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# Shedding of the Glycoprotein from Vesicular Stomatitis Virus-Infected Cells

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In a culture of Chinese hamster ovary cells infected with vesicular stomatitis virus, there is specific shedding of viral antigens into the medium. This shedding appears to be unrelated to progeny formation or to cell lysis. Although all five of the virus-specific proteins are detected in the extracellular soluble fraction, the major antigen is the Gs protein. This protein has a molecular weight of 54,000. Indirect analysis of the content of sialic acid as well as peptide analysis of the Gs and G proteins of vesicular stomatitis virus suggest that the Gs protein is derived from the G protein by proteolysis. Both proteins are hydrophobic when analyzed by charge-shift electrophoresis. The presence of phenylmethylsulfonyl fluoride in the culture medium or the removal of serum from the culture medium partially reduces the shedding of Gs protein. Increased shedding of the Gs protein is seen when there is an unstable M or matrix protein synthesized by a temperature-sensitive mutant, *ts*G31. These results indicate that the G protein is cleaved at the cell surface, thus releasing Gs protein into the medium. Furthermore, the stability of G protein at the cell surface appears to be dependent on its association with the M protein.

Vesicular stomatitis virus (VSV) is an enveloped RNA-containing virus with five structural proteins, of which one, the G protein, is glycosylated and found on the surface of the virion. The virion envelope is derived from the plasma membrane (21). It is believed that just internal to the envelope is the matrix or membrane (M) protein and the viral core, which consists of RNA, N, L, and NS proteins (31).

During infection by VSV, virus-specific polypeptides are released into the culture medium as soluble antigens (2, 4, 13). Soluble VSV antigens in the extracellular environment represent about 3% of the total VSV proteins in an infected culture (14, 22). The major VSV soluble antigen is a glycoprotein, Gs, and is readily identified because it is antigenically related to G protein and has a faster mobility on sodium dodecyl sulfate (SDS)-polyacrylamide gels than the G protein (13, 22). This glycoprotein Gs represents one-sixth the amount of G protein associated with the infected cell surface and is shed whether or not there is inhibition of the synthesis of progeny virions (22).

Although glycoproteins of murine leukemia virus have been detected as soluble antigens (1, 29), few studies have concentrated on how these antigens reach the extracellular environment. The studies reported here focus on one of the soluble antigens of VSV, the Gs protein, with the purpose of determining how Gs protein is shed from infected cells and what controls the stability of G protein at the cell surface. In addition, comparison of the virion-associated G protein and Gs protein is presented.

(This paper is based on studies submitted by S.P.L. to the Faculty of Arts and Sciences, Division of Medical Sciences of Harvard University, in partial fulfillment of the requirements for the Ph.D. degree. Preliminary presentation of some of these results was made at the 1977 ICN-UCLA Symposium on Cell Surface Carbohydrates and Biological Recognition.)

## MATERIALS AND METHODS

The San Juan strain of the Indiana serotype of VSV and Chinese hamster ovary (CHO) cells have been described in detail (28). *ts*G31 was obtained from Craig Pringle, Glasgow, Scotland.

Infection of cells, incorporation of radioactive precursors, and the fractionation of the infected cultures into three fractions (cellular, virion-associated, and soluble antigens) have been previously described (22).

**Polyacrylamide gel electrophoresis of VSV proteins.** Separation of proteins on slab gels has been described (22). Briefly, soluble viral antigens obtained from cell-free and virion-free culture media were analyzed on Laemmli 7.5% SDS-polyacrylamide gels (19). Electrophoresis was carried out at 100 V for 6 h to detect $^{35}$S-labeled material; the gels were dried in a Hoefer slab gel dryer (Hoefer Scientific Instruments,

San Francisco, Calif.) and exposed to X-ray film. Autoradiograms were scanned with a Gilford recording spectrophotometer (Elkhart, Ind.). To detect unlabeled proteins, gels were stained with 0.25% Coomassie brilliant blue in a solution containing methanol-water-acetic acid (5:1:5) and destained in a solution containing 20% methanol and 7% acetic acid.

**Tryptic peptide analysis by ionophoresis.** [$^{35}$S]methionine-labeled G and Gs proteins were first purified on Laemmli 7.5% SDS-polyacrylamide gels as described above. Bands representing the G and Gs proteins were cut out of the gel using the developed X-ray film as a template. Tryptic digests of G and Gs proteins were made by digesting the protein-containing gel pieces directly in 1 ml of 1% ammonium bicarbonate containing 50 μg of trypsin (25). Digestion proceeded at 37°C for 24 h with constant agitation. The gel pieces were placed in fresh digestion solution and allowed to incubate (4 h) until less than 10% acid-precipitable radioactivity remained in the gel pieces. Protein in the digestion solutions was pooled, lyophilized, and washed two times.

Ionophoresis on Whatman 3MM paper at pH 3.5 for 3 h at 3,000 V was carried out in a Savant high-voltage electrophoresis apparatus. Radioactivity was determined by counting 1-cm strips of 3MM paper immersed in Bray solution (3).

