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Endo-β-*N*-Acetylglucosaminidase H Sensitivity of Precursors to Herpes Simplex Virus Type 1 Glycoproteins gB and gC

ELIZABETH A. WENSKE, MICHAEL W. BRATTON, AND RICHARD J. COURTNEY*
Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37996

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The endoglycosidase endo-β-*N*-acetylglucominidase H (endo H) was used to examine the nature of the oligosaccharides associated with the herpes simplex virus type 1 glycoproteins gA, gB, and gC. Immunoprecipitates from detergent extracts of infected cells, using monospecific antisera to gAB and gC, were treated with endo H. The low-molecular-weight precursor to gC, pgC(105), was found to be sensitive to endo H. Removal of the endo H-sensitive oligosaccharide chains from pgC(105) resulted in a protein with an apparent molecular weight of 75,000. In contrast, the fully glycosylated gC was not sensitive to endo H treatment. These results suggested that the oligosaccharide chains of pgC(105) were primarily of the simple high-mannose type. Both gA and gB were sensitive to endo H treatment; however, gB appeared to be only partially susceptible, whereas [^{3}H]mannose-labeled gA was not detectable after endo H treatment. These results indicated that gB contained both complex- and simple-type oligosaccharides, and gA contained only simple-type oligosaccharides. An accumulation of the high-mannose glycoproteins pgC(105) and gA was observed in monensin-treated infected cells with a concomitant inhibition of gB and gC. Glycoproteins gA and pgC(105) synthesized in the presence of monensin were also sensitive to endo H treatment.

Structural studies of viral glycoproteins containing asparagine-linked oligosaccharides reveal that the *N*-linked oligosaccharide side chains have a common biosynthetic origin: a high-mannose precursor oligosaccharide whose synthesis begins while linked to a carrier dolichol (14, 23, 33). The completed high-mannose oligosaccharide chains are then transferred en bloc from the dolichol to the nascent growing polypeptide. The newly glycosylated proteins are then transported to the Golgi, where the oligosaccharide side chains may be further modified to become high-mannose and complex glycans of mature glycoproteins (9, 10, 22, 28).

The biosynthesis of herpes simplex virus type 1 (HSV-1) glycoproteins includes the synthesis of five glycoproteins, gA, gB, gC, gD, and gE (1, 26, 27). The glycoproteins gA and gB have been demonstrated to be antigenically similar, with gA being a precursor of gB (5, 8). The precursor form of gC, pgC(105), has an approximate molecular weight of 105,000 (27). The shift in molecular weight observed in the conversion of pgC(105) to gC is due in part, but not completely, to the addition of sialic acid to pgC(105) (3). The precursor to gD, pgD(52), contains an oligomannosyl core which is processed by glycosylation and sialylation to a larger, more heterogenous, oligosaccharide on the fully glycosylated glycoprotein gD (6).

Little is known about the characteristics of the intermediates involved in the biosynthesis of the high-molecular-weight HSV-1 glycoproteins; therefore, this investigation was carried out in an attempt to determine whether the synthesis of these HSV-1 glycoproteins follows a pattern of synthesis similar to those of glycoproteins of other viral systems. We have investigated the biosynthesis of glycoproteins gA, gB, and gC in an attempt to identify and characterize intermediates which occur during their biosynthesis.

Using endo-β-*N*-acetylglucominidase H (endo H) (30, 31), we have been able to further characterize the oligosaccharide structures of the glycosylated HSV-1 glycoproteins gA, gB, and gC and their precursors. Identification of high-mannose oligosaccharides can be made by the susceptibility of a glycoprotein to the action of endo H (10, 22, 35). Endo H cleaves between the two proximal *N*-acetylglucosamine residues of the high-mannose-type oligosaccharides (31). Complex-type carbohydrate side chains are resistant to endo H, and oligosaccharides which are intermediates in processing show intermediate sensitivities (22, 29, 30, 34).

The processing of HSV-1 glycoproteins was

further studied by use of the ionophore monensin. Monensin is a linear polyether able to equilibrate Na^+/K^+ levels and shows selectivity for Na^+ (21). Monensin appears to inhibit the transport of glycoproteins from the Golgi to the plasma membrane, but its effect on the actual glycoprotein processing differs among glycoproteins (11, 13, 32). It has been reported that the vesicular stomatitis virus G glycoprotein is fully processed in the presence of monensin; however, it is not transported to the plasma membrane (11). In contrast, the proteolytic cleavage of Sindbis virus glycoproteins PE2 and E2 was inhibited in ionophore-treated cells along with the inhibition of the migration of PE2 to the plasma membrane (11). Monensin was used as a tool to investigate whether the HSV-1 glycoproteins gA, gB, and gC were fully processed or blocked at an intermediate step in the presence of monensin.

