ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1984, p. 566–570
0066-4804/84/050566-05$02.00/0

Vol. 25, No. 5

Effect of (*E*)-5-(2-Bromovinyl)-2′-Deoxyuridine on Synthesis of Herpes Simplex Virus Type 1-Specific Polypeptides

SCOTT A. SIEGEL,[1]* MICHAEL J. OTTO,[1]† ERIK DE CLERCQ,[2] AND WILLIAM H. PRUSOFF[1]

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510,[1] and Katholieke Universiteit Leuven, B-3000 Leuven, Belgium[2]

Received 24 October 1983/Accepted 24 February 1984

The antiherpesvirus agent (*E*)-5-(2-bromovinyl)-2′-deoxyuridine caused marked alterations in the synthesis and processing of several herpes simplex virus type 1 (HSV-1)-infected-cell polypeptides. Analogous to other thymidine analogs, there was a dose-dependent decrease in several β and γ polypeptides and an accumulation of HSV-1 thymidine kinase. In contrast to the action of other thymidine analogs, there were alterations in α polypeptides, including an increase in the synthesis and phosphorylation of infected-cell polypeptide 4b and a decrease in the synthesis of infected-cell polypeptide 27. The phosphorylation of several other HSV-1 phosphoproteins was mildly inhibited. (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine inhibited the glycosylation of the major HSV-1 glycoproteins, and this activity appeared to be independent of the incorporation of the drug into the viral DNA. Thus, the alterations in HSV-1 polypeptide expression appear to be due to the presence of the drug in a low-molecular-weight form as well as its presence in the viral DNA. This suggests that this analog or a phosphorylated derivative might act as an inhibitor of an enzyme(s) responsible for posttranslational modification of polypeptides.

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVdU) is a potent and selective antiherpesvirus agent (2, 7). The biochemical basis for the antiviral activity of BVdU and other nucleoside analogs has been the subject of intense investigation (2, 6, 20, 22; W. H. Prusoff, T. S. Lin, W. R. Mancini, M. J. Otto, S. A. Siegel, and J. J. Lee, *in* R. T. Walker and E. De Clercq, ed., *Targets for the Design of Antiviral Agents*, in press). Many of these compounds are incorporated into the viral DNA, resulting in changes in both physical and functional properties of this DNA (1, 8, 9, 14). In addition, nucleoside analogs or their phosphorylated derivatives or both are known to inhibit a variety of enzymatic activities necessary for viral reproduction (6, 21).

The basis for the selectivity of BVdU resides in its enhanced affinity for herpes simplex virus type 1 (HSV-1)-encoded thymidine and thymidylate kinase activities, since phosphorylation of BVdU appears to be essential for antiviral activity (4, 11). The triphosphate derivative of BVdU is a competitive inhibitor of HSV-1 DNA polymerase, with respect to the substrate dTTP (2, 13). BVdU is incorporated into the viral DNA, and the extent of incorporation correlates with a reduction in the number of infectious particles and with a reduced stability of the DNA (14). In addition, the glycosylation of bovine herpesvirus 1 proteins is inhibited by BVdU (15), in agreement with the findings presented in this study for HSV-1.

Recent work in our laboratory has focused on changes in HSV-1-infected-cell polypeptide (ICP) expression induced by the thymidine analogs 5-iodo-2′-deoxyuridine (IdUrd), 5-iodo-5′-amino-2′,5′-dideoxyuridine (AIdUrd), and 5′-amino-2′,5′-dideoxythymidine (5′-AdThd) (17). The present study examines changes in the synthesis of HSV-1 polypeptides induced by the thymidine analog BVdU. In addition, polypeptides synthesized by HSV-1 containing BVdU in the DNA (BVdU substituted), with drug-free media, are examined. A comparison is made with the effects of IdUrd, AIdUrd, and 5′-AdThd on protein synthesis.

MATERIALS AND METHODS

Cells and virus. Vero cells were grown in Dulbecco modified minimal essential medium supplemented with 10% fetal calf serum. The CL-101 strain of HSV-1 was passaged at a multiplicity of 0.01 PFU per cell and plaque assayed on Vero cells.

