JOURNAL OF VIROLOGY, Dec. 1984, p. 953–959
0022-538X/84/120953-07$02.00/0

Vol. 52, No. 3

# Analysis of Three Late Varicella-Zoster Virus Proteins, a 125,000-Molecular-Weight Protein and gp1 and gp3

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Received 21 June 1984/Accepted 2 September 1984

**Two monoclonal antibodies were prepared against varicella-zoster virus proteins. One of the monoclonal antibodies (10.2) reacted only with the nuclei of infected cells and immunoprecipitated one nonglycosylated late viral protein (125,000 molecular weight). The other monoclonal antibody (19.1) with neutralizing activity, reacted with membrane antigens of infected cells and with the varicella-zoster virus envelope and immunoprecipitated two late major viral glycoproteins (gp1 and gp3). Synthesis of the 125,000-molecular-weight protein, gp1, and gp3 began at 20 to 22 h postinfection, 2 h after the peak of viral DNA synthesis, and continued until 29 h postinfection, when the first progeny virus appeared in infected cells. Pulse-chase experiments showed that during pulse-labeling, only gp1 was detected, whereas during the chase period, gp1 as well as gp3 was detected in infected cells. Under nonreducing conditions, gp3 migrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a 130,000-molecular-weight protein as compared with the 62,000-molecular-weight species obtained when gels were resolved under reducing conditions. This finding indicates that gp3 is a dimer that is disulfide linked.**

Varicella-zoster virus (VZV), a member of the human herpesvirus family, causes chicken pox and shingles. According to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) data, the VZV virion appears to contain 30 to 33 structural proteins, including 7 glycosylated proteins (2, 10–14, 19, 21–23, 27). The major viral glycoproteins detectable in VZV-infected cells have apparent molecular weights of 115,000 (115K) to 118K (gp1), 80 to 100K (gp2), 62 to 64K (gp3), and 55K (gp5). Okuno et al. (19) have studied three of the VZV glycoproteins and demonstrated that a 75 and a 49K virus-induced protein are the precursors of gp2 and gp5, respectively, and that gp3 is the cleavage product of gp1. They have suggested that the precursor proteins are synthesized in infected cells and glycosylated and expressed on the infected cell surface membranes and on the VZV virion envelope as gp2, gp3, and gp5.

The study of VZV-induced proteins in infected cells has been hampered by low virus yields in tissue culture cells. The development of monoclonal antibodies (MAbs) against viral proteins has facilitated the detection and characterization of virus-specific proteins in infected cells. In this report, we describe the SDS-PAGE analysis of three VZV-induced proteins by immunoprecipitation with MAbs. The results show that a nonglycosylated 125K viral protein as well as gp1 and gp3 are late viral proteins that are synthesized after DNA replication and that gp3 is located on the envelope of the VZ virion. The results also show that gp1 is detected during pulse-labeling, whereas during the chase period, gp1 and gp3 are detected in infected cells.

## MATERIALS AND METHODS

**Cells and virus.** VZV was propagated in BSC-1 cells by cocultivation of semiconfluent cultures with trypsinized infected cells as described (7). VZV was titered by inoculation of monolayer cells with serial dilutions of Dounce-homogenized (150 strokes), infected cells. After a 3-h adsorption period at 37°C, the cells were washed twice with serum-free minimal essential medium (MEM) and overlaid with MEM containing 2% fetal bovine serum (FBS). At 4 to 6 days after infection, cells were fixed with 10% formalin and stained with 0.1% cresyl violet, and plaques were counted. The virus titer obtained in BSC-1 cells was $10^3$ to $10^4$ PFU per ml.

**Preparation of MAbs.** BALB/c mice were immunized subcutaneously with purified viral particles as previously described (7, 28) and boosted intravenously 3 to 4 days before they were killed. The spleens were removed, and splenocytes were fused with SP2/0-Ag14 mouse myeloma cells by the method of Kohler and Milstein (18), as modified by Gerhard (6). Briefly, a mixture of washed, packed myeloma cells and splenocytes (at a ratio of 1:3) was treated with 50% polyethylene glycol (PEG 1000), and the cell suspension was gradually diluted by doubling the volume each minute. The final cell suspension was diluted in selective medium containing hypoxanthine-aminopterin-thymidine and cultivated ($10^6$ cells per ml) in Costar microtiter plates. An enzyme-linked immunosorbent assay was used to screen for cultures secreting anti-VZV antibodies as previously described (28). Positive hybridomas were cloned by being grown by a limiting dilution method, grown in tissue culture, and inoculated into pristane-primed mice. Ascitic fluids were harvested and stored at −20°C as described (6). Mouse and rabbit anti-VZV antisera were prepared as previously described (28). Anti-VZV MAbs were assayed by indirect immunofluorescence on both acetone-fixed and unfixed VZV-infected cells (9) with undiluted culture fluid containing the MAb and a 1:10 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Cappel Laboratories, Downington, Pa.). The isotype of each MAb was determined by immunofluorescence on VZV-infected cells incubated with undiluted culture fluid containing the MAb followed by incubation with rabbit isotype-specific serum (a gift from W. Gerhard) and finally a 1:10 dilution of fluoresce-

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in isothiocyanate-conjugated goat anti-rabbit immunoglobulin G.