MATERIALS AND METHODS

Cells. Human embryonic lung (MRC-5) fibroblasts and mouse L929 cells were grown in Eagle medium supplemented with 10% fetal bovine serum and 0.075% sodium bicarbonate. Virus stocks were grown in MRC-5 cells, and all virus titrations were conducted with Vero cell monolayers.

Virus. The viruses used in this study were the KOS strain of HSV-1 (25) and *ts*J12, a temperature-sensitive mutant of HSV-1 strain KOS (15), which was kindly provided by Priscilla Schaffer (Sidney Farber Cancer Institute, Boston, Mass.).

Materials. Monensin was purchased from Calbiochem, La Jolla, Calif. Stock solutions of 1 mM were prepared by dissolving the ionophore in absolute ethanol and were stored at −70°C. Final dilutions were made in Eagle medium just before use.

Endo H was purchased from the Division of Laboratories and Research, New York State Department of Health, Albany, N.Y. Stock solutions of endo H (0.5 IU/ml) were made in 10 to 20 mM sodium citrate buffer (pH 5.64) and stored at −70°C.

Isotopically labeled D-[2-^{3}H]mannose (specific activity, 10.4 Ci/mmol), D-[1-^{14}C]glucosamine (specific activity, 55.5 mCi/mmol), and L-[^{35}S]methionine (specific activity, 1,440 Ci/mmol) were purchased from Amersham Corp., Arlington Heights, Ill.

Infection, labeling, and harvesting of cells. Cells cultured in 60- or 100-mm plastic petri dishes were infected with virus at an input multiplicity of 10 to 20 PFU/cell. After a 1-h adsorption at 37°C, the inoculum was removed, the monolayers were washed twice with warm phosphate-buffered saline, and maintenance medium was added. Isotopically labeled [^{3}H]mannose (20 μCi/ml), [^{14}C]glucosamine (5 μCi/ml), or [^{35}S]methionine (5 μCi/ml) was added at 4 h postinfection. When cells were isotopically labeled, Eagle medium supplemented with 1× amino acids without methionine–1× glutamine–2% donor calf serum–0.225% $NaHCO_3$ was used. At appropriate times the cells were scraped into the medium with a rubber policeman and washed twice in phosphate-buffered saline. The pelleted cells were suspended in water (5×10^6 to 1×10^7 cells per 0.2 ml), frozen, thawed, and disrupted by ultrasonic vibration.

Detergent extraction. Infected-cell extracts were prepared by extraction of infected cells with 1% sodium deoxycholate–1% Tween 40 for 0.5 h, with constant agitation at 37°C. Insoluble material was removed by centrifugation at 100,000 × *g* for 1 h. The resulting soluble proteins (supernatant) were used as the antigen for the immunoprecipitation assays.

Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis and molecular weight determinations. Details of the methods used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have been previously described (20). All of the slab gels contained 8.6% acrylamide cross-linked with 0.23% *N,N'*-diallyl tartardiamide as described by Gibson and Roizman (7).

Standard ^{14}C-labeled protein markers for molecular weight determinations were obtained from New England Nuclear Corp., Boston, Mass., and contained myosin (molecular weight, 200,000), globulins (150,000), phosphorylase *b* (92,500), ovalbumin (46,000), carbonic anhydrase (30,000), and lactoglobulin A (18,367). The major capsid protein of HSV-1 (154,000) was also included as a protein standard. A mixture (3,000 cpm) of these markers was coelectrophoresed with immune precipitated glycoproteins treated with and without endo H on 8.6% acrylamide gels cross-linked with *N,N'*-diallyl tartardiamide.

Antisera. Monospecific antisera to HSV-1-purified glycoproteins gAB and gC were used throughout this study. The purification of the glycoproteins and the characterization of the monospecific antisera prepared to the purified glycoproteins have been previously described (4, 5). Briefly, immune sera were prepared in rabbits to the gC and gAB glycoprotein components of HSV-1. Immunoprecipitation experiments demonstrated that the antisera were monospecific, i.e. anti-gC only reacting with the gC and pgC(105) glycoproteins and anti-gAB only reacting with glycoproteins gA and gB (4, 5).

