Assembly and Processing of the Disulfide-Linked Varicella-Zoster Virus Glycoprotein gpII(140)

EDUARDO A. MONTALVO AND CHARLES GROSE*

Departments of Microbiology and Pediatrics, University of Iowa, Iowa City, Iowa 52242

Received 20 February 1987/Accepted 9 June 1987

Varicella-zoster virus (VZV) specifies the synthesis of at least four families of glycoproteins, which have been designated gp1, gpII, gpIII, and gpIV. In this report we describe the assembly and processing of VZV gpII, a structural protein of an apparent Mr of 140,000, which is the homolog of gB of herpes simplex virus. For these studies, we used two anti-gpII monoclonal antibodies which exhibited both complement-independent neutralization activity and inhibition of virus-induced cell-to-cell fusion. Pulse-chase labeling experiments identified a 124,000-Mr intermediate which was chased to the mature 140,000-Mr product when analyzed in nonreducing gels; in the presence of a reducing agent, the native gp140 was cleaved into two closely migrating species (gp66 and gp68). The biosynthesis of VZV gpII was further analyzed in the presence of the following inhibitors of glycoprotein processing: tunicamycin, monensin, castanospermine, swainsonine, and deoxymannojirimycin. All intermediate and mature forms were digested with endoglycosidases H and F, neuraminidase, and O-glycanase to further define high-mannose, complex, and O-linked glycans. Finally, the addition of sulfate residues was investigated. This characterization of VZV gpII revealed the following results. (i) gp128 and gp124 were early high-mannose forms, (ii) gp126 was an intermediate form with complex N-linked oligosaccharides, (iii) gp130 was a later intermediate with both N-linked and O-linked glycans, and (iv) the mature product gp140 contained a mixture of N-linked and O-linked glycans which were both sialated and sulfated. Further investigations indicated that gpII sulfation was inhibited by tunicamycin and castanospermine but not by deoxymannojirimycin or swainsonine. We also concluded that VZV gpII displayed many biological and biochemical properties similar to those of its herpes simplex virus homolog gB.

The genome of varicella-zoster virus (VZV) encodes at least four families of glycoproteins. As with other herpesviruses, the glycosylated products are found in both the enveloped virion and the membrane of the VZV-infected cell (for a review, see reference 15a). In previous studies from several laboratories, these glycoproteins were designated by different classification schemes. To avoid further confusion, a consensus was reached on a common nomenclature for the VZV glycoproteins, based on a Roman numeral designation, with priority determined by the date of identification of the corresponding structural gene (3). The classification document contains a listing of all prior publications relating to the identification of VZV glycoproteins. Since gp98 (98 × 10^3; also called VZV gC) was the first viral glycoprotein to be genetically mapped (12), it was assigned the first numeral, VZV gpI. Based on preliminary genetic data, gp140 and gp18 were designated gpII and gpIII, respectively. A fourth glycoprotein has now been identified, whereas the fifth remains a hypothetical gene (4, 5).

This report describes biological properties and biochemical analyses of VZV gpII. The disulfide-linked glycoprotein was first identified by our laboratory in 1984, on the basis of immunoreactivity profiles of two murine monoclonal antibodies (17). Both of these antibodies precipitate two fucosylated species (a minor gp124 and a prominent gp66) under reducing conditions; when the same reaction is carried out in the absence of 2-mercaptoethanol, only one prominent fucosylated protein is observed at 140,000 Mr. The same phenomenon is apparent when a preparation of purified virions is analyzed; under reducing conditions, a lower-molecular-weight [H]glucose-labeled protein (gp66) is detected in the fractionated polypeptide profile, whereas under nonreducing conditions, gp66 is replaced by a band at 140,000 Mr. Antibodies to this disulfide-linked glycoprotein complex appear in the sera of children shortly after primary VZV infection (chicken pox) (17, 37). Therefore, this viral glycoprotein appears to be an immunodominant species in the humoral immune response to naturally acquired disease. The structural gene which encodes VZV gpII has been mapped by hybrid selection and in vitro translation to the HindIII D fragment near the center of the U1 genomic region (22). In this paper, we characterize, in detail, the glycomoieties and the processing steps associated with the assembly of this glycoprotein, which is the homolog of herpes simplex virus (HSV) glycoprotein gB (4). For these experiments, we have used several newly discovered inhibitors of glycoprotein processing events. One important outcome of this investigation is the demonstration that a neutralization epitope is detectable in an early high-mannose form of VZV gpII.

