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Inhibition of Glycosylation of Bovine Herpesvirus 1 Glycoproteins by the Thymidine Analog (*E*)-5-(2-Bromovinyl)-2′-Deoxyuridine

VIKRAM MISRA,* RANDAL C. NELSON, AND LORNE A. BABIUK

Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

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(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVdU) was phosphorylated by the bovine herpesvirus 1 (BHV-1)-induced thymidine kinase and subsequently incorporated into viral DNA, resulting in DNA that was more dense than DNA from untreated cells. Incorporation of the drug did not result in the termination of replicating BHV-1 DNA molecules since radioactively labeled DNA synthesized in drug-treated and untreated cells sedimented at similar rates in alkaline sucrose gradients. No differences were observed in the electrophoretic mobility of [^{35}S]methionine-labeled viral polypeptides synthesized in treated and untreated cells, although [^{3}H]glucosamine-labeled viral glycoproteins synthesized in treated cells were of a lower molecular weight than those in untreated cells. In BVdU-treated cells, unlike untreated cells, immature neutral and basic precursors of the mature viral glycoproteins accumulated. Although BVdU-treated and untreated cells contained similar amounts of virus, very little virus was released into the culture supernatant from BVdU-treated cells. Our results suggest that BVdU partially inhibits the glycosylation of BHV-1 glycoproteins. BVdU-sensitive glycosylation, however, is not necessary for expression of these glycoproteins on the surface of infected cells since the glycoproteins could be labeled on intact cells with ^{125}I and because BVdU-treated cells remained sensitive to antibody-dependent, cell-mediated cytotoxity mediated by anti-BHV-1 serum. The phosphorylation of BVdU was a prerequisite for its effect on glycosylation since the glycoproteins of a thymidine kinase-deficient mutant of BHV-1 were not affected.

In recent years, a number of nucleosides which selectively inhibit the replication of herpesviruses have been developed (3–5, 8, 21, 25). (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVdU) is such a compound (6). The replications of herpes simplex virus type 1 (6, 7), varicella-zoster virus, and bovine herpesvirus 1 (BHV-1) (28) are sensitive to this drug. In virus-infected cells, BVdU is phosphorylated by the virus-induced thymidine kinase (TK). The deoxynucleotide triphosphate is then, at least in herpes simplex virus type 1-infected cells (1, 2), accepted as a substrate for the virus-induced DNA polymerase and is incorporated into viral DNA.

While examining viral polypeptides synthesized in herpesvirus-infected cells treated with inhibitors of viral DNA synthesis, we observed that BVdU-treated cells contained aberrant viral glycoproteins (17). This suggested that, in addition to interfering with some phase of viral DNA synthesis, BVdU may have an effect on the glycosylation of viral proteins.

The data in this report suggest that BVdU partially inhibits the glycosylation of BHV-1 glycoproteins. The intracellular phosphorylation of the nucleoside by the virus-induced TK was necessary for this effect since viral mutants that induce low levels of TK synthesized normal glycoproteins in the presence of the drug.

MATERIALS AND METHODS

Drugs. BVdU was obtained from E. DeClercq. Cystosine arabinoside (AraC) and phosphonoformic acid (PFA) were purchased from Sigma Chemical Co., St. Louis, Mo.

Virus and cells. The P8-2 strain of BHV-1 and its TK$^-$ derivative (28) were grown in Madin-Darby bovine kidney (MDBK) cells. Details of cell growth and virus infection have been published elsewhere (4, 16).

Analysis of infected-cell DNA by centrifugation in isopycnic gradients. MDBK cells in six-well plastic dishes (Costar, Cambridge, Mass.) were either mock infected or infected with BHV-1 at a multiplicity of infection of 10 PFU per cell. BVdU was added at a concentration of 10 μg/ml to the inoculum of some of the cultures, and AraC was added at a concentration of 50 μg/ml to the inoculum of others. After infection, cultures were overlaied with 1 ml of Eagle minimum

essential medium containing 2% fetal bovine serum. At 6 h after infection, 10 μCi of [*methyl*-^{3}H]thymidine (Amersham, Oakville, Ontario, Canada; 25 Ci/mmol) was added to the infected cultures and 2 μCi of [^{14}C]thymidine (Amersham; 55.7 μCi/mmol) was added to mock-infected cultures. At 20 h after infection, cells were collected, and infected and uninfected cultures were mixed, washed once with TE buffer (0.01 M Tris [pH 8.0], 0.001 M EDTA), and suspended in 1 ml of TNE buffer (0.01 M Tris [pH 8.0], 0.15 M NaCl, 0.001 M EDTA). Proteinase K and sodium lauryl sarcosine (Sigma Chemical Co.) were added to 100 μg/ml and 1 mg/ml, respectively. After standing for 24 h at room temperature, the lysates were made up to a density of 1.72 g/ml with CsCl and centrifuged at 35,000 rpm for 72 h in a Beckman Ty 50 Ti rotor. The gradients were then fractionated, macromolecules in each fraction were precipitated with 10% cold trichloracetic acid (TCA), and the amounts of ^{14}C and ^{3}H in each fraction were determined in a Beckman LS 800 scintillation counter.

