Analysis of Immunoprecipitated Surface Glycoproteins in Measles Virions and in Membranes of Infected Cells

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Measles viral envelope proteins were immune precipitated from membranes of infected cells and from purified virus and analyzed by polyacrylamide gel electrophoresis. Under reducing conditions, specific precipitates contained two major polypeptide bands, designated virus glycopeptides 1 and 2 (VGP-1 and VGP-2). Both polypeptides appeared to be glycosylated, as indicated by their incorporation of [^{14}C]glucosamine in infected cells. VGP-2 appeared as a single band in specific precipitates of infected cells and as a double band in precipitates of purified virus. Trypsin treatment of infected cells showed that reduced VGP-2 may be composed of two unrelated polypeptides. One may be F_1 , which is unglycosylated, and the other may correspond to the proteolytic cleavage product of VGP-1, which is glycosylated. The relation of VGP-1 and VGP-2 to smaller surface antigens (X and Y) obtained by tryptic treatment of infected cells remains to be elucidated. In cells taken at various times postinfection and analyzed for viral membrane proteins, VGP-1 was detected at all times, indicating that the input virus VGP-1 was inserted into the cell and could not be differentiated from newly synthesized VGP-1. VGP-2 was not detectable before 24 h postinfection. In precipitates of cells 4 h postinfection and of infected cells incubated at pH 5.8, an additional polypeptide band migrated immediately ahead of VGP-1. We conclude that VGP-2 (molecular weight, 42,000) possibly consists of two components, one of which is the tryptic cleavage product of VGP-1 and the other of which is the unglycosylated polypeptide, F_1 .

Two functions are associated with the measles viral envelope: attachment, for either hemagglutination (HA) or initiation of infection, and cell membrane fusion, which underlies the characteristic cytopathic effects caused by the virus in cultured cells. From studies of the paramyxoviruses, it might be anticipated that two glycopolypeptides, corresponding with each of these functions, would be detectable in measles viral envelopes (5, 12-16). However, analysis of viral structural proteins by polyacrylamide gel electrophoresis (PAGE) has yielded conflicting results in this regard. Hall and Martin (2, 3) reported that the viral envelope contained two glycopolypeptides, with molecular weights of 69,000 and 53,000. Hardwick and Bussell (4) indicated that two glycopolypeptides which were found in the measles viral envelope under non-reducing conditions represented, respectively, an aggregate of the HA monomer linked by disulfide bonds and the F polypeptide with a molecular weight of 60,000. In PAGE under reducing conditions, the HA polypeptide appeared to have a molecular weight of 76,000, and the F protein was cleaved into two polypeptides, F_1 (molecular weight, 42,000) and F_2 (molecular

weight, 20,000). Mountcastle and Choppin (10) reported that only a single glycopolypeptide, with a molecular weight of 80,000, was detected on PAGE analysis of each of four strains of measles virus under reducing conditions. Likewise, Wechsler and Fields (17) found a single glycopolypeptide, with a molecular weight of 80,000, in cytoplasmic extracts of cells infected with measles virus as well as in purified virions. We have extended these observations by searching for virus-coded protein(s) expressed in the membranes of infected cells and destined to be incorporated into nascent viral envelopes. We used lactoperoxidase radioiodination to label cell membrane proteins, followed by detergent solubilization and specific immune precipitation, a technique which has been successfully applied to the analysis of viral proteins on herpesvirus-infected cells (J. C. Glorioso, L. A. Wilson, T. W. Fenger, and J. W. Smith, *J. Gen. Virol.*, in press).