MATERIALS AND METHODS

Cells and virus. The Mewo strain of human melanoma cells (HMC) was grown at 37°C in Eagle minimum essential medium containing 10% fetal bovine serum, 1% nonessential amino acids, 0.002 M glutamine, 100 U of penicillin per ml, and 100 µg of streptomycin per ml (MEM-FBS). The VZV-32 strain, obtained from passage 9 virus stock, was used for all experiments (15).

* Corresponding author.
Infection of cells, intrinsic radiolabeling, and protein analyses. Infection of cells and intrinsic radiolabeling with [5,6-3H]fucose (specific activity, 40 Ci/mmol), [35S]methionine (specific activity, 1,265 Ci/mmol), and [2-14C]glucosamine hydrochloride (specific activity, 200 mCi/mmol) have been previously described in detail (15, 28). To study sulfation, VZV-infected monolayers were incubated for 24 h in MEM-FBS supplemented with 0.5 mCi of [35S]SO4 per ml (specific activity, 25 to 40 Ci/mg). Cell monolayers were harvested by the addition of either 2.5 (for 75-cm2 monolayers) or 1 ml (for 25-cm2 monolayers) of RIPA buffer (0.15 M NaCl, 0.05 M Tris buffer, pH 7.4, containing 1% Nonidet P-40, 1% deoxycholate, and 0.1% sodium dodecyl sulfate [SDS]). Radioimmune precipitation and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were carried out by previously described methods (16, 17). In some experiments, we precipitated viral glycoproteins with biotinylated monoclonal antibody and streptavidin-agarose (D. R. Gretch, M. Suter, and M. F. Stinski, Anal. Biochem., in press). The apparent molecular weights of the precursor glycoproteins were estimated by their migration in SDS-PAGE compared with known-molecular-weight marker proteins, with the knowledge that this method may yield an apparent Mr for glycoproteins which is different from the true Mr.

Neutralization assay and fusion inhibition experiments. The plaque reduction technique to quantitate neutralization activity has been previously described in detail (16). For fusion inhibition experiments, HMC monolayers grown on 8-well LabTek tissue culture slides (Miles Scientific Div., Miles Laboratories, Inc., Naperville, Ill.) were inoculated with 100,000 trypsin-dispersed VZV-infected cells. Ascites-fluid antibody (total protein, 35 mg/ml; diluted 1:10 and 1:1,000 in MEM-FBS) was added at 2, 4, 12, and 24 h postinfection. At 48 h postinfection, cell monolayers were fixed for 30 min in 0.15 M NaCl-Formalin (9:1, vol/vol) and air dried; then the monolayers were stained by being submerged in diluted Wright-Giemsa stain for 45 min.

Digestion with glycosidases. Methods for digestion of VZV glycoproteins with endo-beta-N-acetylglucosaminidase H (Endo H), endo-beta-N-acetylglucosaminidase F (Endo F), endo-alpha-N-acetylgalactosaminidase (O-glycanase), and neuraminidase have been previously described (28, 30, 32, 36). As in previous experiments (EC Laboratories, Elkhart, Ind.) was added at a concentration of 0.04 U/ml, while Endo F (EC 3.2.1; New England Nuclear Corp., Boston, Mass.) was added at a concentration of 25 U/ml. The final concentrations of O-glycanase (Genzyme Corp., Boston, Mass.) and neuraminidase from Clostridium perfringens (EC 3.2.1.18; Sigma Chemical Co., St. Louis, Mo.) were 60 and 1 U/ml, respectively.

