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Inhibition of Viral RNA Methylation in Herpes Simplex Virus Type 1-Infected Cells by 5′ S-Isobutyl-Adenosine

B. JACQUEMONT* AND J. HUPPERT

Unite de Virologie, INSERM, Groupe de Recherche 33-CNRS, 69008 Lyon, France

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5′ S-isobutyl-adenosine (SIBA), a structural analogue of S-adenosylhomocysteine, reversibly blocks the multiplication of herpes simplex type 1 virus. In the presence of SIBA, viral protein synthesis is inhibited. After removing SIBA the synthesis of proteins starts rapidly again. The new polypeptides are mainly α proteins (Honess and Roizman, J. Virol. 14:8-19, 1974), normally the first to be synthesized after infection. The rapid synthesis of proteins after release of inhibition seems to be directed by mRNA formed in the presence of SIBA as indicated by experiments using actinomycin D but which was undermethylated as shown by analysis of methyl groups on RNA. SIBA inhibits the methylation of mRNA and especially that of the 5′ cap. Capping of mRNA thus seems to be essential for efficient translation. The analogue affected various methylations to different extents.

Methylation of cellular or viral mRNA's seems to be a general event necessary for expression of the specific function. This was first established with several RNA or DNA viruses, for example, reovirus (19), simian virus 40 (13), adenovirus (5), or vaccinia (21). In addition, it has been shown that in vitro methylation of the 5′-terminal nucleotide of cellular mRNA is necessary for its translation (2). The tRNA also has to be methylated in order to be active. The tRNA methyl transferase activity of some neoplastic tissues and cells is increased in comparison with that of normal tissues (4, 10). The process of RNA methylation is important because it introduces several supplementary possibilities for regulation at the levels of transcription and translation.

Most of the available information comes from experiments using in vitro (cell-free) systems that allow a good analysis of the biochemical events involved in methylation, but which may or may not correspond to the situation in living cells.

In this paper we will describe experiments performed in vivo on cells in culture. We used HEp2 cells, herpesvirus type 1 [HSV-1(F)], and 5′ S-isobutyl-adenosine (SIBA), an analogue of S-adenosylhomocysteine (SAH). In vivo, S-adenosylmethionine (SAM) is the main donor of methyl groups. SAH, the product of the methyl transfer reaction, inhibits methylation but is itself normally metabolized either into degradation products or back to SAM. The analogue SIBA, however, is not metabolized. It was selected from a series of SAM or SAH analogues which have been synthesized and described (7). SIBA was shown to block in vitro the methylation of *Escherichia coli* tRNA (7) and to prevent in vivo the oncogenic transformation of chicken embryo cells infected with Rous sarcoma virus (17). We will show that SIBA inhibits reversibly HSV production by blocking the methylation and especially the capping at the 5′ end of viral mRNA.

MATERIALS AND METHODS

Solutions and chemicals. Standard saline citrate (1x SSC) consisted of 0.15 M sodium chloride and 0.015 M sodium citrate. Reticulocyte standard buffer (RSB) consisted of 0.01 M sodium chloride, 2.5 mM magnesium chloride, and 0.01 M Tris-hydrochloride, pH 7.5. Crystallized DNase free of RNase was from Worthington Biochemicals; [5-^{3}H]uridine (specific activity, 24 Ci/mmol) and [^{32}P]phosphate were from Commissariat à l'Energie Atomique (France), [*methyl*-^{3}H]methionine (14 Ci/mmol), [^{35}S]methionine (647 mCi/mmol), and ^{14}C-labeled amino acids (50 μCi/mmol) were obtained from New England Nuclear; actinomycin D was a gift of Merck, Sharpe and Dohme; and SIBA was a gift of Lederer and Gero (Gif/Yvette, France).

Cells and virus. The procedures for propagation and maintenance of human epidermoid carcinoma no. 2 (HEp2) cells and production, assay and properties of the F strain of herpes simplex virus HSV-1(F) were described elsewhere (11).

Radiolabeling and purification of enveloped virus particles. Cells infected with 10 PFU of HSV-1 per cell were labeled from 4 to 48 h postinfection with ^{14}C-labeled amino acids (1 μCi/ml), in medium 199 containing one-fourth of the normal concentration of amino acids. Enveloped virions were purified from infected cells as described by Spear and Roizman

(20) except that virions obtained after dextran banding were used directly.

