Sulfation of the Human Immunodeficiency Virus Envelope Glycoprotein

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Sulfation is a posttranslational modification of proteins which occurs on either the tyrosine residues or the carbohydrate moieties of some glycoproteins. In the case of secretory proteins, sulfation has been hypothesized to act as a signal for export from the cell. We have shown that the human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein precursor (gp160) as well as the surface (gp120) and transmembrane (gp41) subunits can be specifically labelled with $^{35}\text{SO}_4^{2-}$. Sulfated HIV-1 envelope glycoproteins were identified in H9 cells infected with the IIIB isolate of HIV-1 and in the cell lysates and culture media of cells infected with vaccinia virus recombinants expressing a full-length or truncated, secreted form of the HIV-1 gp160 gene. *N*-glycosidase F digestion of $^{35}\text{SO}_4^{2-}$ -labelled envelope proteins removed virtually all radiolabel from gp160, gp120, and gp41, indicating that sulfate was linked to the carbohydrate chains of the glycoprotein. The $^{35}\text{SO}_4^{2-}$ label was at least partially resistant to endoglycosidase H digestion, indicating that some sulfate was linked to complex carbohydrates. Brefeldin A, a compound that inhibits the endoplasmic reticulum to Golgi transport of glycoproteins, was found to inhibit the sulfation of the envelope glycoproteins. Envelope glycoproteins synthesized in cells treated with chlorate failed to incorporate $^{35}\text{SO}_4^{2-}$. However, HIV glycoproteins were still secreted from cells in the presence of chlorate, indicating that sulfation is not a requirement for secretion of envelope glycoproteins. Sulfation of HIV-2 and simian immunodeficiency virus envelope glycoproteins has also been demonstrated by using vaccinia virus-based expression systems. Sulfation is a major determinant of negative charge and could play a role in biological functions and antigenic properties of HIV glycoproteins.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS and related disorders (5, 13, 27, 38). The HIV-1 envelope glycoprotein mediates attachment of the virus to cells via the cellular receptor, CD4 (24, 29, 30, 40). Previous studies on the transport of HIV envelope proteins indicate that they are first synthesized as a polyprotein precursor (gp160), the majority of which is retained in an intracellular compartment and undergoes subsequent degradation (1, 43, 50, 52). A fraction of gp160 is proteolytically cleaved in the Golgi complex to yield the transmembrane (gp41) and surface (gp120) subunits of the mature protein (9, 11, 15, 36, 47). gp120 and gp41 are expressed on the surfaces of infected cells and are incorporated into budding virions (48). Following proteolytic cleavage, gp120 and gp41 remain associated via noncovalent interactions which appear to be sufficiently weak to allow the shedding large amounts of gp120 from the surfaces of cells into the extracellular environment (42, 44). The envelope glycoproteins are highly glycosylated, with carbohydrate moieties estimated to account for one-half of the molecular weight of gp120 (1, 43).

Sulfation is a posttranslational modification of proteins that occurs in virtually all animal cells and has been hypothesized to play a role in the transport of secretory proteins from the cell (20). Other possible functions of sulfation include contributions to cell differentiation and cell-cell interactions (17). Sulfation is also a major determinant of negative charge. Inorganic sulfate is transferred from a carrier molecule, 3'-phosphoadenosine 5'-phosphosulfate, to proteins by sulfotransferases located in the *trans* Golgi network (TGN) (18, 20). Two types of protein sulfation

modifications have been reported: the addition of sulfate to tyrosine, and the addition of sulfate to the carbohydrate moieties of glycoproteins. The V1 capsid protein of polyomavirus is modified by tyrosine sulfation (28). Carbohydrates have been found to be modified by the addition of sulfate to *N*-acetylglucosamine or terminal mannose residues (31, 39, 53). Sulfation of the carbohydrate moieties of viral envelope glycoproteins has also been reported (19, 31, 37, 39).

We have investigated the incorporation of sulfate into the envelope glycoproteins of HIV-1 and other lentiviruses. The association of the sulfate with the HIV-1 envelope glycoprotein has been examined by using enzymatic deglycosylation. We have also determined the effects of brefeldin A (BFA) and chlorate on envelope glycoprotein synthesis, sulfation, and transport, using recombinant vaccinia viruses which express full-length or truncated, secreted forms of the envelope glycoproteins.