Inhibition of glycoprotein biosynthesis and processing. HMC monolayers (25 cm2) were inoculated with a 1:3 volume of trypsin-dispersed VZV-infected cells. At 10 h postinfection, the medium was replaced with fresh MEM-FBS containing one of the following inhibitors of glycoprotein biosynthesis or processing: 2.5 µg of tunicamycin (Calbiochem-Behring, San Diego, Calif.) per ml, 100 to 250 µg of castanospermine (Genzyme) per ml, 1.5 to 5.0 mg of swainsonine (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml, 1.0 to 4.0 mM deoxyxamnoinojirimycin (Genzyme), or 1.0 to 1.5 µM monensin (Calbiochem-Behring). At 2 h after the addition of inhibitor, 25 µCi of 2-[14C]amino acids or 16.6 µCi of 2-[14C]glucosamine hydrochloride or 500 µCi of [35S]methionine was added to the culture medium. After an additional 24-h incubation at 32°C, cell monolayers were harvested by dislodging cells into RIPA buffer. Untreated infected HMC monolayers served as a control.

RESULTS

Neutralization assays and inhibition of fusion. The functions of several herpesvirus membrane glycoproteins have been elucidated elsewhere (36). Some of the biologically important functions ascribed to these molecules include (i) attachment to cell surface, (ii) penetration into host cell, and (iii) induction of cell-to-cell fusion. To ascertain a possible role for VZV gplI, we first tested our two previously described anti-gplI monoclonal antibodies (17) for their ability to inhibit plaque formation in VZV-infected cell monolayers. In these assays, cell-free virus and antibody were first mixed together, allowed to react, and then added to an uninfected HMC monolayer (16). Each of the two monoclonal antibodies (clones 15 and 158) was capable of reducing VZV plaque formation by greater than 50% when tested at a dilution of 1:10,000. These titers were unchanged by the addition of guinea pig serum as a source of complement.

In addition to our neutralization assays, we also tested our panel of antibodies for their ability to inhibit the syncytial formation which is characteristic of VZV cytopathology in an in vitro system. In contrast to the neutralization experiments, cell monolayers were first infected with virus before exposure to antibody. Diluted (1:10 or 1:1,000) ascites monoclonal antibodies was added to infected monolayers at various times postinfection and then allowed to react until a rating from 3 to 4+ for VZV cytopathology was observed in untreated control wells. The photographs shown in Fig. 1 are characteristic of the results obtained from these experiments. At the lower dilution of antibody tested (panel A), 100% fusion inhibition was observed when anti-gplI antibody was added at 2, 4, or 12 h postinfection. Clumping of infected cells by antibody was typically seen in these monolayers. When antibody was added at 24 h postinfection, no inhibition of CPE was observed. In monolayers containing a 1:1,000 dilution of anti-gplI antibody (panel B), syncytial formation was detectable but markedly less than that observed in cultures with no monoclonal antibody (panel C). As an additional control experiment, we incubated infected cell cultures in medium supplemented with anti-gpl monoclonal antibody (clone 3B3 [16]) but found no inhibition of cell-to-cell fusion (photograph not shown).

Pulse-chase labeling experiments. We previously reported that native gplI(140) is cleaved to a 66,000-Mr glycoprotein in the presence of 2-mercaptoethanol (17). In these earlier experiments, [3H]fucose-labeled viral antigen was immunoprecipitated with anti-gp140 monoclonal antibody and examined under both nonreducing and reducing conditions. To further investigate the relationship between gp140 and gp66, the kinetics of gp140 synthesis were examined by pulse-chase labeling experiments. Monolayers of VZV-infected cells were pulse-labeled with [35S]methionine for 10, 20, and 30 min and then chased with unlabeled medium for 1, 2, 4, and 12 h. Lysates prepared from the infected monolayers were immunoprecipitated with anti-gp140 monoclonal antibody and examined under both nonreducing and reducing conditions in an SDS-PAGE system. In nonreducing gels, a 124,000-Mr intermediate was chased to a 140,000-Mr final product (Fig. 2A); the intensity of the gp140 band diminished after a 12-h chase (results not shown). In a reducing-gel system (Fig. 2B), the 124,000-Mr precursor was chased to two lower-molecular-weight peptides, designated
gp66 and gp68. Furthermore, the appearance of gp140 (nonreduced; lanes 5 and 6) correlated with the appearance of the 66,000-molecular-weight bands (66K bands) and 68K bands (lanes 12 and 13) in reducing gels. Thus, the experiments suggested that gp140 was a disulfide-linked heterodimer composed of a 66K and a 68K subunit. It is apparent that the nonglycosylated 100,000-Mr polypeptide backbone (4, 22) was not visualized (Fig. 2), even after a 1-month exposure of the fluorogram.