**Peptide analysis by limited proteolysis in SDS and analysis by gel electrophoresis.** Peptide maps of virion-associated G protein and Gs protein were generated according to the procedures described by Cleveland et al. (6). [$^{35}$S]methionine-labeled virion and soluble proteins were first purified on a Laemmli 7.5% SDS-polyacrylamide gel. Bands (2 by 5 mm) containing the G and Gs proteins were excised as described for tryptic peptide analysis and placed into 10 ml equilibration buffer (0.125 M Tris, pH 6.8; 0.1% SDS; 1 mM EDTA) and incubated at 22°C for 30 min. Gel slices were then placed into sample wells of a Laemmli 15% SDS-polyacrylamide gel. The top of the gel consisted of a 3% acrylamide stacking gel of 5-cm length. To each sample well was added 10 μl of well filler (equilibration buffer containing 20% glycerol), 25 μg of bovine serum albumin (BSA), and 10 μg of α-chymotrypsin or *Staphylococcus aureus* V-8 protease in equilibration buffer containing 10% glycerol. Gel pieces were immediately subjected to continuous electrophoresis at 40 V for 18 h, without any interruption as the material passed from the stacking gel into the 15% polyacrylamide gel. With this procedure, digestion occurred in the stacking gel during electrophoresis. Then the gel was stained with Coomassie blue, dried, and exposed to X-ray film.

**Materials.** The radioactive precursor L-[$^{35}$S]methionine at 452 Ci/mM was obtained from New England Nuclear, Boston, Mass. Trypsin (treated with diphenylcarbamoyl chloride) and neuraminidase (*Vibrio cholerae*) were purchased from Calbiochem, La Jolla, Calif. *S. aureus* V-8 protease was purchased from Miles Laboratories, Oberlin, Ohio, and α-chymotrypsin, Triton X-100, and sodium deoxycholate were obtained from Sigma Chemical Co., St. Louis, Mo. Agarose was purchased from Bausch and Lomb, Rockland, Maine.

BSA, catalase, ovalbumin, γ-globulin, and alcohol dehydrogenase were kind gifts from Frank Ramig. Rabbit anti-VSV serum was heat-inactivated serum obtained from rabbits immunized intraperitoneally three times with 1 ml of purified VSV (11). Actinomycin D was a kind gift of Merck Sharp and Dohme, Inc., Rahway, N.J. All other chemicals and reagents were obtained as described previously (12, 22).

## RESULTS

**Soluble, extracellular proteins produced by VSV-infected cell cultures.** Extracellular viral proteins are usually harvested from the culture medium after the culture has been clarified of infected cells and virions. Because the medium contains serum globulins, it has been difficult to obtain clean separations of some of the soluble viral proteins and to quantitate their amounts. Two methods for overcoming these problems are to increase the specific activity of the labeled viral proteins or to immunoprecipitate the viral proteins by specific antisera.

Figure 1 shows the extracellular soluble VSV proteins that have been highly labeled with [$^{35}$S]methionine. Without immunoprecipitation it was possible to detect in the soluble fraction all of the virus-specific proteins. The kinetics of their release indicate the L, G, N, and M proteins were shed from the cell at about equal rates up to 2 h. The NS protein did not appear to increase over time. The most dramatic increase was in the Gs protein, which became the major soluble protein within 2 h.

Because Gs protein was found in the largest amount and because the kinetics of its appearance in the extracellular medium differed from the other proteins, further studies focused on this antigen.

**Molecular weight determination of the Gs protein.** The Gs protein migrates faster than the G protein in SDS gels (13). To determine the molecular weight of the Gs protein in the absence of serum globulins, the material in the soluble fraction was immunoprecipitated and co-electrophoresed with standard marker proteins. Under these conditions, the G protein has an apparent molecular weight of 65,000 and the Gs protein appears as 54,000 (Fig. 2). The other viral polypeptides, N, NS, and M, are also shown.

The N protein (50,000) and NS protein (48,000) migrate as predicted by their published molecular weight (32). The molecular weight of the G protein, recently estimated at 67,000 (16), is somewhat uncertain because of its carbohydrate content. Glycoproteins may migrate anomalously in SDS-containing gels because their carbohydrate portion does not bind SDS (24). Even so, the difference between the Gs and G proteins suggests a significant alteration of