BVdU-substituted virions were prepared by infecting confluent monolayers of Vero cells in 175-mm^2 flasks with 3 PFU of stock virus per cell in 5 ml of medium. After adsorption, the infecting medium was removed and 15 ml of fresh medium containing 0.0, 0.3, or 0.6 μM BVdU was added. The infected cells were then incubated for 20 h at 37°C, after which the BVdU-substituted virus was harvested and plaque assayed on Vero cells. The inclusion of 0.3 and 0.6 μM BVdU in the media resulted in, respectively, a 50 and 90% reduction in the yield of infectious virus. An increase in the density of the BVdU-substituted HSV-1 DNA on CsCl gradients was observed, and this was found to be in good agreement with an earlier report from our laboratory (14). Based on this earlier work, the DNA of the BVdU-substituted virions contained averages of 15 and 22% BVdU in place of thymidine when passaged in media containing 0.3 and 0.6 μM BVdU, respectively.

A thymidine kinase-deficient (TK$^-$) strain of HSV-1, CL-101, was generously supplied by Wilma P. Summers, Yale University School of Medicine, New Haven, Conn.

Chemicals. L-[^{35}S]methionine and [^{14}C]glucosamine were obtained from Amersham Corp., Arlington Heights, Ill., and [^{14}C]mannose and $^{32}P_i$ were obtained from New England Nuclear Corp., Boston, Mass. All other chemicals were of reagent grade.

Labeling and extraction of HSV-1 polypeptides. The labeling and extraction of HSV-1 and cellular polypeptides were accomplished by the method of Otto et al. (17). Briefly, confluent monolayers of 2 × 10^5 Vero cells in wells (16 mm

* Corresponding author.
† Present address: Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

in diameter) were infected at a multiplicity of infection of 20 PFU per cell. After 1 h of adsorption, the inocula were replaced with fresh media containing 2% fetal calf serum and the appropriate concentration of BVdU. At this point, 10 μCi of L-[^{35}S]methionine, 5 μCi of [^{14}C]glucosamine, 5 μCi of [^{14}C]mannose, or 20 μCi of $^{32}P_i$ was added. The infected cells were allowed to incubate until 24 h postinfection, at which time the media were carefully removed and the cells were lysed with 50 μl of sodium dodecyl sulfate (SDS) sample buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol, 62.5 mM Tris-hydrochloride [pH 6.8]). The cell-virus lysates were then transferred to 1.5-ml microfuge tubes and stored at −20°C. Mock-infected cells were treated as described above but were not infected with virus. For the short-term label experiments, the infected cells were labeled with 20 μCi of L-[^{35}S]methionine for 2-h intervals and then harvested as above.

Labeling and extraction of cells infected with BVdU-substituted virions at a multiplicity of infection of 20 PFU per cell were performed exactly as described above, except no drug was added to the media.

SDS-PAGE. Polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli (12). Gels were cast as either 9% acrylamide or a linear gradient from 7.5 to 12.5% acrylamide. Fixed and dried gels were analyzed by contact autoradiography, and the bands were quantitated with a Joyce-Loebl scanning densitometer. Approximate molecular weights were determined by comparison with a set of radiolabeled markers included in each gel. ICP numbers were assigned to the various polypeptides based on the order of increasing mobility and molecular weight as defined by Morse et al. (16).

RESULTS

Effect of BVdU on the synthesis of HSV-1 ICPs. HSV-1 was grown in the presence or absence of various concentrations of BVdU and labeled with L-[^{35}S]methionine, and the cell lysates were analyzed by SDS-PAGE. There was a significant increase in the labeling of ICPs 4b, 35, and 36, whereas the other polypeptides listed showed a decrease in labeling (Table 1). In addition to the results presented in Table 1, the short-term label experiments (Fig. 1a and b) revealed a BVdU-induced reduction in the rates of accumulation of ICP 2 (molecular weight, 196,000 [196K]), ICP 27 (57K), ICP 32 (51K), and ICP 34 (49K). Analysis of the densitometer scans of Fig. 1a revealed that the shut off of ICP 4b, an α polypeptide, was delayed by ca. 2 h in the presence of BVdU. There was also a general reduction in the L-[^{35}S]methionine labeling of the BVdU-treated, infected cells from 12 to 20 h postinfection (Fig. 1b). BVdU induced no significant alterations in the L-[^{35}S]methionine labeling of other HSV-1 ICPs.