MATERIALS AND METHODS

Cells and virus. Vero cells in roller bottles were washed with Dulbecco phosphate-buffered saline, pH 7.2 (PBS), and inoculated with measles virus (LEC strain) in minimal essential medium without serum at

a multiplicity of infection of 0.1. After adsorption at 37°C for 2 h, the cells were overlaid with minimal essential medium containing 1% fetal calf serum. Three to four days after infection, when extensive cytopathic effect was observed, the medium was removed and clarified by centrifugation at $1,200 \times g$ and then at $55,000 \times g$ for 70 min. Viral pellets were either purified further or, when used for viral passage, suspended by Dounce homogenization in minimal essential medium without serum. Vero cells (10^7) to be radioiodinated or labeled with [^{14}C]glucosamine were washed with PBS and inoculated at a multiplicity of infection of 10 with concentrated measles virus in minimal essential medium without serum. After adsorption as above, the cells were overlaid and incubated at 37°C. Viral infectivity and neutralization were measured by standard plaquing techniques on Vero cell monolayers. HA and HA inhibition were titrated at 37°C in microtiter V plates, using rhesus monkey erythrocytes.

Antisera. Purified measles virus suspended in Freund complete adjuvant was injected into rabbits via toe pads at weekly intervals for a total of four injections. Animals were bled out 10 days after the final injection. Sera were heat inactivated and absorbed three times with washed monolayers of Vero cells and subsequently clarified by centrifugation at $1,200 \times g$ for 10 min. Absorbed rabbit antisera gave HA inhibition titers of 2,560 and commensurate neutralization (plaque reduction) titers. Immunoglobulin G of antiserum to measles virus prepared in rhesus monkeys was obtained through K. C. Hsu, Columbia University (New York, N.Y.), from the Division of Biologics Standards, National Institutes of Health (Bethesda, Md.). The simian antiserum contained specific HA inhibition antibody to measles virus to a titer of 512.

Purification of virus. Measles virus concentrated by centrifugation at $55,000 \times g$ was suspended by Dounce homogenization in saline-Tris-EDTA (STE) buffer (0.01 M Tris-hydrochloride, 0.1 M NaCl, and 0.001 M EDTA, pH 7.4), layered onto a 25% sucrose barrier on top of a 50% sucrose cushion, and centrifuged at $189,000 \times g$ for 45 min in an SW 50.1 rotor. Material which banded above the 50% sucrose cushion was collected, diluted in STE, and centrifuged to a pellet. Suspended virus was then layered over a 10 to 50% (wt/wt) sucrose gradient and centrifuged in an SW 27 rotor at $81,500 \times g$ for 4 h. The virus band from the middle of the gradient was diluted with STE and centrifuged as before. The pellet was suspended in PBS for radioiodination or in sample buffer for electrophoretic analysis. Purified viral preparations contained 5×10^7 PFU and 20,480 HA units per ml. Electron microscopic observation of negatively stained preparations revealed characteristic pleomorphic and generally spherical particles.

Radiolabeling. Cells and virus were surface iodinated by techniques previously described (9; Glorioso et al., *J. Gen. Virol.*, in press). Vero cells (10^7) were washed in PBS without Ca^{2+} and Mg^{2+} and dispersed with Tris-EDTA buffer (0.01 M Tris, 0.14 M NaCl, and 0.5 mM EDTA, pH 7.2). Cells were again washed three times with PBS and finally resuspended in 1.0 ml of PBS containing 10^{-5} M potassium iodide. So-

dium [^{125}I]iodide (0.5 mCi, Amersham/Searle) was mixed with 60 μl of PBS and 60 μl of 10 μM NaS. Lactoperoxidase (0.12 mg) (Sigma, EC 1.11.1.7) was dissolved in 60 μl of PBS. The [^{125}I] and lactoperoxidase solutions were mixed with cells, and seven successive 15- μl portions of 1.3 mM H_2O_2 were added at 2-min intervals. Ten milliliters of 5 mM L-cysteine-HCl was added to terminate the reaction, and the cells were centrifuged and washed four times with PBS. Over 95% of the cells remained viable, as shown by trypan blue exclusion tests. Purified measles virus was suspended in PBS and iodinated with 0.25 mCi of [^{125}I], 0.7 mg of lactoperoxidase, and seven successive 15- μl portions of 1.3 mM H_2O_2 . After terminating the reaction with L-cysteine, the virus was centrifuged at $55,000 \times g$ for 70 min. The pellet was suspended in PBS and treated with nonionic detergent Nonidet P-40 (NP-40).

