JOURNAL OF VIROLOGY, Feb. 1978, p. 687-692
0022-538X/78/0025-0687$02.00/0

Vol. 25, No. 2
Printed in U.S.A.

# Glycoproteins of Measles Virus Under Reducing and Nonreducing Conditions

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Received for publication 18 July 1977

Measles virus has two glycoproteins. The larger glycoprotein (HA) is composed of 76,000-dalton subunits that are bound by disulfide bonds. The smaller glycoprotein (F) appears to contain a glucosamine-rich portion that is linked to an unglycosylated protein by disulfide bonds.

The two glycoproteins and associated biological activities of Sendai virus (7, 16, 18), simian virus 5 (14), and Newcastle disease virus (15) have been well characterized. Two distinct antigenic determinants have been identified on the surface of measles virions (12), but there are conflicting data concerning the identification and number of glycoproteins of measles virions analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (2, 5, 6). Although measles virus glycoproteins appear to be similar to glycoproteins of other paramyxoviruses, we have found some significant differences that disagree with previous reports on measles virus glycoproteins by Hall and Martin (5, 6).

When structural proteins of Sendai virus (9, 11, 13), Newcastle disease virus (8), and other paramyxoviruses (1) were examined under nonreducing conditions, the glycoprotein patterns on SDS-polyacrylamide gels differed from those patterns observed under reducing conditions, suggesting that disulfide bonds play an important role in the glycoprotein structure of these viruses. Because of the usefulness of this approach with other paramyxoviruses, we examined reduced and nonreduced preparations of measles virus proteins on SDS-polyacrylamide gels.

Vero cells were grown in plastic roller bottles (Corning Glass Works, Corning, N.Y.) at 37°C in medium consisting of 50% Earle based minimal essential medium (MEM) and 50% Hanks based MEM (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% fetal bovine serum (Microbiological Associates Inc., Bethesda, Md.). Confluent cell monolayers were infected with the Edmonston strain of measles virus at a multiplicity of infection of 0.1. The virus was allowed to adsorb for 2 h, after which time the inoculum was removed and fresh medium containing 5% serum was added. Virus used in these studies could be neutralized by measles hyperimmune monkey serum (lot 3-2017, Microbiological Associates).

Viral glycoproteins were labeled at 45 h postinfection by adding 20 μCi of [$^3$H]glucosamine (Amersham/Searle, Arlington Heights, Ill.; 20 Ci/mmol) per ml of medium, and virus was harvested 13 to 18 h later. The labeling medium consisted of Earle balanced salt solution (with the glucose concentration reduced by 70%) supplemented with vitamins, essential and nonessential amino acids for Eagle MEM (Flow Laboratories, Inc., Rockville, Md.) 2 mM L-glutamine, and 5% fetal bovine serum (Microbiological Associates). The pH was adjusted to 7.2 with 7.5% sodium bicarbonate. Viral proteins were labeled by adding 5 μCi of $^{14}$C-protein hydrolysate (Amersham/Searle; 55 mCi/milliatom of carbon) or 20 μCi of L-$^3$H-amino acid mix (New England Nuclear Corp., Boston, Mass.) per ml of medium (50% Earle based MEM and 50% Hanks based MEM, Grand Island Biological Co.) 40 to 45 h postinfection, and virus was harvested 20 to 25 h later.

Virus was purified from the medium over infected cell cultures, which was clarified by centrifuging at 12,000 × *g* for 10 min. The virus was sedimented onto a 60% (wt/wt) sucrose cushion by centrifugation at 27,000 rpm for 180 min in an SW27 rotor. Virus was recovered by puncturing the bottom of the tube and collecting drops. The virus preparation was diluted approximately 1:4 in phosphate-buffered saline, layered on top of a 30 to 60% (wt/wt) discontinuous sucrose gradient, and centrifuged at 40,000 rpm for 90 min in an SW41 rotor. Virus at the 30-60% interface was collected, diluted approximately 1:10 with phosphate-buffered saline, and centrifuged for 60 min at 40,000 rpm in a type 40 rotor. The pellet was resuspended in 0.5 ml of growth medium containing 1% serum and stored at −70°C.