TABLE 1. Effect of BVdU on L-[^{35}S]methionine labeling of native HSV-1 ICPs[a]

ICP no.	Approx. mol wt ($\times 10^{-3}$)	% Labeling at BVdU concn (μM)[b]		
		0.3	0.6	1.3
4b	158	112	175	219
24	67	96	83	69
35	43	125	128	148
36	40	100	110	122
40	31	94	73	69
41	30	82	68	56
43	22	40	<20	<20
44	21	38	<20	<20

[a] Data were calculated from densitometer scans of native HSV-1-infected cell cultures labeled from 1 to 24 h postinfection and analyzed by SDS-PAGE.

[b] Numbers represent the percentage of labeling of a particular polypeptide compared with a control having no BVdU.

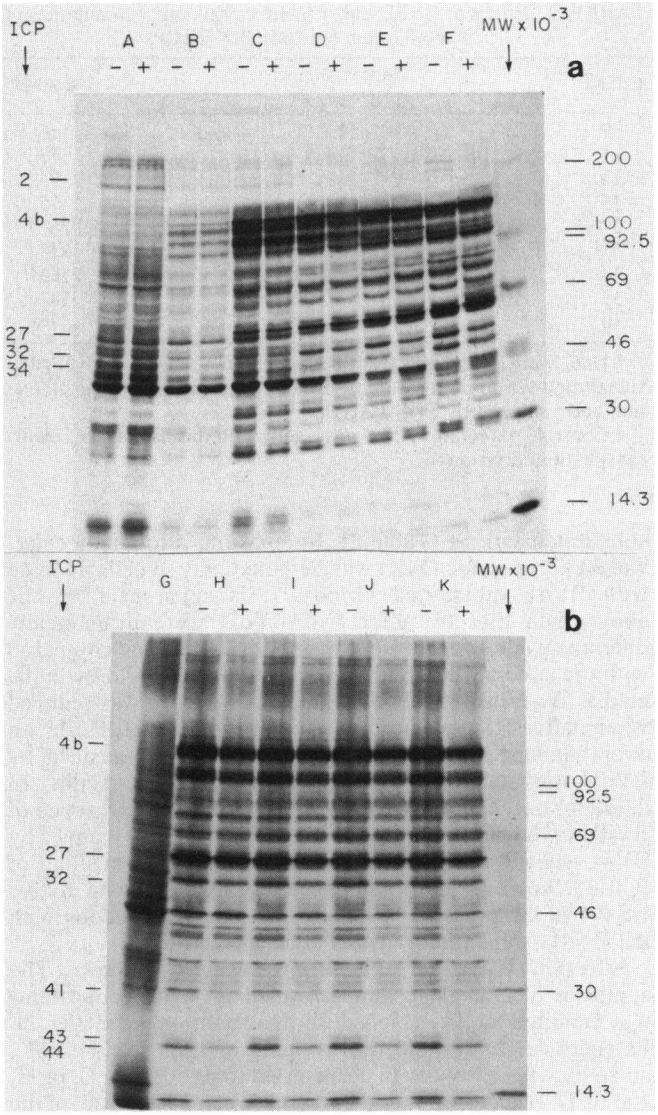

FIG. 1. Short-term labeling of native HSV-1 ICPs with L-[^{35}S]methionine when the virus was grown in the presence (+) or absence (−) of 0.6 μM BVdU. Cells were labeled for 2-h intervals from 1 to 20 h postinfection. A linear gradient polyacrylamide gel (7.5 to 12.5%) was used. (a) Lane A, 1 to 3 h (mock infected); lane B, 1 to 3 h; lane C, 3 to 5 h; lane D, 5 to 7 h; lane E, 7 to 9 h; lane F, 9 to 11 h. (b) Lane G, 12 to 14 h (mock infected, no BVdU); lane H, 12 to 14 h; lane I, 14 to 16 h; lane J, 16 to 18 h; lane K, 18 to 20 h. MW, Molecular weight.