Analysis of DNA on alkaline sucrose gradients. MDBK cells in 35-mm-diameter, six-well Costar dishes were infected as outlined above. At 6 h after infection, untreated cultures were labeled with 2 μCi of [^{14}C]thymidine, and cultures treated with 10 μg of BVdU per ml were labeled with 25 μCi of [^{3}H]thymidine per ml. After an additional 15 h, the cells were collected and washed with TNE buffer. The cells from the BVdU-treated and untreated wells were either suspended separately or mixed and suspended in 0.5 ml of TNE buffer. The cells were lysed by adding sodium lauryl sarcosine and proteinase K to 0.2% and 200 μg/ml, respectively. After standing at room temperature for 1 h, the lysates were layered onto 0.5 ml of lysing solution (0.5 M NaOH, 0.2 M EDTA, 0.1% sodium lauryl sarcosine) placed on a 32.5-ml, 5 to 25% (wt/vol) sucrose gradient. Sucrose solutions were made in 0.1 M NaOH–0.9 M NaCl–0.01 M EDTA. After standing for 5 h at 4°C, the gradients were centrifuged for 9 h at 22,000 rpm in the buckets of a Beckman SW28 rotor. Fractions (2 ml each) were collected from the bottom of the tubes, the macromolecules were precipitated with TCA, and the radioactivity associated with each fraction was measured.

Quantitation of extracellular virus. MDBK cells were infected and treated with BVdU as outlined previously. At 6 h after infection, the tissue culture supernatant was replaced with 1 ml of methionine-deficient minimum essential medium containing 50 μCi of [^{35}S]methionine (Amersham; 1,420 Ci/mmol). At 20 h after infection, the infected cells and the tissue culture supernatant were collected and separated by centrifugation. The infected cells were suspended in 1 ml of TNE buffer and ultrasonically disrupted, and the cellular debris was removed by centrifugation. The cell lysate, or the tissue culture supernatant (0.5 ml), was layered on an 11-ml, linear 20 to 50% potassium tartrate gradient in TNE buffer and centrifuged at 80,000 × *g* for 1.5 h at 4°C. Fractions (0.2 ml each) were collected directly onto glass fiber filters. The filters were washed successively with 10% TCA and with 95% ethanol and then dried, and the radioactivity associated with each filter was determined.

Preparation of antigen for immunoprecipitation. Radioactively labeled cells were suspended in RIPA buffer (0.05 M Tris-hydrochloride [pH 7.0], 0.15 M NaCl, 1% sodium deoxycholate, 1% Triton X-100) and sonicated on ice for 30 s at a setting of 4 on a Sonifier cell disrupter (Ultrasonics Inc., Plainsview, N.Y.). The cell debris was removed by centrifugation at 80,000 × *g* for 1 h, and supernatant was used immediately for immunoprecipitation. Inclusion of 0.1 mM phenylmethylsulfonyl fluoride in RIPA buffer did not alter the pattern of immunoprecipitation.

Immunoprecipitation. A 10-μl amount of ascites fluid containing monoclonal antibody against BHV-1 glycoprotein (17) was added to BHV-1 antigen in 200 μl of RIPA buffer. After the mixture stood overnight at 4°C to allow antigen-antibody complexes to form, the immunoglobulin G fraction of rabbit anti-mouse immunoglobulin (Cappel Laboratories, Cochranville, Pa.) was added, and incubation continued for a further 4 h. Ten milligrams of Protein A-Sepharose Cl-4B beads (Pharmacia Fine Chemicals, Uppsala, Sweden) in 100 μl of RIPA buffer was added. After 45 min, the Sepharose beads were washed four times with RIPA buffer containing 0.1% sodium dodecyl sulfate (SDS), suspended in 50 μl of sample buffer (0.06 M Tris-hydrochloride [pH 6.8], 0.15 M β-mercaptoethanol. 0.00125% bromophenol blue, 1.25% SDS, 12.5% glycerol), and heated in boiling water for 4 min.

Analysis of proteins by PAGE. For analysis of proteins by polyacrylamide gel electrophoresis (PAGE), samples were electrophoresed in the presence of SDS through polyacrylamide gels (13, 16), using a 15-cm vertical electrophoresis apparatus (Richter Scientific, Vancouver, British Columbia, Canada). Gels containing ^{125}I- or ^{35}S-labeled samples were autoradiographed on 3M film. Gels containing ^{3}H and ^{14}C were soaked in En^{3}Hance (New England Nuclear Corp., Lachine, Quebec, Canada) and fluorographed on preflashed film.

