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EFFECTS OF NUCLEOSIDE ANALOGUES ON THE EXPRESSION OF HERPES SIMPLEX TYPE 1-INDUCED PROTEINS

MICHAEL J. OTTO*, J.J. LEE and WILLIAM H. PRUSOFF

Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, U.S.A.

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Exposure of herpes simplex virus type 1 (HSV-1)-infected Vero cells to the nucleoside analogues 5-iodo-5'-amino-2',5'-dideoxyuridine (AIdUrd), 5-iodo-2'-deoxyuridine (IdUrd) or 5'-amino-2',5'-dideoxythymidine (5'-AdThd) resulted in altered expression of HSV-1-induced proteins. Infected cell proteins (ICPs) synthesized in the presence of the nucleoside analogues were compared by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis to ICPs from non-drug-treated cells and it was found that there was no effect on HSV-1-induced α proteins but β and γ proteins were reduced as much as 60%. There were three exceptions: ICP 35 (M_r = 46,000) and ICP 39 (M_r = 36,000) were not reduced and ICP 36 (M_r = 42,000) was increased during drug treatment. Progeny virions were isolated from drug-treated infected Vero cells and were compared to progeny isolated from control cells with respect to their polypeptide make-up and for their ability to induce HSV-1 proteins in non-drug-treated Vero cells. The progeny virus from drug-treated cells exhibited altered protein patterns on SDS-polyacrylamide gels with respect to control HSV-1. The progeny virions from AIdUrd- or IdUrd- but not from 5'-AdThd-treated cells were defective in their abilities to induce proteins upon subsequent infection of non-drug-treated Vero cells. Two unusual phosphoproteins were detected; one with an apparent moleculaf weight of 30,000 was induced by progeny virus from AIdUrd-treated cells and another at approximately 69,000 was induced by progeny virus from 5'-AdThd-treated cells.

nucleoside analogues herpes simplex virus type 1 proteins

INTRODUCTION

The antiviral activities of 5'-amino-2',5'-dideoxythymidine (5'-AdThd), 5-iodo-5'-amino-2',5'-dideoxyuridine (AIdUrd) and 5-iodo-2'-deoxyuridine (IdUrd) against herpesvirus have been established in cell culture as well as in animal studies [1,20,22,23,27]. However, the mechanism(s) by which these nucleoside analogues exert their antiviral effect is yet to be fully established. The phosphorylated derivatives of the halogenated analogues are found in progeny viral DNA and the reduction in the yield of infectious virions most closely correlates with the degree of substitution for thymidine in the DNA [9]. If has been suggested that this substitution in the viral genome results in a deleterious effect on gene expression [26] and, in fact, studies with IdUrd indicated that incorporation of the analogue into pseudorabies virus [16] or herpesvirus [28,29] resulted

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in a significant impairment of infectious particle formation and/or maturation. Apparently, with pseudorabies virus this was due to the inability of the IdUrd-substituted genome to code for the synthesis of functional proteins required for viral assembly [16]. In addition, studies by Buettner and Werchau [3] revealed that IdUrd replacement of thymidine in DNA was related to the reduced production of simian virus (SV)-40 viral coat protein, as well as the suppression of viral DNA synthesis. Kan-Mitchell and Prusoff [15] have reported that exposure of adenovirus type 2-infected cells to IdUrd resulted in the inhibition of late viral proteins but not earlier virus-induced proteins. Exposure of vaccinia virus-infected cells to a related analogue, 5-bromo-2′-deoxyuridine, caused an inhibition in the synthesis of a major viral core component which paralleled the inhibition of progeny virions [24].

These observations led us to investigate the effects of AIdUrd and of 5′-AdThd on the synthesis and the modification of individual HSV-1-induced polypeptides, and compare them to the effects observed with IdUrd.

MATERIALS AND METHODS

Cells and virus

Vero cells were grown in Dulbecco's modified minimal essential medium supplemented with 10% calf serum (Grand Island Biological Company). The CL-101 strain of HSV-1 [7] was passaged at a multiplicity of 0.01 plaque-forming units (p.f.u.)/cell and plaque assayed on Vero cells.

Chemicals

5-Iodo-5′-amino-2′,5′-dideoxyuridine (AIdUrd) and 5′-amino-2′,5′-dideoxythymidine (5′-AdThd) were synthesized by Dr. T.-S. Lin [20,21]. 5-Iodo-2′-deoxyuridine was purchased from Sigma Chemical Company. L-[^{35}S]Methionine (1300 Ci/mmol) and [^{14}C]glucosamine (61 mCi/mmol) were obtained from Amersham Corporation, ortho-[^{32}P]phosphate was purchased from New England Nuclear and [^{14}C]amino acids were purchased from Schwartz Mann.