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†† Robert H. Bussell died on 16 July 1976 at the age of 48. His students and colleagues deeply mourn his departure.

Labeled virions were disrupted under reducing conditions by immersion in a boiling-water bath for 2 min in 0.01 M sodium phosphate (pH 7.2), 5% β-mercaptoethanol, 4% SDS, 8 M urea, 4% glycerol, and 0.005% bromophenol blue and immediately layered on gels. Under nonreducing conditions, the β-mercaptoethanol was omitted. Gels (approximately 0.6 by 10 cm) consisted of 7.5% acrylamide and 0.2% bisacrylamide in 0.1% SDS–0.1 M sodium phosphate (pH 7.2) and were polymerized with TEMED (*N,N,N',N'*-tetramethylethylenediamine) and ammonium persulfate. The electrode buffer consisted of 0.1 M sodium phosphate (pH 7.5) and 0.1% SDS. Electrophoresis was carried out at a constant voltage of 40 V for 9.5 h. Alternatively, measles virus proteins were examined by electrophoresis in a Tris-glycine-buffered system as described by Hall and Martin (5). After electrophoresis, gels were removed from the glass tubes, frozen at −20°C, and sliced into 1-mm pieces. Each gel slice was incubated overnight at 37°C with 3 ml of toluene-Omnifluor containing 3% protosol (New England Nuclear) and counted in a liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Molecular weights were estimated by comparing relative migration of virion proteins to the migration of five standard markers (25,000 to 130,000 daltons) in the same or in an adjacent gel, and in all cases the major nucleocapsid subunit was used as an internal standard.

To elute viral polypeptides, gel pieces were macerated with a spatula and placed in 2 ml of 0.01 M Tris-hydrochloride (pH 7.5) and 1% SDS. Samples were agitated at 38°C for about 15 h and then centrifuged at 12,000 × *g* for 10 min to remove gel fragments. The supernatant was lyophilized and resuspended for electrophoresis. We estimate the recovery of radioactivity in such treatments to be about 50%.

When labeled measles virions were disrupted and subjected to electrophoresis under reducing conditions on polyacrylamide gels, five major polypeptides (HA, P, NC, $F_1$, and M) were observed (Fig. 1A). Of the major polypeptides, only HA (76,000 daltons) contained glucosamine. These data agree with a previous report from this laboratory (2) and demonstrate that measles virus differs from other paramyxoviruses, which have two major glycoproteins when resolved under reducing conditions (3). However, our data disagree with the experiments described by Hall and Martin (5, 6), who reported that two major virus glycoproteins were resolved by electrophoresis of virions disrupted under reducing conditions. In addition to the major glycoprotein, we have consistently observed heterogeneous, low-molecular-weight, [$^3$H]glucosamine-labeled components of approximately 20,000 daltons, designated $F_2$. Although there was always an appreciable amount of glucosamine-labeled material at the top of the gel, the amino acid label found at the top of the gel represented only 4.5% of the total amino acid radioactivity. Therefore, it is unlikely that a major glycoprotein failed to enter the gel. The P protein has an apparent molecular weight of 62,000 and has been shown to be a nucleocapsid-associated phosphoprotein in measles virus (S. J. Robbins and R. H. Bussell, unpublished data) and in canine distemper virus (2). The NC protein is the major nucleocapsid subunit (2) and has a molecular weight of 58,000. The protein designated $F_1$ migrates with an apparent molecular weight of 42,000. By analogy with other paramyxoviruses, the protein designated M (37,000 daltons) is the membrane protein.