Two-dimensional PAGE analysis. For two-dimensional PAGE analysis, the technique of O'Farrell (18) was used, with the modifications described by Garrels (9). Frozen cell pellets were thawed and suspended in a solution containing 0.5 volume of 0.015 M Tris (pH 8.8) and 0.05 volume of a 0.5 M Tris (pH 8.8) buffer containing 1 mg of DNase I and 0.5 mg of RNase A per ml, and 0.1 M $MgCl_2$ was then added. After the preparation stood on ice for 5 min, 1 mg of solid urea per μl of lysate was added, followed by 15 μl of lysis buffer (10 M urea, 4% Nonidet P-40, 0.5 mM lysine hydrochloride, 0.1 M dithiothreitol, 0.25% SDS, 4.5% glycerol, 0.05 ml of ampholytes per ml). Polypeptides were separated along the first dimension in gels containing 9 parts of Biolyte (Bio-Rad Laboratories, Mississauga, Ontario, Canada; pH 5 to 7) and 1 part of Biolyte (pH 3 to 10) and along the second dimension by SDS-PAGE on 7.5% gels. The gels were then stained, soaked in En^{3}Hance, dried, and fluorographed.

Iodination of cell surface proteins. For iodination of cell surface proteins, a technique modified from that of Glorioso and Smith (10) was used. Infected or mock-infected cells were harvested, washed once with Hanks balanced salt solution (HBSS) plus 2% fetal bovine serum and once with HBSS containing KI at a concentration of 10^{-5} M. The cells were then counted in a hemacytometer after staining with trypan blue, and only cultures with greater than 95% viable cells were used. The cells (2×10^6) were suspended in 0.49 ml of HBSS plus 10^{-5} M KI and mixed with 25 μl of a

2% solution of β-D-glucose, 100 μl of Enzymobeads (Bio-Rad Laboratories), and 0.5 mCi of $Na^{125}I$ (New England Nuclear). After the mixture stood at room temperature for 30 min, 10 ml of HBSS plus 10^{-4} M KI was added. The cells were then pelleted and washed three times with HBSS plus 10^{-4} M KI. The cells retained their viability throughout the procedure. Soluble antigen was then prepared from the cells as outlined above.

ADCC. Details of the antibody-dependent cell-mediated cytotoxicity (ADCC) assay have been described previously (22). Briefly, bovine kidney cell monolayers were infected at a multiplicity of infection of 1 and labeled with $Na_2{}^{51}CrO_4$ (New England Nuclear). At the end of virus adsorption and labeling, the various concentrations of BVdU were added. After 16 h, the labeled infected cells were removed by mild trypsinization, enumerated, and used as target cells in the assays. Effector cells were polymorphonuclear cells obtained from the mammary gland (27). All assays were performed in microtiter plates at an effector/target cell ratio of 25:1 and a final anti-BHV-1 antiserum concentration of 1/50. The assays were carried out for 6 h at 37°C in a humidified CO_2 incubator. At the end of the assay, 50% of the supernatant fluids were removed for assaying radioactivity, and the percent specific release was computed by the formula described previously (22). Controls contained either antibody-sensitized target cells in minimum essential medium or nonsensitized target cells plus polymorphonuclear cells. The inclusion of up to 100 μg of BVdU per ml in the cytotoxicity assays did not alter the level of killing of target cells infected in the absence of BVdU.

RESULTS

Incorporation of BVdU into viral DNA. In a recent communication (28) we presented data that indicated that the replication of BHV-1 in tissue cultures is sensitive to BVdU at concentrations of as low as 10 to 100 ng/ml. Furthermore, BVdU is phosphorylated by the BHV-1-induced TK, whereas the TK of the host cell phosphorylates the nucleoside to a much lesser extent. To determine whether the phosphorylated nucleoside inhibited viral DNA synthesis or was incorporated into it, radioactive DNA synthesized in BVdU-treated and untreated BHV-1-infected cells was examined on isopycnic CsCl gradients. Unlike AraC, an inhibitor of DNA synthesis, BVdU did not significantly inhibit the synthesis of viral DNA (Fig. 1). Viral DNA synthesized in BVdU-treated cells was more dense, suggesting incorporation of the bromine-containing nucleotide, and formed a wider band than that of untreated cells. However, the total amount of TCA-precipitable radioactivity in viral DNA from BVdU-treated cells was about 65% of that in untreated cells.