Preparation of viral DNA. The procedure of preparation and purification of viral DNA from nucleocapsids was the same as previously described (11), except that the final product was digested with RNase. Then DNA was repurified by isopycnic banding in CsCl to suppress any possible contamination by cellular DNA.

Labeling of RNA and proteins synthesized by infected cells. Confluent HEp2 cell monolayers were infected with 50 PFU/cell. After 2 h at 37°C with agitation, the monolayers were rinsed with 5 ml of maintenance medium per flask, replenished with the same volume of maintenance medium, and reincubated at 37°C. At the end of the labeling period, the cells were rinsed three times with ice-cold phosphate-buffered saline and mechanically stripped off the flask. Cells with labeled RNA were opened with 0.5% deoxycholate, treated with DNase (50 μg/ml) and solubilized with 0.5% sodium dodecyl sulfate (SDS). Cells with labeled proteins were solubilized in a small volume of 2% SDS, 5% β-mercaptoethanol, 0.05 M Tris-hydrochloride, pH 7, for subsequent electrophoresis on 9% polyacrylamide gels (6). The times at which labeled precursors were added or removed are stated in hours after the exposure of cells to virus.

RNA purification. To extract total RNA from infected cells, the pellet was suspended in RSB, lysed by the addition of sodium deoxycholate (0.5% final concentration), and digested with RNase-free DNase (50 μg/ml, room temperature) to diminish viscosity. The material was then extracted with SDS, phenol, and chloroform, precipitated with ethanol, dissolved in RSB buffer, and then extracted again as previously.

Hybridization of labeled RNA to DNA on filters. RNA was hybridized to DNA from HSV-1 and from *E. coli* fixed on nitrocellulose filters (9). The DNAs were fixed to 25-mm Schleicher and Schuell B6 filters (12). Small disks were punched out and placed in 200 μl of hybridization buffer (0.75 M NaCl, 5 mM EDTA, 0.25% SDS, 0.01 M Tris-hydrochloride, pH 7.5) containing undenatured RNA. After incubation for 20 h at 66°C, the disks were removed, washed two times for 30 min in 2x SSC, digested with RNase A (25 μg/ml) for 1 h at room temperature, washed, and dried, and the radioactivity was counted. The nonspecific counts fixed on *E. coli* DNA were subtracted.

Chromatography of methylated nucleotides. Samples of RNA were dissolved in 0.3 N potassium hydroxide and hydrolyzed overnight at 37°C, chilled samples were neutralized by the addition of cold 0.5 N perchloric acid, and the resulting precipitate was removed by centrifugation at 3,000 rpm for 20 min. The supernatant was neutralized, diluted with 10 mM Tris-hydrochloride, pH 7.5, containing 7 M urea, and was applied to a DEAE-Sephadex column (0.9 by 15 cm; A25, Pharmacia) equilibrated with the same buffer. The column was washed with buffer and samples were eluted with 200 ml of a 0 to 0.4 M NaCl linear gradient in 10 mM Tris-hydrochloride, pH 7.5, and 7 M urea (15). A known mixture of nucleotides with different phosphate charges was added as controls.

Chromatographic determination of methylated bases. Samples of RNA or nucleotides were dissolved in 88% formic acid and hydrolyzed for 2 h at 100°C. The volumes of the samples were brought back to 20 μl after evaporation to dryness and chromatographed by thin-layer chromatography for 4 h on silica gel plates with ethyl acetate, methanol, water, 88% formic acid (100:25:20:1; vol/vol). Known methylated and nonmethylated bases were used as controls (14).

RESULTS

Effect of SIBA on cell survival and on herpesvirus production. HEp2 cells in monolayer cultures were overlaid with medium containing 1 mM SIBA and were incubated at 37°C. This concentration of the drug was the minimal amount necessary for efficient inhibition of protein synthesis and corresponded also to the maximum solubility of the product. At 0.5 mM only partial inhibition took place. At various times the medium was changed, to remove the inhibitor, and cells were further cultivated to estimate survival and multiplication. Contact with SIBA for 12 h had no effect on later cell multiplication. However, after contact for longer periods the cells survived for several days but no longer multiplied.