Labelling and extraction of HSV-1 or cell proteins

Vero cells were grown to confluency in 16 mm diameter wells (2×10^5 cells) and infected with a multiplicity of infection (m.o.i.) of 20–50 p.f.u. of HSV-1 per cell. After an adsorption period of 1 h at 37°C, cells were rinsed once with fresh medium and 1 ml medium containing 2% dialyzed calf serum, and 25 μM IdUrd (8.8 μg/ml) or 800 μM AIdUrd (200 μg/ml) or 400 μM 5′-AdThd (96 μg/ml) or no drug, was added to each well. These concentrations of drugs were selected to give a 2-log reduction in the yield of infectious virus. At the times indicated either 20 μCi L-[^{35}S]methionine for 1 h or 2.5 μCi [^{14}C]glucosamine for 12–24 h or 80 μCi ortho[^{32}P]phosphate for 3–18 h was added to each well. After the labelling period the monolayer was gently rinsed with ice-cold phosphate-buffered saline (PBS) and the cells were lysed with 0.1–0.15 ml of SDS sample buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol, 62.5 mM Tris-HCl; pH 6.8). The cell lysates were boiled for 5 min and frozen at -20°C until analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Preparation of immediate early HSV-induced polypeptides

Confluent monolayers of Vero cells (2×10^5 cells) were infected at 10 p.f.u./cell in 0.1 ml medium containing 50 μg/ml cycloheximide. After 1 h adsorption, the monolayers were washed to remove unattached virus and 1 ml medium containing 50 μg/ml cycloheximide and 800 μM AIdUrd, 400 μM 5′-AdThd, 25 μM IdUrd or no drug was added. Cells were incubated at 37°C for 5 additional hours, at which time the cycloheximide was removed by washing with fresh serum-free medium. Labelling of proteins was carried out by exposing these monolayers to serum-free medium containing 10 μCi [^{35}S]methionine/ml with or without the appropriate antiviral drug for 1 h. Labelled proteins were harvested and prepared for SDS-PAGE as described above.

SDS-PAGE

Slab gel electrophoresis was performed using the method of Laemmli [17] or the modification of Gibson and Roizman [10] using *N,N′*-diallytartardiamide (DATD) instead of *N,N*-methylene-bis-acrylamide. Gels were cast with 5%, 8.5%, 10% or 15% acrylamide or as a linear gradient from 5–15% acrylamide as indicated in the text. The gels were dried after electrophoresis and analyzed by contact autoradiography using Kodak film. The bands appearing on the autoradiograph were quantitated using Joyce-Loebel scanner densitometer and integrating the area under the peaks. Accurate quantitation of protein bands required the utilization of many gels at various concentrations of acrylamide which allowed for the best resolution of specific proteins.

Preparation of labelled virions

Vero cells in 75 mm^2 flasks (1.5×10^7 cells) were infected with HSV-1 (m.o.i. 20) as described above. After a 1 h adsorption period at 37°C, medium containing 20 μCi/ml [^{14}C]amino acids, 2% dialyzed calf serum and 25 μM IdUrd or 800 μM AIdUrd or 400 μM 5′-AdThd was added to each flask. Untreated and drug-treated virus were prepared essentially as described by Spear and Roizman [30]. Briefly, cells infected for 24 h were harvested by scraping and centrifugation at 500 × *g* for 10 min. The cell pellet was resuspended in hypotonic buffer (20 μM Hepes, pH 7.5, 1 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM $CaCl_2$) and allowed to swell on ice for 10 min. The cells were Dounce homogenized and the nuclei pelleted by centrifugation at 10,000 × *g*. The supernatant containing virus was loaded onto 10–22% linear gradients of Dextran 10 in 1 mM sodium-phosphate buffer, pH 7.4. Centrifugation was carried out for 60 min at 100,000 × *g* in a SW 50.1 rotor. The virus-containing bands were located visually, collected, adjusted to 60% (w/v) sucrose, placed in centrifuge tube and overlaid with a step gradient of sucrose (40%, 20%, 10%) in 1.0 mM Tris-HCl, pH 7.0. The gradients were centrifuged for 15–18 h at 100,000 × *g* and the material banding at the interface between 60% and 40% sucrose was removed. This material was centrifuged at 100,000 × *g* for 60 min to pellet the virus. The pellets were resuspended in 0.5 ml 1.0 mM Tris-HCl, pH 7.5, and 0.5 M urea, layered over 4.5 ml 15% sucrose and centrifuged at 100,000 × *g* for 90 min. The resulting viral pellets were prepared for SDS-PAGE as described above. The purity of the virus prepara-

tions was checked by electron microscopic examination and by monitoring the disappearance of labelled ([^{3}H]amino acids) proteins from uninfected cells which had been added at the beginning of the purification.