**Virus neutralization.** Neutralization was performed by the constant virus-varying serum technique (26) and also by the enhanced neutralization method described by Asano et al. (1). Briefly, VZV-infected BSC-1 cells were Dounce homogenized and incubated for 60 min at 37°C with tissue culture fluid containing 1:2 to 1:320 dilutions of MAb. A 1:3 dilution of rabbit anti-mouse immunoglobulin was added for an additional 30 min at 37°C. BSC-1 cells, grown in Costar plates, were inoculated with 0.5 ml ($2 \times 10^2$ PFU/ml) of each preincubated sample. After adsorption for 3 h at 37°C, the inoculum was replaced with MEM containing 2% FBS, and the cultures were incubated at 37°C for 7 to 8 days. Cells were then fixed and stained, and plaques were counted.

**Time course of viral DNA synthesis and virus replication.** BSC-1 cells ($10^5$) were infected with VZV at a multiplicity of 0.001 PFU/cell. After a 3-h adsorption period, cells were washed twice with serum-free MEM and overlaid with MEM containing 2% FBS. Infected cells were pulse-labeled with 50 μCi of [*methyl*-$^3$H]thymidine (specific activity, 50 to 100 Ci/mmol; Amersham Corp.) per ml for 2-h intervals during a 28-h infection period. After being pulse-labeled the cells were lysed in TNE buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 5 mM EDTA) containing 2% SDS. The DNA was extracted twice with phenol and three times with chloroform–*n*-butanol (24:1) and precipitated with 2.2 volumes of 100% ethanol and with 1/10 total volume of 3 M sodium acetate at −20°C. After centrifugation at 12,000 × *g* for 30 min at 4°C in a Sorvall SS34 rotor, the DNA pellet was suspended in 0.5 ml of 0.5 N NaOH, heated in boiling water for 10 min, cooled on ice, and neutralized with 0.5 ml of 1 M Tris. The DNA was precipitated with ethanol, resuspended in TE (50 mM Tris [pH 7.5], 1 mM EDTA), sonicated for 20 s, heated in boiling water for 10 min, and cooled on ice. The $^3$H-labeled DNA was then hybridized to a 9.7-kilobase-pair (kb) *Bam*HI-cleaved VZV DNA (pVBC)-cloned fragment (8). The VZV DNA-cloned fragment (5 μg) was denatured with 0.5 N NaOH for 30 min at room temperature and neutralized with 1 M Tris. Denatured DNAs were immobilized on BA85 nitrocellulose filters (Schleicher & Schuell Co., Keene, N.H.) which had been previously soaked in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate). The filters were washed with 2× SSC, baked at 80°C for 2 h, incubated in prehybridization buffer for 16 h at 50°C, and hybridized to $^3$H-labeled DNA ($10^5$ cpm) for 72 h at 50°C by the methods of Wahl et al. (25) and Stabel et al. (24). The filters were washed with several changes of 2× SSC–0.1% SDS at 65°C for a total of 2 h, air dried, and assayed for radioactivity. Radioactivity after hybridization of $^3$H-labeled DNA ($10^5$ cpm) from uninfected cells to VZV DNA-cloned fragment was 188 cpm. To determine the time course of virus replication, monolayer cell cultures ($10^6$) were infected with VZV at a multiplicity of 0.001 PFU/cell. After a 3-h adsorption period, cells were washed twice with serum-free MEM and overlaid with MEM containing 2% FBS. At various times after infection, cultures were harvested, and titers of virus were determined as described above.