For metabolic labeling of glycoproteins, Vero cell monolayers infected with measles virus for 24 h were washed with PBS and overlaid with minimal essential medium containing 1% of the normal concentration of glucose, twice the normal concentration of nonessential amino acids, 2% dialyzed fetal calf serum and 5 μCi of D-[1- ^{14}C]glucosamine hydrochloride (ICN Pharmaceuticals, Inc.) per ml (1a). At 48 h postinfection, the cells were washed with PBS, removed from the flask with STE buffer, and washed with PBS. Membranes were solubilized with NP-40, and immune precipitates were obtained as described below.

Membrane solubilization and immune precipitation. Radioiodinated or [^{14}C]glucosamine-labeled cells or virus were mixed with 0.5% NP-40 containing 1 mM phenylmethylsulfonylfluoride, a protease inhibitor, and incubated at 25°C for 15 min with occasional shaking. Nuclei and particulate matter were removed by centrifugation at $1,000 \times g$ for 10 min. Supernatants were then centrifuged at $85,000 \times g$ for 1 h in the SW 50.1 rotor. The high-speed supernatants were mixed with antiserum and stored at 4°C overnight. The mixture was layered over 1.0 ml of 20% sucrose (wt/wt) in PBS and centrifuged at $85,000 \times g$ for 1 h. The precipitated protein was recovered from the bottom of the tube and analyzed by PAGE.

PAGE. Immune precipitates of NP-40-treated membranes or viral envelopes were solubilized with 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol or SDS only by heating them at 100°C for 4 min. Discontinuous SDS-slab gels (3% stacking and 9% separating) were run at 50 mA (7; Glorioso et al., *J. Gen. Virol.*, in press). Gels were then fixed, stained with Coomassie brilliant blue, and destained (8). Autoradiograms were prepared by exposing Kodak NS 2T No-Screen X-ray film to dried gels. Estimates of polypeptide molecular weights were done in the same gel system, using β -galactosidase, lactoperoxidase, bovine serum albumin (fraction V), catalase, glutamate dehydrogenase, fumarase, ovalbumin, pepsin, and trypsin as standards.

RESULTS

PAGE analysis of immune precipitates from cells infected with measles virus and from purified virions. Uninfected Vero cells

or cells 36 h after infection were washed and iodinated. After NP-40 treatment and centrifugation, the supernatants were divided into three portions and mixed, respectively, with simian antiviral immunoglobulin G, rabbit antiserum to measles virus, or normal rabbit serum. Precipitates were subjected to electrophoresis, and gels were examined by autoradiography. Two bands, designated virus glycopolypeptides 1 and 2 (VGP-1 and VGP-2), were detected in specific precipitates of cells 36 h postinfection (Fig. 1, lanes 1 and 2), but not in those of uninfected cells (lanes 7 and 8). An additional band of high molecular weight, apparently of cellular origin and designated cellular polypeptide (CP), was present in both infected and uninfected cell precipitates with rabbit antiserum (lanes 2 and 8), but not in precipitates with rhesus monkey antiserum (lanes 1 and 7). Normal rabbit serum (lanes 3 and 9) yielded no precipitate. By comparing lanes 1 and 4 with lanes 2 and 5, it is evident that monkey antiserum immunoglobulin G, although specific, did not precipitate as much viral protein as the rabbit antiserum, which was therefore used in all other experiments. Specific precipitates of radioiodinated, purified virus (Fig. 1, series B) showed two major bands which

corresponded to those seen in precipitates of infected cells. The cell surface protein (CP) was much less prominent in precipitates of virus than in precipitates of normal or infected cells. In specific precipitates of purified virus (lane 5), VGP-2 appeared as two closely associated bands rather than as the single band seen in precipitates of cell membranes.

Expression of viral membrane antigens at various times postinfection. To determine the time postinfection at which viral antigens became detectable in cell membranes, replicate cultures of Vero cells were inoculated with measles virus at a multiplicity of infection of 10. After incubation periods of from 4 to 54 h, cell samples were radioiodinated and NP-40 treated, and soluble proteins were mixed with antibody. Autoradiograms of these precipitates after PAGE analysis are shown in Fig. 2. It is apparent that VGP-1 and VGP-2 were expressed together on the cell surface as early as 24 h postinfection; however, at earlier times VGP-2 was not detected. Two polypeptide bands were present in the 4-h sample, one migrating in the position of VGP-1 and the other (VGP-1a) migrating slightly faster. By 12 h, however, VGP-1a was no longer evident.

