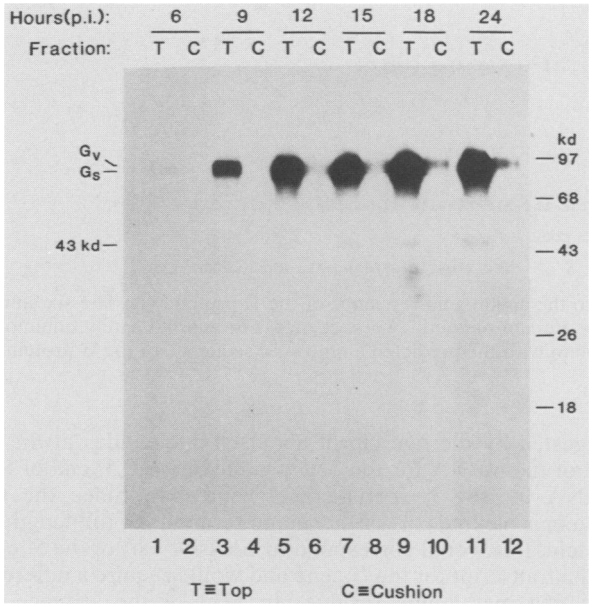

FIG. 2. Time of appearance of the G_s protein in culture fluids after infection. Culture fluids were harvested from RS virus-infected cells at various times after infection, and virions were resolved from G_s proteins in discontinuous gradients as described in the legend to Fig. 1. Samples of the top (T) gradient fractions and the fractions atop the sucrose cushions (C), which were expected to contain the G_s proteins and virions, respectively, were analyzed for virus-specific glycoproteins by radioimmunoprecipitation assay and discontinuous SDS-PAGE as described in the legend to Fig. 1.

Appearance of the G_s protein in fluids of cells incubated with TM. To determine whether N-linked oligosaccharides on the G protein are essential to the appearance of the G_s protein in culture fluids, infected cultures were incubated with TM, and [^{3}H]glucosamine-labeled glycoproteins in fluids were analyzed by SDS-PAGE (Fig. 3). G protein was released from cells incubated with TM (Fig. 3A, lane 1), but it had an apparent molecular size that was 6 to 8 kDa smaller than the protein from an untreated culture (lane 2). The 97-kDa host glycoprotein, immunoprecipitated by the guinea pig hyperimmune serum to RS virus (Fig. 1C), was not produced or radiolabeled in TM-treated, mock-infected cultures (lane 3); it would therefore not be present in TM-treated, virus-infected cultures. After separation of soluble proteins and virions in culture fluids on a discontinuous sucrose gradient, [^{3}H]glucosamine-labeled viral glycoproteins were immunoprecipitated and then analyzed by SDS-PAGE (Fig. 3B). A G_s protein (74 kDa) was abundant in the top fractions of the gradient (lanes 5 and 6). A very small amount of G_v protein (82 kDa), apparently associated with virions or aggregates, may have been present (lane 1). The G_v protein was about 8 kDa larger than the G_s protein. That addition of N-linked oligosaccharides was inhibited in these cultures was supported by the absence of cytopathic effects and of [^{3}H]glucosamine-labeled F_1 and F_2 proteins in immunoprecipitates of infected cell lysates (data not shown), observations that have been reported by others (7, 20). The 39-kDa glycoprotein that was present only in a soluble form (Fig. 3B, lane 6) is likely to be the analog of the 43- to 45-kDa protein that was present in cultures not treated with TM (Fig. 1C). These results show that N-linked oligosaccharides, present on both forms of the G protein, are not required for the production and release of the soluble form of this protein from intact cells.

Amino-terminal sequence of the G_s protein. We hypothesized that the G_s protein is shorter than the full-length G protein and soluble because it lacks a portion of the amino terminus, believed to be the site of membrane anchorage (28, 37). To test this hypothesis, the amino terminus of purified G_s protein was sequenced. The preparation of G_s protein contained one heterogeneously-migrating G_s band by SDS-PAGE (data not shown). Edman degradation of the purified G_s preparation through six cycles yielded two amino acids, in approximately equimolar concentrations, at each cycle (Fig. 4). This suggested that there were two different amino termini in the preparation. It was not possible, however, to assign each amino acid to one or the other sequence. By visual inspection, it was possible to align an amino-terminal sequence, starting with the Asn residue, with amino acids 66 through 71 of the predicted amino acid sequence of the G protein of the Long strain (15). The remaining hexapeptide terminal sequence then aligned exactly with residues 75 through 80 of the G protein (Fig. 4B). These assignments of the two hexapeptide termini to the G protein were confirmed by using the Quest IntelliGenetics Program. These results suggested (i) that this G_s preparation did not contain full-length G protein, (ii) that G_s proteins have two different amino termini, and (iii) that G_s proteins lack either 65 or 74 residues that are part of the amino terminus of the full-length G protein.