MATERIALS AND METHODS

Reagents. Brefeldin A was obtained from Epicenter Technologies (Madison, Wis.). Sodium chlorate was purchased from Aldrich Chemical Co. (Milwaukee, Wis.). 5'-Bromo-2'-deoxyuridine, *N*-glycosidase F (PNGase F), and endoglycosidase H (endo H) were obtained from Boehringer Mannheim (Indianapolis, Ind.).

Cells and viruses. TK⁻ 143 cells were obtained from the American Type Culture Collection and grown in Dulbecco's modification of Eagle's medium containing 10% newborn calf serum and 25 μg of 5'-bromo-2'-deoxyuridine per ml. H9 cells were also obtained from the American Type Culture Collection and were grown in RPMI supplemented with 15% fetal calf serum. The IIIB isolate of HIV-1, a kind gift from John Kappes, was used to persistently infect H9 cells as

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described previously (14). Vaccinia virus recombinants VV-K1 (22), VV-SC11 and VV-env-1 (35), VV-env-4 (16), VV-ST and VV-ROD (32), VV-ST2 (33), and VV-SIV_{1A11} (41) were grown as described previously (23).

Protein expression. Confluent TK⁻ 143 cells in 35-mm dishes were infected with recombinant vaccinia viruses at a multiplicity of infection of 5. For radiolabelling, the cells were starved for 30 min in deficient Eagle's medium and then metabolically labelled with either 50 μ Ci of [³⁵S]methionine-cysteine (Met-Cys) (New England Nuclear) per ml or 100 μ Ci of Na₂³⁵SO₄ (ICN) per ml in Met-Cys-deficient or SO₄²⁻-deficient Eagle's medium, respectively, for various time periods. Cells were lysed in a buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, and 1% Nonidet P-40. Lysates were precleared with *Staphylococcus aureus* preloaded with normal human antisera, and immunoprecipitations were then carried out by using pooled HIV-positive antisera and protein A-agarose beads (Pierce) as described previously (35). Immunoprecipitated proteins were resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and autoradiography.

PNGase F and endo H digestions. Immunoprecipitated samples were removed from the agarose beads by boiling the samples for 5 min in 10 μ l of sample loading buffer containing 1% SDS, 10% glycerol, 5% mercaptoethanol, and 62 mM Tris (pH 6.8). Aliquots of eluted protein samples were deglycosylated with 0.4 U of PNGase F for 17 h at 37°C in 100 μ l of buffer containing 1% *N*-octylglucoside, 50 mM Tris (pH 7.4), and 10 mM EDTA. Other aliquots were treated with 5 mU of endo H in 100 μ l of buffer containing 1% *N*-octylglucoside, 10 mM Tris (pH 7.4), 100 mM NaCl, and 1% SDS for 17 h at 37°C. The samples were then precipitated with 10 volumes of acetone for 2 h at -20°C, and pellets were redissolved in sample loading buffer (35) and subjected to SDS-PAGE.

RESULTS

HIV-1 envelope glycoproteins are sulfated. A time course of sulfate incorporation was performed to establish that the HIV-1 envelope glycoproteins are sulfated and to determine the time of peak sulfate incorporation. TK⁻ 143 cells were infected with VV-env-1, a recombinant vaccinia virus which expresses the complete HIV-1 envelope gene, or VV-SC11, a recombinant vaccinia virus which expresses β -galactosidase. Figure 1A shows the incorporation of sulfate into both gp160 and gp120 at various intervals postinfection. The peak level of sulfate incorporation was found to occur between 8 and 12 h postinfection. Both gp160 and gp120 were found in the sulfate-labelled samples; however, in the amino acid-labelled sample, the predominant envelope species found was gp160 (Fig. 1B). The observation that only gp160 is seen in the amino acid pulse-labelled sample, whereas both gp160 and gp120 are seen in the sulfate pulse-labelled sample, indicates that sulfate incorporation is a posttranslational event. However, the finding that a fraction of gp160 is sulfated indicates that the sulfotransferase enzyme is present at a location proximal to the protease which causes cleavage of the envelope glycoprotein. To verify that HIV-1 envelope glycoproteins incorporate sulfate during viral infection, persistently HIV-IIIB-infected H9 cells were metabolically labelled with ³⁵SO₄²⁻, and viral proteins were examined by SDS-PAGE (10% polyacrylamide gel). HIV-1-specific proteins with apparent molecular masses of 160 and 120 kDa were immunoprecipitated from sulfate-labelled, infected cell lysates by pooled HIV-1-positive sera (Fig. 2, lane 2). gp160,