HSV-1 containing BVdU-substituted DNA (ca. 22% BVdU for thymidine) was also used to infect Vero cells in the absence of any added drug. There was an increase of almost sixfold in the L-[^{35}S]methionine labeling of ICP 40, as well as a significant increase in the labeling of ICPs 27, 31, 34, and 35 (Table 2). There was also a 23 to 26% reduction in the labeling of ICPs 9, 11, 12, and 15 synthesized by BVdU-

TABLE 2. Effect of BVdU-substituted virions on accumulation of L-[^{35}S]methionine labeled HSV-1 ICPs[a]

ICP no.	Approx. mol wt ($\times 10^{-3}$)	% Control[b]
9	119	76
11	114	77
12	111	74
15	104	75
27	58	267
31	52	223
34	48	163
35	47	187
40	31	574

[a] Data were calculated from densitometer scans of 22% BVdU-substituted virion-infected cultures labeled from 1 to 24 h postinfection and analyzed by SDS-PAGE.

[b] Control, Infecting virus passaged with no BVdU in media (unsubstituted virions).

substituted virions (Table 2). In addition, a low-molecular-weight polypeptide (14K) was detected only in cells infected with BVdU-substituted virions. It is apparent from the above data that the effects of BVdU were dramatically different when present only in the viral DNA as compared with the effects when BVdU was present initially only in the media. Whereas the labeling of ICPs 27 and 40 was reduced when native HSV-1 was grown in the presence of BVdU, an overabundance of these polypeptides was synthesized by BVdU-substituted virions grown in drug-free media. In addition, polypeptides that were affected by the presence of BVdU in the media were synthesized in a normal manner by BVdU-substituted virions and vice versa. The labeling of ICP 35, however, was increased by infecting with native HSV-1 in the presence of BVdU and also by infecting with the BVdU-substituted virions in drug-free media.

Effect of BVdU on HSV-1 glycoprotein synthesis. The synthesis of [^{14}C]glucosamine-labeled HSV-1 glycoproteins was inhibited by BVdU in a dose-dependent manner (Fig. 2). Polypeptides with molecular weights of 122K, 112K, 66K, and 60K, which appear to correspond to pg A/B, pg C, pg E, and pg D, respectively, decreased between 17 and 56% in the presence of 1.3 μM BVdU.

The synthesis of [^{14}C]mannose-labeled HSV-1 glycoproteins with molecular weights of 110K, 85K, 76K, 63K, and 54K, which appear to correspond to pg C, g E, pg E, g D, and pg D, respectively, was also inhibited by BVdU in a dose-dependent manner (Fig. 3). A low-molecular-weight (44K), [^{14}C]mannose-labeled polypeptide increased with the dosage of BVdU (Fig. 3), suggesting that this species may be a precursor of one of the inhibited higher-molecular-weight glycoproteins.

To determine the relationship between BVdU incorporation into HSV-1 DNA and its effect on viral glycoprotein synthesis, we inoculated cell cultures with BVdU-substituted virions and determined the extent of viral glycoprotein synthesis by SDS-PAGE. All experiments were performed at a multiplicity of infection of 20 PFU per cell in drug-free media. A control sample inoculated with native HSV-1 was included in each experiment. Duplicate determinations were made for each of two independent stocks for both the 15 and 22% BVdU-substituted virions. In contrast to the effects of BVdU on native HSV-1 glycoproteins, there was no inhibition of the 24-h [^{14}C]glucosamine or [^{14}C]mannose labeling of glycoproteins encoded by the BVdU-substituted virions. The same BVdU-substituted virions did, however, show