**Radioactive labeling and purification of virus.** Cells ($10^8$)

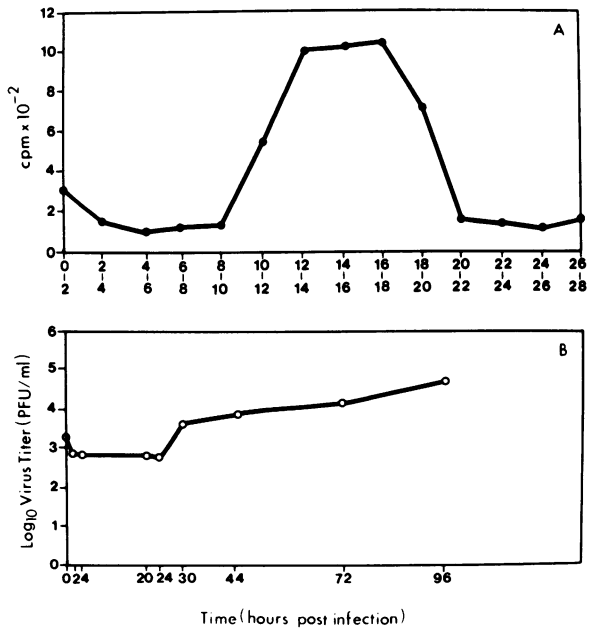


FIG. 1. (A) Time course of VZV DNA synthesis. Infected cells were pulse-labeled with [$^3$H]thymidine for 2-h intervals during a 28-h infection period. Labeled cell DNAs were extracted, immobilized onto nitrocellulose filters, and hybridized to a 9.7-kb *Bam*HI-cleaved VZV-DNA (pVBC)-cloned fragment (8) as described in the text. (B) Time course of VZV replication in BSC-1 cells. Cultures of VZV-infected cells were harvested at various times after infection, and virus titers were determined by plaque assay.

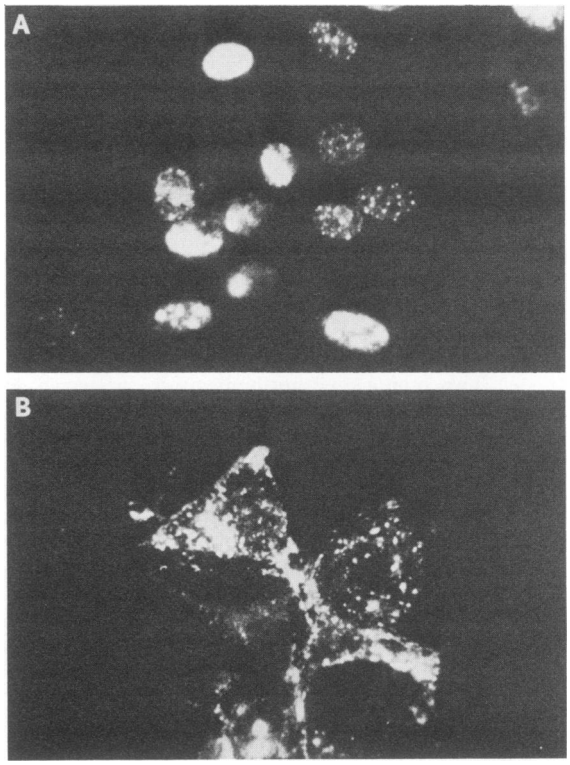


FIG. 2. Indirect immunofluorescence staining patterns of VZV-infected BSC-1 cells which were reacted with MAbs. Cells grown on cover slips were infected with VZV, and virus-specific antigens were detected with MAbs and fluorescein-conjugated goat anti-mouse immunoglobulin G as described in the text. (A) Acetone-fixed infected cells which were reacted with MAb 10.2 show only nuclear fluorescence, and (B) unfixed infected cells which were reacted with MAb 19.1 show membrane fluorescence. Magnification, ×300.

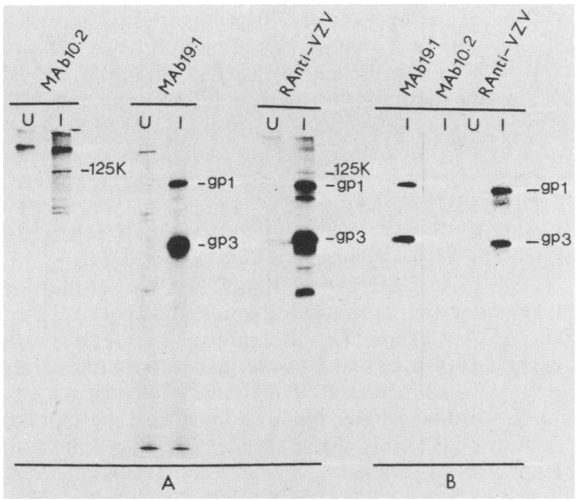


FIG. 3. Immunoprecipitation of viral proteins with monoclonal MAbs. Uninfected (U) and VZV-infected (I) cells were labeled with 50 μCi of [$^{35}$S]methionine per ml (A) or 60 μCi of [$^{3}$H]mannose per ml (B). Cell lysates were prepared, reacted with MAb 10.2, MAb 19.1, or rabbit anti-VZV serum (RAnti-VZV), and analyzed (50,000 to 100,000 cpm) by SDS–10% PAGE as described in the text. gp1 and gp3 represent two major VZV glycoproteins with 62 and 115K, respectively. Lactate dehydrogenase (140K), phosphorylase *b* (94K), bovine serum albumin (67K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (20.1K), and α-lactalbumin (14.4K) were used as internal size markers.