Radioimmune precipitation assays. The details for the radioimmune precipitation assays have been previously described (5). Diluent used for radioimmune precipitation was TNE (10 mM Tris [pH 7.4], 100 mM NaCl, 1 mM EDTA), containing 0.1% Triton X-100. Samples of 25 μl of solubilized cell extract were reacted with 25 μl of rabbit antisera in a final volume of 100 μl for 1 h at 37°C and for an additional 6 h at 4°C. *Staphylococcus aureus* cells (20 μl) (Pansorbin; Calbiochem-Behring, La Jolla, Calif.) were then added and incubated for an additional 12 h. Immune complexes were pelleted by centrifugation at 6,000 × *g* for 20 min, and the precipitates were washed three times and solubilized in 35 μl of 2% SDS–2% mercaptoethanol and analyzed by SDS-PAGE.

Endo H digestion of immunoprecipitated glycoproteins. Glycoproteins immunoprecipitated with the monospecific antisera were eluted from the staphylococcal cells by incubating the samples in 0.8% SDS for 20 min at 37°C followed by boiling for 30 s. The staphylococcal cells were removed by centrifugation. Samples to be treated with and without endo H were diluted to 0.2% SDS and made 50 mM with respect to sodium citrate buffer (pH 5.5). Endo H (6 μl of 0.5 IU/ml) was added to samples for endo H treatment, and the appropriate volume of water was added to the

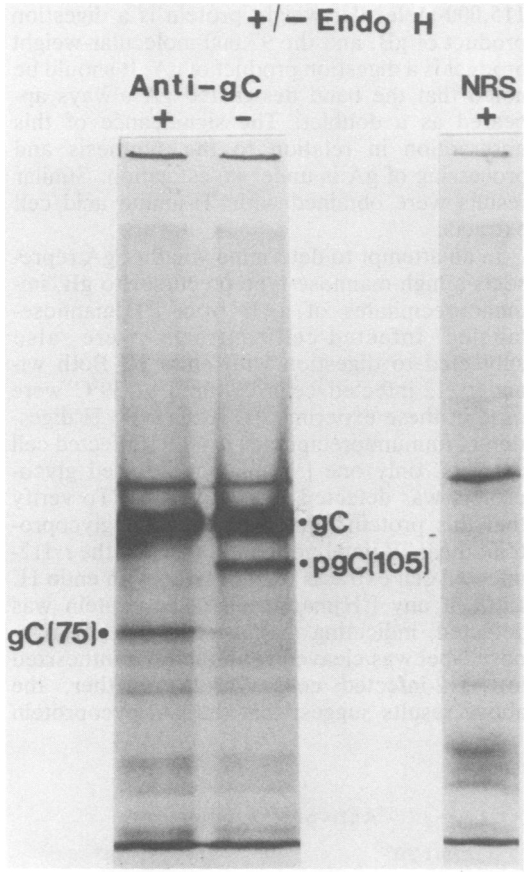

FIG. 1. Endo H treatment of anti-gC immunoprecipitated proteins from HSV-1-infected MRC-5 cell extracts. Infected cells were labeled with [^{35}S]methionine from 4 to 24 h postinfection and incubated at 37°C. Immunoprecipitates were solubilized, incubated in the presence (+) or absence (−) of endo H, and prepared for SDS-PAGE analysis as described in the text. Normal rabbit serum (NRS) was included as a control.

control samples. After being incubated at 37°C for 6 h, the samples were precipitated with 1 ml of cold acetone and centrifuged at 13,000 × *g* for 8 min, using a Fisher model 235A microfuge. The pellet was resuspended in 35 μl of 2% SDS–2% mercaptoethanol and stored at −20°C until it was analyzed by SDS-PAGE.