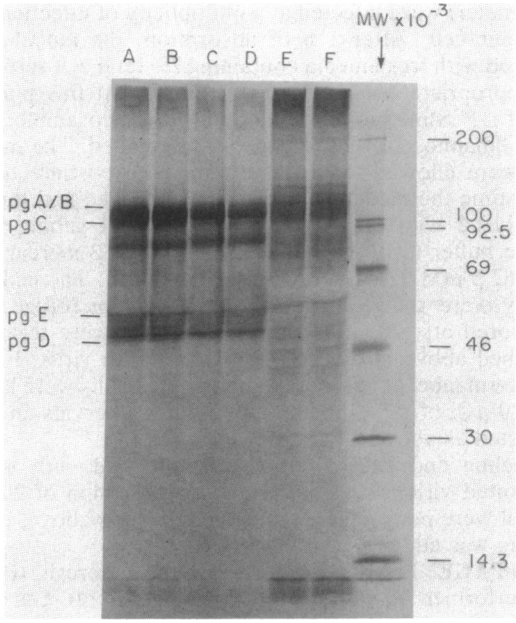

FIG. 2. Synthesis of [^{14}C]glucosamine-labeled HSV-1 glycoproteins in the presence or absence of BVdU. Lane A, no BVdU; lane B, 0.3 μM BVdU; lane C, 0.6 μM BVdU; lane D, 1.3 μM BVdU; lane E (mock infected), no BVdU; lane F (mock infected), 1.3 μM BVdU. A linear gradient polyacrylamide gel (7.5 to 12.5%) was used. MW, Molecular weight. HSV-1 glycoproteins are identified in the left margin according to the principles of the Cold Spring Harbor workshop (5).

marked alterations in the pattern of other L-[^{35}S]methionine-labeled ICPs when grown in drug-free media (Table 2).

To determine whether the phosphorylation of BVdU is necessary for the inhibition of viral glycoproteins, we inoculated cell cultures with 20 PFU of TK$^-$ HSV-1 per cell and subsequently labeled these cultures with [^{14}C]glucosamine for 24 h. SDS-PAGE analysis revealed no inhibition of viral glycoproteins in the range of 0 to 1.3 μM BVdU, suggesting that phosphorylation of BVdU by the HSV-1 thymidine kinase is a prerequisite for the inhibition of HSV-1 glycoprotein synthesis.

Effect of BVdU on HSV-1 phosphoprotein synthesis. The extent of phosphorylation of HSV-1 ICPs 4a (155K), 4c (165K), 19 (80K), 20 (75K), and 22 (61K) was mildly inhibited by BVdU, reaching a maximum of 24 to 26% inhibition in the presence of 1.3 μM BVdU (Fig. 4). The extent of phosphorylation of ICP 4b (160K), however, increased in a dose-dependent manner (Fig. 4), analagous to an increase in L-[^{35}S]methionine labeling of the corresponding band (Table 1).

DISCUSSION

The synthesis and processing of several HSV-1 polypeptides is markedly affected by the nucleoside analog BVdU. Since there are HSV-1 ICPs that increase as well as decrease in the presence of BVdU, a transport phenomenon is unlikely. In support of this idea, the uptake of [^{14}C]glucosamine by HSV-1-infected Vero cells is not inhibited by BVdU (data not shown). The increase in ICP 36 induced by BVdU is similar to an increase in the same polypeptide induced by AIdUrd, IdUrd, and 5'-AdThd (17). Since this polypeptide is the herpesvirus-encoded thymidine kinase and the drugs are all thymidine analogs, a study of the mechanism of drug

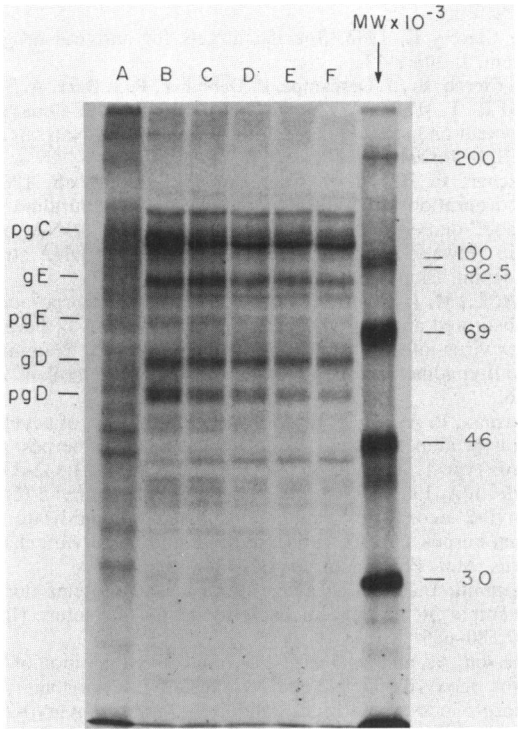

FIG. 3. Synthesis of [^{14}C]mannose-labeled HSV-1 glycoproteins in the presence or absence of BVdU. Lane A (mock infected), no BVdU; lane B, no BVdU; lane C, 0.3 μM BVdU; lane D, 0.6 μM BVdU; lane E, 0.9 μM BVdU; lane F, 1.3 μM BVdU. A polyacrylamide gel (9%) was used. MW, Molecular weight. HSV-1 glycoproteins are identified in the left margin according to the principles of the Cold Spring Harbor workshop (5).