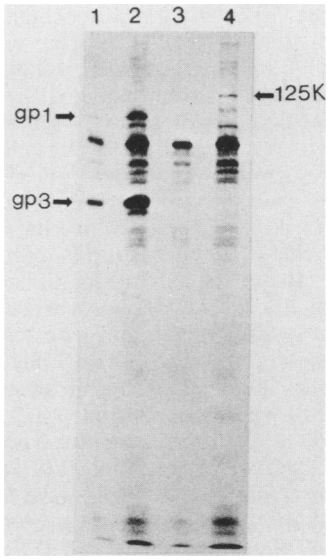


FIG. 5. Effect of PAA on synthesis of VZV-induced proteins (125K, gp1, gp3). Cells ($10^6$) were infected with VZV (0.001 PFU/cell) and labeled with [$^{35}$S]methionine (50 μCi/ml) for 30 h in the absence (lanes 2 and 4) or presence of 200 μg of PAA per ml (lanes 1 and 3). Infected-cell lysates were prepared and immunoprecipitated with MAb 19.1 (lanes 1 and 2) or MAb 10.2 (lanes 3 and 4), and the whole immunoprecipitated materials from untreated and PAA-treated cultures were analyzed by SDS–10% PAGE. Gels were stained, destained, dried, and exposed to X-ray films for 7 days.

were infected with VZV at a multiplicity of 0.001 PFU/cell. After a 3-h adsorption period, cells were washed with serum-free MEM and overlaid with MEM containing 1/10 of the concentration of normal methionine, 2% FBS, and 50 μCi of [$^{35}$S]methionine (specific activity, 1,450 Ci/mmol; Amersham) per ml for 72 h at 37°C. Cells were scraped into tissue culture medium and centrifuged at 2,000 × *g* for 20 min at 4°C. The cell pellet was suspended in TNE buffer and Dounce homogenized (50 strokes), and nuclei were removed by centrifugation at 800 × *g* for 10 min. The cytoplasmic fraction was layered onto 20% sucrose (in TNE buffer) and centrifuged at 120,000 × *g* in a Beckman SW28 rotor for 30 min at 4°C. The pellet was suspended in TNE and banded twice in 10 to 50% sucrose (at 55,000 × *g* in a Beckman SW28 rotor for 60 min at 4°C) and once in 10 to 50%

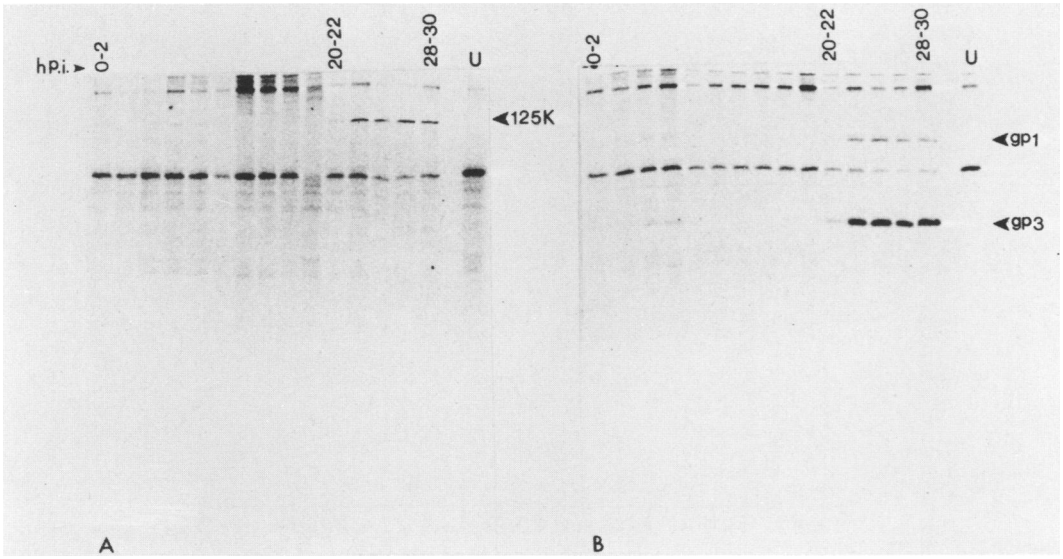


FIG. 4. Time course synthesis of VZV-induced proteins (125K, gp1, gp3) in infected BSC-1 cells. Cells were infected with VZV (0.001 PFU/cell) and pulse-labeled with [$^{35}$S]methionine (100 μCi/ml) for 2-h intervals during a 30-h infection period. Cell lysates were prepared and virus-specific proteins were immunoprecipitated with MAb 10.2 (A) or MAb 19.1 (B) and analyzed (30,000 to 50,000 cpm) by SDS–10% PAGE as described in the text. U, Uninfected cells labeled for 30 h.

potassium tartrate (160,000 × *g* in a Beckman SW41 rotor for 80 min at 4°C) density gradients. The virus pellet was suspended in TNE, and viral proteins (100,000 to 200,000 cpm) were separated by sodium dodecyl SDS-PAGE.