Effect of BVdU on premature DNA chain termination. It has been suggested that incorporated analogs that lack a 3'-OH group may inhibit DNA replication by causing nascent strands of DNA to terminate (15), others may slow the process of chain elongation. In an attempt to determine whether BVdU exerted a similar effect, we analyzed the size of single-stranded DNA synthesized in the presence and absence of BVdU. No major differences were observed in the behavior of DNA from drug-treated and untreated cells analyzed on the same alkaline sucrose gradient (Fig. 2).

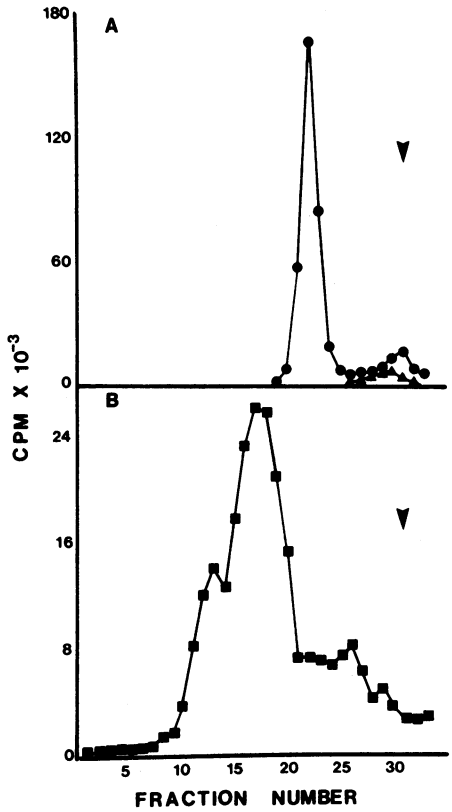

FIG. 1. Incorporation of BVdU into viral DNA. (A) Mock-infected cells and untreated BHV-1-infected cells (●) or BHV-1-infected cells treated with 50 μg of AraC per ml (▲). (B) Mock-infected cells and uninfected cells treated with 10 μg of BVdU per ml (■). DNA from all cells was extracted and analyzed in CsCl gradients. TCA-precipitable radioactivity associated with the gradient fraction was counted on glass fiber filters. Mock-infected cells were labeled with [^{14}C]thymidine, whereas infected cells were labeled with [^{3}H]thymidine. Arrows indicate the position of the [^{14}C]thymidine-labeled, uninfected-cell DNA marker.

Proteins synthesized in cells treated with BVdU. We next examined proteins synthesized in untreated BHV-1-infected cells and infected cells treated with either BVdU, AraC, or PFA (Fig. 3). PFA specifically inhibits herpesviral DNA polymerases, whereas AraC inhibits viral, as well as host cell, DNA synthesis. In infected cells arrested in the pre-DNA synthesis phase with these inhibitors, the synthesis of "early"

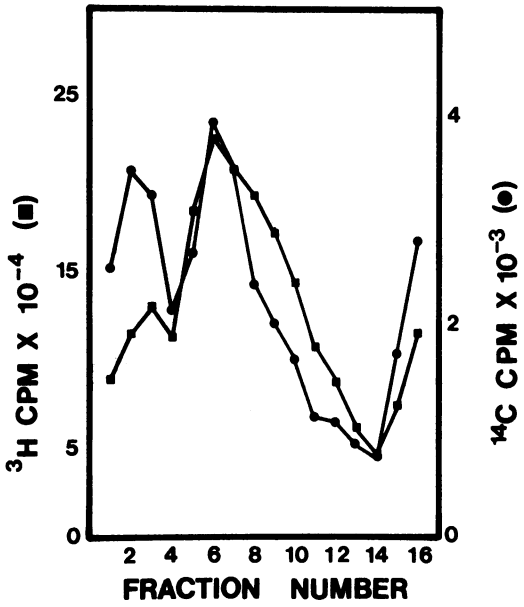

FIG. 2. Effects of BVdU on the size of single-stranded DNA synthesized in infected cells. BHV-1-infected untreated cells were labeled with [^{14}C]thymidine, and cells treated with 10 μg of BVdU per ml were labeled with [^{3}H]thymidine. At 13 h after infection, cells were harvested, mixed, and analyzed on an alkaline sucrose (5 to 25%) gradient. Symbols: ■, BVdU-treated cells; ●, untreated cells.