To determine whether any measles virus proteins are linked by interchain disulfide bonds, measles virions were disrupted without β-mercaptoethanol and subjected to electrophoresis (Fig. 1B). To establish a correspondence between peaks observed with virus disrupted under reducing and nonreducing conditions, we excised three sections from gels of virus disrupted under nonreducing conditions. Protein was eluted from these gel sections, exposed to β-mercaptoethanol, and subjected to electrophoresis. The first five fractions of a gel of nonreduced measles virion proteins labeled with $^3$H-amino acids gave rise to peaks corresponding to HA and P when treated with β-mercaptoethanol and resubjected to electrophoresis (Fig. 2A). The HA and P polypeptides may form nonspecific disulfide linkages under nonreducing conditions. The protein eluted from fractions 6 to 10 of a gel of nonreduced virion polypeptides labeled with [$^3$H]glucosamine (Fig. 1B) gave rise to only one peak corresponding to HA when subjected to electrophoresis (Fig. 2B). When this experiment was performed on virions labeled with $^{14}$C-amino acids, the same result was obtained, indicating that the nonreduced form of HA did not contain unglycosylated polypeptides. Therefore, it appears that two or more 76,000-dalton HA glycoproteins are linked by disulfide bonds to form a large complex observed when virions are disrupted under nonreducing conditions. To examine the [$^3$H]glucosamine-labeled component that comigrates with the major nucleocapsid subunit under nonreducing conditions, viral polypeptides labeled with [$^3$H]glucosamine and $^{14}$C-amino acids were eluted from gel fractions 38 to 48 (Fig. 1B), treated with β-mercaptoethanol, and subjected to electrophoresis (Fig. 2C).