DISCUSSION

A soluble form of the G protein of RS virus is shed from HEp-2 cells infected with the Long or 18537 strain before the appearance of viral progeny and virus-induced cytopathology. The G_s protein lacks at least 65 amino-terminal residues of the full-length G protein. We propose that the G_s protein

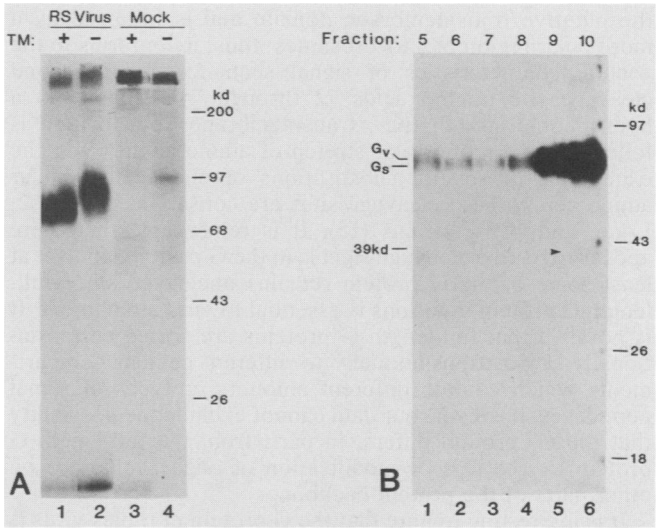

FIG. 3. Appearance of the G_s protein in the fluids of cells incubated with TM. Fluids of RS virus-infected cultures were replaced 3 h postinfection with MEM that contained 2 μg/ml of TM, 30 μCi of [^{3}H]glucosamine per ml, and 0.1% bovine albumin. At 24 h postinfection, culture fluids were collected. (A) Glycoproteins of mock- or virus-infected cultures which were incubated with (+) or without (−) TM were analyzed by SDS-PAGE. (B) Soluble proteins were separated from virions in fluids of TM-treated, virus-infected cultures by centrifugation in a discontinuous gradient, and virus-specific glycoproteins in fractions 5 through 10 were identified by a radioimmunoprecipitation assay and SDS-PAGE. 39 kd, Putative analog of 43- to 45-kDa protein.

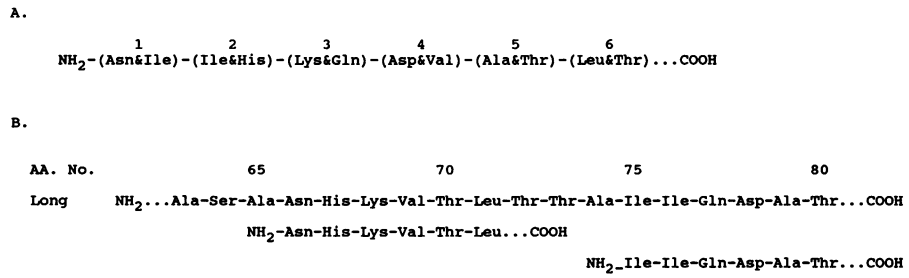

FIG. 4. Amino-terminal sequences of purified G_s protein and alignment to the amino acid sequence of the G protein. (A) The six amino acids at the amino terminus of purified G_s protein were determined by the Edman method; two sequences, in approximately equimolar amounts, were obtained. (B) Possible alignments of amino termini of G_s protein with the predicted amino acid sequence of the G protein of the Long strain.

is soluble because it lacks the transmembrane domain, believed to be located near the amino terminus (28, 37). This membrane orientation (i.e., type II membrane protein) is shared by the HN protein of the paramyxovirus SV5 (14) and the influenza virus neuraminidase (9, 24), but, to our knowledge, ours is the first report of shedding of a type II viral glycoprotein from infected cells.