RESULTS

Effects of antiviral drugs on protein synthesis in infected cells

Total protein synthesis in infected cells was analyzed by incorporation of [^{35}S]methionine or [^{14}C]amino acids into TCA-precipitable material in 30 min at various times after infection in the presence or absence of drugs. At concentrations of drugs which gave a 2-log reduction in the yield of HSV-1 (25 μM IdUrd, 800 μM AIdUrd, 400 μM 5′-AdThd) no effect was observed on protein synthesis in uninfected cells, nor was protein synthesis in infected cells significantly reduced in the presence of the drugs (Fig. 1). In order to investigate the effect of drugs on the synthesis of individual proteins, [^{35}S]-methionine-labelled polypeptides from HSV-1-infected Vero cells were examined by SDS-PAGE and autoradiography specific infected cell proteins (ICPs) were identified by migration on SDS gels and by kinetics of synthesis [12–14] (see Methods for details). The inhibition was greatest with IdUrd and less with both AIdUrd and 5′-AdThd (Table 1). The degree of inhibition appeared to be related to the time of synthesis of the ICPs. Proteins designated as α proteins were not affected or only slightly reduced. Proteins designated β or γ in general were reduced significantly, with γ proteins being reduced

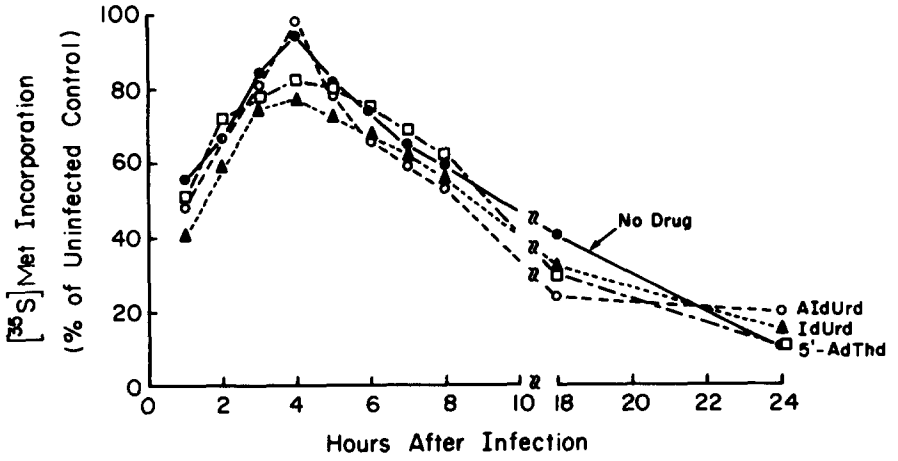

Fig. 1. Protein synthesis in HSV-1-infected Vero cells in the presence or absence of nucleoside analogues. Cells were infected with 10 p.f.u./cell at 0 h and fresh medium with or without drugs (800 μM AIdUrd, 400 μM 5′-AdThd or 25 μM IdUrd) was added at 1 h. At indicated times, cells were labelled for 30 min with [^{35}S]methionine and trichloroacetic acid-precipitable counts were determined and plotted as percent of uninfected controls. Each point is the average of three separate experiments.

TABLE 1

Amount of protein synthesized (% of amount from untreated infected cells) with nucleoside treatment[a]

ICP No.[b]	Kinetic class	AIdUrd	IdUrd	5'-AdThd
4		99 ± 3	95 ± 5	100 ± 2
0		100 ± 2	96 ± 6	100 ± 2
27	α	100 ± 2	100 ± 2	100 ± 2
22 h		100 ± 2	100 ± 2	100 ± 2
34 h		102 ± 2	101 ± 2	103 ± 2
6		76 ± 3	46 ± 14	70 ± 10
8		85 ± 3	79 ± 2	84 ± 4
26		70 ± 5	76 ± 3	75 ± 5
29/30	β	86 ± 2	83 ± 3	89 ± 7
36		123 ± 6	106 ± 3	113 ± 4
39		100 ± 2	100 ± 3	100 ± 2
40/41		66 ± 4	75 ± 6	99 ± 3
5		65 ± 2	55 ± 8	66 ± 6
10		82 ± 3	80 ± 4	82 ± 2
11/12		55 ± 5	89 ± 6	90 ± 3
20		85 ± 7	83 ± 7	95 ± 6
23		58 ± 3	64 ± 4	70 ± 2
32	γ	46 ± 6	35 ± 4	44 ± 2
35 h		100 ± 2	100 ± 2	100 ± 2
37		79 ± 6	83 ± 4	100 ± 5
43		77 ± 3	68 ± 6	87 ± 3
44		69 ± 4	58 ± 6	86 ± 6

[a] Proteins were labelled with [^{35}S]methionine and analyzed as described in Methods. For identification of kinetic class, a 1-h labelling period at various times after infection was used as described in Methods. For quantitation of total β and γ proteins synthesized, cells were exposed to label 2–24 h after infection; and for α proteins, a 1-h labelling period was used as described in Methods.

[b] h = identified as host protein by Honess and Roizman [12].

more than β proteins. The reduction in synthesis of ICPs, as measured by pulse-chase experiments, was not the result of a change in the kinetics of synthesis, or leakage from the cell but rather an overall decrease in the amount of protein synthesized (data not shown).

There appeared to be at least two exceptions to the observed inhibition of protein synthesis. ICP 36 (M_r = 42,000) was increased and ICP 39 (M_r = 36,000) was not reduced during drug treatment. Fig. 2 shows the radioactivity incorporated into ICP 36 during 30 min labelling periods at different times after infection in the presence or absence of the drugs. The increase in ICP 36 appeared to be due to a higher level of synthesis late in infection.