Glycosylation of viral membrane proteins. To determine whether VGP-1 and VGP-2 were glycosylated, Vero cells labeled with [14 C]glucosamine between 24 and 48 h postinfection or after mock infection were treated with NP-40, and proteins were precipitated. Specifically precipitated VGP-1 and VGP-2 had been labeled with [14 C]glucosamine under these conditions (Fig. 3, lane A), being found in positions

FIG. 1. SDS-PAGE analysis of viral membrane proteins. Cells 36 h after infection (series A), purified measles virus (series B), and uninfected cells (series C) were radioiodinated, and membrane proteins were solubilized with NP-40. After centrifugation, supernatants from each series were divided into three aliquots and mixed with 0.2 ml of reference antiserum immunoglobulin (lanes 1, 4, and 7), 0.5 ml of rabbit antiserum to measles virus (lanes 2, 5, and 8), or 0.5 ml of normal rabbit serum (lanes 3, 6, and 9). Immune precipitates were then analyzed by SDS-PAGE. Total precipitated proteins were subjected to electrophoresis when simian antiserum and normal serum were used, whereas approximately 250,000 cpm was applied to the gel when proteins precipitated with rabbit antiserum were analyzed.

FIG. 2. Immune precipitation of viral proteins at various times after measles virus infection. Cells infected for the indicated times were radioiodinated and then treated with NP-40. Immune precipitates were analyzed by SDS-PAGE.

FIG. 3. Immune precipitates of viral membrane proteins labeled with [14 C]glucosamine. Infected or mock-infected cells were labeled between 24 and 48 h postinfection. After NP-40 treatment and centrifugation, viral proteins were immune precipitated and analyzed by SDS-PAGE. (Lane A) Cells infected with measles virus and labeled with [14 C]glucosamine. (Lane B) Cells infected with measles virus and radioiodinated. (Lane C) Uninfected cells labeled with [14 C]glucosamine.

corresponding exactly with VGP-1 and VGP-2 in precipitates of radioiodinated cells (lane B). The CP was not detected in infected cells, but was heavily labeled with [14 C]glucosamine in uninfected control cells (lane C).

Molecular weight determinations. The molecular weights of VGP-1 and VGP-2 were determined by concurrent analysis of standard proteins (listed in Materials and Methods) along with solubilized immune precipitates from cells 42 h after infection (not shown). Approximate values of 76 and 42 megadaltons were obtained for VGP-1 and VGP-2, respectively.

Neuraminidase treatment of radioiodinated cells infected with measles virus. Since measles virus HA contains N-acetylneuraminic acid (1), we attempted to determine

what effect neuraminidase treatment might have on the electrophoretic mobility of the viral glycopolypeptides. Cells 42 h after infection with measles virus were radioiodinated, washed with PBS, and treated with *Vibrio cholerae* neuraminidase (50 U) for 1 h at 37°C in complete PBS at either pH 7.2 or pH 5.8. Controls without neuraminidase were included. Immune precipitates of membrane proteins were prepared and analyzed by PAGE-autoradiography. Neuraminidase treatment at pH 7.2 had no apparent effect on the position of either VGP-1 or VGP-2 (Fig. 4 lanes D and E). However, an additional band (VGP-1') was detected ahead of VGP-1 in specific precipitates of infected cells exposed to pH 5.8 (Fig. 4, lanes B and C), whether or not they had been treated with neuraminidase. VGP-1 and VGP-1' (Fig. 4) were in the approximate positions of VGP-1 and VGP-1a (Fig. 2) seen in the electrophoretic profile of cells 4 h postinfection. In these same precipitates (Fig. 4, lanes B and C), two additional polypeptide bands, designated M and N, were detected which were unrelated to neuraminidase treatment and