However, the above experiments did provide an answer to why we had previously observed only one 66,000-Mr product in the reduced gels. Both cleavage products incorporated [3H]glucosamine, while only one had sufficient [3H]fucose moieties for visualization under our fluorographic conditions (Fig. 2B, lanes 8 and 15). In our earlier paper, we had prepared only [3H]fucose-labeled VZV-infected cell extracts (17).

Effect of inhibitors of glycoprotein processing on gp11 biosynthesis. We have examined the effect of three inhibitors of glycoprotein processing on the formation of mature gp11. The inhibitors included swainsonine, castanospermine, and deoxymannojirimycin. These inhibitors prevent the formation of N-linked complex glycans, by interfering with various trimming and processing events (9). In particular, swainsonine inhibits Golgi mannosidase II (10), whereas deoxymannojirimycin and castanospermine exert at least part of their effect on mannosidase 1 and glucosidase 1, respectively (11, 32). In addition to the above inhibitors, we also investigated the effects of the antibiotic tunicamycin and the ionophore monensin on the biosynthesis and processing of VZV gp11 (9, 26).

For these experiments, monolayers of infected cells were grown in the presence of an inhibitor and then radiolabeled as described in Materials and Methods. The cultures were harvested, solubilized with detergents, and analyzed by immunoprecipitation reactions. Fig. 3 is an example of our findings under nonreduced conditions. Lane 1 contained [3H]fucose-labeled antigen. Lane 2 contained untreated [35S]methionine-labeled glycoproteins precipitated by antibody 151; the locations of the two major bands gp11/140 and gp124 are designated in the right-hand margin. In contrast to lanes 1 and 2, no proteins were visible in the immunoprecipitate of the tunicamycin-treated sample shown in lane 3. Since tunicamycin inhibits all N-linked glycosylation reactions, monoclonal antibody 151 probably bound poorly, if at all, to the nonglycosylated precursor of gp11. Alternatively, since nonglycosylated analogs of glycoproteins may be more susceptible to intracellular proteases (26), the altered gp11 products synthesized in the presence of tunicamycin may be degraded and thus undetectable in these experiments. These negative findings reaffirmed similar negative results in the pulse-chase labeling experiments (Fig. 2).

The effects of castanospermine and swainsonine on glycoprotein biosynthesis are displayed in the profiles in Fig. 3, lanes 4 and 5. After a castanospermine block, gp124 was replaced by a more slowly migrating product (M, 128,000), while the major product migrated slightly faster than did gp11/140 in the control lane. The effect of swainsonine was an even more subtle event, viz., only a slight decrease in the apparent molecular weight of the major component (gp140) was observed. Likewise, the deoxymannojirimycin block minimally altered the electrophoretic mobility of forms gp140 and gp124 (lane 5). By comparison, monensin exerted a more dramatic effect (lane F): formation of the mature gp140 was completely blocked. In its place was a 135,000-Mr form, as well as gp124. The relative amount of the 135,000-Mr product was dependent upon the completeness of the monensin block, i.e., with higher concentrations of the ionophore, there was a greater accumulation of gp124 and less of the higher-molecular-weight product (data not shown).

Another side effect of growth of the viral glycoprotein in the presence of inhibitors of glycoprotein processing was demonstrable (Fig. 3, lanes 5 and 6). Both deoxymannojirimycin and swainsonine blocks led to increased amounts of cleavage of the native gp140, with the appearance of two products at approximately 60,000 daltons. These lower-molecular-weight proteins were virtually never seen under the usual conditions of glycoprotein biosynthesis, as analyzed in nonreducing gels (Fig. 3, lane 2; Fig. 2, lanes 5 to 7). nor were the 60,000-Mr products seen in VZV-infected cultures treated with either castanospermine or monensin (Fig. 3, lanes 4 and 7), which inhibit different stages of glycoprotein biosynthesis.