Although the mechanism and cellular location of G_s protein production are unknown, it does appear that full-length G protein is cleaved at either of two specific sites in the formation of the G_s protein. These sites have structural elements and sequences which are characteristic of signal sequences, recognized by signal peptidases (22, 25, 31, 32). In both cases, the sequence on the amino-terminal side of the cleavage site (and at the carboxy terminus of the deleted polypeptide) contains a small, neutral residue (Ala) at position -1 (amino acids 65 and 74) and a small, neutral residue (Ala at residue 63 and Thr at residue 72) at the -3 position. The sequence at residues 63 through 65 is contained within the putative transmembrane domain and is followed by a more polar region of 5 to 7 residues; thus, it conforms to the general characteristics of signal sequences. The second cleavage site (amino acids 72 through 74) is close to a hydrophobic stretch (i.e., transmembrane domain) and is followed by a more polar stretch of amino acids. With the exceptions of several substitutions of chemically similar amino acids, these cleavage sites are conserved in the A2, Long, and 18537 strains (15). It is remarkable that there appear to be two cleavage signals in the G protein, and yet at least some of the G protein remains uncleaved since full-length G protein in virions is essential to viral attachment. It is possible that full-length G proteins are sorted cotranslationally or posttranslationally to different cellular compartments which contain different amounts or types of signal peptidases; however, our data cannot exclude the possibility that the G_s protein differs, in part, from the full-length G protein by the loss or modification of oligosaccharides on other parts of the protein backbone.

It is interesting to note that the G protein of rabies virus is cleaved between amino acids residues 447 and 448, 58 residues from its carboxy terminus (1, 6), and that the sequence next to the cleavage site (and at the C terminus of the nascent G_s protein; -Ala-Gly-Ala-COOH) appears to be a consensus sequence for a signal peptidase. This observation is interesting in light of the fact that G protein of rabies is a type I membrane glycoprotein.

It is possible that the G_s protein of RS virus is generated by translation of a truncated transcript of the gene for the G protein. That the RS viral transcriptase may stop transcription and reinitiated elsewhere on the genomic template is suggested by the overlap of about 60 nucleotides at the 3′ end of the mRNA for the 22K protein and the 5′ end of the mRNA for the L (polymerase) gene (4a). Since the G_s protein is lacking part of the amino terminus of full-length G protein, this would correspond to a loss of part of the 5′ end of the transcript for the G gene and would require a different initiating methionine residue. In light of the absence of translocation and glycosylation of mutants of influenza virus neuraminidase, a type II membrane protein which lacks a membrane anchorage domain (25, 30), it is doubtful that truncated forms of the G protein would be translocated across membranes, glycosylated, and exported from cells.

Soluble forms of glycoproteins have been described for vesicular stomatitis (16, 23), rabies (6), herpes simplex (4), and murine leukemia (2) viruses. The secreted glycoproteins of rabies and vesicular stomatitis viruses differ from their full-length forms by the absence of 58 and about 60 amino acids, respectively, at their carboxy termini (3, 6). Although a soluble form of the 70-kDa glycoprotein of murine leukemia virus has been found as free (non-virion-associated, non-antibody-complexed) glycoprotein in the sera of New Zealand black mice (29), it is not known whether release of soluble viral glycoproteins during infection of humans or experimental animals is a general phenomenon. If soluble forms of viral glycoproteins are released during infection, then it would be important to study their possible roles in absorbing neutralizing antibodies, in mediating immunopathological mechanisms, or perhaps in saturating viral receptors on susceptible cells or antigen receptors on immune cells. In light of the report that immunization of mice with the G_s protein of rabies virus does not protect them against lethal challenge (6), it will be interesting to determine whether the G_s protein of RS virus is immunogenic, or possibly tolerogenic, and whether it can protect experimental animals against RS viral challenge.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant R01 AI22389 from the National Institutes of Health. J.L.P. was partially supported by U.S. Army grant DAMD17-87-C-7004.

We thank Ed Walsh for providing hyperimmune rabbit serum to the G protein of the 18537 strain of RS virus. Discussions with Max Nibert concerning cleavage sites of proteases were invaluable. We thank Karen Duhamel for typing the manuscript.