effect on this polypeptide may provide insight into the regulation of the herpesvirus thymidine kinase gene. BVdU also inhibits ICPs 40, 41, 43, and 44 in a fashion similar to that of AIdUrd and IdUrd (17), suggesting that these drugs may have common sites of action or of incorporation into the viral genome. In contrast to the effects of AIdUrd, IdUrd, and 5'-AdThd, in which no α polypeptides are altered (17), the synthesis of ICP 27 is decreased, and the synthesis and phosphorylation of ICP 4b are increased by BVdU. The increase in synthesis of ICP 4b appears to be due, at least in part, to a delay in the shut off of this polypeptide. This indicates that BVdU may have an indirect effect on the cascade regulation of HSV-1 protein synthesis by delaying the onset of HSV-1 β and γ protein synthesis through the stimulation of a regulatory α protein. The nucleoside analog 9-(2-hydroxyethoxymethyl)guanine (acyclovir) also causes a stimulation in the accumulation of HSV-1 ICP 4, presumably owing to an enhanced rate of synthesis (10). These results indicate that the HSV-1 phosphoprotein ICP 4 is subject to alterations by antiviral nucleoside analogs and that this may affect the regulation of HSV-1 protein synthesis. Further study into the regulatory role of this α polypeptide and the specific consequences of its alteration by antiviral agents may provide insight into the relationship between the specific events affected by antiviral agents and the resulting reduction in infectious-virus yield.

The changes in HSV-1 polypeptide expression when native HSV-1 is grown in the presence of BVdU are remarkably different from the changes when BVdU-substituted virions are employed. This suggests that the drug may exert an effect on polypeptide synthesis independent of its incorporation into the viral DNA. This point is further exemplified by the finding that HSV-1 glycoprotein synthesis is inhibited by the presence of BVdU in the media but not by the presence of BVdU only in the viral DNA. Since the pathways of glycoprotein synthesis involve transfer of sugar moieties through nucleotide sugar intermediates, it is possible that BVdU or a phosphorylated derivative may inhibit one or more of the glycosyl transferase enzymes. BVdU itself may be transformed into a nucleotide sugar, resulting in a nonproductive or inhibitory moiety. Indeed, the pyrimidine analog 5-fluorouracil, when administered to certain cell lines, has been found in a nucleotide sugar form (18, 19). Studies are now under way to determine the specific point(s) of BVdU inhibition of the polypeptide glycosylation pathway. Since the HSV glycoproteins are known to be a determinant of virion infectivity, possibly by functioning in the attachment or penetration stages (5), inhibition of these glycoproteins might result in the production of a lower number of infectious particles or virions with impaired infectivity or both. It also appears that phosphorylation of BVdU by the HSV-1 thymidine kinase is necessary for inhibition of viral glycoprotein synthesis, thus limiting this effect to virus-infected cells. Therefore, a BVdU-induced inhibition of HSV-1 glycoproteins may provide a basis for the increased antiviral selectivity and efficacy of BVdU compared with those of other thymidine analogs.

The synthesis of HSV-1 glycoproteins is also inhibited by acyclovir in a dose-dependent manner (10), although the mechanism of inhibition remains unclear. The role of BVdU as a glycosylation inhibitor for bovine herpesvirus 1 has been suggested by Misra et al. (15), which is in agreement with the