RESULTS

Endo H digestion of HSV-1 glycoproteins gC and pgC. To characterize the complexity of the oligosaccharide side chains of glycoproteins pgC(105) and gC, immunoprecipitates from isotopically labeled HSV-1-infected cell extracts were analyzed. HSV-1-infected MRC-5 cells were isotopically labeled with [^{35}S]methionine from 4 to 24 h postinfection, and the whole cell fraction was harvested at 24 h postinfection. The isotopically labeled cell extracts were immunoprecipitated with the monospecific anti-gC rabbit serum. The immunoprecipitates were washed, solubilized, and then incubated with or without endo H. The PAGE profiles of these samples are shown in Fig. 1. In the absence of endo H the fully glycosylated gC (molecular weight 130,000) and the partially glycosylated precursor pgC(105) (105,000) were clearly resolved. When endo H was present, the pgC(105) glycoprotein was not detectable; however, a new protein with an apparent molecular weight of 75,000 was observed. In contrast, the migration of the fully glycosylated gC was not affected by the presence of endo H. The [^{35}S]methionine-labeled protein, which migrated with a slower electrophoretic mobility than that of gC and which was detectable in the immune precipitates with anti-gC or normal rabbit serum, probably represents the major capsid polypeptide of HSV-1. The presence of this protein, which was nonspecifically precipitated, served as a good control for demonstrating the lack of detectable protease activity in the endo H preparation.

Experiments were also conducted with HSV-1-infected cell extracts isotopically labeled with [^{3}H]mannose and immunoprecipitated with anti-gC (Fig. 2). As observed in Fig. 1, gC was not affected by the action of endo H. However, the [^{3}H]mannose-labeled pgC(105) was not detectable in the samples treated with endo H (Fig. 2, lane 1). Based on endo H sensitivity and on the migration of the immunoprecipitated proteins as analyzed by SDS-PAGE, these results suggest that (i) gC has oligosaccharides of the complex type; (ii) pgC(105) has *N*-linked oligosaccharides of the simple high-mannose type, and (iii) the nonglycosylated form of gC has an apparent molecular weight of 75,000.

Endo H digestion of HSV-1 glycoproteins gA and gB. Previously, we have presented data suggesting that gA represents the partially glycosylated precursor to gB (5). To characterize the complexity of the oligosaccharide side chains of glycoproteins gA and gB, MRC-5 cells were infected with either HSV-1 (designated wt, or wild type) or *ts*J12 and isotopically labeled with [^{35}S]methionine from 4 to 24 h postinfection. The infected cells were cultured at 39°C, the nonpermissive temperature for *ts*J12. The isotopically labeled cell extracts were immunoprecipitated with monospecific anti-gAB rabbit serum. The immunoprecipitates were washed, solubilized, and then incubated with or without endo H, followed by analysis by SDS-PAGE (Fig. 3). In the absence of endo H, gA and gB from wt-infected cells migrated at a position in the gel which corresponded to apparent molecular weights of 110,000 and 120,000, respectively. However, when the immunoprecipitates of gAB

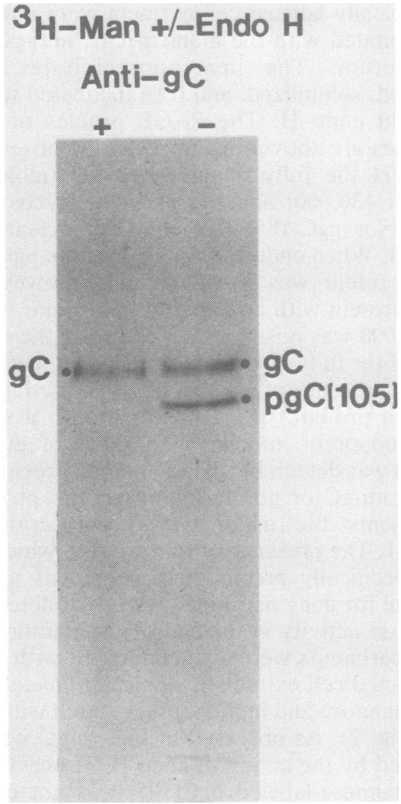

FIG. 2. Endo H treatment of anti-gC immunoprecipitated proteins from HSV-1-infected MRC-5 cell extracts. Infected cells were labeled with [^{3}H]mannose from 4 to 24 h postinfection and incubated at 37°C. Immunoprecipitates were solubilized, incubated in the presence (+) or absence (−) of endo H, and prepared for SDS-PAGE analysis as described in the text.