LITERATURE CITED

1. **Anilionis, A., W. H. Wunner, and P. J. Curtis.** 1981. Structure of the glycoprotein gene in rabies virus. Nature (London) **294**:275–278.
2. **Bolognesi, D. P., and A. J. Langlois.** 1975. Polypeptides of

mammalian oncornaviruses. IV. Structural components of murine leukemia virus released as soluble antigens in cell culture. Virology **68**:550–555.

3. **Chatis, P. A., and T. G. Morrison.** 1983. Characterization of the soluble glycoprotein released from vesicular stomatitis virus-infected cells. J. Virol. **45**:80–90.
4. **Chen, A. B., T. Ben-Porat, R. J. Whitley, and A. S. Kaplan.** 1978. Purification and characterization of proteins excreted by cells infected with herpes simplex virus and their use in diagnosis. Virology **91**:234–242.

4a. **Collins, P. L., R. A. Olmsted, M. K. Spriggs, P. R. Johnson, and A. J. Buckler-White.** 1987. Gene overlap and site-specific attenuation of transcription of the viral polymerase L gene of human respiratory syncytial virus. Proc. Natl. Acad. Sci. USA **84**: 5134–5138.

5. **Collins, P. L., and G. W. Wertz.** 1985. The envelope-associated 22K protein of human respiratory synical virus: nucleotide sequence of the mRNA and a related polytranscript. J. Virol. **54**:65–71.
6. **Dietzschold, B., T. J. Witkor, W. H. Wunner, and A. Varrichio.** 1983. Chemical and immunological analysis of the rabies soluble glycoprotein. Virology **124**:330–337.
7. **Fernie, B. F., G. Dapolito, P. J. Cote, and J. L. Gerin.** 1985. Kinetics of synthesis of respiratory syncytial virus glycoproteins. J. Gen. Virol. **66**:1983–1990.
8. **Fernie, B. F., and J. L. Gerin.** 1982. Immunochemical identification of viral and nonviral proteins of the respiratory syncytial virus virion. Infect. Immun. **37**:243–249.
9. **Fields, S., G. Winter, and G. G. Brownlee.** 1981. Structure of the neuraminidase gene in human influenza virus A/PR/8/34. Nature (London). **290**:213–217.
10. **Gruber, C., and S. Levine.** 1983. Respiratory syncytial virus polypeptides. III. The envelope-associated proteins. J. Gen. Virol. **64**:825–832.
11. **Gruber, C., and S. Levine.** 1985. Respiratory syncytial virus polypeptides. IV. The oligosaccharides of the glycoproteins. J. Gen. Virol. **66**:417–432.
12. **Gruber, C., and S. Levine.** 1985. Respiratory syncytial virus polypeptides. V. The kinetics of glycoprotein synthesis. J. Gen. Virol. **66**:1241–1247.
13. **Hendricks, D. A., K. Baradaran, K. McIntosh, and J. L. Patterson.** 1987. Appearance of a soluble form of the G protein of respiratory syncytial virus in fluids of infected cells. J. Gen. Virol. **68**:1705–1714.
14. **Hiebert, S. W., R. G. Paterson, and R. A. Lamb.** 1985. Hemagglutinin-neuraminidase protein of the paramyxovirus simian virus 5: nucleotide sequence of the mRNA predicts the N-terminal membrane anchor. J. Virol. **54**:1–16.
15. **Johnson, P. R., M. K. Spriggs, R. A. Olmstead, and P. L. Collins.** 1987. The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. Proc. Natl. Acad. Sci. USA **84**:5625–5629.
16. **Kang, C. Y., and L. Prevec.** 1971. Proteins of vesicular stomatitis virus. III. Intracellular synthesis and extracellular appearance of virus-specific proteins. Virology **46**:678–690.
17. **Kim, H. W., J. O. Arrobio, C. D. Brandt, B. C. Jeffries, G. Pyles, J. L. Reid, R. M. Chanock, and R. H. Parrott.** 1973. Epidemiology of respiratory syncytial virus infection in Washington, D.C. I. Importance of this virus in different respiratory tract disease syndromes and temporal distribution of infection. Am. J. Epidemiol. **98**:216–225.