FIG. 1. Time course of sulfation of the envelope glycoprotein. TK⁻ 143 cells infected with VV-env-1 were labelled with 100 μ Ci of ³⁵SO₄²⁻ per ml (A) or 50 μ Ci of [³⁵S]Met-Cys per ml (B) for 2 h, and cells were lysed at the indicated times postinfection. VV-SC11 cells were lysed at 16 h postinfection as a control. Immunoprecipitated cell lysates were analyzed by SDS-PAGE (10% polyacrylamide gel) and fluorography.

gp120, and various forms of the capsid proteins were found in immunoprecipitates from the amino acid-labelled infected cell lysates (Fig. 2, lane 4). Lanes 1 and 3 are radiolabelled, immunoprecipitated cell lysates from uninfected H9 cells. These results demonstrate that the HIV-1 envelope glycoproteins incorporate sulfate in virus-infected cells as well as in recombinant expression systems.

Sulfation of other lentivirus glycoproteins. TK⁻ 143 cells were infected with several recombinant vaccinia viruses expressing HIV-1, HIV-2, or simian immunodeficiency virus (SIV) glycoproteins to determine whether the glycoproteins of other lentiviruses are also sulfated. Viruses used include VV-ST, VV-ST2, and VV-ROD (vaccinia virus recombinants which express HIV-2 envelope glycoproteins), VV-SIV_{1A11} (a vaccinia virus recombinant which expresses the SIV envelope glycoprotein), VV-env-1, and VV-SC11. Infected cells were labelled with 100 μ Ci of ³⁵SO₄²⁻ per ml or 50 μ Ci of [³⁵S]Met-Cys per ml at 10 h postinfection for 4 h prior to lysing of the cells. Cell lysates were immunoprecipitated with HIV-2-positive antisera (obtained from the AIDS repository) with the exception of the VV-env-1-infected cell lysate, for which pooled HIV-1-positive antisera were used. As shown in Fig. 3A, the amino acid-labelled immunopre-

FIG. 2. Incorporation of sulfate into the HIV-IIIB envelope glycoprotein. H9 (lanes 1 and 3) and persistently HIV-IIIB-infected H9 (lanes 2 and 4) cells were metabolically labelled with 50 μ Ci of [³⁵S]Met-Cys per ml or 100 μ Ci of ³⁵SO₄²⁻ per ml for 6 h. Immunoprecipitated cell lysates were analyzed by SDS-PAGE (10% polyacrylamide gel) and fluorography.

FIG. 3. Modification of other lentiviral envelope glycoproteins by the addition of sulfate. Vaccinia virus-infected TK⁻ 143 cells were metabolically labelled with 100 μ Ci of $^{35}\text{SO}_4^{2-}$ per ml or 50 μ Ci of [^{35}S]Met-Cys per ml for 4 h at 10 h postinfection. Immunoprecipitated cell lysates were resolved by SDS-PAGE (10% polyacrylamide gel). (A) Precursor (Pre) and surface (SU) units of envelope glycoproteins immunoprecipitated from cells infected with VV-SC11 (lane 1), VV-env-1 (lane 2), VV-ST (lane 3), VV-ST2 (lane 4), VV-SIV_{IA11} (lane 5), and VV-ROD (lane 6); (B) transmembrane (TM) units of envelope glycoproteins immunoprecipitated from cells infected with VV-SC11 (lane 1), VV-env-1 (lane 2), VV-ST (lane 3), VV-ST2 (lane 4), VV-ROD (lane 5), and VV-SIV_{IA11} (lane 6) (4-week exposure).

cipitates yielded precursor and surface subunit proteins of the expected molecular weights, which vary among the different lentiviruses. All of the envelope glycoproteins examined were found to be labelled with sulfate, as evidenced by the fluorograph. The level of [^{35}S]Met-Cys incorporation into envelope glycoproteins was higher than the level of $^{35}\text{SO}_4^{2-}$ incorporation, as demonstrated by the increased intensity of the bands in the amino acid-labelled lanes compared with the sulfate-labelled lanes (Fig. 3A). On prolonged exposure of the gel, the transmembrane proteins were also found to be labelled with sulfate (Fig. 3B). These results indicate that sulfation is a motif shared by envelope glycoproteins of several lentiviruses and that both the surface subunit and transmembrane unit cleavage products are sulfated.