To test the effect of SIBA on HSV multiplication, HEp2 cells were infected, the inhibitor was added 2 h later, and the amount of virus produced was measured at 12 and 24 h postinfection (Table 1). Virus production was decreased by 88.4 and 98.2%, at 12 and 24 h, respectively, as compared with controls. When SIBA was removed after 24 h, virus production started again, but it was delayed; none was observed at 24 h, and at 72 h the yield was 75% of the control. SIBA inhibits HSV production reversibly.

Effect of SIBA on viral RNA synthesis. SIBA, like adenosine and adenosine analogues (16), was shown to reduce the cell permeability for nucleotides and to modify the intracellular pool. Therefore, it was not possible to estimate RNA synthesis by a straightforward measure of radioactive uridine incorporation into RNA.

As the permeability of the cell to [^{3}H]uridine is affected by the presence of SIBA, the effect of the drug on cellular RNA synthesis was carried out on cells whose intracellular pool had been equilibrated with [^{3}H]uridine before the addition of the drug. To cells infected for 7 h, [^{3}H]uridine was added and SIBA was introduced either simultaneously or 30 min later. Intracellular acid-soluble and acid-insoluble radioactivities were measured after 30, 60, and 90

TABLE 1. *Effect of SIBA on herpes simplex type 1 replication*

Sample	Virus produced (PFU/ml)			
	Time of contact with SIBA (h)		Time of incubation after removal of SIBA (h)	
	12	24	24	72
Control	1.9×10^7	2.9×10^7	2.1×10^7	8.5×10^7
+ SIBA	0.22×10^7	0.052×10^7	0.096×10^7	6.3×10^7

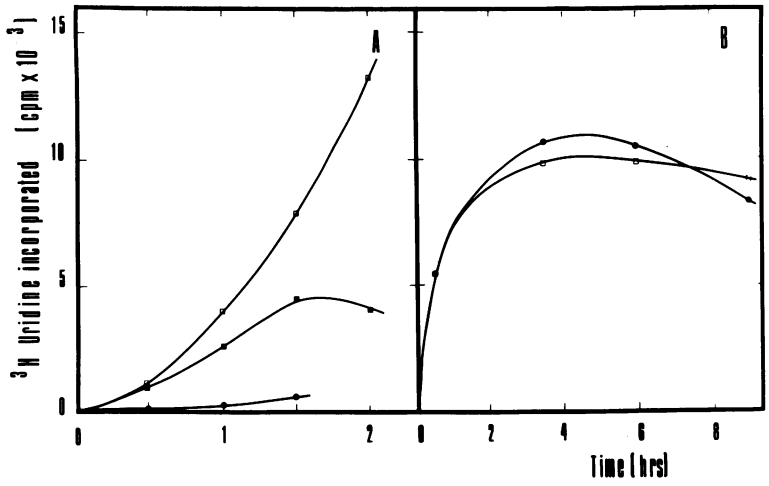

FIG. 1. *Effect of SIBA on uridine incorporation by HSV-1-infected cells. HEp2 cells were infected with HSV-1 (50 PFU/cell). (A) At 7 h postinfection [^{3}H]uridine (10 μCi/ml) was added; 30, 60, 90, and 120 min later acid-insoluble radioactivity of cells was determined. Open squares, no SIBA; closed circles, 1 mM SIBA was added simultaneously with [^{3}H]uridine; closed squares, SIBA added 30 min after [^{3}H]uridine. (B) At 2 h postinfection [^{3}H]uridine (10 μCi/ml) was added; 30 min later, [^{3}H]uridine was removed and fresh medium containing 1 mM SIBA was added. At the time indicated acid-insoluble radioactivity was determined. Open squares, no SIBA; closed circles, SIBA-treated cells.*

min of labeling. Portions from solubilized cells were precipitated with trichloroacetic acid and filtered on membrane filters (HAWP, Millipore Corp.). The trichloroacetic acid was removed from the filtrate by repeated extraction with ether and a portion was counted. Trichloroacetic acid on filters was removed by washing with ethanol and the filters were counted. SIBA added simultaneously decreased the acid-soluble and -insoluble radioactivities in the cells by 95%. However, when 30 min were allowed for [^{3}H]uridine to penetrate before the addition of the drug, incorporated acid-insoluble counts were reduced only by about 30% (Fig. 1A). In another experiment, [^{3}H]uridine was removed after 30 min, and cells were washed and incubated further in the presence of SIBA. No difference was observed between treated and untreated cells in the incorporation of [^{3}H]uridine into acid-insoluble material (Fig. 1B). This suggests that RNA synthesis continued in the presence of SIBA and that the drug provoked a change in permeability for uridine.