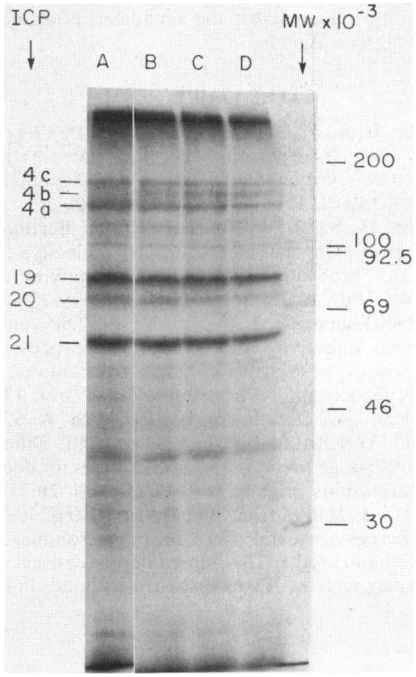

FIG. 4. Synthesis of $^{32}P_i$-labeled HSV-1 phosphoproteins in the presence or absence of BVdU. Lane A, no BVdU; lane B, 0.3 μM BVdU; lane C, 0.6 μM BVdU; lane D, 1.3 μM BVdU. A polyacrylamide gel (9%) was used. MW, Molecular weight.

results obtained with HSV-1. Interestingly, the synthesis of L-[^{35}S]methionine-labeled polypeptides is unaltered by BVdU in the bovine herpesvirus 1 system, whereas it is markedly altered in the HSV-1 system. This is not a totally unlikely result since the synthesis of L-[^{35}S]methionine-labeled polypeptides is under direct viral genomic control and is hence subject to species differences. The glycosylation of viral polypeptides, however, is thought to be accomplished by a cellular pathway (3). Thus, it is more likely to be affected by BVdU in the same manner regardless of the species of infecting virus.

The role of BVdU as an inhibitor of polypeptide phosphorylation appears unlikely since there is an increase in the phosphorylation of ICP 4b concomitant with the decrease in phosphorylation of the other ICPs.

In conclusion, the nucleoside analog BVdU caused marked alterations in the pattern of HSV-1 polypeptide expression. These changes appeared to depend on the presence of BVdU in a low-molecular-weight form as well as BVdU incorporation into the viral DNA. The inhibition of viral glycoprotein synthesis appeared to be independent of BVdU incorporation into the viral DNA. The antiviral effects of BVdU through its selective interaction with the HSV-1 thymidine kinase and DNA polymerase and its subsequent incorporation into the viral DNA can only be augmented by an auxiliary mode of inhibition affecting viral glycoprotein synthesis. The relative importance of the various drug-sensitive biochemical transformations necessary for viral reproduction has yet to be determined, and the successful elucidation of these parameters could provide direction for the development of more potent and selective antiviral agents.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants CA 05262 and T32 CA 09085 from the National Cancer Institute.

We gratefully acknowledge the technical assistance of Evelyn Lentz and Kathleen Woods.