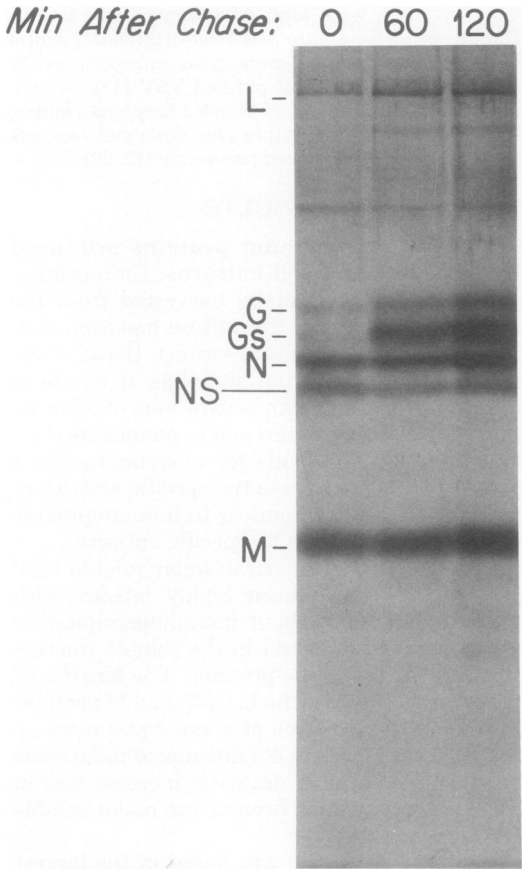


FIG. 1. *Kinetics of shedding of soluble VSV proteins from infected cells. A sample of 6 × 10⁷ CHO cells was infected with VSV at a multiplicity of 20 PFU/cell and incubated at 34°C. At 2.5 h after infection, [³⁵S]methionine (750 μCi) was added to the infected cell culture. After 60 min, 2 × 10⁷ cells were removed and placed at 4°C before harvest. The remainder was incubated with 1,000× nonradioactive methionine in excess of the radioactive material for 60 min, at which time 2 × 10⁷ cells were harvested. This process was repeated at 120 min. Infected cells were fractionated, and equal aliquots of the soluble proteins were analyzed on 7.5% polyacrylamide gels as described in the text.*

either the carbohydrate or the protein moiety of the G protein to give rise to Gs protein.

**Extent of glycosylation of the G and Gs proteins.** The G and Gs proteins can be readily labeled with glucosamine (13, 22). To further compare the extent of glycosylation between G and Gs protein, these glycoproteins were tested for the presence of sialic acid. The removal of sialic acid can be detected indirectly by digestion of the glycoprotein with neuraminidase, which results in an increase in the migration rate of glycoproteins on SDS gels (17).

Figure 3 shows the slightly increased mobility of G protein after neuraminidase digestion of virion-associated proteins (slot d) when compared with control undigested samples (slots e and f). A similar change in Gs protein was barely discernible after neuraminidase digestion (slot a); the overloading of these slots (a-c) with unlabeled serum proteins obscured the real mobility of the G and Gs proteins. Immunoprecipitation of a similarly neuraminidase-treated soluble fraction yielded a small but discernible difference in migration from the untreated sample (Fig. 4).

Such an alteration in migration might be interpreted to be due to proteolytic cleavage, except that the neuraminidase used in this experiment was free of any detectable proteolytic activity and did not affect migration of the other viral proteins, L, N, NS, and M. Because sialic acid residues have been characterized as terminal sugars on the carbohydrate chains of the VSV G protein (5, 9), the similarity of behavior between the G and Gs proteins after neuraminidase treatment suggests that the Gs protein has

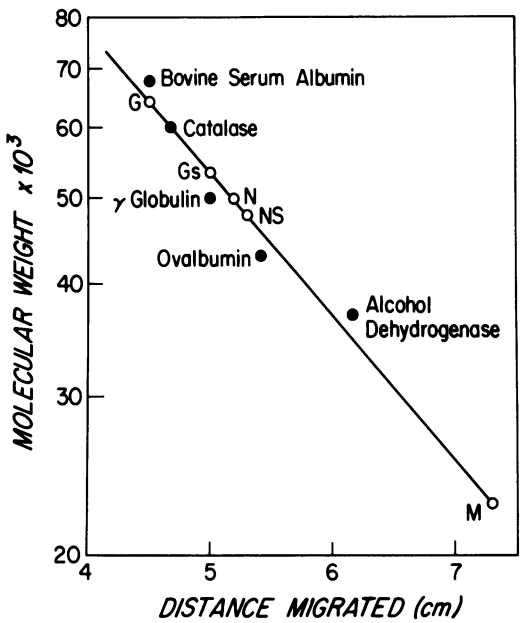


FIG. 2. *Molecular weight of the Gs protein. Soluble [³⁵S]methionine-labeled proteins from VSV-infected cells were mixed with rabbit anti-VSV serum at a final concentration of 1:100 and allowed to incubate for 18 h at 4°C. The precipitated material was washed with phosphate-buffered saline and resuspended for analysis on 7.5% polyacrylamide gels. For comparison, ³⁵S-labeled whole virions were also analyzed. Five protein standards each at 10 μg were analyzed in separate gel slots. They were BSA (68,000); catalase (60,000); γ-globulin (heavy chain) (50,000); ovalbumin (43,000); and alcohol dehydrogenase (37,000).*