In order to verify the effect of SIBA on viral RNA synthesis, cells infected for 7 h were labeled during 1 h with ^{32}P (25 μCi/ml) in medium without phosphate, in the presence of SIBA added at the same time as the label, or in its absence. Increasing quantities of ^{32}P-labeled RNA were hybridized to a constant amount of HSV-1 DNA fixed on filters. The hybridization was very similar in both samples (Fig. 2). Therefore we conclude that SIBA, at least for a time, does not affect viral RNA synthesis to a large extent.

Effect of SIBA on viral protein synthesis. SIBA was added at 2 h after infection and protein synthesis was estimated by 30-min pulse-labeling with ^{14}C-labeled amino acids (0.5 μCi/ml). At 10 h after infection protein synthesis was inhibited by 98% (Fig. 3). We verified experimentally that SIBA itself does not modify the pool of the intracellular amino acids. When the drug was removed at this time and the cells were fed with fresh medium containing five times the normal concentration of methionine,

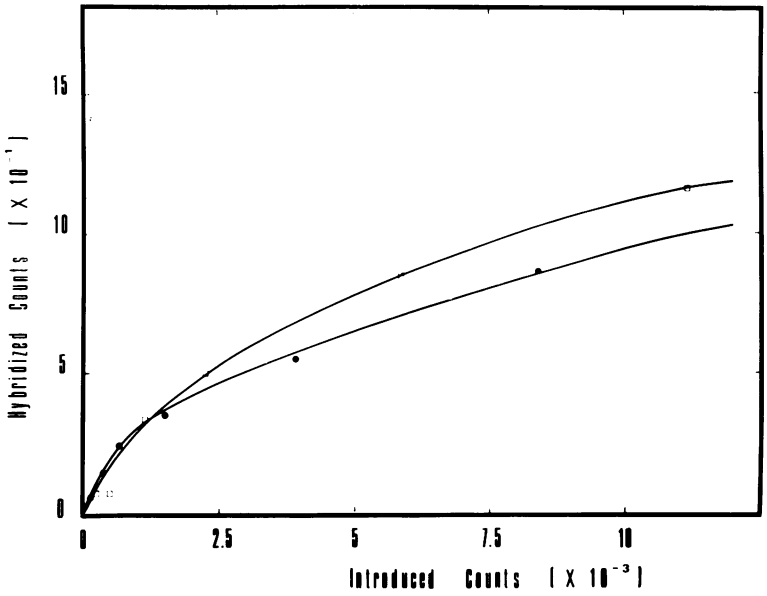

FIG. 2. *Hybridization of labeled RNA from HSV-1-infected cells to HSV-1 DNA on filters. HEp2 cells were infected as described in the legend of Fig. 1. At 7 h postinfection, ^{32}P (25 μCi/ml) was added; 1 h later RNA was extracted and purified. (Specific viral RNA before hybridization represents only a fraction of the total counts, the rest being cellular.) Increasing amounts of counts were hybridized to filters containing a constant amount (1 μg/filter) of unlabeled viral DNA. RNase-resistant counts were plotted against the total number of counts introduced. Symbols: Open squares, RNA from infected cells without SIBA; closed circles, RNA from infected cells labeled in the presence of SIBA added at 7 h.*