Digestion of gp140 with Endo H and Endo F. After determining that gp140 was a disulfide-linked mature glycoprotein, structural analyses of the glycoprotein and the individual subunits were performed. We began by examining the susceptibility of gp140 to two endoglycosidases which cleave
N-linked oligosaccharides, Endo H and Endo F. Endo H cleaves predominantly high-mannose oligosaccharide chains, whereas both high-mannose and complex-type oligosaccharides are sensitive to enzymatic cleavage by Endo F (25). To this end, [35S]methionine-labeled VZV-infected cell lysates from untreated and inhibitor-treated cultures were immunoprecipitated with monoclonal antibody to gp140, washed, and suspended in the appropriate enzyme buffer. Samples were then incubated in the presence or absence of endoglycosidase. Endo H digestion of mature gp140 from untreated cultures caused a modest shift in relative mobility.

![Image](http://jvi.asm.org/)
corresponding to a loss of approximately 3,500 daltons (Fig. 4). Treatment of the intermediate gp124 with the same endoglycosidase led to an even greater change in molecular weight; a new band of approximately 100,000-M, was found immediately above the location of gp1140. The 100K protein corresponded in molecular weight to the polypeptide backbone, as estimated from the DNA sequence of the glycoprotein gene (4).

When the glycoproteins in the castanospermine- and swainsonine-treated cultures were subjected to similar treatment, the shift in electrophoretic mobility of the final products in lanes 4 to 7 appeared to be slightly greater than in the untreated cultures (Fig. 4, lanes 2 to 3). This finding is to be expected since the two inhibitors prevent formation of complex-type glycans, which are Endo H resistant. Likewise, the decrease in molecular weight of the 128,000-M, intermediate form in the castanospermine-treated culture clearly demonstrated that (i) the 128,000-M, protein was another high-mannose intermediate, and (ii) the deglycosylated form was the same molecular weight as the 100K backbone protein was. Endo H digestion of the glycoproteins isolated from monensin-treated cultures led to similar conclusions about the relative Endo H sensitivity of the higher (135K) and lower (124K) forms (lanes 8 to 9).

Since hydrolysis with Endo F is performed under reducing conditions, gp1140 cannot be analyzed with this enzyme. Rather, we subjected the reduced products to Endo F digestion and observed reductions in the molecular weights of gp124, as well as gp68 and gp66 (fluorogram not shown). These studies confirmed that N-linked glycans were present in the reduced forms, as would be expected from the above results after Endo H digestion of the native gp1140.

**Digestion with neuraminidase and endo-alpha-N-acetylgalactosaminidase.** To determine whether gp11 contained O-linked glycans like its HSV homolog gB (21), digestions with an endoglycosidase specific for O-linked oligosaccharides, endo-alpha-N-acetylgalactosaminidase (O-glycanase), were carried out. Immunoprecipitates of radiolabeled VZV anti-

**FIG. 5.** Treatment of native gp11 with neuraminidase and O-glycanase. Untreated VZV-infected cultures as well as similar cultures grown in the presence of castanospermine (CASTANO) or monensin were radiolabeled with [14C]methionine. Anti-gp11 immunoprecipitates from the three cultures were subjected to successive digestions with neuraminidase (NEU) and O-glycanase (OGL). The order of digestion for each set of samples is indicated at the bottom of each lane. SDS-PAGE was carried out under nonreducing conditions. The location of native gp1140 is indicated in the [14C]fucose-labeled VZV-infected-cell lysate (Fuc) lane 1.

**FIG. 6.** Treatment of the gp11 cleavage products with neuraminidase and O-glycanase. Immunoprecipitation of gp11 from an untreated VZV-infected culture was performed as described in the legend to Fig. 5. Samples of the gp1140 complex were sequentially digested with neuraminidase and O-glycanase, as indicated at the bottom of lanes 2 to 4. Each sample was then analyzed by SDS-PAGE under reducing conditions. The closely migrating glycoproteins gp126 and gp124 are designated by closed circles beside lane 2, whereas only gp124 is similarly marked beside lanes 3 and 4. The [14C]fucose-labeled reduced form (gp66) of gp11 is designated in the lysate in lane 1. Both gp11 cleavage products (gp68 and gp66) are visible in lanes 2 and 3.
addition of neuraminidase to the two lower-molecular-weight forms, both decreased slightly in size (lane 3). Subsequent treatment with O-glycanase caused a marked increase in mobility of gp68 (lane 4). The last result supported the positive findings after O-glycanase digestion of native gp140 (Fig. 5).