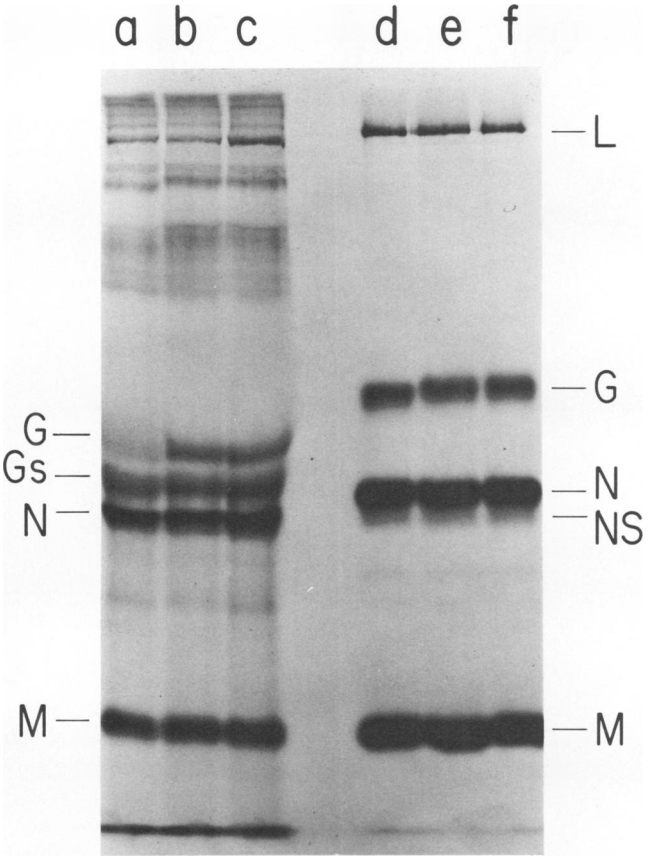


FIG. 3. *Neuraminidase digestion of G and Gs proteins. [$^{35}$S]methionine-labeled soluble proteins and virions of VSV were incubated with 12.5 U of neuraminidase for 30 min at 37°C. After digestion, the proteins were separated on a 7.5% polyacrylamide gel. (a) Soluble proteins, incubated with neuraminidase; (b) soluble proteins, incubated without neuraminidase; (c) soluble proteins, unincubated; (d) virions, incubated with neuraminidase; (e) virions, incubated without enzyme; (f) virions, unincubated.*

a full complement of carbohydrates and is as fully glycosylated as the G protein.

**Peptide analysis of VSV glycoproteins.** To determine whether the Gs protein is a proteolytic cleavage product of the G protein, peptides of G and Gs proteins were compared by two different methods. In one case, [$^{35}$S]methionine-labeled tryptic peptides were separated in one dimension by paper electrophoresis. The arrow in Fig. 5 points to the major difference between G and Gs proteins. The other differences, at 16 and 37 cm, between G and Gs proteins were not reproducible from experiment to experiment.

Another way to compare the peptides of G and Gs proteins was to generate them by limited proteolysis in SDS and separation on polyacrylamide gels (6). The oligopeptide pattern of G and Gs proteins treated with α-chymotrypsin and *S. aureus* V-8 protease are shown in Fig. 6 and 7, respectively. For each peptide digestion, 25 μg of BSA was added to maintain an equivalent enzyme-to-substrate ratio. The stained gel indicated that BSA was cleaved into the same number of fragments whether it was codigested with G or Gs proteins (data not shown).

Chymotrypsin cleaves after tryptophan, phenylalanine, tyrosine, and, to a lesser extent, leucine and methionine residues. The peptides generated by chymotrypsin digestion are shown in Fig. 6. The spectrophotometer tracing of the autoradiogram shows a difference in the migration of two peptides of Gs protein, which are indicated by the vertical lines. The undigested BSA marker, shown by the arrow, indicates peptides larger than 68,000 daltons. *S. aureus* V-8 protease cleaves after glutamic acid and aspartic acid residues (6). Comparison of G with Gs proteins after V-8 digestion showed a difference in the migration of one peptide, indicated by a

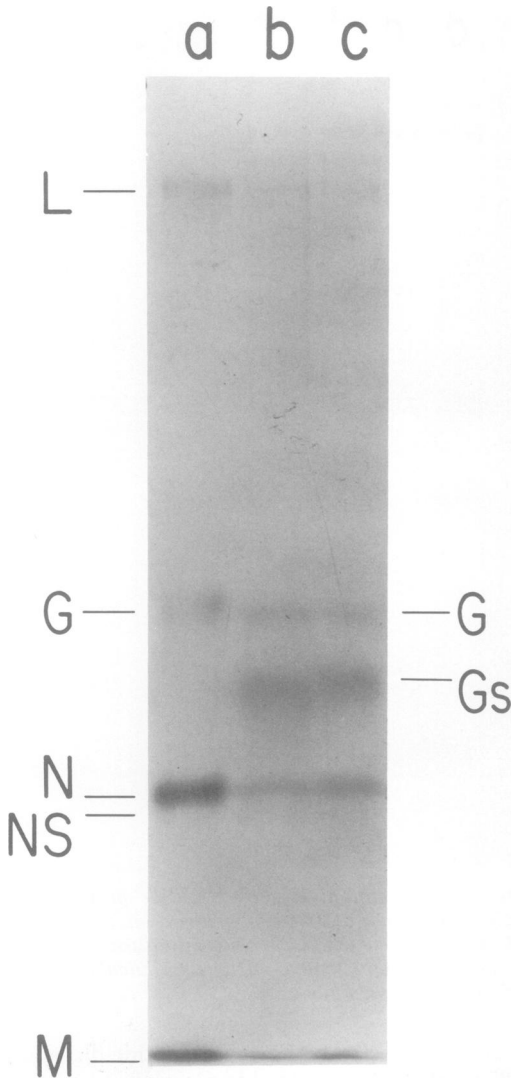


FIG. 4. *Neuraminidase digestion of immunoprecipitated Gs protein. [$^{35}$S]methionine-labeled soluble proteins of VSV were digested with 12.5 U of neuraminidase for 30 min at 37°C. Then the proteins were precipitated with rabbit anti-VSV serum (1:100) at 4°C for 18 h. The precipitated material was washed with phosphate-buffered saline and then separated on a 7.5% polyacrylamide gel. (a) VSV marker proteins; (b) soluble proteins, incubated with neuraminidase; (c) soluble proteins, incubated without neuraminidase.*