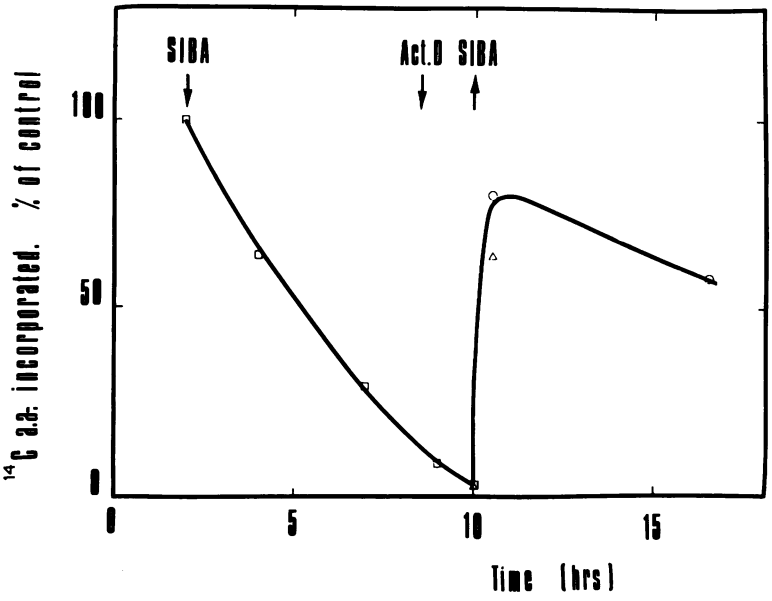

FIG. 3. *Effect of SIBA on ^{14}C-labeled amino acid incorporation. HEp2 cells were infected with HSV-1 (50 PFU/cell). A mixture of ^{14}C-labeled amino acids (0.5 μCi/ml) was added at indicated times and 30 min later acid-insoluble radioactivity was counted. To some cultures SIBA was added 2 h after infection and removed at 10 h. Actinomycin D, when used, was added at 8.5 h. The counts incorporated by SIBA-treated cells are expressed as the percentage of the corresponding samples of untreated infected cells. Symbols: Open squares and open circles, no actinomycin D; open triangles, actinomycin D.*

amino acid incorporation started again and reached about 80% of the control value. It was possible that the postinhibitional synthesis could be directed by a "de novo" transcribed viral mRNA. To verify this hypothesis, RNA synthesis was blocked by actinomycin D (10 μg/ml) added 1.5 h before removal of SIBA and was maintained thereafter. Under these conditions amino acid incorporation was observed reaching 64% of the control values (Fig. 3).

The viral proteins synthesized after release from 19 h of SIBA inhibition were analyzed by electrophoresis on SDS-acrylamide gels. It can be seen (Fig. 4) that polypeptides 4, 27 and 6, 8 were synthesized in a larger amount than in untreated infected cells. These are the so-called

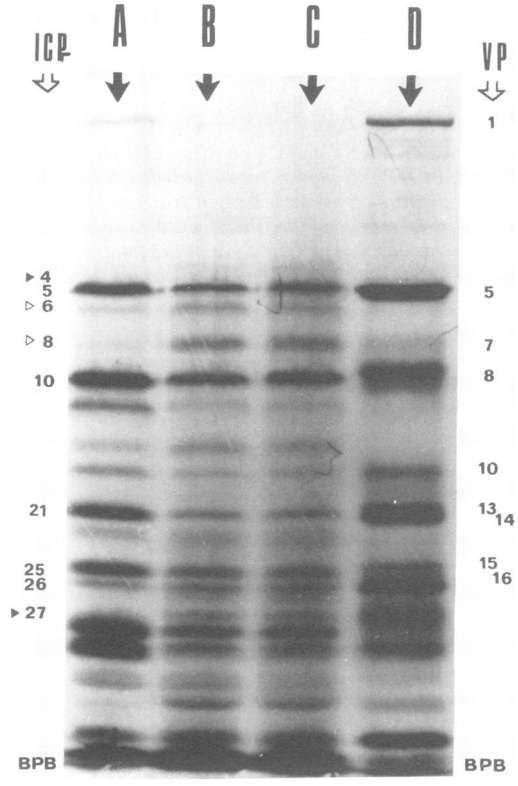

FIG. 4. *(A) Autoradiogram of a 9% polyacrylamide gel slab containing electrophoretically separated polypeptides from HSV-1-infected cells labeled with ^{14}C-labeled amino acids between 19 and 19.5 h postinfection; (B) cells treated with SIBA from 2 to 19 h and labeled for 30 min between 19 and 19.5 h postinfection; (C) cells as for (B) but actinomycin D was present in the medium at the moment of reversion; (D) viral proteins from purified enveloped virions. Arrows to the left of the figure indicates ICP present in greater amount in SIBA-treated than in untreated infected cells. Abbreviations; VP, viral proteins; ICP, infected cell proteins according to Honess and Roizman (8).*

α and β polypeptides (8), the earliest groups of viral polypeptides made in the infected cells. The electrophoresis pattern was similar when SIBA was removed in the presence or absence of actinomycin D.