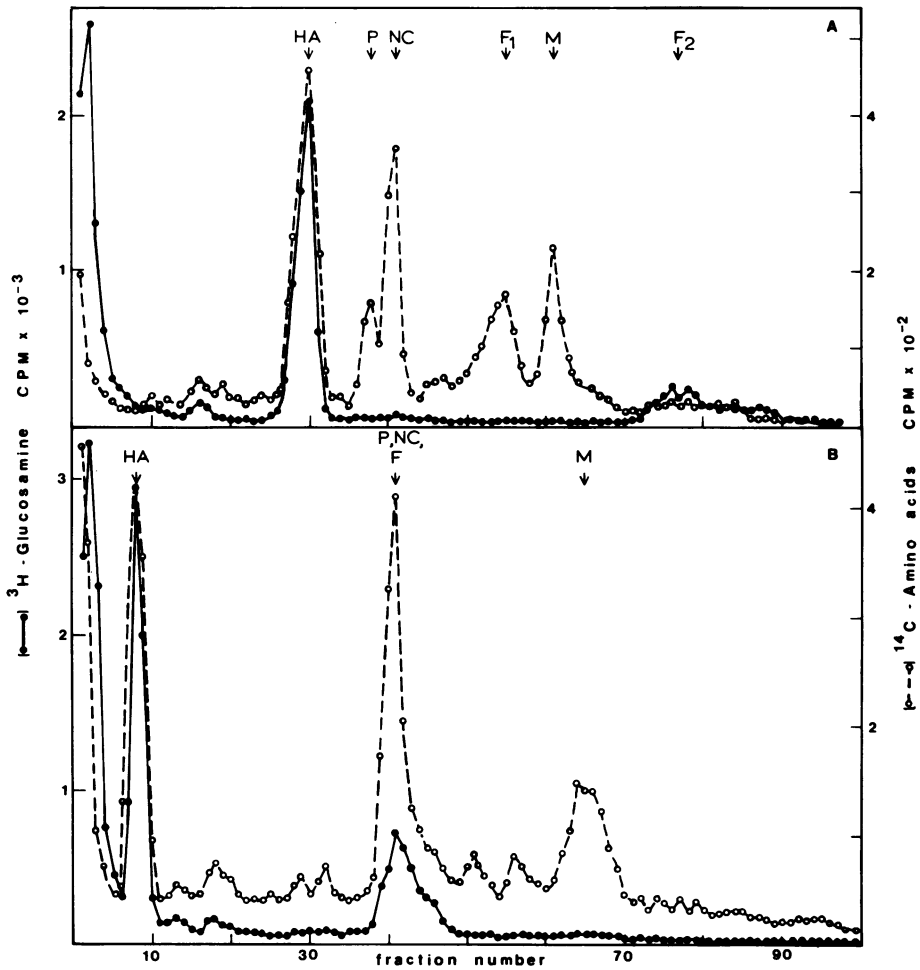


FIG. 1. *SDS-polyacrylamide gel electrophoresis of [$^3$H]glucosamine-labeled and $^{14}$C-amino acid-labeled measles virion proteins under (A) reducing and (B) nonreducing conditions.*

We observed three major polypeptides corresponding to peaks P, NC, and $F_1$. The glucosamine label was predominantly found as heterogeneous, low-molecular-weight, glucosamine-rich material, designated $F_2$. Apparently, F is cleaved to form unglycosylated $F_1$ and glucosamine-rich $F_2$ when disulfide bonds are broken. Attempts to label $F_1$ with [$^3$H]fucose were also unsuccessful.

When virus is disrupted under nonreducing conditions, the P and M polypeptides migrate slightly faster than when virus is disrupted under reducing conditions, which results in P polypeptide comigrating with NC polypeptide under nonreducing conditions. This is probably due to intrachain disulfide bonds in P and M (4).

The reduced form of HA can be resolved into two peaks when subjected to electrophoresis in the Tris-glycine-buffered gel system of Hall and Martin (5; Fig. 3A). This indicates that there may be two species of HA glycoproteins. The pattern observed with the Tris-glycine-buffered system on virus disrupted under nonreducing conditions (Fig. 3B) is similar to the gel pattern of Fig. 1B.

We conclude from our results that measles virus has two glycoproteins. The HA glycoprotein exists as a complex of two or more polypeptide subunits, which are linked by disulfide bonds. This is analogous to the result of Ozawa and Asano (13), who showed that the HA of Sendai virus exists as a large complex under nonreducing conditions. By analogy with other paramyxoviruses, the HA glycoprotein of measles virus is expected to have hemagglutination activity. The F glycoprotein of measles virus