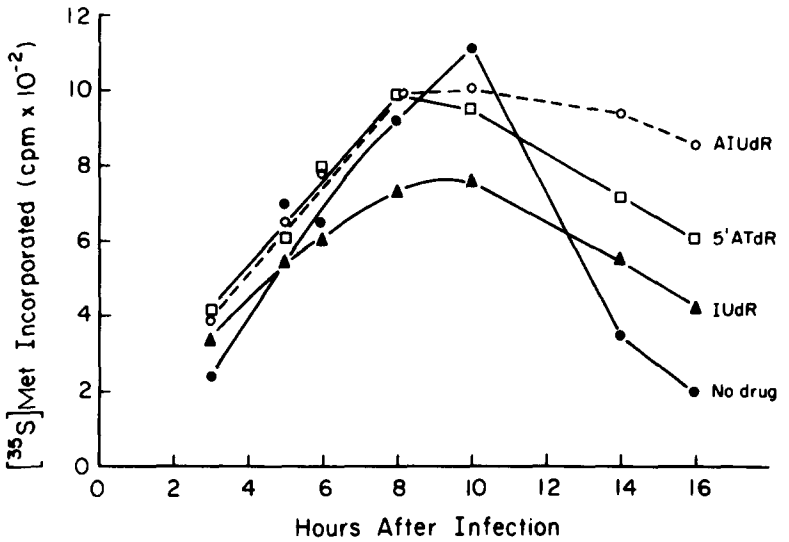

Fig. 2. Kinetics of synthesis of ICP 36 in the presence or absence of drug. Cells were infected and exposed to drugs as described in Fig. 1. The amount of label incorporated in 30 min into band 36 at the indicated times was determined by cutting the band from the gel and counting the slice in a Beckman liquid scintillation counter.

Synthesis of infected cell proteins in Vero cells infected with HSV-1 derived from drug-treated cells

HSV-1 was grown in Vero cells in the presence of the nucleoside analogues AIdUrd, IdUrd, or 5′-AdThd. The harvested virions (i.e. second generation virions) were tested for their ability to induce and/or encode infected cell proteins (ICPs) in non-drug-treated Vero cells. Since drug treatment resulted in reduced yields of virus (no drug: 1.4×10^8 p.f.u./ml; AIdUrd: 7×10^5 p.f.u./ml; IdUrd: 7×10^5 p.f.u./ml; and 5′-AdThd: 2×10^6 p.f.u./ml), second generation virus was purified and concentrated by centrifugation as described in Methods. Labelling with [^{35}S]methionine revealed that the synthesis of all ICPs was reduced in cells infected with second generation virions, with AIdUrd–HSV-1 or 5′-AdThd–HSV-1 being more efficient in inducing viral proteins than IdUrd–HSV-1 (Fig. 3). It should be noted that, although the cells were infected at the same multiplicity of infection as determined by plaque assay, second generation drug-substituted virions had increased particle to p.f.u. ratios: no drug treatment 300 : 1, AIdUrd-substituted virions 1500 : 1, IdUrd-substituted 1900 : 1, 5′-AdThd-substituted 800 : 1. In addition, second generation drug-substituted virions were much slower in producing plaques, requiring as long as 72 h for plaque development (vs. 48 h for non-drug-treated virions prepared in the same manner).