**Radioactive labeling of infected-cell proteins.** Cells ($10^5$) were infected with VZV (0.001 PFU per cell) and, after the adsorption period, were washed and pulse-labeled for 2-h intervals during a 30-h infection period with [$^{35}$S]methionine (100 μCi/ml). For pulse-chase experiments, cells ($10^5$) were infected and labeled at 20 and 21 h postinfection (p.i.) with [$^{35}$S]methionine (100 t0 150 μCi/ml) for 10 and 20 min. After being pulse-labeled, cells were either washed with cold PBS and harvested or washed three times with serum-free MEM, overlaid with MEM containing 2% FBS, incubated at 37°C, and harvested after 30, 60, 90, 120, and 240 min. In addition, infected cells ($10^7$) were labeled with 50 μCi of [$^{35}$S]methionine per ml or 60 μCi of [2-$^3$H]mannose (specific activity 10 to 20 Ci/mmol; Amersham) per ml for 72 h. To separate early and late viral proteins, infected cells were labeled for 30 h with [$^{35}$S]methionine (50 μCi/ml) in the presence of 200 μg of a DNA inhibitor, phosphonoacetic acid (PAA), per ml.

Labeled cells were scraped into tissue culture medium and centrifuged at 2,000 × *g* for 20 min at 4°C. The cell pellet was washed in cold PBS and disrupted at 4°C for 2 h in 5 ml of lysis buffer (0.01 M sodium phosphate [pH 7.6], 0.1 M NaCl, 1% Triton X-100, 0.5% deoxycholate, and 0.1% SDS). The cell lysate was centrifuged at 2,000 × *g* for 20 min at 4°C, and the supernatant was centrifuged at 175,000 × *g* in a Beckman SW65 rotor for 2 h at 4°C. The supernatants were stored at −70°C until used for immunoprecipitation.

**Immunoprecipitation.** Before immunoprecipitation of viral proteins, 1-ml portions of VZV-infected-cell lysates were incubated for 20 h at 4°C with 10 μl of normal rabbit or mouse serum and 50 μl of a 10% Formalin-fixed suspension of protein A-containing *Staphylococcus aureus* Cowan I (16). After centrifugation at 2,000 × *g* for 20 min, VZV-specific proteins were immunoprecipitated by incubation at 4°C for 20 h in the presence of 20 μl of rabbit anti-VZV serum or 100 μl of tissue culture fluid containing MAbs. Finally, 50 μl of a 10% *S. aureus* suspension was added, and after 2 h at 4°C, absorbed immune complexes were washed three times with lysis buffer, suspended in TNE buffer, and analyzed by SDS-PAGE.

**SDS-PAGE.** Samples containing 50,000 to 100,000 cpm were suspended in 3× sample buffer (150 mM Tris [pH 7.0], 6% SDS, 15% 2-mercaptoethanol, 0.03% bromophenol blue), heated in boiling water for 4 min, and analyzed by 10% SDS-PAGE as described by Laemmli (17). Electrophoresis was carried out for 16 h at 50 V per gel at room temperature. The gels were stained in 50% methanol–7% acetic acid–2% Coomassie brilliant blue and destained in 10% methanol–7% acetic acid. Gels were processed for fluorography (En$^3$Hance; New England Nuclear Corp., Boston, Mass.), dried, and exposed to Kodak X-Omat AR film at −70°C.

## RESULTS

**Time course of VZV DNA synthesis and virus replication.** VZV-infected cells were pulse-labeled with [$^3$H]thymidine for 2-h intervals during a 28-h infection period and labeled-cell DNAs were hybridized to a VZV DNA-cloned fragment (pVBC) which does not share sequence homology with cell DNA (8). The results showed that viral DNA synthesis begins at 10 to 12 h p.i. and peaks at 16 to 18 h p.i. (Fig. 1A). Virus titration by plaque assay of VZV-infected cells harvested at various times after infection showed that the first progeny virus appears at about 29 h p.i. and that the number of infectious virions slowly increases throughout a 96-h infection period (Fig. 1B).