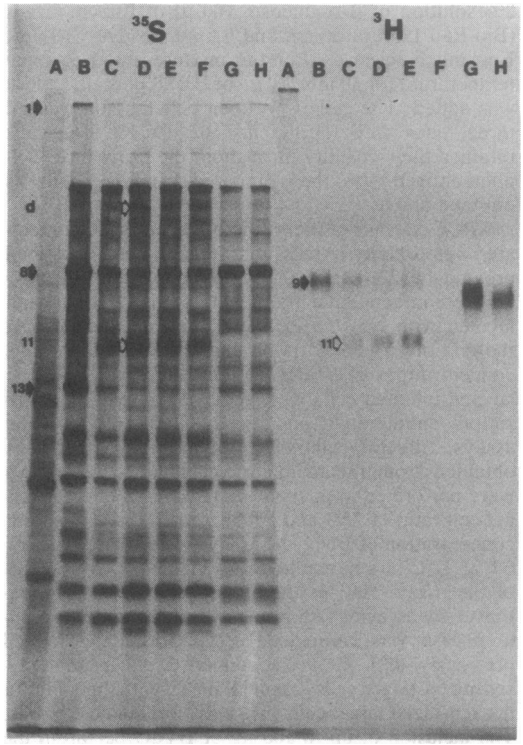

FIG. 3. Effect of PFA, AraC, or bromovinyl deoxyuridine on proteins and glycoproteins synthesized in BHV-1-infected cells. Mock-infected cells (lane A), BHV-1-infected cells (lane B), or infected cells treated with 100 μg of PFA per ml (lane C), 500 μg of PFA per ml (lane D), 50 μg of AraC per ml (lane E), 100 μg of AraC per ml (lane F), 5 μg of BVdU per ml (lang G), or 20 μg of BVdU per ml (lane H) were labeled with either [^{3}H]glucosamine or [^{35}S]methionine. At 20 h after infection, the cells were harvested and analyzed on a 7.5% polyacrylamide gel.

proteins (β) persists, and "late" proteins (γ) are synthesized in low concentrations (16). No differences were observed (Fig. 3) in the electrophoretic mobility of the [^{35}S]methionine-labeled nonglycosylated polypeptides from cultures treated with any of the antiviral drugs. In contrast, the [^{3}H]glucosamine-labeled glycoproteins from BVdU-treated cells (Fig. 3, lanes G and H) migrated faster than did the corresponding polypeptides from untreated cultures or cultures treated with PFA and AraC. In addition, although the pattern of protein synthesis in AraC- and PFA-treated cells was characteristic of cells arrested in the early phase of protein synthesis (16, 17) (i.e., the synthesis of early polypeptides d and 11 persisted, whereas late polypeptides 8 and 9 were synthesized in lesser amounts), the pattern of BVdU-treated cells was similar to that of "unarrested" untreated cells.

Analysis of glycoproteins synthesized in BVdU-treated cells by two-dimensional electrophoresis. To confirm observations that BVdU interfered with the synthesis of viral glycoproteins, we analyzed [^{3}H]glucosamine-labeled polypeptides from treated and untreated cells by two-dimensional electrophoresis (Fig. 4). As reported previously by our group (16) and other workers (11, 14), the major glycoproteins in untreated cells appeared as multiple spots, where each set of spots presumably represented molecules of the same glycoprotein that differed in the extent of glycosylation. Spots with more carbohydrate residues (darker on the autoradiogram) appeared to have more acidic isoelectric points, and in untreated cells most of these spots, especially those of glycoprotein 9, were clustered at the acidic end of the gel. Multiple spots also appeared on the autoradiograms of infected cells treated with BVdU, except that the spots tended to cluster in the neutral or basic range. This suggested that the glycosylation of proteins in these cells did not proceed to completion.

Effect of BVdU on the expression of glycoproteins on the surface of infected cells. To determine whether BVdU, in addition to interfering with glycosylation, could also prevent the expression