Effect of SIBA on methylation. HSV-infected cells were treated with 1 mM SIBA at 2 h postinfection and at 8 h were labeled for 30 min simultaneously with [*methyl*-^{3}H]methionine (10 μCi/ml) and [^{35}S]methionine (2 μCi/ml). [^{35}S]methionine will be specifically incorporated into protein for protein synthesis, whereas the [*methyl*-^{3}H]methionine will be used both as a methyl donor and for protein synthesis. Therefore the difference between the percent inhibition of ^{3}H and ^{35}S incorporation into proteins from cells in the presence and absence of SIBA gives a measure of the effect of the drug on protein methylation and on protein synthesis. Adenosine and guanosine (10^{-4} M) and sodium formate (20 mM) were added to the medium to reduce the incorporation into the purine ring. Methionine concentration of the medium was reduced to one-tenth the usual concentration to increase the labeling efficiency.

Proteins were separated from lipids and nucleic acids by extraction with chloroform-methanol (2:1; vol/vol), hydrolyzed with 0.5 N $HClO_4$ at 80°C, and sedimented and dissolved in alkali. RNA was extracted and purified. The ^{35}S radioactivity was used to follow the elimination of proteins.

From data summarized in Table 2 the following is seen in the presence of SIBA. (i) Protein synthesis (estimated by acid-insoluble ^{35}S counts) was reduced by 41.3% in normal medium and by 63.5% in medium poor in methionine. (ii) Newly synthesized proteins were methylated (presence of ^{3}H counts). This methylation was only slightly inhibited: 6.8% or 4.7% in normal or low methionine medium, respectively (difference between acid-insoluble ^{3}H and ^{35}S counts). (iii) RNA methylation was inhibited by 65.4% (^{3}H counts on purified RNA in the absence of ^{35}S).

We conclude that SIBA inhibits protein synthesis and RNA methylation roughly to the same extent, but that protein methylation is only slightly affected.

The next question therefore was whether SIBA acts on all methyl receptors in RNA or whether it has a selective effect on some nucleotides.

Effect of SIBA on site of RNA methylation. Infected cells were pulse-labeled with [*methyl*-^{3}H]methionine as previously but at various times after the addition of SIBA. The absence of protein contamination on the RNA was insured

TABLE 2. *Inhibition of proteins and RNA methylation by SIBA*

Substance	SIBA	Concn of methionine in the medium	cpm of:		Inhibition of (%):	
			^3H	^{35}S	Protein synthesis[a] (%)	Methylation (%)
Proteins	−	Normal	1,581	3,436		
	+	Normal	821	2,016	41.3	6.8[b]
	−	1/10th	15,290	30,377		
	+	1/10th	4,857	11,078	63.5	4.7[b]
RNA	−	1/10th	605	4		
	+	1/10th	209	3		65.4[c]

[a] Calculated from ^{35}S incorporation.
[b] Calculated by difference between the percent inhibition of ^3H and ^{35}S incorporation into proteins.
[c] Calculated from ^3H incorporation.

by extensive treatment with Pronase. This experiment shows (Fig. 5) that RNA methylation was inhibited with a maximum efficiency at 7 h.

To determine the position of the methyl group in the RNA molecule, two types of experiments were carried out: (i) acid hydrolysis of RNA to analyze separated bases; and (ii) alkaline hydrolysis to remove nucleotides not included in the cap specific for mRNA (15).

A *methyl*-^3H-labeled RNA extracted from cells treated with SIBA for 7 h and from untreated controls was hydrolyzed with HCOOH and chromatographed as described in Materials and Methods. Radioactivity was found in spots with R_f values similar to those of authentic m7G and m6A. In addition, there was also radioactivity that remained at the origin, at the same spot where unresolved methylated pyrimidines were found. Furthermore, inhibition of methylation of m7G was more pronounced (45%) than that of m6A (2%) in the presence of a concentration of SIBA which inhibited total methylation of RNA by 50%.

A similar sample of these RNAs was submitted to alkaline hydrolysis and chromatographed on a DEAE-Sephadex column. The elution with a NaCl gradient provided, in control RNA, five peaks corresponding to phosphate charges: −2, −3, −4, −5, and −6 (Fig. 6A) and only two peaks (charges −2, −3) in RNA from SIBA-treated infected cells (Fig. 6B). The inhibition of methylation was 28.7% and 27% for peaks −2 and −3, respectively, and 100% for the others.

The capping, that is, the formation on the 5′ end of the mRNA molecule of a structure of type m7G(5′)ppp(5′)X^{m}pY^{m}pNp nucleotide, appeared to be the structure most susceptible to the action of SIBA. HSV mRNA normally contains a cap at the 5′ end (1).