**Monoclonal antibodies against VZV proteins.** Two MAbs were prepared against VZV proteins. MAb 10.2 (immuno-

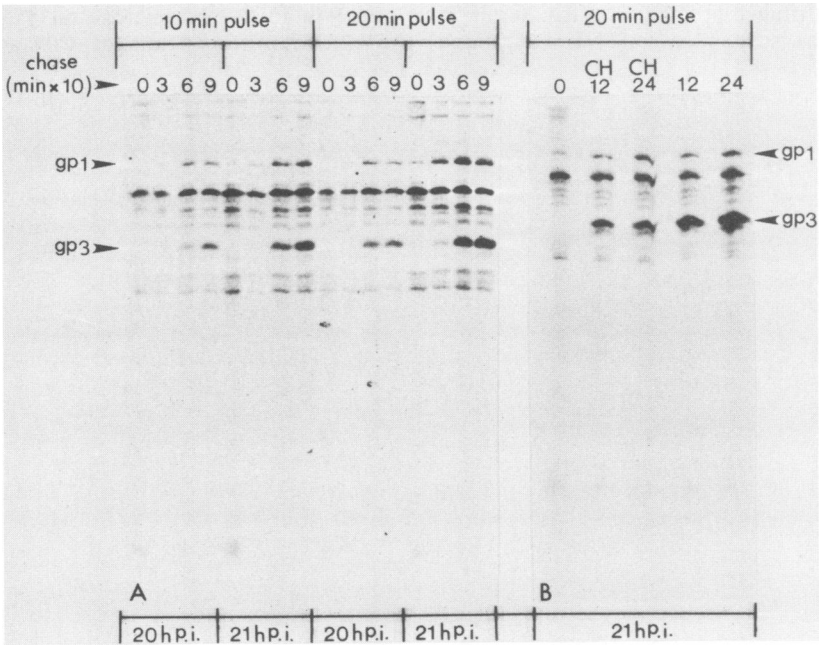


FIG. 6. Pulse-chase experiments of VZV-infected cells immunoprecipitated with MAb 19.1. (A) Cells were infected with VZV (0.001 PFU/cell) and labeled at 20 and 21 h p.i. with [$^{35}$S]methionine (150 μCi/ml) for 10 and 20 min. After being pulse-labeled, cells were washed, and the label was chased in normal medium for 30, 60, and 90 min. (B) Infected cells were labeled with [$^{35}$S]methionine for 20 min and chased in normal medium for 120 and 240 min in the presence (50 μg/ml) or absence of cycloheximide (CH). Infected-cell lysates were prepared, reacted with MAb 19.1, and analyzed by SDS–10% PAGE.

globulin G, subclass 1) reacted only with nuclear antigens of fixed, infected cells (Fig. 2A), whereas MAb 19.1 (immunoglobulin G, subclass 2) reacted with unfixed infected cells (Fig. 2B) indicating that it recognized viral antigens on the plasma membrane. Neutralization of VZV with MAb 19.1 resulted in a plaque reduction of 50 to 75%, indicating the neutralizing capacity of this MAb.

MAbs were further characterized by immunoprecipitation and SDS-PAGE. VZV-infected cell proteins were labeled with [$^{35}$S]methionine, and cell lysates were immunoprecipitated with MAbs. MAb 10.2 immunoprecipitated a virus-specific protein with an apparent molecular weight of 125K, and MAb 19.1 immunoprecipitated two viral proteins with apparent molecular weights of 115K (gp1) and 62K (gp3) as shown in Fig. 3A.

Immunoprecipitation of [$^{3}$H]mannose-labeled cell lysates with MAb 10.2 and MAb 19.1 showed that the 125K viral protein is nonglycosylated, whereas the 115K (gp1) and 62K (gp3) viral proteins are glycosylated (Fig. 3B).

Because the latter two proteins correspond in molecular weight to gp1 and gp3, respectively (19, 22), we refer to the 115K protein as gp1 and the 62K protein as gp3.

**Time course synthesis of virus-induced proteins (125K, gp1, and gp3).** Infected cells were pulse-labeled with [$^{35}$S]methionine for 2-h intervals, and viral proteins were immunoprecipitated with MAbs and analyzed by SDS-PAGE. The results showed that the synthesis of 125K, gp1, and gp3 viral proteins began at 20 to 22 h p.i., which was 2 h after the peak of viral DNA synthesis (Fig. 4). These proteins had increased levels of synthesis until 28 to 30 h p.i., when the first progeny virus appeared in the infected cells. In addition to virus-induced proteins (125K, gp1, and gp3), one host cell protein with an apparent molecular weight of 94K was often detected in VZV-infected as well as in uninfected cell lysates immunoprecipitated with MAb 10.2 and MAb 19.1 (Fig. 4). This could be due to the nonspecific binding or cross-reactivity of this protein with MAbs.