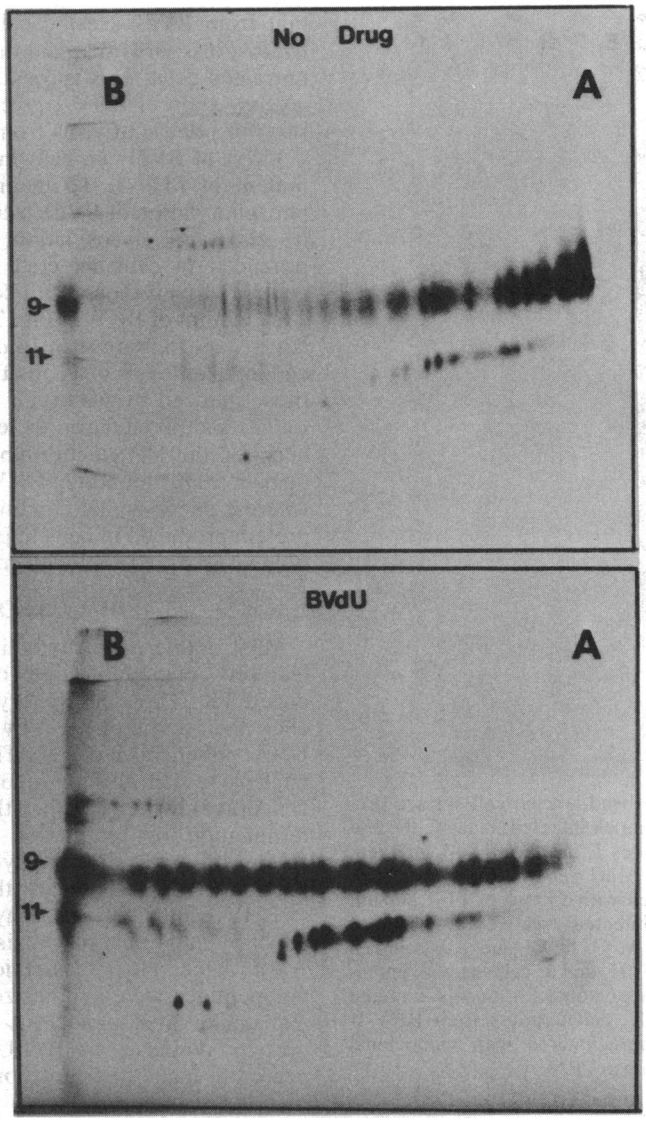

FIG. 4. Analysis of [^{3}H]glucosamine-labeled proteins in BHV-1-infected untreated cells and BVdU-treated cells by two-dimensional electrophoresis. Virus-infected untreated cells or cells treated with 10 μg of BVdU per ml were labeled from 6 h postinfection with [^{3}H]glucosamine. At 20 h after infection, the cells were harvested and analyzed by two-dimensional electrophoresis. A and B refer to the acidic and basic ends of the first dimension. A fraction of each sample was placed in a lane on the left-hand side of the gel and analyzed directly along the second dimension by SDS-PAGE. The numbers 9 and 11 refer to the BHV-1 glycoproteins GVP9 and GVP11 (16, 17) separated by SDS-PAGE.

of BHV-1 glycoproteins on the surface of infected cells, two approaches were taken. First, we iodinated the surface proteins of infected untreated cells or cells treated with BVdU. BVdU-treated cells contained all of the surface proteins of untreated cells (Fig. 5). However, all iodinated surface proteins in these cells had a higher electrophoretic mobility than those of untreated cells. Under similar conditions, tunicamycin, a compound known to inhibit the addition of carbohydrate side chains linked by N glycosydic linkages, completely inhibited the expression of viral glycoprotein on the cell surface (23).

Second, we examined the ability of anti-BHV-1 serum to mediate cytolysis of infected drug-treated and untreated cells by ADCC. Both untreated and BVdU-treated cells were sensitive to cytolysis (Table 1), whereas tunicamycin-treated cells were not (data not shown). This again suggested that BVdU-induced inhibition of

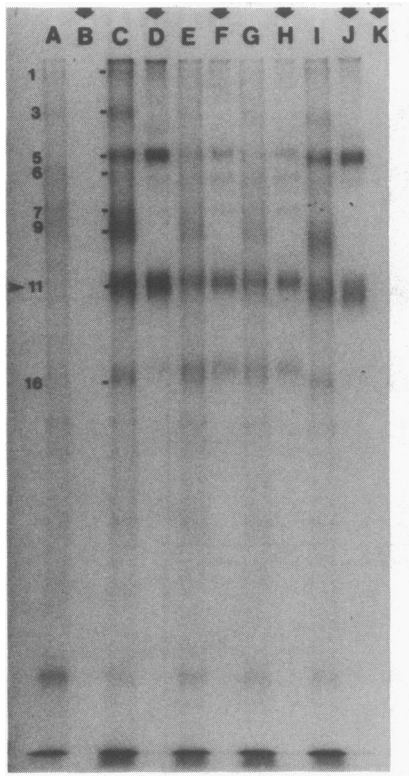

FIG. 5. ^{125}I-Enzymobead-labeled cell surface proteins. Lanes A and B, mock infected; lanes C, D, and K, BHV-1 infected; lanes E and F, BHV-1 infected, treated with 75 μg of AraC per ml; lanes G and H, BHV-1 infected, treated with 150 μg of PFA per ml; lanes I and J, BHV-1 infected, treated with 7.5 μg of BVdU per ml. Lanes A, C, E, G, and I, whole-cell extracts; lanes B, D, F, H, and J, cell extracts immunoprecipitated with monoclonal antibodies directed against GVP11; lane K, cell extracts from BHV-1-infected cells immunoprecipitated with monoclonal antibodies directed against VP23.

glycosylation is different from that mediated by tunicamycin in that the former does not prevent the expression of the glycoprotein on the cell surface.