18. **Kim H. W., J. G. Canchola, C. D. Brandt, G. Pyles, R. M. Chanock, K. Jensen, and R. H. Parrott.** 1969. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am. J. Epidemiol. **89**:422–434.
19. **Kingsbury, D. W., M. A. Bratt, P. W. Chippin, R. P. Hanson, Y. Hoska, V. TerMeulen, E. Norrby, W. Plowright, R. Rott, and W. H. Wunner.** 1978. Paramyxoviridae. Intervirology **10**:137–152.
20. **Lambert, D. M., and M. W. Pons.** 1983. Respiratory syncytial virus glycoproteins. Virology **130**:204–214.
21. **Levine, S., R. Klaiber-Franco, and P. R. Paradiso.** 1987. Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. J. Gen. Virol. **68**:2521–2524.
22. **Lipp, J., and B. Dobberstein.** 1986. The membrane-spanning segment of invariant chain (1) contains a potentially cleavable signal sequence. Cell **46**:1103–1112, 1122.
23. **Little, S. P., and A. S. Huang.** 1977. Synthesis and distribution of vesicular stomatitis virus-specific polypeptides in the absence of progeny production. Virology **81**:37–47.
24. **Markoff, L. J., and C.-J. Lai.** 1982. Sequence of the influenza A/Udorn/72 (H3N2) virus neuraminidase gene as determined from cloned full length DNA. Virology **119**:288–297.
25. **Markoff, L., B.-C. Lin, M. M. Sveda, and C.-J. Lai.** 1984. Glycosylation and surface expression of the influenza virus neuraminidase requires the N-terminal hydrophobic region. Mol. Cell. Biol. **4**:8–16.
26. **Norrby, E., M. A. Mufson, and H. Sheshberadaran.** 1986. Structural differences between subtype A and B strains of respiratory syncytial virus. J. Gen. Virol. **67**:2721–2729.
27. **Pressman, B. C.** 1976. Biological application of ionophores. Annu. Rev. Biochem. **45**:501–630.
28. **Satake, M., J. E. Coligan, N. Elango, E. Norrby, and S. Venkatessan.** 1985. Respiratory syncytial virus envelop glycoprotein (G) has a novel structure. Nucleic Acids Res. **13**:7795–7812.
29. **Strand,. M., and J. T. August.** 1976. Oncoronavirus envelope glycoproteins in serum of mice. Virology **75**:130–144.
30. **Sveda, M. M., L. J. Markoff, and C.-J. Lai.** 1982. Cell surface expression of the influenza virus hemagglutinin requires the hydrophobic carboxy-terminal sequences. Cell **30**:649–656.
31. **von Heijne, G.** 1983. Patterns of amino acids near signal-sequence cleavage sites. Eur. J. Biochem. **133**:17–21.
32. **von Heijne, G.** 1984. How signal sequences maintain cleavage specificity. J. Mol. Biol. **173**:243–251.
33. **Waechter, C. M., and W. J. Lennarz.** 1976. The role of polyprenol-linked sugars in glycoprotein synthesis. Annu. Rev. Biochem. **45**:95–112.
34. **Walsh, E. E., M. W. Brandriss, and J. J. Schlesinger.** 1985. Purification and characterization of the respiratory syncytial virus fusion protein. J. Gen. Virol. **66**:409–415.
35. **Walsh, E. E., and J. Hruska.** 1983. Monoclonal antibodies to respiratory syncytial virus proteins: identification of the fusion protein. J. Virol. **47**:171–177.
36. **Walsh, E. E., J. J. Schlesinger, and M. W. Brandriss.** 1984. Purification and characterization of GP90, one of the envelope glycoproteins of respiratory syncytial virus. J. Gen. Virol. **65**: 761–767.
37. **Wertz, G. W., P. L. Collins, Y. Huang, C. Gruber, S. Levine, and L. A. Ball.** 1985. Nucleotide sequence of the G protein gene of human respiratory syncytial virus reveals and unusual type of viral membrane protein. Proc. Natl. Acad. Sci. USA **82**:4075–4079.
38. **Wright, P. F., R. B. Belshe, H. W. Kim, L. P. Van Voris, and R. M. Chanock.** 1982. Administration of a highly attentuated live respiratory syncytial virus vaccine to adults and children. Infect. Immun. **37**:397–400.