were treated with endo H, there was an increase in the migration rate of both gA and gB. The apparent molecular weights of the proteins designated gB and gA were determined to be 115,000 and 97,000, respectively. To determine whether these two faster-migrating proteins were correctly designated as gA and gB, the experiment was repeated, using the temperature-sensitive mutant *ts*J12. Previously it has been reported that at the nonpermissive temperature (39°C) *ts*J12-infected cells synthesized gA, but not gB (15). As expected, in the absence of endo H, only gA was immunoprecipitated. After endo H digestion of the anti-gAB immunoprecipitated proteins obtained from the [^{35}S]methionine-labeled *ts*J12-infected cell extract, a 97,000-molecular-weight protein was detectable. However, the 115,000-molecular-weight endo H digestion product detected in wt-infected cells was not detected in *ts*J12-infected cells. Based on these results we suggest that the 115,000-molecular-weight protein is a digestion product of gB, and the 97,000-molecular-weight product is a digestion product of gA. It should be noted that the band designated gA always appeared as a doublet. The significance of this observation in relation to the synthesis and processing of gA is under investigation. Similar results were obtained with ^{3}H-amino acid cell extracts.

In an attempt to determine whether gA represents a high-mannose-type precursor to gB, immunoprecipitates of gAB from [^{3}H]mannose-labeled infected-cell extracts were also subjected to digestion with endo H. Both wt- and *ts*J12-infected cells cultured at 39°C were used in these experiments. After endo H digestion of immunoprecipitates from wt-infected cell extracts, only one [^{3}H]mannose-labeled glycoprotein was detected (Fig. 4, lane 1). To verify that this protein represented the gB glycoprotein, the gAB immunoprecipitate from the *ts*J12-infected cell extracts was digested with endo H. Little if any [^{3}H]mannose-labeled protein was detected, indicating that most of the [^{3}H]mannose label was cleaved from the gA synthesized in *ts*J12-infected cells. Taken together, the above results suggest that the gA glycoprotein

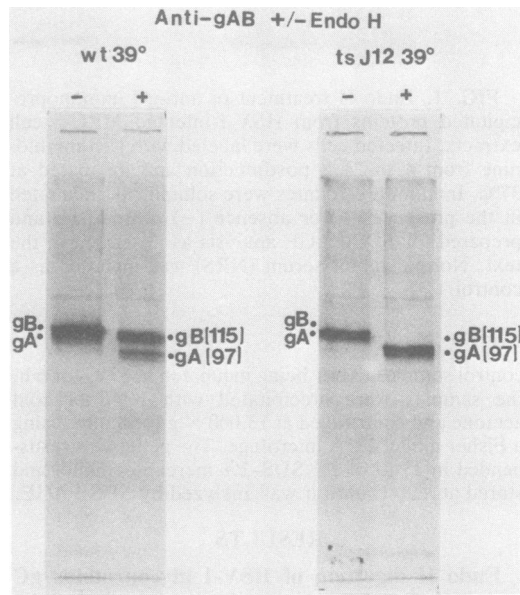

FIG. 3. Endo H treatment of anti-gAB immunoprecipitated proteins from wt- and *ts*J12-infected MRC-5 cell extracts. Infected MRC-5 cells were labeled with [^{35}S]methionine from 4 to 24 h postinfection and incubated at 39°C. Immunoprecipitates were solubilized, incubated in the presence (+) or absence (−) of endo H, and prepared for SDS-PAGE analysis as described in the text.