FIG. 4. Neuraminidase treatment of uninfected cells and cells infected with measles virus. Lanes A, B, and C represent, respectively, immune precipitates, after radioiodination, of neuraminidase-treated uninfected cells, neuraminidase-treated infected cells, and infected control cells, all of which were incubated at pH 5.8. Lanes D and E show gel profiles of immune precipitates, after iodination, of infected cells incubated at pH 7.2 either in the presence (lane D) or in the absence (lane E) of neuraminidase. Lanes A, B, and C and lanes D and E represent two separate electrophoretic runs. M and N are possible derivatives of VGP-1 or VGP-1'.

apparently resulted solely from prior exposure of the cells to buffer at pH 5.8.

Tryptic digestion of infected cells. Vero cells 42 h after infection with measles virus were iodinated as before and washed with PBS. Three samples of 10^7 cells each in 1 ml of PBS were treated, respectively, with 1, 10, or 20 μ g of trypsin for 10 min at 37°C. After addition of soy bean inhibitor, the cells were washed three times with PBS and pelleted. The first supernatant (tryptic digest) was centrifuged at $8,000 \times g$ for 60 min, and the high-speed supernatant was then incubated at 4°C with viral antiserum to precipitate any viral proteins which might have been cleaved from the cells by trypsin. Trypsinized cells were treated with NP-40 containing 1 mM phenylmethylsulfonyl fluoride, and soluble membrane proteins were precipitated. PAGE analysis of immune precipitates of trypsinized infected cells showed that the specific activity of VGP-2 was apparently enriched at the expense of VGP-1 (Fig. 5, lanes C and D), giving a pattern different from precipitates of untrypsinized infected cells (lane B). Trypsinization of infected cells also resulted in the appearance of two diffuse bands (X and Y, lanes C and D) which migrated ahead of VGP-2 and which were not found in immune precipitates of trypsinized, uninfected cells (lane B). In precipitates from the tryptic digest of infected cells (lanes E and F), a band migrating to the position of VGP-2 was seen along with traces of band Y, the lower-molecular-weight component already noted in precipitates of trypsinized cells. The PAGE pattern of the cellular polypeptide in the precipitates of trypsinized infected (lanes C and D) and uninfected (lane B) cells comprised two components, in contrast to the single band obtained with untrypsinized cells.

PAGE analysis of viral membrane proteins under nonreducing conditions. Immune precipitates of radioiodinated, infected cells were solubilized with SDS without 2-mercaptoethanol and heated. In autoradiograms of the nonreducing gels, a high-molecular-weight polypeptide was seen above the cellular polypeptide. No VGP-2 was present in its reduced position, but a double band (VGP-2_{NR}) appeared in a position corresponding to about 60 and 57 megadaltons. In Fig. 6, the designations CP_R, VGP-1_R, and VGP-2_R refer, respectively, to the locations of CP, VGP-1, and VGP-2 determined by electrophoresis of infected cell precipitates under reducing conditions in an adjacent lane.

DISCUSSION

Under reducing conditions, SDS-PAGE analysis of purified radioiodinated measles virus (Fig. 1, series B) revealed the presence of two poly-

FIG. 5. Trypsin treatment of radioiodinated cells and immune precipitation of proteins in supernatant (digest) and cell-associated proteins. (Lanes A and B) Proteins precipitated from the tryptic digest and the NP-40-soluble cell fraction, respectively, obtained from trypsinized (20 μ g) uninfected cells. (Lanes C and D) Proteins solubilized with NP-40 from infected cells after treatment with 10 and 20 μ g of trypsin, respectively. (Lanes E and F) Precipitates from tryptic digests obtained from infected cells treated with 10 and 20 μ g of trypsin, respectively. (Lane G) Untrypsinized, infected cells. Lane G cannot be quantitatively compared with lanes C, D, E, and F and only indicates the positions of VGP-1 and VGP-2. CP includes a cellular polypeptide plus possible tryptic cleavage product; X and Y are possible cleavage products of VGP-1.