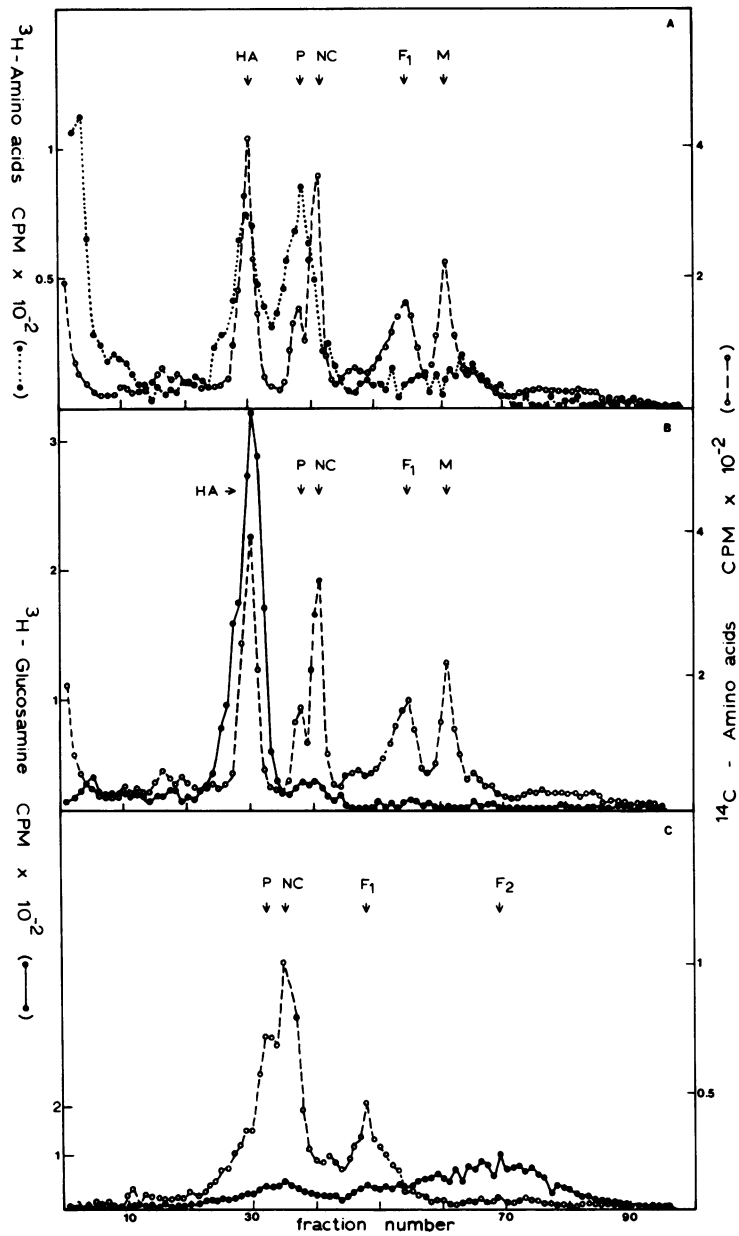


FIG. 2. *SDS-polyacrylamide gel electrophoresis under reducing conditions of $^{3}H$-amino acid-labeled protein eluted from fractions 1 to 5 of a nonreducing gel (A). The glycoprotein labeled with [$^{3}H$]glucosamine under peak HA was eluted from a nonreducing gel and rerun under reducing conditions (B). The $^{14}C$-amino acid markers (A and B) were obtained from whole virus subjected to electrophoresis simultaneously in an adjacent gel. The [$^{3}H$]glucosamine- and $^{14}C$-amino acid-labeled polypeptides under the P, NC, F peak were eluted from a nonreducing gel and subjected to electrophoresis for 6.5 h under reducing conditions (C). Molecular weights were determined by measuring the relative migration to standards in the same and adjacent gels.*

differs from that of other paramyxoviruses because it appears to be glycosylated only in the nonreduced form. Upon reduction, the glucosamine-containing portion, $F_2$, is liberated from the remaining, apparently unglycosylated portion ($F_1$). By analogy with other paramyxoviruses, the F glycoprotein is expected to cause cell fusion.