vertical line in Fig. 7. For both proteases, the peptides of the Gs protein that differ from those of the G protein migrated faster and therefore indicated that they were of a smaller molecular weight. The differences in molecular weight did not account for the 10,000- to 11,000-dalton difference between G and Gs proteins; however, only [$^{35}$S]methionine-labeled peptides were detected in these experiments. Nevertheless, these results support the hypothesis that proteolytic cleavage of the G protein resulted in the formation of the Gs protein.

**Charge-shift electrophoresis of G and Gs proteins.** Treatment of virions with other pro-

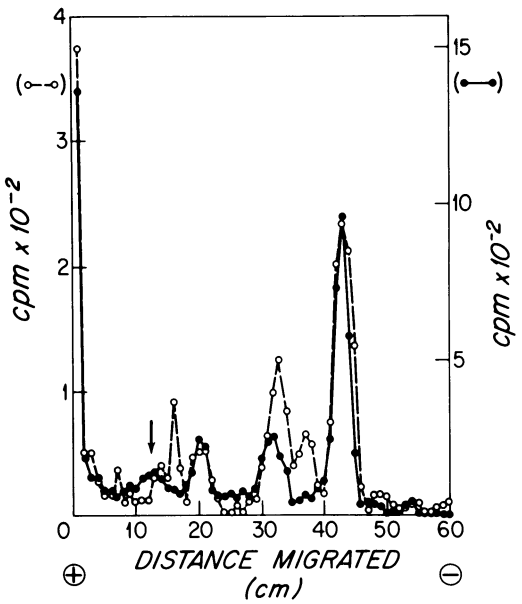


FIG. 5. *Comparison of tryptic peptides of G and Gs protein. [$^{35}$S]methionine-labeled G and Gs proteins were purified on 7.5% polyacrylamide gels, digested with trypsin, and subjected to ionophoresis according to the procedures described in the text.* (O– – –O) *G protein;* (●——●) *Gs protein.*

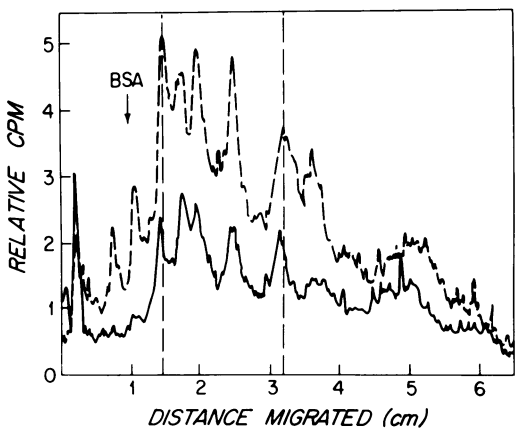


FIG. 6. *Comparison of peptides of G and Gs proteins generated by limited digestion with α-chymotrypsin. [$^{35}$S]methionine-labeled G and Gs proteins were excised from SDS-polyacrylamide gels and subjected to limited proteolysis by chymotrypsin in a 15% polyacrylamide gel as described in the text.* (——) *G protein;* (– – –) *Gs protein;* (↓) *undigested BSA marker.*

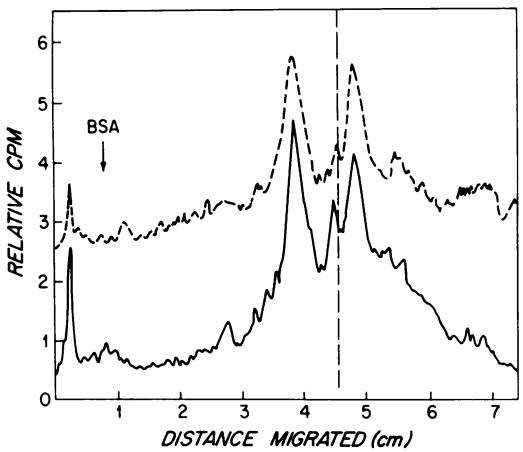


FIG. 7. *Comparison of peptides of G and Gs proteins generated by limited digestion with S. aureus V-8 enzyme. [$^{35}$S]methionine-labeled G and Gs proteins were excised from an SDS-polyacrylamide gel and digested with V-8 enzyme as described in the text. (——) G protein; (- - -) Gs protein; (↓) undigested BSA marker.*

teases cleaves the G protein at the membrane (26, 27), resulting in a hydrophobic fragment which is left imbedded in the membrane. If the Gs protein resulted from the cleavage of G at a similar site, then the Gs protein should be less hydrophobic than the G protein. To test this, the G and Gs proteins were subjected to charge-shift electrophoresis. This technique, introduced by Helenius and Simons (10), allows the comparison of hydrophobic and hydrophilic proteins in detergent solutions. When a hydrophobic protein associates with a nonionic detergent (Triton X-100), it will migrate according to its charge in 1% agarose. If a charged detergent (sodium deoxycholate) is also added to this protein, a shift in migration due to the net charge by association with the ionic detergent will occur. A comparison of electrophoretic shift in migration should indicate a difference in the hydrophobic nature of the protein.