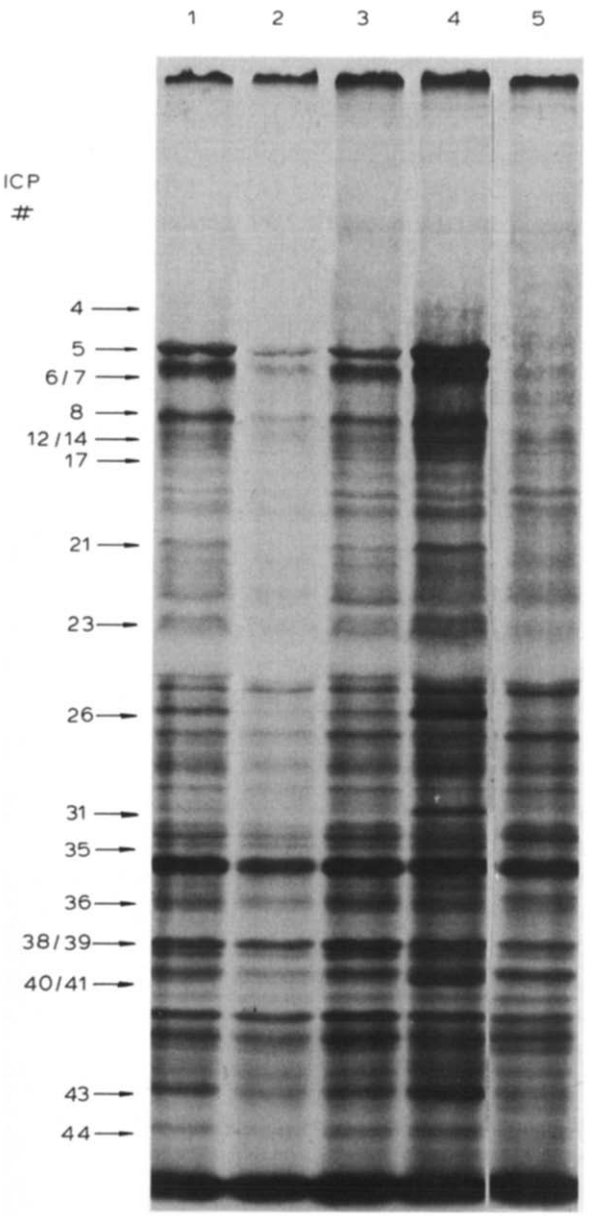

Fig. 3. Autoradiogram of electrophoretically separated [^{35}S]methionine-labelled proteins from Vero cells infected with HSV-1. Cells were infected at 10 p.f.u./cell in the absence of drugs with HSV-1 derived from cells treated with drugs. Label was present 2–12 h after infection. Samples were prepared for electrophoresis as described and separated on a gradient gel of 5–15% acrylamide. Lysates from cells infected with second generation HSV-1 from 5'-AdThd-treated cells (1), IdUrd-treated cells (2), AIdUrd-treated cells (3), untreated cells (4), or from uninfected cells (5).

Virion proteins of HSV-1 from drug-treated Vero cells

HSV-1-infected cells were labelled with [^{35}S]methionine or [^{14}C]amino acids in the presence or absence of AIdUrd, IdUrd or 5′-AdThd and the progeny virus was purified and analyzed by SDS-PAGE and autoradiography. When equal numbers of particles were analyzed, the protein patterns of virus from drug-treated cells showed a decrease in many bands, although some bands were unaffected or were significantly increased (Fig. 4). With AIdUrd–HSV-1 two proteins appeared to be present at higher levels than control, VP 7 (238% increase) and VP 19c (111% increase). Another protein band, VP 19e, was increased in preparations of 5′-AdThd–HSV-1 (160%). The protein pattern with IdUrd–HSV-1 appeared normal in distribution of bands, although the amount of label in the bands was less than control. In addition, protein band VP 5/6 was significantly reduced with AIdUrd–HSV-1 and 5′-AdThd–HSV-1. These unusual protein patterns suggest that abnormal assembly or maturation of the virions is taking place in the presence of the drugs.

Modification of infected cell proteins

Since small changes in HSV-1-induced proteins could be reflected in the ability of the proteins to be processed to glycoproteins or phosphoproteins, the incorporation of ortho [^{32}P]phosphate or [^{14}C]glucosamine into electrophoretically separated ICPs was investigated. The results of labelling with ortho[^{32}P]phosphate are shown in Fig. 5. Treatment with IdUrd resulted in no significant reduction in the amount of label incorporated into ICPs. Second generation virions derived from IdUrd-treated cells induced the synthesis of only a slight amount of the phosphoproteins ICPs 4 and 6 (Fig. 5, lane 6). Treatment with AIdUrd or 5′-AdThd resulted in no significant reduction in the incorporation of ^{32}P, whereas second generation virions from AIdUrd (lane 5) or 5′-AdThd (lane 7) treated cells produced abnormal patterns of phosphoproteins. Not only was intensity of labelling reduced in both instances, but also ICP 0/8 was missing and a new band, or an increased phosphorylation of a minor band, appeared with an approximate molecular weight of 30,000 in cells infected with AIdUrd-second generation virions, and ICPs 22, 39 and 44 were missing and a new band appeared at approximately 69,000 MW in cells infected with 5′-AdThd-second generation virions.

Modification by glycosylation of the ICPs was monitored by incorporation of [^{14}C]-glucosamine (Fig. 6). Glycosylation was reduced most by treatment with AIdUrd or IdUrd and less by exposure to 5′-AdThd. Infection with second generation virions resulted in reduced glycoprotein synthesis. The greatest reduction was observed with infection by virions derived from IdUrd-treated cells and less with virions derived from AIdUrd or 5′-AdThd-treated cells. The reduced synthesis of glycoproteins therefore parallels the general reduction in all ICPs synthesized in the second generation systems.