Effect of BVdU upon release of virus from infected cells. To determine whether BVdU significantly reduced the amount of virus produced in BHV-1-infected cells, we compared the amount of intracellular and extracellular virus in BVdU-treated cultures with that in untreated cultures. Infected cells were labeled with [^{35}S]methionine, and the culture supernatant and cells were separated. The radioactive virus associated with these fractions was then quantitated after banding in potassium tartrate gradients. BVdU-treated and untreated cells contained comparable levels of intracellular virus (Fig. 6). In contrast, the tissue culture supernatant from BVdU-treated cells contained about fivefold less virus than did the supernatant from untreated cells. This suggests that the complete glycosylation of BHV-1 glycoproteins is necessary for release of virus from infected cells.

Effect of BVdU on cells infected with a TK$^-$ mutant of BHV-1. To determine whether the phosphorylation of BVdU was a prerequisite for its effect on glycosylation, the glycoproteins produced in cells infected with BHV-1 were compared with those from cells infected with a TK$^-$ mutant of BHV-1 (Fig. 7). This mutant (28) is resistant to more than 10 μg of BVdU per ml and induces levels of TK that are less than 3% of those induced by wild-type BHV-1. BVdU (10 μg/ml) had no effect on the electrophoretic mobility of the [^{3}H]glucosamine-labeled polypeptides of a TK$^-$ mutant of BHV-1 (Fig. 7, lane E), whereas under similar conditions the viral glycoprotein produced in cells infected with the wild-type virus were of a lower molecular weight.

DISCUSSION

Most antiherpetic thymidine analogs are selectively phosphorylated by herpesvirus-induced TK. These phosphorylated analogs then exert their antiviral effect by inhibiting the viral DNA polymerase (1, 3). The phosphorylated nucleoside can also be incorporated into viral DNA (2, 5), where it is then thought to cause the termination or retardation of nascent DNA chains (15) or lead to the synthesis of aberrant peptides. Our data suggest that BVdU, at least in BHV-1-infected cells, may exert its antiviral effect by additional mechanisms.

All detectable [^{35}S]methionine-labeled viral polypeptides were synthesized in treated cells, and unlike AraC- and PFA-treated cells, viral protein synthesis in BVdU-treated cells appeared to make the transition from the early to

TABLE 1. ADCC of BHV-1-infected cells grown at various BVdU concentrations

BVdU concn[a]	% Lysis[b]
0	35
1	21
5	20
10	16
25	17
100	18

[a] BVdU was added at the various concentrations (micrograms per milliliter) immediately after virus adsorption and was maintained throughout.

[b] Values represent percent specific release computed as described in the text and represent the mean of quadruplicate assays. The effector/target cell ratio was 25:1, and the assay time was 6 h in the presence of a 1/50 dilution of anti-BHV-1 serum. The nonspecific release was 8.9%.

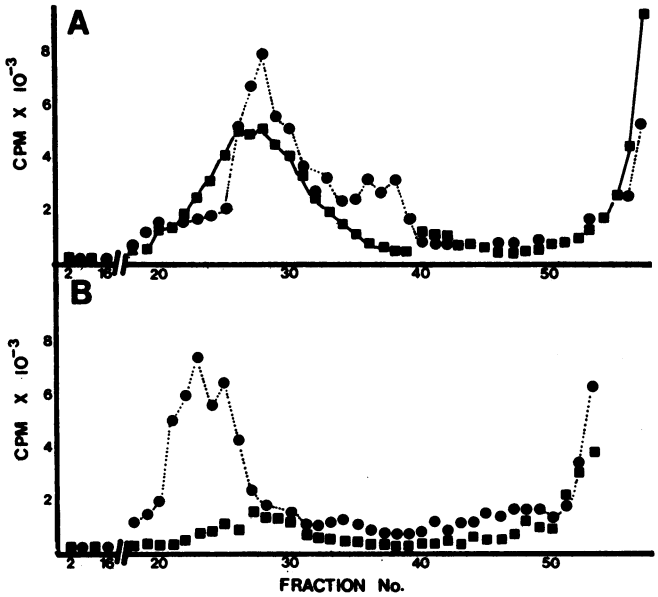

FIG. 6. Sedimentation of extracellular and intracellular virus from BVdU-treated and untreated cells through potassium tartrate gradients. Infected untreated cells (●) or cells treated with BVdU (■) were labeled with [^{35}S]methionine from 6 h after infection. At 20 h after infection, sonicated cell extracts (A) or tissue culture supernatant (B) was analyzed on 20 to 50% potassium tartrate gradients. Fractions were collected directly onto glass fiber filters which were then washed with TCA and counted for radioactivity.

the late phase (16, 17). This further supported the observation that viral DNA synthesis in these cells was not inhibited. In addition, electron micrographs of BVdU-treated cells (data not shown) contained numerous viral particles, suggesting that virions were assembled in these cells.