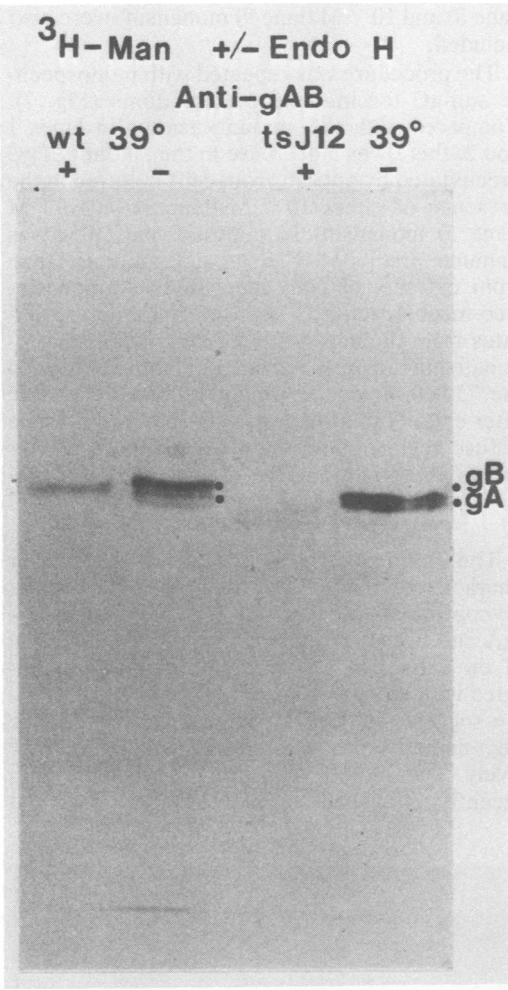

FIG. 4. Endo H treatment of anti-gAB immunoprecipitated proteins from wt- and *ts*J12-infected MRC-5 cell extracts. Infected cells were labeled with [^{3}H]mannose from 4 to 24 h postinfection and incubated at 39°C. Immunoprecipitates were solubilized, incubated in the presence (+) or absence (−) of endo H, and prepared for SDS-PAGE analysis as described in the text.

has mostly the simple- or high-mannose-type oligosaccharides, which are sensitive to endo H digestion. In contrast, gB contains both complex- and simple-type oligosaccharide chains.

Radioimmune precipitation of HSV-1 glycoproteins from monensin-treated infected cells. The sensitivity of the HSV glycoproteins to endo H digestion (described above) suggested that gA and pgC(105) were high-mannose precursors to gB and gC, respectively. Since monensin has been shown to block the processing of Sindbis virus glycoproteins at the high-mannose stage (11), it was of interest to determine whether an accumulation of pgC(105) and gA occurred in infected cells incubated in the presence of the ionophore. Cell extracts of infected cells incubated in the presence or absence of monensin were first analyzed by SDS-PAGE. HSV-1-infected L-cells were incubated in 10^{-7} and 10^{-8} M monensin for 24 h postinfection and labeled with [^{14}C]glucosamine 4 to 24 h postinfection. At 24 h postinfection the cells were harvested, and the solubilized cell extracts were analyzed by SDS-PAGE (Fig. 5). In the presence of 10^{-7} or 10^{-8} M monensin the appearance of the gB and gC glycoproteins was significantly reduced. This was especially apparent with a monensin concentration of 10^{-7} M. In contrast, it appeared that gA, pgC(105), and pgD(52) were synthesized and detectable in greater amounts than those of cells cultured in the absence of the inhibitor.

To verify whether the gA and pgC(105) made in the presence of monensin do contain high-mannose oligosaccharides, immune precipitates

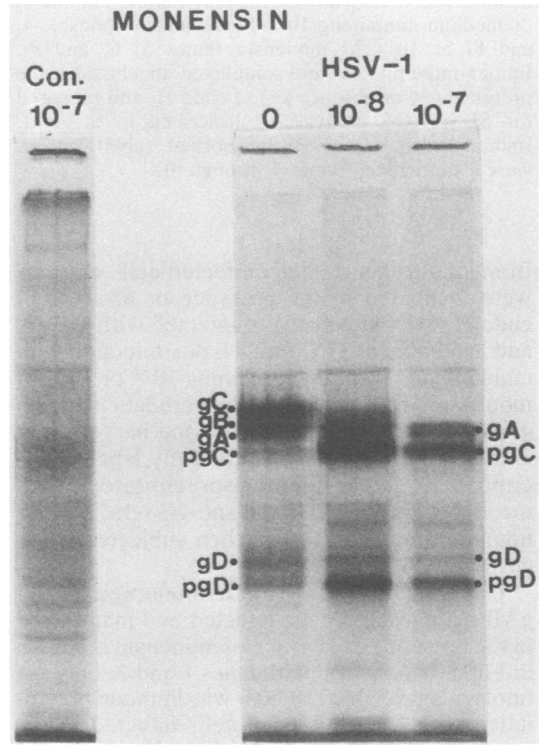

FIG. 5. Effect of monensin on HSV-1 glycoprotein synthesis in L-cells. HSV-1-infected L-cells were incubated in 10^{-7} or 10^{-8} M monensin and labeled with [^{14}C]glucosamine 4 to 24 h postinfection. Cell extracts were solubilized and analyzed by SDS-PAGE. Uninfected cells (Con. 10^{-7}) and infected cells incubated in the absence of monensin (HSV-1, 0) were included as controls.