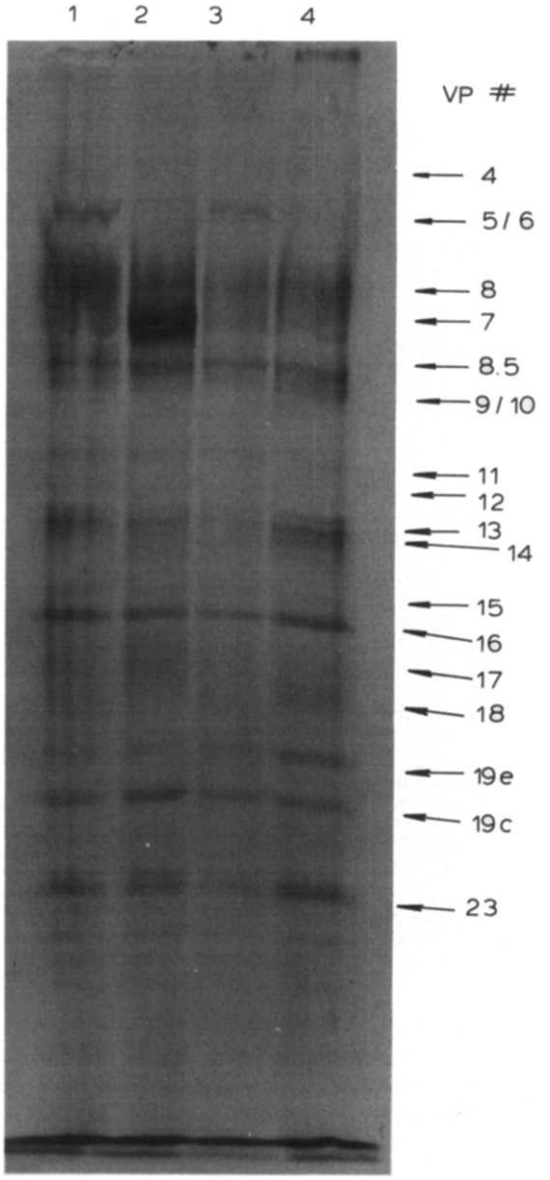

Fig. 4. Autoradiograms of electrophoretically separated [^{35}S]methionine-labelled virion proteins. Cells were infected, drug-treated and labelled as described in Fig. 2. Virions were harvested, purified and particle number determined. Equal numbers of particles were analyzed by SDS-PAGE as described in the text. Virion proteins are designated by number. 1) Virions from untreated cells. 2) Virions from AIdUrd-treated cells. 3) Virions from IdUrd-treated cells. 4) Virions from 5'-AdThd-treated cells.

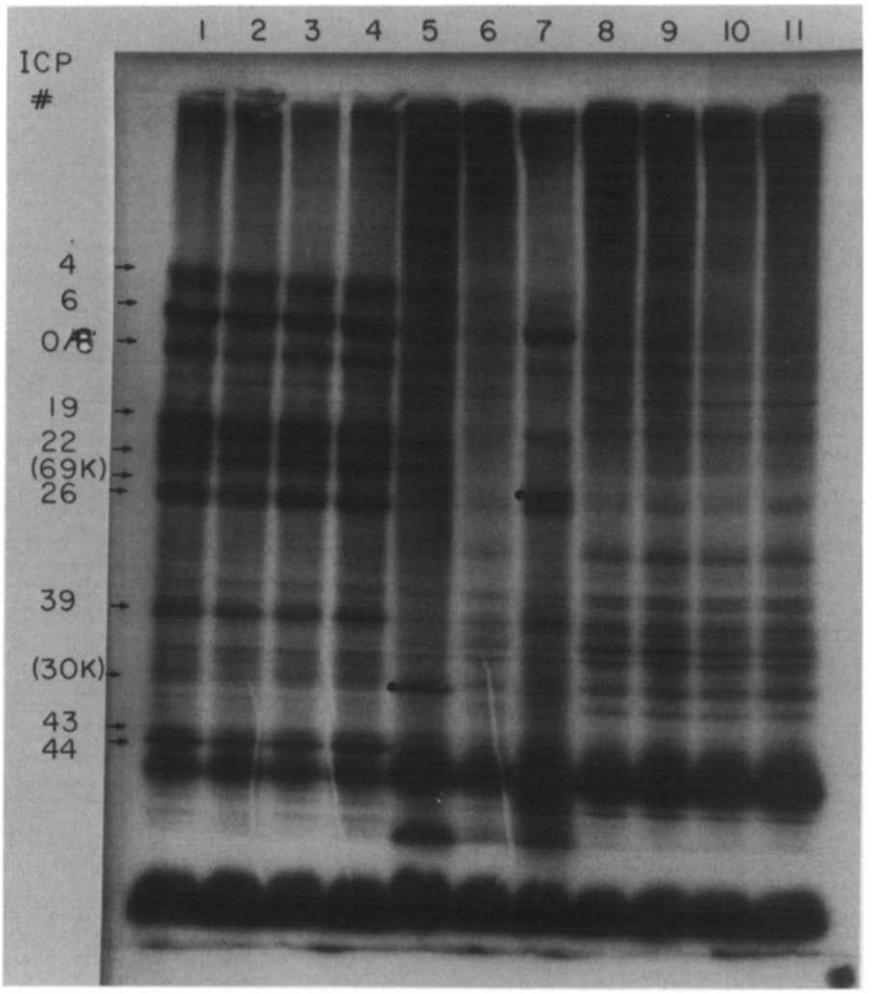

Fig. 5. Autoradiogram of electrophoretically separated ^{32}P-labelled proteins from infected (lanes 1–7) or uninfected (lanes 8–11) Vero cells. Cells were labelled 2–18 h after infection with 80 μCi/ml $^{32}P_i$ in the presence or absence of drugs or virus. SDS-lysates of cells were analyzed by electrophoresis and autoradiography as described in the text. 1) HSV-1 + AIdUrd. 2) HSV-1 + IdUrd. 3) HSV-1 + 5′-AdThd. 4) HSV-1 + no drug. 5) Second generation HSV-1 from AIdUrd-treated cells. 6) Second generation HSV-1 from IdUrd-treated cells. 7) Second generation HSV-1 from 5′-AdThd-treated cells. 8) Uninfected + no drug. 9) Uninfected + AIdUrd. 10) Uninfected + IdUrd. 11) Uninfected + 5′-AdThd.