Figure 8 shows the autoradiogram of virus-associated and soluble polypeptides subjected to such charge-shift electrophoresis. The G and Gs

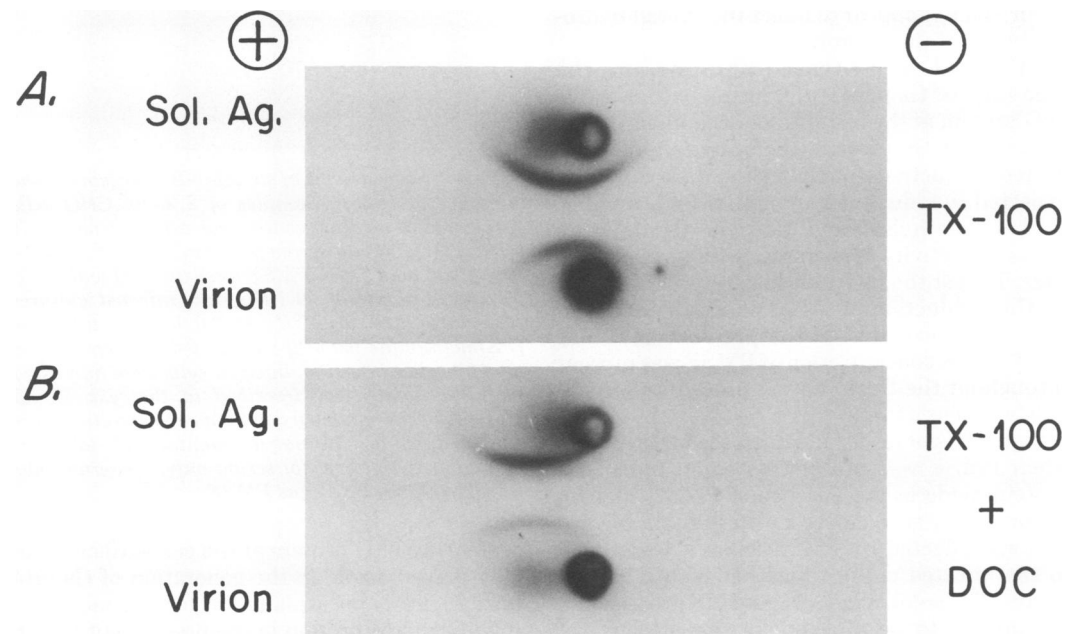


FIG. 8. *Charge-shift electrophoresis of VSV soluble antigens and structural proteins. [$^{35}$S]methionine-labeled soluble proteins and VSV marker proteins were subjected to charge-shift electrophoresis essentially as described by Helenius and Simons (10). Agarose electrophoresis was performed at room temperature in 1% agarose in glycine buffer (0.05 M glycine, 0.1 M sodium chloride) on glass microscope slides using a water-cooled chamber. The 1% agarose and running buffer contained either 0.5% Triton X-100 (TX-100) alone or 0.5% Triton X-100 and 0.25% sodium deoxycholate (DOC). Radioactive samples (10,000 cpm) of soluble antigens and virions were incubated for 30 min at 37°C in 20 μl of glycine buffer containing 2% Triton X-100 alone or 2% Triton X-100 and 1% sodium deoxycholate. After preparation of the agarose plates, wells were punched out, and 10 μl of radioactive samples was added to each well. Electrophoresis was performed at 100 V for 3 h. After electrophoresis, a rectangular slit was made between the wells with a razor blade. Rabbit anti-VSV serum (50 μl) was added to the slit to identify the G and Gs proteins, and the plates were incubated at room temperature for 18 h. The agarose plates were then soaked in sodium borate buffer (0.4% sodium chloride and 0.4% sodium borate) for 18 h, dried, and exposed to X-ray film (5 days).*

proteins, which were presumably the major proteins solubilized by Triton X-100, reacted with antibody and showed up as precipitin arcs in the agarose. To demonstrate the identity of the major protein in the precipitin arc, this band was removed from the agarose gel and the material in it was subjected to electrophoresis on a 7.5% SDS-polyacrylamide gel together with VSV marker proteins. The only labeled protein detected in the precipitin arcs was G or Gs protein (data not shown). The shifted precipitin lines of both the virion-associated G protein and Gs protein when subjected to electrophoresis in the presence of sodium deoxycholate was reproducible. When another charged detergent, cetyltrimethylammoniumbromide, was used instead of sodium deoxycholate, both Gs and G moved towards the negative pole.