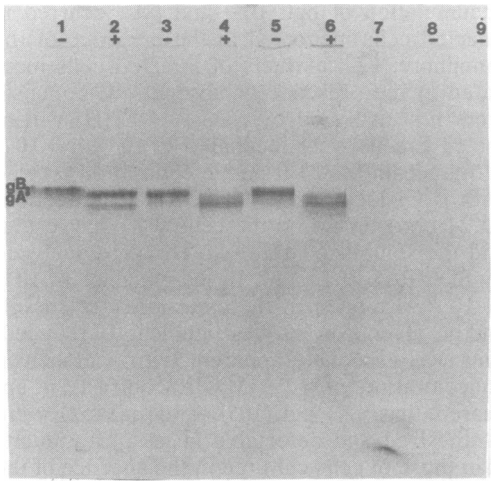

FIG. 6. Endo H treatment of anti-gAB immunoprecipitated proteins from HSV-1-infected MRC-5 cells incubated in the absence or presence of 10^{-6} or 10^{-7} M monensin. HSV-1-infected cells were labeled with [^{35}S]methionine from 4 to 24 h postinfection and incubated at 37°C. After adsorption, the cells were incubated in monensin-free medium (lanes 1, 2, and 7) or medium containing 10^{-6} M monensin (lanes 3, 4, and 8) or 10^{-7} M monensin (lanes 5, 6, and 9). Immunoprecipitates were solubilized, incubated in the presence (+) or absence (−) of endo H, and prepared for SDS-PAGE analysis as described in the text. Immunoprecipitations, using normal rabbit serum, were also included (lanes 7 through 9).

from monensin-treated infected-cell extracts were incubated in the presence or absence of endo H. MRC-5 cells were infected with HSV-1 and incubated at 37°C for 24 h postinfection with maintenance medium containing 10^{-6} or 10^{-7} M monensin. The infected cells were labeled from 4 to 24 h with [^{35}S]methionine and harvested at 24 h postinfection. The isotopically labeled glycoproteins were immunoprecipitated with monospecific anti-gAB and anti-gC sera, and the immune precipitates were then subjected to digestion with endo H.

The effect of endo H on immunoprecipitated gAB from MRC-5 cells infected and maintained in the presence or absence of monensin is shown in Fig. 6. Compared with lanes 1 and 2, only gA (molecular weight, 110,000) was immune precipitated with anti-gAB from cells infected in the presence of either 10^{-6} M (lane 3) or 10^{-7} M (lane 5) monensin. However, this gA was sensitive to the action of endo H and was reduced in molecular weight to approximately 97,000 after endo H treatment. Results of immune precipitations, using normal rabbit serum reacted with solubilized extracts of HSV-1-infected cells (lane 7) and infected cells treated with 10^{-6} M (lane 8) and 10^{-7} M (lane 9) monensin, were also included.

The procedure was repeated with monospecific anti-gC for immune precipitations (Fig. 7). Compared with the amounts shown in lanes 1 and 2, there was a decrease in the amount of gC precipitated by anti-gC from cells cultured in the presence of either 10^{-6} M (lane 3) or 10^{-7} M (lane 5) monensin. In contrast, pgC(105) was immune precipitated in greater amounts than from extracts of cells incubated in monensin-free medium (lane 1). Anti-gC immune precipitates from monensin-treated cells were also incubated in the presence of endo H. In addition to the 75,000-molecular-weight protein detectable after endo H treatment of pgC(105), there was a diffuse region of proteins which had a slower electrophoretic mobility.

DISCUSSION

The purpose of this study was to further characterize the HSV-1 high-molecular-weight glycoproteins (gB and gC) and their precursors [gA and pgC(105)]. Based on the effect of endo H on individual glycoproteins immunoprecipitated with monospecific antisera to gC and gAB, we suggest that pgC(105) and gA represent the high-mannose precursors to gC and gB, respectively. These conclusions are in agreement with recently published data of Serafini-Cessi and

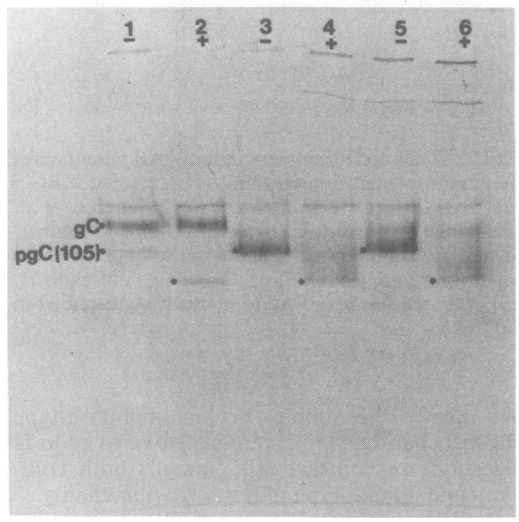

FIG. 7. Endo H treatment of anti-gC immunoprecipitated proteins from infected MRC-5 cells incubated in the absence (lanes 1 and 2) or presence of 10^{-6} M (lanes 3 and 4) or 10^{-7} M (lanes 5 and 6) monensin. The procedure used was the same as that described in the legend to Fig. 6. Immunoprecipitates were solubilized, incubated in the presence (+) or absence (−) of endo H, and prepared for SDS-PAGE analysis as described in the text.