**Sulfation of viral glycoproteins.** Because of previous studies (18) which indicated that sulfation occurred on HSV glycoproteins including HSV gB, the homolog of VZV gp1, we investigated whether this posttranslational modification was seen within VZV-specified glycoproteins. To this end, we added $^{35}$SO$_4$ to culture medium overlying infected cultures, as well as to uninfected HMC monolayers. Very few sulfated forms were visible in uninfected cell cultures; however, in VZV-infected cells, it was evident that the predominant glycoprotein species gp1 was highly sulfated, while lesser amounts of the isotope comigrated with gp11 and gp111 (fluorogram not shown). To substantiate that the $^{35}$S-labeled band at 140,000 M, was actually gp11, we prepared detergent-solubilized extracts for analysis by immunoprecipitation with monoclonal antibody 151. The immunoprecipitate contained $^{35}$S-labeled gp11 (Fig. 7A, lane 2). We also added $^{35}$SO$_4$ to VZV-infected cultures containing the previously described inhibitors of glycoprotein biosynthesis and processing (lanes 3 to 7). In the presence of tunicamycin or monensin, sulfation of gp11 was markedly inhibited; likewise, sulfation was only minimally detectable after castanospermine block. However, addition of swainsonine or deoxymannojirimycin to the medium of infected cell cultures had little effect on subsequent sulfation of the final viral glycoprotein product.

Sulfation of the glycans of gp11 was further analyzed by digestion with neuraminidase and O-glycanase. Treatment of the $^{35}$S-labeled glycoprotein with neuraminidase reduced the molecular weight in a manner similar to that described earlier in these Results (Fig. 7B). Further treatment of desialated gp11 by O-glycanase led to the loss of a detectable radioactive band. The last result suggested that an appreciable amount of sulfation occurred on O-linked glycomoieties which were released by endo-alpha-N-acetylgalactosaminidase.

**DISCUSSION**

We have now studied the biosynthesis of the three major VZV glycoproteins: gp1(98), gp111(118), and gp1111(140). The predominant viral glycoprotein within the VZV-infected cell membrane is the phosphorylated species gp1 (15a, 29). The backbone of this mature product is a 73,000-M, polypeptide, to which both N-linked and O-linked glycans are attached (30). Since the final product is Endo H resistant, all of the N-linked oligosaccharides appear to be processed to complex chains. The least prominent viral glycoprotein in the infected-cell membrane is gp111, an 118,000-M, product which contains predominantly N-linked oligosaccharides of the complex type (28). The high-mannose intermediate form of gp111 is a 94K product built upon a 79K backbone. Finally, gp11 described herein has been the most difficult of the three major VZV glycoproteins to characterize because of its susceptibility to cleavage. Recent studies have shown that the imputed polypeptide backbone contains 868 amino acids; after glycosylation has been completed, the mature glycoprotein appears to be cleaved between amino acids 431 to 432, when placed under reducing conditions (4, 22).

A schematic representation of the assembly and processing of VZV gp1111(140) is shown in Fig. 8. The molecular weight of the polypeptide backbone (approximately 100,000) was derived from the VZV genomic sequence data of Davison and Scott (4). This estimate was supported by our results from Endo H digestion experiments in which gp1124 was reduced to a 100,000-M, product. The first detectable glycoprotein species was seen in cultures treated with caste-
nospermine (128,000 M\(_{r}\): this precursor was subsequently trimmed to 124,000 M\(_{r}\). Both the 128K and 124K products represented high-mannose forms, which were sensitive to treatment with Endo H. The slightly heavier 126,000-M\(_{r}\) product included some glycans which were fucosylated and sialated, i.e., probably a hybrid glycoprotein. The intermediate viral products were further processed by the addition of O-linked glycans, several of which probably contained sialic acid and sulfate residues, to build a 140,000-M\(_{r}\) native product. This mature glycoprotein was subsequently cleaved into subunits of similar molecular weight (ca. 60,000), which were discernible after disruption of interchain disulfide bonds by 2-mercaptoethanol (17, 31).