Rather than demonstrate a loss of a hydrophobic portion of the G protein during the generation of the Gs protein, these results, surprisingly, demonstrated that both proteins were highly hydrophobic. The loss of a 10,000- to 11,000-dalton piece of protein, if it did in fact occur, did not appear to affect the overall hydrophobicity of the G protein.

**Effect of a protease inhibitor on the cleavage of Gs protein.** If proteolytic cleavage of G protein at the cell surface accounted for the shedding of Gs protein, then any inhibition of proteolytic activity at the cell surface might be expected to reduce the amount of Gs protein.

Phenylmethylsulfonyl fluoride (PMSF) inhibits the activity of proteases with serine-active sites. To test the effect of this protease inhibitor on the production of Gs protein, VSV-infected cells were incubated in the presence of 2 mM PMSF. This concentration of PMSF was present throughout the fractionation procedure for soluble antigens as well.

Figure 9 (slot c) shows a decrease in Gs protein when PMSF was present, as compared to Gs protein made in the absence of PMSF (slot b). Surprisingly, an increase in the soluble M protein was effected by PMSF when it was added to the infected culture together with fetal calf serum. The soluble L, G, N, and NS proteins did not appear to be affected by the presence of PMSF. For comparison, virion marker polypeptides are shown in slot a. When the serum was replaced with BSA (10 μg/ml) and PMSF, Gs protein and the other soluble proteins were also reduced (slot d). Soybean trypsin inhibitor in the medium affected Gs protein similarly (data not shown).

Although there was never a complete inhibition of the appearance of Gs protein in the extracellular environment as a result of inhibiting proteolytic activity, these data suggest that

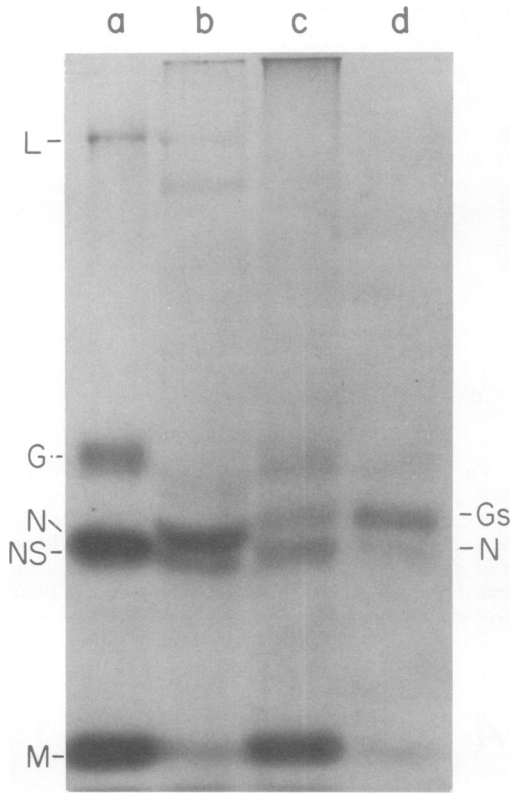


FIG. 9. *Appearance of soluble VSV proteins in the presence of PMSF. Samples of 2 × 10⁷ CHO cells suspended in medium containing either 2% fetal calf serum, 2% fetal calf serum plus 2 mM PMSF, or BSA (10 μg/ml) plus 2 mM PMSF were infected with VSV at a multiplicity of 20 PFU/cell. Infected cultures were incubated at 34°C. At 2.0 h after infection, [³⁵S]methionine (50 μCi) was added to each sample. At 5.0 h after infection, infected cells were harvested and fractionated as described in the text. Equal aliquots of the soluble fraction of each infection were analyzed on a 7.5% polyacrylamide gel. (a) VSV marker polypeptides; (b) serum only; (c) serum plus PMSF; and (d) BSA plus PMSF.*

proteolysis of G protein at the cell surface probably played a role in the generation of Gs protein. There is no explanation for the increased shedding of M protein in the presence of PMSF together with serum. The variability of the amount of soluble M protein from experiment to experiment has been observed before (22).

**Stability of G protein at the cell surface.** One factor influencing the shedding of G protein may involve the inability of G protein to recognize M protein at the plasma membrane and thereby result in a rapid loss of G from the cell surface (22). To further test this hypothesis, CHO cells were infected with a temperature-sensitive mutant, *ts*G31, which has been shown

to have a lesion affecting the M protein (15, 20). Virus-specific proteins made at permissive (31°C) and nonpermissive (38°C) temperatures were fractionated into progeny virions and soluble proteins and analyzed by SDS-polyacrylamide gel electrophoresis.

Figure 10 shows that Gs protein was produced in somewhat greater amounts at the nonpermissive temperature (slot d) than at the permissive temperature (slot c). The virion fraction made at 38°C contained very little M protein, but G protein was present (slot b). When this fraction was analyzed on sucrose-containing gradients, virions at 600S were not detected, and only nucleocapsids were measured (S. P. Little and G. M. Clinton, unpublished data; 30).