Campadelli-Fiume (24). After endo H treatment of HSV-1-infected whole cell extracts labeled with [^{14}C]glucosamine, these authors reported the disappearance of bands corresponding to gA, pgC(105), and pgD(52) and the appearance of new bands. The correlation of these new bands which appeared after endo H treatment with the individual glycoproteins gB, gC, or gD was not reported. Person et al. (18) have also reported that a precursor designated pgB was sensitive to endo H treatment; however, the partial sensitivity of gB was not discussed.

The accumulation of endo H-sensitive gA and pgC(105) along with the inhibition of gB and gC in infected cells treated with monensin further demonstrated that gA and pgC(105) represent high-mannose precursors. These results are in agreement with the mode of action of monensin in which the transport of glycoproteins may be blocked at the Golgi and subsequent processing of the glycoproteins is inhibited (11, 32). Since not all of the gA and pgC(105) from monensin-treated cells were equally sensitive to the action of endo H, some processing of gA and pgC(105) may have occurred in the presence of monensin. Other studies have reported that in the presence of monensin, limited removal of mannose residues does occur (11). The removal of these carbohydrates may therefore affect the susceptibility of the oligosaccharides to endo H digestion (31).

After endo H digestion, the [^{35}S]methionine-labeled gA had an apparent molecular weight of 97,000. This molecular weight is similar to that observed for the nonglycosylated gAB synthesized in tunicamycin-treated cells (manuscript in preparation) and would suggest that gA contains only the high-mannose-type oligosaccharides which are cleaved by endo H. In contrast, gB appeared to be partially sensitive to endo H and, therefore, may contain both simple- and complex-type oligosaccharides analogous to the mature E2 glycoprotein of Sindbis, which also contains both simple- and complex-type oligosaccharide side chains (2, 22).

At no time did gC appear to be susceptible to the action of endo H, suggesting that the *N*-linked oligosaccharides of gC are of the complex type. This is in agreement with Cohen et al. (3), who demonstrated the presence of sialic acid on gC but not on pgC(105). Sialic acid is normally the terminal sugar of complex-type oligosaccharides but is not present in simple-type oligosaccharides. The disappearance of [^{35}S]methionine-labeled pgC(105) after endo H digestion along with the appearance of a 75,000-molecular-weight protein would suggest that the 75,000 protein may represent the nonglycosylated gC polypeptide. This protein has a lower molecular weight than was obtained for a gC-related protein isolated from tunicamycin-treated infected cells (19; E. A. Wenske and R. J. Courtney, manuscript in preparation); however, the gC protein synthesized in tunicamycin-treated cells contains O-linked oligosaccharides (manuscript in preparation) and therefore may not represent the true nonglycosylated gC polypeptide. The presence of O-linked oligosaccharides on gC has also been suggested by Olofsson et al. (16, 17). It is conceivable that pgC(105) does not contain O-linked oligosaccharides; therefore, the majority of pgC(105) is digested by endo H to a 75,000-molecular-weight polypeptide. As suggested above, it also appears that pgC(105) may undergo some processing in the presence of monensin. These results could be explained in two ways, either monensin allows a partial processing of pgC(105) to gC thus producing glycoproteins with various sensitivities to endo H, or there are other oligosaccharides, other than *N*-linked oligosaccharides, insensitive to endo H which are present on the pgC(105) glycoproteins synthesized in the presence of monensin. Both possibilities are being considered and are under further investigation.

During the progress of our studies with monensin, we learned that Johnson and Spear (12) had obtained similar results with respect to the effects of monensin on the processing of HSV-1 glycoproteins. We recently learned that K. Kousoulas, D. Bzik, and S. Person have also investigated the effect of monensin on HSV-1 glycoprotein synthesis (submitted for publication).

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