DISCUSSION

The exposure of HSV-1-infected Vero cells to AIdUrd, 5′-AdThd or IdUrd results in the altered expression of HSV-1-induced β and γ proteins. Unlike other nucleoside analogues which have been shown to inhibit γ protein synthesis by inhibiting DNA

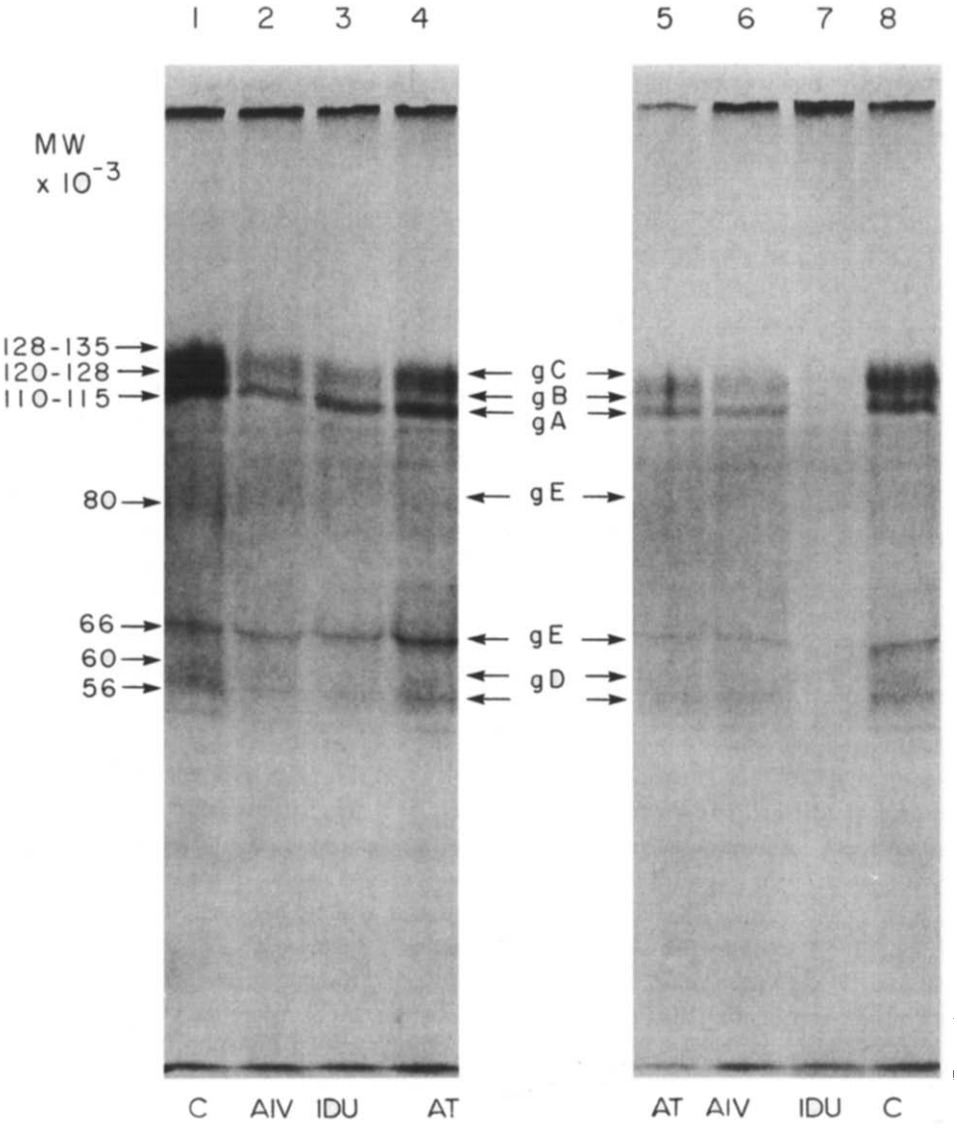

Fig. 6. Autoradiogram of electrophoretically separated [^{14}C]glucosamine-labelled proteins from cells infected by first generation HSV-1 in the presence or absence of drugs (lanes 1–4) or by second generation HSV-1 in the absence of drugs (lanes 5–8). Cells were labelled 2–18 h after infection and SDS lysates were analyzed by electrophoresis and autoradiography as described in the text. 1) HSV-1 + no drug. 2) HSV-1 + AIdUrd. 3) HSV-1 + IUdR. 4) HSV-1 + 5′-AdThd. 5) Second generation 5′-AdThd–HSV-1. 6) Second generation AIdUrd–HSV-1. 7) Second generation IdUrd–HSV-1. 8) Second generation no drug–HSV-1.