Transport of full-length and truncated HIV-1 glycoproteins. Sulfation of proteins has been proposed to act as a signal for the export of secretory proteins from the cell (20). VV-env-4 is a vaccinia virus recombinant which expresses a soluble, truncated form of the envelope glycoprotein (gp160tr) which is secreted into the media of infected cells (16). We wished to determine whether sulfated forms of gp160tr are exported from the cell and to investigate the possible role of sulfation in the transport of secreted envelope glycoproteins. Media and lysates were collected from $^{35}\text{SO}_4^{2-}$ - and [^{35}S]Met-Cys-labelled TK⁻ 143 cells infected with VV-env-1 or VV-env-4, and immunoprecipitated proteins were analyzed by SDS-PAGE (10% polyacrylamide gel) (Fig. 4). Sulfate-labelled gp160 and gp120 were immunoprecipitated from lysates, and gp120 was present in the medium of VV-env-1-infected cells. Sulfated gp160tr and its cleavage product, gp120, were both immunoprecipitated from the medium of cells infected with VV-env-4, but only a low level of sulfate-labelled gp160tr was identified in cell lysates. This result indicates that the

FIG. 4. Transport of sulfated glycoprotein. TK⁻ 143 cells infected with VV-env-1 (lanes 1, 3, 5, and 7) and VV-env-4 (lanes 2, 4, 6, and 8) were labelled with 100 μ Ci of $^{35}\text{SO}_4^{2-}$ per ml or 50 μ Ci of [^{35}S]Met-Cys per ml for 4 h at 10 h postinfection. Media and cell lysates from infected cells were immunoprecipitated and subject to SDS-PAGE (10% polyacrylamide gel) and fluorography.

gp160tr molecules which are secreted into the medium are more highly sulfated than the cell-associated forms.

To further examine the role of sulfation in the transport of viral glycoproteins, cells expressing viral glycoproteins were treated with chlorate, an inhibitor of ATP-sulfurylase, the first enzyme in 3'-phosphoadenosine 5'-phosphosulfate biosynthesis. Chlorate treatment has been reported to produce 99% inhibition of sulfation when used with reduced amino acid concentrations (3, 20). Sodium chlorate was added to the media at a final concentration of 0, 1, or 10 mM at 10 h postinfection, 30 min prior to labelling of cells, and throughout the labelling period. In this experiment, the cells were labelled in SO_4^{2-} -deficient and 99% Met-Cys-deficient Eagle's media as described by Baeuerle and Huttner (3). As shown in Fig. 5, chlorate had no effect on the level of amino acid-radiolabelled envelope glycoproteins found in the medium of cells infected with VV-env-1 or VV-env-4. There was, however, marked inhibition of sulfate incorporation, which is apparent at a concentration of 1 mM chlorate and appears to be virtually complete at 10 mM chlorate. Even under conditions of complete inhibition of sulfation, at 10 mM chlorate, gp160tr was still found to be secreted into the medium (Fig. 5B) and gp120 was also present in the medium (Fig. 5A). These results indicate that sulfation is not a requirement for the secretion of HIV envelope glycoproteins. Chlorate was also examined for its ability to inhibit

FIG. 5. Chlorate inhibition of sulfation. TK⁻ 143 cells were infected with VV-env-1 (A) or VV-env-4 (B). At 10 h postinfection, the cells were starved in SO_4^{2-} -deficient and 99% Met-Cys-deficient Eagle's media containing 0 (lanes A), 1 (lanes B), or 10 (lanes C) mM chlorate for 0.5 h and then metabolically labelled with 100 μ Ci of $^{35}\text{SO}_4^{2-}$ per ml or 50 μ Ci of [^{35}S]Met-Cys per ml for 10 h in media of the same composition. Cell lysates and media were immunoprecipitated and resolved by SDS-PAGE (10% polyacrylamide gel) and fluorography.