peptide bands, approximately corresponding in molecular weight to the HA and F₁ polypeptides previously reported by others (4). In our system, the second, smaller polypeptide (probably F₁), which we designated VGP-2, comprised two closely associated components, with molecular weights of 43,500 and 42,000, rather than a single band (Fig. 1, lane 5). However, in similar analyses of membranes of infected cells in SDS reducing gels, VGP-2 appeared as a single component (Fig. 1, series A; Fig. 2). When nonreducing PAGE was used to analyze similar precipitates, two closely migrating bands were resolved at approximately 60,000 and 55,000 daltons (Fig. 6). This raises the possibility that reduced VGP-2 from infected cells may be composed of two unrelated components with nearly identical electrophoretic mobilities. Under nonreducing conditions, the two peptides of different sizes may be linked by disulfide bonds to the portion of VGP-2 represented in reducing gels. One of the

FIG. 6. Gel of immune precipitates run under non-reducing conditions. Precipitates of radioiodinated cells were solubilized by heating at 100°C for 4 min in 2% SDS without 2-mercaptoethanol and subjected to electrophoresis as before. VGP-2_{NR} is a dual band, representing VGP-2 under nonreducing conditions. CP_R, VGP-1_R, and VGP-2_R designate the usual positions of these polypeptides in reducing gels. Two exposures of the same nonreducing gel are shown.

nonreduced polypeptides may correspond to F protein (molecular weight, 60,000) (4), and the other may be an enzymatic cleavage product of VGP-1. Alternatively, reduced VGP-2 may represent a single polypeptide species which is linked in the nonreduced state to peptides which differ in size or extent of glycosylation.

PAGE analysis of immune precipitates from cells at various times postinfection revealed a sequential change in the expression of VGP-1 and VGP-2 on the cell surface. At all times analyzed, VGP-1 was detected in immune precipitates. Since nascent VGP-1 was unlikely to be present in cell membranes at 4 h, this protein was probably inserted during virus penetration. Therefore, no distinction could be made between input VGP-1 and any newly synthesized polypeptide. At 4 h postinfection, a band which migrated directly ahead of VGP-1 (VGP-1a, Fig. 2) was also resolved by PAGE. This may represent VGP-1 which was altered either during attachment of virus to receptors or during subsequent penetration. VGP-2 was not detected in immune precipitates until 24 h postinfection,

which may represent the lapse of time required for expression of nascent VGP-2 and possibly VGP-1.

Treatment of measles virus-infected cells with neuraminidase at pH 7.2 or 5.8 did not alter the viral proteins, once formed (Fig. 4). However, simply exposing infected cells to the lower pH changed the electrophoretic migration of VGP-1, which, under reducing conditions, appeared as a double band. The difference in electrophoretic mobility between the paired bands was approximately equivalent to that observed between the polypeptides in the dual VGP-1 band seen in precipitates of cells 4 h postinfection (Fig. 2). The relationship of the polypeptides designated M and N (Fig. 4) to VGP-1 and VGP-2 was not ascertained. If the appearance of VGP-1' was due to an alteration of VGP-1, then M and/or N may also arise from the same or a similar process.

Immune precipitates of trypsinized, radioiodinated infected cells gave patterns in which VGP-2 was enriched at the expense of VGP-1 and in which VGP-1 was greatly reduced (Fig. 5, lanes C and D). This finding suggests that VGP-2, or a component thereof, may be derived from VGP-1 as a result of proteolytic activity occurring in the cell membrane during viral maturation. VGP-2 in tryptic digests of infected cells (Fig. 5, lanes E and F) had the same mobility as the corresponding components which remained bound to the membrane (Fig. 5, lanes C and D). This suggests not only that the protein was detached as an entity but also that the antigenic determinants reactive with viral antibody were distal to the site of tryptic hydrolysis. From these results it still could not be determined whether VGP-2 of the cell fraction and that of the tryptic digest were components of the same polypeptide. Trypsinization of radioiodinated VGP-1 and VGP-2 which had been eluted from preparative gels resulted in further degradation of both molecules to peptides of even smaller molecular weight (results not shown), indicating that the viral polypeptides were susceptible to more extensive proteolytic degradation after isolation than when still in the membrane.