To differentiate between early and late viral proteins, an inhibitor of viral DNA synthesis, PAA, was used. Cells were infected with VZV (0.001 PFU /cell) in the presence of different concentrations (50, 100, 150, and 200 μg/ml) of PAA for 72 h. Infected-cell DNA was extracted, immobilized on nitrocellulose filters, and hybridized to a $^{32}$P-labeled VZV DNA-cloned fragment (pVBC). No detectable hybridization

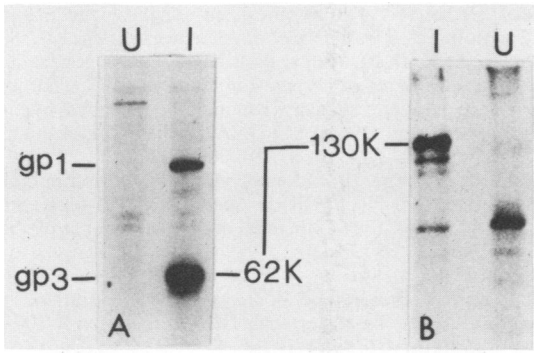


FIG. 7. SDS-PAGE of immunoprecipitated gp3 under reducing (A) and nonreducing (B) conditions. Cells were infected with VZV (0.001 PFU/cell) and labeled with [$^{35}$S]methionine (100 μCi/ml) at 28 to 30 h p.i. Uninfected- and infected-cell lysates (U and I, respectively) were prepared, immunoprecipitated with MAb 19.1, and analyzed by SDS–10% PAGE. Under nonreducing conditions, in which 2-mercaptoethanol was excluded from sample buffer, gp3 (62K) forms a 130K protein band on SDS-PAGE.

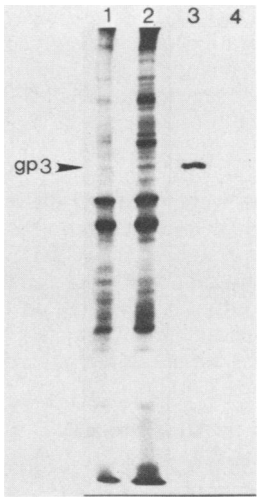


FIG. 8. SDS-PAGE of viral nucleocapsid proteins (lane 1), whole-virion proteins (lane 2), and whole-virion and nucleocapsid lysates immunoprecipitated with MAb 19.1 (lanes 3 and 4). VZV-infected cells were labeled with [$^{35}$S]methionine (50 μCi/ml) for 72 h, and VZV virions were purified by sucrose and potassium tartrate gradients as described in the text. To prepare nucleocapsids, purified virions were resuspended in 1 ml of TNE buffer containing 0.5% Nonidet P-40 and 1% 2-mercaptoethanol. After incubation at 37°C for 1 h, the suspension was sonicated for 10 s and layered onto 20% sucrose and centrifuged at 70,000 × *g* in a Beckman SW41 rotor for 60 min at 4°C; the pellet was suspended in TNE buffer. The nucleocapsids and whole virions (100,000 cpm) were analyzed by SDS–10% PAGE (lanes 1 and 2). Whole virions and nucleocapsids were suspended in lysis buffer and after 2 h at 4°C, the suspensions were centrifuged at 2,000 × *g* for 20 min, and the supernatants were centrifuged at 175,000 × *g* in a Beckman SW65 rotor for 2 h at 4°C. The lysates (supernatants) were immunoprecipitated with MAb 19.1 and analyzed by SDS–10% PAGE (lanes 3 and 4).

of viral DNA to infected-cell DNA was observed at 100, 150, and 200 μg of PAA per ml (results not shown), indicating inhibition of viral DNA synthesis at these concentrations. Synthesis of the 125K viral protein was completely inhibited in the presence of PAA at 200 μg/ml, and the synthesis of gp1 and gp3 was significantly reduced (Fig. 5) indicating that these proteins are late viral proteins and are synthesized after viral DNA replication.

**Pulse-chase labeling of VZV-induced proteins.** Since MAb 19.1 reacts with both gp1 and gp3, pulse-chase experiments were conducted to determine whether any relationship exists between these viral glycoproteins. Infected cells were labeled with [$^{35}$S]methionine for 10 and 20 min at 20 and 21 h p.i. After being pulse-labeled, cells were washed, and the label was chased in normal medium for 30, 60, 90, 120, and 240 min. In addition, cycloheximide (50 μg/ml) was added to the medium after pulse-labeling to inhibit further protein synthesis (3). Cells were then harvested, and cell lysates were immunoprecipitated with MAb 19.1 and analyzed by SDS-PAGE. Figure 6 shows that during either a 10- or 20-min pulse-labeling period, only gp1 was detected in infected cells; however, both gp1 and gp3 were detected in infected cells during a 30-min chase period. These results are similar to those reported previously (19). No precursor protein was detected for the 125K protein when cells were labeled for 20 min and chased for 30, 60, and 90 min (data not shown).

Analysis of infected-cell lysates immunoprecipitated with MAb 19.1 under nonreducing conditions, in which 2-mercaptoethanol was excluded from the sample buffer, revealed a

130K protein in SDS-PAGE (Fig. 7), but not the 62K (gp3) protein band, indicating that 62K glycoproteins (gp3) are disulfide linked. No change in electrophoretic mobility of the 125K protein was observed in SDS-PAGE under nonreducing conditions (data not shown).