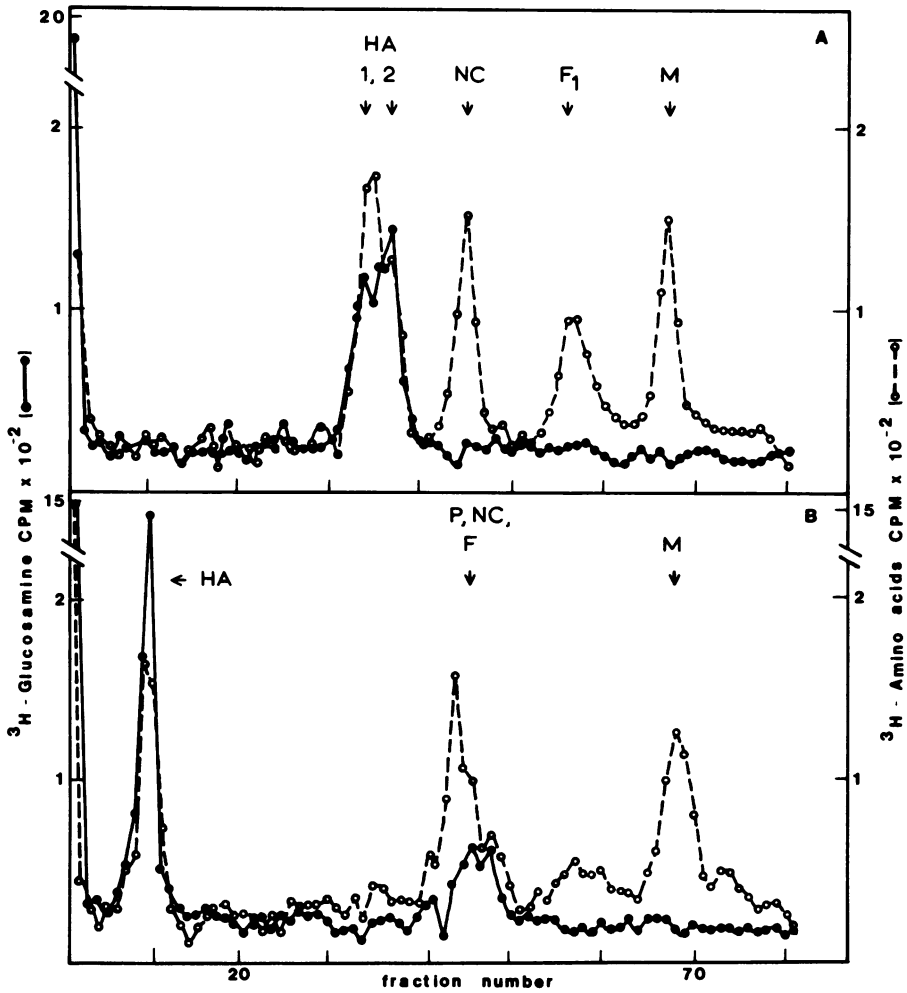


FIG. 3. *SDS-polyacrylamide gel electrophoresis of [$^3$H]glucosamine-labeled and $^{14}$C-amino acid-labeled measles virions in a Tris-glycine-buffered system under (A) reducing and (B) nonreducing conditions. Glucosamine- and amino acid-labeled samples were run on separate gel.*

The results we have obtained for the disulfide-linked structure of measles virus F glycoprotein fit into a scheme which has been observed for Sendai virus. Scheid and Choppin (16) and Homma and Ohuchi (7) have shown that the fusion glycoprotein of Sendai virus can be cleaved to $F_1$ and $F_2$. Shimizu et al. (18) have shown that when fusion protein isolated from Sendai virions was disrupted under reducing conditions, it gave rise to two peaks, designated VP4, and small glucosamine-rich glycoprotein (SGP) of approximately 15,000 daltons. Shimizu et al. concluded that VP4 and SGP were associated throughout purification because both were found at the same isoelectric point after electrofocusing of isolated fusion glycoprotein. In view of the results we have obtained for measles virus, we propose that VP4 and SGP correspond to $F_1$ and $F_2$, respectively, of measles virus and are linked by disulfide bonds under nonreducing conditions (J. M. Hardwick and H. O. Stone, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, S30, p. 284). Scheid and Choppin have recently reported that two disulfide-linked subunits, $F_1$ and $F_2$, constitute the F glycoprotein of Sendai virus, Newcastle disease virus, and simian virus 5 (17).

Our results differ significantly from those of Hall and Martin (5, 6) in two respects. First, the electrophoretic patterns of virions disrupted under reducing conditions differ markedly (compare Fig. 2A of reference 6 and our Fig. 3A). Second, Hall and Martin observed two major glycoprotein peaks when virions were disrupted

under reducing conditions, but we have observed only one major glycoprotein peak and heterogeneous, low-molecular-weight, glucosamine-rich material. We do not know how to account for the differences in results; perhaps they reflect actual differences between our strain of measles virus and the virus used by Hall and Martin. During preparation of this manuscript, Mountcastle and Choppin reported results that are consistent with our finding of only one major glucosamine-labeled protein under reducing conditions (10).

We thank G. R. Reeck for critical reading of the manuscript.

This work was supported by Public Health Service research grant AI-11741 from the National Institute of Allergy and Infectious Diseases and by University of Kansas grant 4133.

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