Trypsinization of infected cells resulted in the appearance of two smaller components (X and Y) (Fig. 5, lanes C and D). Component X (34,000 daltons) remained associated with the membrane, whereas component Y (28,000 daltons) was found in both the cell fraction and the tryptic digest. The relationship between these polypeptides is currently being evaluated, using tryptic digest mapping. As suggested previously, reduced VGP-2 from precipitates of infected cells may be composed of two unrelated polypeptides. Based on the data obtained from tryp-

sin treatment of cells, we hypothesize that one component of reduced VGP-2 could be F₁, which is unglycosylated, whereas the other component, which is labeled with [¹⁴C]glucosamine, may be the tryptic cleavage product of VGP-1. Additional unpublished data have indicated that trypsin treatment of infected cells does not simply degrade VGP-1 to low-molecular-weight products and leave VGP-2 intact. When immune precipitates from trypsin-treated infected cells were analyzed in a nonreducing gel, polypeptides were resolved which migrated in the same positions as VGP-1 and VGP-2. This suggested that, even in the face of limited proteolysis, disulfide bonds still held together polypeptide structures of the approximate molecular sizes of VGP-1 and VGP-2.

The largest polypeptide resolved from immune precipitates of both infected and uninfected cells had a molecular weight of approximately 200,000, a value which corresponds with that of cell surface proteins, such as the "large external transforming sensitive" protein (6) or the omega protein found in many cell lines (8, 11). Large external transforming sensitive protein is readily removed from the cell surface by mild protease treatment, a fact which argues against the identification of CP as large external transforming sensitive protein. On the other hand, the Ω protein is insensitive to protease and has a molecular weight (206,000) which corresponds closely to that of CP. The origin of the antibody to CP in the viral antiserum is uncertain. It is possible that the purified virus used for immunization of rabbits contained traces of CP, either as a contaminant in the viral preparation or even as a host component of the viral envelope (Fig. 1, lane 5). In either case, less CP, relative to VGP-1, was precipitated from purified virus than from infected cells by rabbit antiserum to virus. Surprisingly, the level of antibody to CP was not much reduced by extensive absorption with normal cell monolayers. The reasons for this discrepancy are not clear. Simian antibody to virus did not precipitate any CP either from virus grown in Vero cells (Fig. 1, lane 4) or from membranes of infected Vero cells (Fig. 1, lanes 1 and 7). This may be explained by the fact that viral antibody had been evoked by infection in the homologous species. The specificity of the antibody for virus-coded components is further substantiated by the results of similar analyses of HeLa cells infected with measles virus, from which only VGP-1 and VGP-2, and no CP, were precipitated with rabbit hyperimmune serum to virus (results not shown).

In uninfected cells, CP was labeled with [¹⁴C]glucosamine. However, in infected cells, in

which VGP-1 and VGP-2 were both labeled, CP was unlabeled. Since glucosamine can be converted metabolically to amino acids, infected cells labeled with [³H]fucose, which is not metabolically converted, were examined. Preliminary results (not shown) failed to confirm the glycosylation of VGP-2, since only VGP-1 was labeled with [³H]fucose. However, unlike the results with [¹⁴C]glucosamine, CP was labeled with [³H]fucose in both infected and uninfected cells. It is possible that glycosylation of CP is diminished by measles virus infection, which specifically inhibits incorporation of glucosamine and perhaps other sugars. The fact that VGP-1 and VGP-2 were labeled with [¹⁴C]glucosamine but only VGP-1 was labeled with fucose suggests that the component of VGP-2 which is derived from VGP-1 is glycosylated but contains few if any fucose residues. An alternative explanation is that glucosamine is converted to amino acids, with the result that all or a portion of the radiolabel in VGP-2 appears in the polypeptide part of the molecule. The latter hypothesis is not entirely valid, for if glucosamine were converted to amino acids, other viral proteins along with VGP-1 and VGP-2 would be labeled and detected in immune precipitates. This pattern was not evident in our preparations. Moreover, CP synthesized by infected cells during the labeling period would have been labeled with secondarily derived radioactive amino acids.

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