Viral glycoproteins synthesized in BVdU-treated cells were of a lower molecular weight than those in untreated cells. Analysis of the glycoproteins from treated and untreated cells by two-dimensional electrophoresis supported this observation since these cells appeared to have a higher concentration of partially glycosylated basic and neutral intermediates. This suggests that BVdU may exert at least some of its antiviral effect by interfering with the processing of the neutral, immature forms of the viral glycoproteins to the more mature, complex acidic forms (11). Tunicamycin, an inhibitor which prevents the addition of carbohydrate side chains attached by N-linked glycosydic linkages (23), also only partially inhibits the glycosylation of BHV-1 glycoproteins. In a previous communication (17), we have shown that in tunicamycin-treated cells partially glycosylated versions of all BHV-1 glycoproteins are synthesized. Unlike BVdU, however, tunicamycin inhibits the expression of viral glycoproteins on the surface of infected cells. Monensin, an ionophore that disrupts ionic gradients across cell membranes and consequently prevents the budding of vesicles from the golgi apparatus, interferes with the maturation of herpesvirus glycoproteins and also prevents their expression on the cell surface (12). In contrast, truncated, immature versions of BHV-1 glycoproteins were expressed upon the surface of BVdU-treated cells. The drug-sensitive glycosylation, however, did appear to be necessary for the orderly maturation of the virus since drug-treated cells failed to release significant amounts of virus into the growth supernatant. BVdU, therefore, appears to inhibit steps in glycosylation that are different from those inhibited by tunicamycin or monensin.

The glycosylation of herpesvirus proteins, like that of other viral glycoproteins, is probably initiated as a cotranslation event in the rough endoplasmic reticulum. The first step of this process is the en bloc transfer of an activated oligosaccharide from a dolichol-linked intermediate to a peptide sequence in the proteins. The initial step in the synthesis of the dolichol-linked oligosaccharide itself is the transfer of UDP-linked *N*-acetylglucosamine to dolichol phosphate (24, 26). The immature glycoproteins then migrate to the inner nuclear membrane, where they are incorporated into the envelope of budding nucleocapsids. Recent evidence suggests

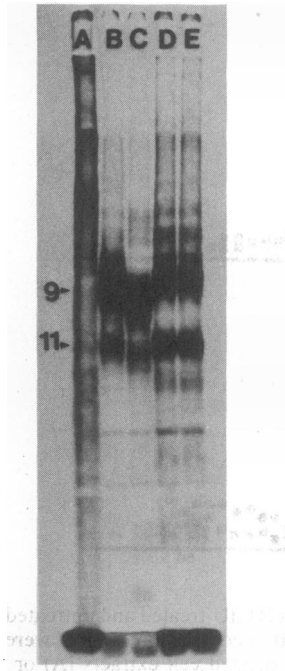

FIG. 7. Effect of BVdU on glycoprotein synthesis in cells infected with BHV-1 or its TK$^-$ mutant. Mock-infected cells (lane A) or cells infected with BHV-1 (lanes B and C) or its TK$^-$ mutant (lanes D and E) were labeled from 6 h after infection with [^{35}S]methionine. A culture in each category (lanes C and E) was incubated with 10 μg of BVdU per ml throughout the infection and labeling period. At 20 h after infection, the cells were harvested and analyzed by SDS-PAGE in 7.5% polyacrylamide gels.

that en route to the exterior of the infected cells, possibly via the golgi apparatus (12), the core oligosaccharides on the immature glycoproteins are trimmed, and new sugars, some attached by O linkages (19), as well as muramic acid and sialic acid residues are added on. Maturation, which also involves UDP-linked sugars (24), appears to be a stepwise process, and the intermediates in the conversion of the neutral or basic forms of the glycoproteins to the acidic, mature forms can be readily separated on two-dimensional gels (11, 16).

We have not been able to elucidate the mechanisms by which BVdU, a thymidine analog, could interfere with glycosylation, although the insensitivity of the glycoproteins of TK$^-$ mutants of BHV-1 to BVdU suggests that phosphorylation of the nucleoside by the virus-induced TK is a prerequisite for this effect. The most plausible possibility is that BVdU-containing nucleotides may compete with uridine-containing nucleotides that are directly involved in carbohydrate metabolism. Although Pogolotti et al. (20) have shown that after uptake a large amount of 5-fluorouracil can be found within cells as fluoro-UDP–hexose complexes, we are unaware of data that suggest that deoxynucleotides can compete with ribo-UDP in the formation of nucleotide-sugar complexes or of pathways that could convert BVdU or its nucleotides to ribonucleotides.

BVdU may inhibit steps in the glycosylation of proteins that are not affected by other inhibitors and thus may provide us with another tool to study this process. We are, therefore, attempting to further elucidate its mechanism of action.

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