**Location of VZV-induced proteins.** To determine the location of the virus-induced proteins on VZV, [$^{35}$S]methionine-labeled VZ virions were purified, the viral envelope was solubilized with mild detergents, and viral nucleocapsids and whole virions were analyzed by SDS-PAGE. Figure 8 (Lanes 1, 2) shows that gp3 was removed by detergent treatment indicating that it is located on the VZV envelope. When whole virions and nucleocapsids were reacted with MAb 19.1, gp3 was immunoprecipitated only from whole virions (Fig. 8, lanes 3, 4).

## DISCUSSION

We have shown that VZV DNA synthesis begins at 10 to 12 h p.i. and peaks at 16 to 18 h p.i., and the first progeny virus appears in BSC-1-infected cells at about 29 h after infection. These results indicate that the VZV replication cycle is longer than that of herpes simplex virus (HSV). HSV DNA synthesis begins at 2 to 3 h after infection and peaks at 4 h p.i. (4, 20), and infectious virions are detected in infected cells at 10 to 13 h p.i. (20). The yield of $10^3$ to $10^4$ PFU of infectious virions per ml for VZV is also lower than that of HSV at $10^8$ to $10^9$ PFU/ml (4).

The study of VZV-induced proteins in infected cells has been hampered by the low virus yields in tissue culture cells, continuation of uninfected cell protein synthesis, and comigration of cellular and viral proteins in SDS-PAGE. Therefore, monoclonal antibodies (MAb 10.2, MAb 19.1) were prepared to study VZV-induced proteins in infected cells. MAb 10.2 reacts only with nuclei of infected cells and immunoprecipitates one nonglycosylated VZV protein (125K). The 125K protein which is detected in infected cells at 20 to 22 h p.i. is a late viral protein. This viral protein does not appear to be disulfide linked, and no precursor protein was detected in infected cells by pulse-chase experiments.

MAb 19.1 has virus-neutralizing activity, reacts with membrane antigens of infected cells, and immunoprecipitates two VZV glycoproteins (gp1 and gp3). These viral proteins are first detected in infected cells at 20 to 22 h p.i., and their synthesis was significantly reduced when infected cells were maintained in the presence of 200 μg of PAA per ml, which inhibits VZV DNA replication in BSC-1 cells (data not shown). This indicates that these proteins are late viral proteins, although it is not clear why the synthesis of the 125K protein was totally inhibited by PAA, whereas that of gp1 and gp3 was only reduced. This differential inhibition of the synthesis of several late viral proteins in the presence of PAA has been reported in herpes simplex virus-infected cells (5, 15).

The results of pulse-chase experiments described in this study showed that during the pulse-labeling, only gp1 is detected, whereas, during the chase period, in the absence or presence of 50 μg of cycloheximide per ml (3), gp1 and gp3 are detected in infected cells, suggesting that gp1 is synthesized in infected cells before gp3. Pulse-chase experiments reported by Okuno et al. (19) have suggested that gp3 is the cleavage product of gp1. However, no reduction in gp1 had been detected during the chase period. In our studies, the autoradiographs also did not show a significant reduction of gp1 during the chase period. If gp1 is the precursor of gp3, the reason the intensity of gp1 is not reduced during the chase period is not known. Peptide mapping experiments may help to determine the structural relationship between gp1 and gp3.

Under nonreducing conditions, gp3 migrates in SDS-PAGE as a 130K protein as compared with the 62K species obtained when gels are resolved under reducing conditions, indicating that, in infected cells, gp3 is a dimer that is disulfide linked. Grose et al. (13) have detected a 140K disulfide-linked VZV glycoprotein in VZV-infected cells as well as in infected human tissues from chicken pox and varicella-zoster patients. Under reducing conditions, the 140K is cleaved into a 66K protein (13). Since the 62K (gp3) detected by MAb 19.1 is disulfide linked and is resolved as a 130K protein band in SDS-PAGE under nonreducing conditions, the 62K (gp3) may be similar to the 66K protein. Immunoprecipitation of purified whole virions and nucleocapsids obtained with MAb 19.1 revealed only gp3 in the whole virions. Thus, gp3 appears to be the mature form of the viral glycoprotein which is found in the envelope. Because the anti-gp3 MAb 19.1 neutralizes virus infectivity by as much as 50 to 75%, it is possible that gp3 plays a major role in the adsorption of the VZ virion to the cell surface in the initial stage of viral infection.

## ACKNOWLEDGMENTS

We thank R. J. Eisenberg and G. H. Cohen for critical reading of the manuscript and W. H. Wunner for helpful discussion. We also thank M. Devlin for technical assistance, A. Gilden for preparation of the photographs, M. Hoffman for editorial review, and J. Bracken and J. DeRitis for preparing the manuscript. This work was supported by Public Health Service grant NS-11037 from the National Institutes of Health and a grant (894-D4) from the National Multiple Sclerosis Society.

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