Another important aspect of this scheme relates to the immunoprecipitability of the individual proteins. That the early high-mannose forms were recognized by the monoclonal antibody, while the precursor polypeptide was not, suggests that the formation of the neutralization epitope occurs shortly after the addition of mannose chains (gp128) and is not dependent on the later glycosylation events illustrated in Fig. 8.

Sulfation of envelope glycoproteins has been reported in many RNA and DNA viruses (19, 33). The major sulfated HSV glycoprotein is gE, whereas lesser degrees of sulfation are observed on gD, gB, and gC (18). However, the effects of inhibitors of glycoprotein processing on sulfation have not been extensively investigated in a herpesvirus system (25). Therefore, we analyzed aspects of the sulfation of VZV gpII in both untreated and inhibitor-treated cultures. When infected cells were grown in medium containing \(^{35}\)SO\(_4\) sulfate was clearly incorporated into VZV gpII. In infected cells treated with either tunicamycin or castanospermine, however, virtually no sulfation was detectable. By contrast, neither swainsonine nor deoxymannojirimycin prevented the sulfation of the viral glycoprotein. These VZV results correlate with prior observations on the effect of swainsonine and castanospermine on the sulfation of influenza A virus hemagglutinin, viz., of the two inhibitors, only swainsonine permitted sulfation to occur (27). This observation led the investigators to conclude that certain early processing steps, e.g., removal of glucose units from the high-mannose core, were required before sulfate groups could be attached to oligosaccharides. Thus, swainsonine and deoxymannojirimycin would not inhibit sulfation because they exert their effect after the glucosyl groups have already been trimmed. Since monensin also prevented sulfation, the location of the sulfotransferase must be distal to the monensin block within the Golgi apparatus. Although not further investigated in this study, we recently determined that another modification of viral glycoproteins, phosphorylation, does not occur on gpII, but appears to be restricted to VZV gpI (29).

With the availability of DNA sequence data on the human herpesvirus genomes, it is now apparent that varying degrees of homology can be found between the glycoproteins encoded by different herpesviruses (4). In particular, VZV gpII is related to gpB of HSV types 1 and 2 (1, 2). Antigenic similarities between these HSV and VZV glycoproteins had been postulated previously because of serologic cross-reactivity detected in both polyvalent sera and monoclonal antibodies (8, 24, 34). HSV type 1 gB, in turn, is known to be antigenically related to glycoproteins of HSV type 2 and other herpesviruses, such as Epstein-Barr virus, equine herpesvirus, and bovine mamillitis virus (14, 35). By comparison of the biologic properties of the individual glycoproteins, we can determine whether this glycoprotein product has highly conserved functions which are retained across species barriers. For example, anti-HSV gB antibodies often have neutralizing activity (6, 36). As shown in this paper and earlier reports (7, 38), some monoclonal and polyclonal antibodies to VZV gpII also neutralize virus in the absence of exogenous complement, whereas other anti-gpII antibodies lack this capability (7, 13, 23). On the basis of studies of mutant HSV strains, HSV gB is thought to play an important role in virus penetration (36). By analogy, the neutralization activity present in anti-gpII antibodies may reflect interference with VZV penetration of the cell. These comparisons are especially important for VZV investigations, because there are no well-defined mutant strains of this closely cell-associated herpesvirus. The two antibodies described in this report also inhibited VZV-induced cell-to-cell fusion; however, they did not mediate antibody-dependent cellular cytotoxicity of VZV-infected fibroblasts by human peripheral blood mononuclear cells (20). Thus, the above observations in several different herpesvirus systems suggest an important role for this glycoprotein in both virus penetration of the cell and virus-induced cell-to-cell fusion. After VZV infection in the human host, therefore, the anti-gpII antibody which develops is most likely directed toward a complement-independent neutralization epitope on gpII (17, 37). This humoral response, in turn, halts further spread of the viral agent.

ACKNOWLEDGMENTS

We thank A. D. Elbein, K. O. Smith, C. J. Gauntt, and M. F. Stinski for helpful discussions. This research was supported by Public Health Service grant AI 22795 from the National Institutes of Health. E.A.M. was a recipient of a Public Health Service fellowship.
of a graduate fellowship from the American Society for Microbiology.

LITERATURE CITED