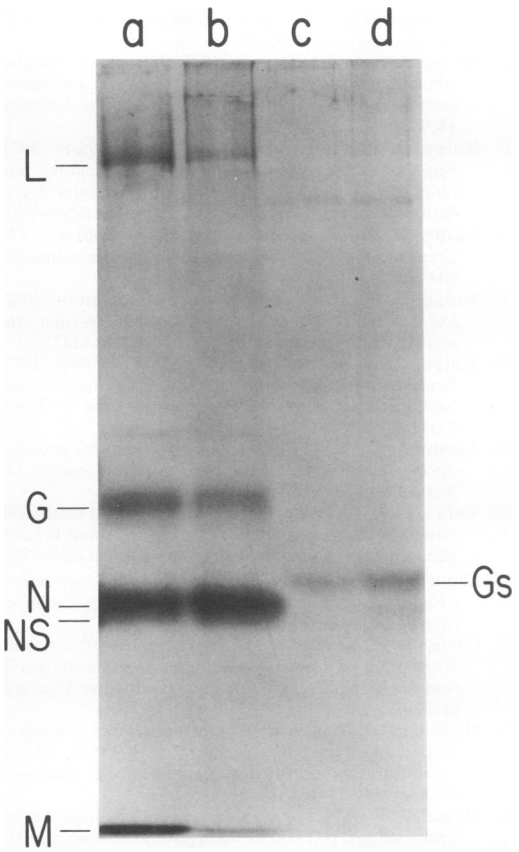


FIG. 10. *Proteins in the virion and soluble fractions of cells infected with tsG31 at permissive and nonpermissive temperatures. A sample of 4 × 10⁷ CHO cells, infected with tsG31 at a multiplicity of 20 PFU/cell, was equally divided and incubated at 31 and 38°C. At 2 h after infection, 50 μCi of [³⁵S]methionine was added to each culture. At 5 h after infection, cells were harvested, fractionated, and analyzed on a 7.5% polyacrylamide gel as described in the text. The film was exposed for 5 days. (a,c) Virions and soluble fractions, respectively, at 31°C; (b,d) virions and soluble fractions, respectively, at 38°C.*

The increase in Gs protein at the nonpermissive temperature was specific for this mutant as well as for a glycoprotein mutant, *ts*045 (22). Wild-type virus (data not shown) or a mutant in the N protein, *ts*G41, did not show any increased shedding of Gs protein at 38°C as compared to 31°C (22).

## DISCUSSION

This study was designed to determine the possible mechanism(s) by which viral glycoproteins reach the extracellular environment as soluble antigens. VSV-infected cells were used because analysis of extracellular soluble VSV proteins indicated the presence of a major antigen, the Gs protein, as well as all of the other virus-specific proteins. Previous studies have ruled out degradation of virions or cell lysis as possible sources for extracellular soluble proteins (22); therefore, these proteins must be shed from infected cells by mechanisms other than that associated with virion morphogenesis and "budding out." Possible mechanisms for shedding in the absence of antiviral antibody include microvesiculation at the cell surface or proteolytic cleavage of those viral proteins that reach the cell surface.

Because most of the VSV proteins appeared intact in the extracellular soluble fraction, they were probably enclosed in small vesicles and subsequently released from the cell. The glycoprotein, on the other hand, appeared not only as intact G protein, but also as the cleavage product of G protein. This major extracellular soluble material, Gs protein, was probably cleaved from the cell surface, where fully glycosylated G protein is known to reside (18).

The instability of G protein at the cell surface may be due to a loss of recognition of M proteins by the G protein as the two associate at the plasma membrane of the infected cell (8). The increased shedding of Gs proteins when there is a lesion in the M protein supports this concept (Fig. 10). Also, such an association on virions has been demonstrated by the cross-linking of G-M heterodimers (7). Therefore, M protein can be envisioned as an anchoring protein which not only stabilizes G protein at the cell surface but also organizes the G protein into patches or aggregates at the cell surface. It is through such aggregations that G protein is likely to be further stabilized and protected from proteolytic cleavage. More detailed kinetic analysis of the appearance of extracellular G and Gs proteins may resolve these steps during the release of these antigens.

The site of cleavage of G protein to Gs protein might be similar to that observed when the

virion is treated with proteases, which results in a hydrophobic fragment imbedded in the membrane (26, 27). Therefore, the Gs protein should be less hydrophobic than the G protein. However, the shift in electrophoretic migration in the presence of nonionic and ionic detergents indicates that the Gs protein retained the hydrophobic nature of the G protein. This most probably means that G protein is very hydrophobic overall. Moreover, the detection of the aggregate of Gs protein sedimenting as 6S (13) probably reflects a hydrophobic association of 3 to 4 Gs molecules.

Previous studies demonstrated that there is significant shedding of virus-specific proteins into the extracellular environment even when the infection is abortive (22). What role these soluble proteins, especially the Gs protein, might play during viral pathogenesis can only be briefly surmised. The proteins can combine with host antibodies or cytotoxic immune cells and, perhaps, block their effectiveness. In addition, the specificity of the G protein in inhibiting cellular macromolecular synthesis has been recently shown (23), and the Gs protein may have similar cytotoxic properties.

## ACKNOWLEDGMENTS

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