FIG. 6. BFA treatment of TK⁻ 143 cells expressing the envelope glycoprotein. TK⁻ 143 cells were infected with VV-env-1, VV-env-4, or VV-SC11 and metabolically labelled with 100 µCi of $^{35}\text{SO}_4^{2-}$ per ml or 50 µCi of [^{35}S]Met-Cys per ml for 4 h at 10 h postinfection. BFA was initially added to cells 30 min prior to labelling and was present throughout the labelling period. (A) Immunoprecipitated cell lysates from [^{35}S]Met-Cys-labelled cells with various concentrations of BFA; (B) immunoprecipitated lysates from $^{35}\text{SO}_4^{2-}$ -labelled cells treated with 10 µg of BFA per ml; (C) immunoprecipitated media from $^{35}\text{SO}_4^{2-}$ - and [^{35}S]Met-Cys-labelled cells treated with 10 µg of BFA per ml.

syncytium formation in VV-env-1-infected CD4⁺ HeLa cells. At concentrations of up to 25 mM chlorate, no reduction in HIV-induced cell fusion was observed (data not shown), indicating that sulfation of the HIV-1 envelope glycoprotein is not a determinant of the fusion activity of the HIV-1 envelope glycoprotein.

BFA treatment inhibits sulfation and transport of the HIV-1 envelope glycoprotein. We examined the effect of BFA, a fungal metabolite which reversibly inhibits protein transport from the endoplasmic reticulum to the Golgi complex (12), on sulfation of the HIV-1 envelope glycoproteins. Vaccinia virus recombinant-infected cells were treated with BFA 30 min prior to and throughout the labelling period. We first examined the effect of BFA treatment on the synthesis and processing of gp160. Figure 6A shows that the synthesis of gp160 and gp160tr was unaffected in VV-env-1- and VV-env-4-infected cells treated with 0, 1, 5, or 10 µg of BFA per ml. There was, as previously reported (11, 36), a lack of gp160 cleavage, which is likely to be due to the inaccessibility of the envelope glycoprotein to the protease under the conditions of BFA treatment. BFA also affected carbohydrate processing of gp160 and gp160tr, as evidenced by the lower apparent molecular weight of the envelope glycoproteins synthesized in the presence of BFA. BFA was found to inhibit the incorporation of sulfate into the HIV envelope proteins, as demonstrated by the absence of radiolabelled gp160, gp120, and gp160tr in lysates of sulfate-labelled vaccinia virus recombinant-infected cells treated with 10 µg of BFA per ml (Fig. 6B). No radiolabelled glycoproteins were detected in the medium of cells treated with 10 µg of BFA per ml, whereas radiolabelled glycoproteins were

readily detected in the medium of untreated cells (Fig. 6C). These results indicate that sulfation of the HIV envelope glycoprotein occurs after transport from the endoplasmic reticulum.

Sulfate is linked to the envelope glycoprotein via carbohydrate moieties. To determine the nature of the sulfate linkage to the envelope glycoproteins, the effect of enzymatic deglycosylation of the HIV-1 envelope glycoproteins was examined. TK⁻ 143 cells were infected with VV-env-1 or VV-SC11 and metabolically labelled for 10 h at 10 h postinfection. Aliquots of immunoprecipitated proteins were treated with the deglycosylating enzyme PNGase F or endo H. After PNGase F or endo H treatment, amino acid-labelled glycoproteins (Fig. 7) yielded deglycosylated proteins of the expected molecular weights (15). In the case of the sulfate-labelled glycoproteins, PNGase F digestion eliminated all of the detectable $^{35}\text{SO}_4^{2-}$ from all three species of the sulfate-labelled envelope glycoproteins, indicating that the sulfate was linked to the carbohydrate moieties of gp160, gp120, and gp41. In the endo H-digested samples, however, there was not complete removal of the $^{35}\text{SO}_4^{2-}$, indicating that sulfate was linked to endo H-resistant carbohydrate moieties. Similar results were also obtained after two rounds of enzymatic digestion to ensure that deglycosylation was complete (data not shown).