synthesis [12], AIdUrd and 5′-AdThd do not significantly reduce herpesvirus-specific DNA synthesis (Otto and Prusoff, manuscript in preparation). Thus the inhibition reported here is probably the result of substitution of the drugs for thymidine in the viral DNA [9]. The substitution of the drugs in the viral DNA, the degree of which corresponds to the degree of reduction in the yield of infectious virus, possibly results in misreading of the new DNA during transcription and replication. Misreading in the coding or non-coding (i.e. regulatory) regions of the viral genome could account for most of the inhibition in protein synthesis (as well as overproduction of ICP 36). Similar results have been reported for IdUrd with other DNA viruses; for example, SV 40 antigen production was inhibited as a result of incorporation of IdUrd [3]; T-2 phage containing IdUrd-substituted DNA was unable to produce normal levels of enzymes [11]; and in pseudorabies virus infection IdUrd treatment resulted in the overproduction of viral antigen [16].

The overproduction of ICP 36 in infected cells treated with these analogues may be the result of a loss of regulation. This is similar to the effect IdUrd has on the synthesis of pseudorabies capsid protein [16]. The synthesis of both proteins continues in drug-treated cells, while it declines in control-infected cells. The herpes-induced polypeptide 36 is interesting for two reasons. It appears to be the only protein overproduced in HSV-1-infected Vero cells in the presence of the drugs and two, it migrates on SDS gels at the position which was identified as the herpes-induced thymidine kinase [14,31]. Indeed, results in our laboratory (Mancini, Otto and Prusoff, Abstract S-43 1982 Ann. Meeting Am. Soc. Micro.) indicate that thymidine kinase activity accumulates in HSV-1-infected LMTK$^-$ cells treated with AIdUrd, or 5′-AdThd to a level comparable to the increase in ICP 36. The exact mechanisms by which this accumulation takes place are unknown at this time, but one can envision a late gene product whose function might be to terminate the expression of genes no longer needed late in infection. The existence of such a gene product has been suggested before, but none has been identified as yet.

In addition to drug effects during first generation protein synthesis, second generation drug-substituted virions exhibit both abnormal virion protein patterns and altered ability to synthesize proteins. The reasons for the apparent abnormal protein composition of drug-substituted virions as determined by SDS-PAGE are unknown at this time. One possibility is photo-activated DNA–protein (capsid protein in particular) cross linking. Since no effort was made to maintain virions in the dark during purification photochemical reactions could easily occur. These reactions involving halogenated pyrimidines in particular have been well documented both in nucleoside–protein interactions [4,5] and in halogenated nucleoside substituted DNA–protein reactions [19]. These reactions can result in covalent linkages between DNA and protein which are not disrupted by processing in SDS. Such complexes would not enter the SDS-polyacrylamide gels and the resulting protein pattern would be deficient in those proteins linked to herpes DNA. We are presently investigating this possibility.

As well as having abnormal virion protein patterns, second generation drug-substituted virions exhibit altered protein synthesis patterns. AIdUrd- or IdUrd-substituted virus

showed a decreased ability to synthesize all viral-induced proteins and glycoproteins. AIdUrd- or 5′-AdThd-substituted virus, in addition, produced unusual phosphoprotein patterns [8]. This was especially interesting with virus from 5′-AdThd-treated cells, since this virus showed no reduction or obvious change in either the [^{35}S]methionine–protein or [^{14}C]glucosamine–glycoprotein patterns. The significance of this is unknown at this time but could indicate changes in the specificity of the viral protein kinase itself or in the proteins which serve as normal substrates for its action. It has been suggested that VP 23 is the protein kinase or at least the functional subunit of the enzyme [18]. Experiments examining in vitro phosphorylations of viral proteins with purified HSV-1 protein kinase [2] may allow us to determine the specific alterations induced by 5′-AdThd.

The level of inhibition of certain β and γ proteins during exposure to the drugs is, by itself, probably not sufficient to account for the 2-log reduction in infectious virus yield. These data suggest that the reduction in virus yield is due to the synthesis of abnormal or non-functional proteins. Obviously, some viral protein functions are lost or altered since ICP 36 is overproduced, infectivity is lost and progeny virions have altered protein patterns and produce unusual phosphoprotein patterns.

Other functions of HSV-1-induced proteins probably are affected by treatment with these drugs, for example: DNA polymerase, alkaline DNAse, or other herpes induced enzyme activities, both those that have been identified as well as those as yet undefined. Any or all of these may be important in the antiviral effects of these drugs. The fact that some proteins are affected to a greater degree than others may be a clue or may merely reflect thymidine rich regions of the viral genome. Further experiments are being done which hopefully will answer these questions.

ACKNOWLEDGEMENTS

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