LITERATURE CITED

1. **Allaudeen, H. S., M. S. Chen, J. J. Lee, E. De Clercq, and W. H. Prusoff.** 1982. Incorporation of E-5-(2-halovinyl)-2′-deoxyuridines into deoxyribonucleic acids of herpes simplex virus type 1-infected cells. J. Biol. Chem. **257**:603–606.
2. **Allaudeen, H. S., J. W. Kozarich, J. R. Bertino, and E. De Clercq.** 1981. On the mechanism of selective inhibition of herpesvirus replication by (E)-5-(2-bromovinyl)-2′-deoxyuridine. Proc. Natl. Acad. Sci. U.S.A. **78**:2698–2702.
3. **Campadelli-Fiume, G., L. Poletti, F. Dall'Olio, and F. Serafini-Cessi.** 1982. Infectivity and glycoprotein processing of herpes simplex virus type 1 grown in a ricin-resistant cell line deficient in *N*-acetylglucosaminyl transferase I. J. Virol. **43**:1061–1071.
4. **Cheng, Y.-C., G. Dutschman, E. De Clercq, A. S. Jones, S. G. Rahim, G. Verhelst, and R. T. Walker.** 1981. Differential affinities of 5-(2-halogenovinyl)-2′-deoxyuridines for deoxythymidine kinases of various origins. Mol. Pharmacol. **20**:230–233.
5. **Cohen, G., I. Halliburton, and R. Eisenberg.** 1981. Glycoproteins of herpesviruses, p. 549–554. *In* A. Nahmias, W. Dowdle, and R. Schinazi (ed.), The human herpesviruses: an interdisciplinary perspective. Elsevier/North-Holland Publishing Co., New York.
6. **De Clercq, E.** 1982. Specific targets for antiviral drugs. Biochem. J. **205**:1–13.
7. **De Clercq, E., J. Descamps, P. DeSomer, P. J. Barr, A. S. Jones, and R. T. Walker.** 1979. (E)-5-(2-bromovinyl)-2′-deoxyuridine: a potent and selective anti-herpes agent. Proc. Natl. Acad. Sci. U.S.A. **76**:2947–2951.
8. **Fischer, P. H., M. S. Chen, and W. H. Prusoff.** 1980. The incorporation of 5-iodo-5′-amino-2′,5′-dideoxyuridine and 5-iodo-2′-deoxyuridine into herpes simplex virus DNA: relationship between antiviral activity and effects on DNA structure. Biochim. Biophys. Acta **606**:236–245.
9. **Fox, L., M. J. Dobersen, and S. Greer.** 1983. Incorporation of 5-substituted analogs of deoxycytidine into DNA of herpes simplex virus-infected or -transformed cells without deamination to the thymidine analog. Antimicrob. Agents Chemother. **23**:465–476.
10. **Furman, P. A., and P. V. McGuirt.** 1983. Effect of acyclovir on viral protein synthesis in cells infected with herpes simplex virus type 1. Antimicrob. Agents Chemother. **23**:332–334.
11. **Fyfe, J. A.** 1982. Differential phosphorylation of (E)-5-(2-bromovinyl)-2′-deoxyuridine monophosphate by thymidylate kinases from herpes simplex viruses types 1 and 2 and varicella zoster virus. Mol. Pharmacol. **21**:432–437.
12. **Laemmli, U.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. Nature (London) **227**:580–685.
13. **Larsson, A., and B. Öberg.** 1981. Selective inhibition of herpesvirus deoxyribonucleic acid synthesis by acycloguanosine, 2′-fluoro-5-iodo-aracytosine, and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. Antimicrob. Agents Chemother. **19**:927–929.
14. **Mancini, W. R., E. De Clercq, and W. H. Prusoff.** 1983. The relationship between incorporation of E-5-(2-bromovinyl)-2′-deoxyuridine into herpes simplex virus type 1 DNA with virus infectivity and DNA integrity. J. Biol. Chem. **258**:792–795.
15. **Misra, V., R. C. Nelson, and L. A. Babiuk.** 1983. Inhibition of glycosylation of bovine herpesvirus 1 glycoproteins by the thymidine analog (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. Antimicrob. Agents Chemother. **23**:857–865.
16. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. J. Virol. **26**:389–410.
17. **Otto, M. J., J. J. Lee, and W. H. Prusoff.** 1982. Effects of nucleoside analogues on the expression of herpes simplex type 1-induced proteins. Antiviral Res. **2**:267–281.
18. **Peterson, M. S., H. A. Ingraham, and M. Goulian.** 1983. 2′-Deoxyribosyl analogues of UDP-N-acetylglucosamine in cells treated with methotrexate or 5-fluorodeoxyuridine. J. Biol. Chem. **258**:10831–10834.
19. **Pogolotti, A. L., Jr., P. A. Nolan, and D. V. Santi.** 1981. Methods for the complete analysis of 5-fluorouracil metabolites in cell extracts. Anal. Biochem. **117**:178–186.
20. **Prusoff, W. H., and S. A. Siegel.** 1983. Selective and non-selective nucleoside inhibitors, p. 40–43. *In* Proceedings XIII Congresso Nazional Societa Italiano di Chemotherapie, vol. 2. Catania, Italy.
21. **Shugar, D.** 1982. Viral encoded enzymes of nucleic acid metabolism and their role in the development of antiviral agents, p. 127–138. *In* G. Akoyunoglou (ed.), Cell function and differentiation, part C. Alan R. Liss, Inc., New York.
22. **Sim, I. S., J. Goodchild, D. M. Meredith, R. A. Porter, R. H. Raper, J. Viney, and H. J. Wadsworth.** 1983. Possible molecular basis for antiviral activity of certain 5-substituted deoxyuridines. Antimicrob. Agents Chemother. **23**:416–421.