DISCUSSION

We have demonstrated that the envelope glycoproteins of the IIB isolate of HIV-1 and the recombinant vaccinia virus VV-env-1 are radiolabelled with inorganic sulfate and that

FIG. 7. Deglycosylation of sulfate-labelled envelope glycoprotein. Immunoprecipitated cell lysates from VV-env-1-infected TK⁻143 cells labelled with 100 μ Ci of $^{35}\text{SO}_4^{2-}$ per ml or 50 μ Ci of [^{35}S]Met-Cys per ml for 10 h at 10 h postinfection were treated with PNGase F (lanes P) or endo H (lanes H) or were untreated (lanes N), and samples were analyzed by SDS-PAGE (10% polyacrylamide gel).

this sulfate is linked to the carbohydrate moieties of the glycoprotein. Sulfated forms of the HIV-1 envelope glycoprotein include the precursor, gp160, and both the gp120 and gp41 cleavage products. In contrast, the HIV-1 Gag proteins of persistently HIV-IIIB-infected H9 cells were not found to incorporate sulfate. Sulfate-labelled immunoprecipitated proteins from TK⁻143 cells infected with VV-K1, a recombinant vaccinia virus which synthesizes Gag proteins, also contained no sulfated proteins (data not shown). Radiolabel incorporation in the $^{35}\text{SO}_4^{2-}$ -labelled samples can be attributed only to the incorporation of inorganic sulfate into proteins, as mammalian cells lack the biochemical pathways necessary to convert inorganic sulfate into amino acids (6). HIV-2 and SIV envelope glycoproteins were also found to be labelled with sulfate. This posttranslational modification of envelope glycoproteins has also been reported for several other enveloped viruses, including the retrovirus Rauscher leukemia virus (19, 31, 37, 39). Chlorate appeared to completely inhibit the sulfation of HIV-1 envelope proteins, with no effect on envelope protein synthesis, proteolytic cleavage, or secretion into the culture media.

Sulfate is present on both the precursor and cleaved forms of the HIV envelope glycoprotein. In contrast, the precursor form of the herpesvirus gD protein is not sulfated (19). The presence of both cleaved (gp120 and gp41) and uncleaved (gp160) forms of sulfated glycoprotein indicates that the sulfotransferases, which are located in the Golgi complex (20), reside at a location proximal to the cellular protease which causes cleavage of the precursor into the surface and transmembrane units and that some gp160 may be cleaved at a very late step in the transport process. Glycosaminoglycan sulfotransferases and tyrosyl sulfotransferases have been specifically localized to the TGN (20, 46). Since BFA inhibits the sulfation of the envelope glycoprotein, our results indicate that the glycoprotein sulfotransferases are also located in the TGN. This result is in agreement with those of Spiro et al. (46), who reported the inhibition of chondroitin sulfate glycosaminoglycan elongation and sulfation of the melanoma proteoglycan core protein with BFA treatment. Endo H digestion of the sulfate-labelled glycoproteins resulted in incomplete removal of the $^{35}\text{SO}_4^{2-}$ label, indicating that sulfate is added, at least in part, to complex carbohydrates which are resistant to endo H digestion. This view is consistent with the idea that sulfation is a late posttranslational protein modification (20), mediated by sulfotransferases in the TGN.

Our results show that the HIV-1 envelope glycoproteins are sulfated and that the sulfate is linked to the carbohydrate moieties of the glycoproteins. Since the carbohydrate components linked to viral glycoproteins are specified by host cell enzymes, host cell-dependent differences may exist in oligosaccharide structures. The oligosaccharide structures of the viral glycoproteins clearly resemble those present on host cell glycoproteins, and evidence has been obtained that they may represent host cell-specific antigens that are incorporated into virus particles (21, 25). In the case of the influenza virus HA glycoproteins, the host antigen activity has been correlated with the presence of a sulfated oligosaccharide linked to the N-terminal region of the HA1 subunit (10, 51). The association of a host cell-specific antigen with lentiviruses has recently been demonstrated in the case of SIV, in studies in which protection of rhesus monkeys against virus challenge involved the induction of an immune response to a host cell-specific antigen (8, 26, 34, 45, 49). It will therefore be of interest to further identify the precise structure of the sulfated viral oligosaccharides and their possible role in induction of an immune response. In regard to the role of sulfation in the transport of secretory proteins from the cell, our results indicate that sulfation is not required for the secretion of the envelope glycoproteins. It is possible that sulfate protects glycoproteins from exoglycosidic degradation or affects the proteolytic processing of the envelope glycoprotein. Sulfate contributes negative charge to the surface of the envelope glycoprotein, and this may affect the interaction of virus with cells or extracellular matrix components. It is also of interest that sulfated components, including the monosaccharide glucosamine-6-sulfate, have been reported to inhibit HIV-induced cell fusion (4) and appear to affect the early stages of virus-cell interaction (2).

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