To establish that the absence of capping was effectively the main cause of inhibition of viral protein synthesis, the eventual methylation of

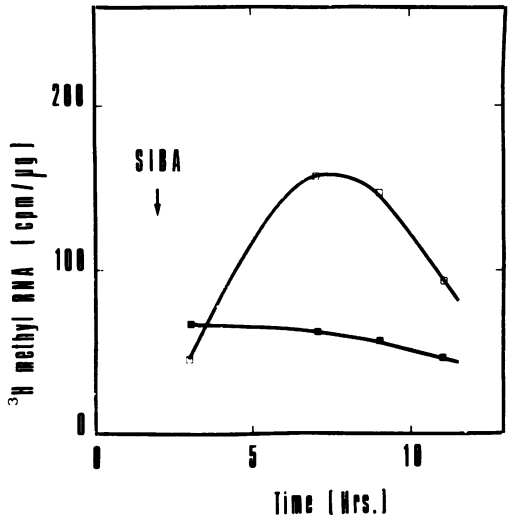

FIG. 5. *Effect of SIBA on RNA methylation. HEp2 cells were infected with HSV (50 PFU/cell). At indicated times, the cells were pulse-labeled (30 min) with [methyl-^3H]methionine (5 μCi/ml) under conditions described in the text. The amount of RNA was estimated from an optical density at 260 nm, and its radioactivity was counted. About 80 μg of RNA was obtained from each culture and the cpm varied from 3,000 to 10,000. Symbols: Open squares, untreated infected cells; closed squares, SIBA added at 2 h postinfection.*

the 5′ terminal of viral mRNA was investigated after reversion of SIBA inhibition in the presence of actinomycin D. The culture of infected cells was treated with SIBA at 2 h, actinomycin D (10 μg/ml) was added at 6 h, and at 7 h the culture was split in two parts. From one, SIBA was removed, whereas it was left over in the other (actinomycin remained in both). RNA was extracted and was alkaline hydrolyzed.

After DEAE-Sephadex separation the radioactivity found in peaks corresponding to phosphate charges −2 and −3 was similar in RNA from both samples; in peaks corresponding to charges −4, −5, and −6 it was 50, 30, and 30%

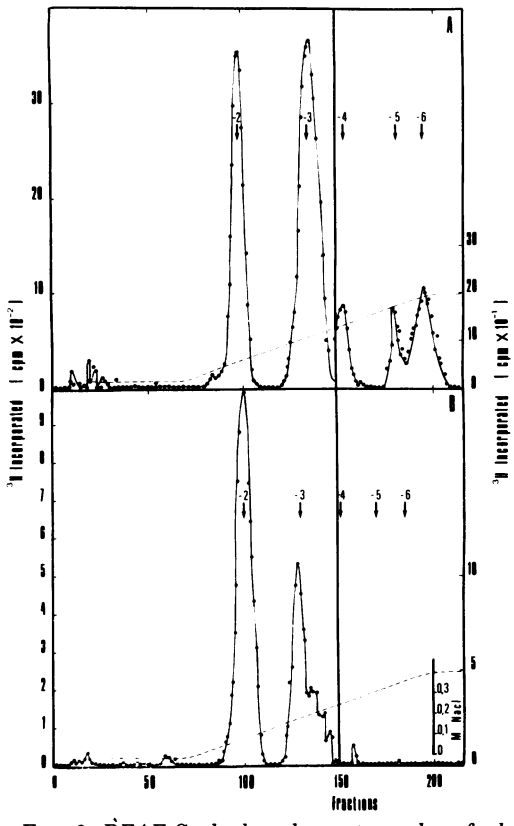

FIG. 6. *DEAE-Sephadex chromatography of alkali-hydrolyzed RNA from infected cells. HEp2 cells were infected with HSV-1 (50 PFU/cell). Cells were labeled with [methyl-^{3}H]methionine (50 μCi/ml) between 6 and 10 h postinfection. Purified RNA was hydrolyzed and chromatographed as described in Materials and Methods. ^{3}H counts of each fraction were determined. Numbers and arrows indicate the position of added marker nucleotides with the corresponding negative phosphate charge. (A) Untreated infected cells; (B) SIBA added at 2 h postinfection.*

higher, respectively, in RNA from culture where SIBA was removed than from those where the drug remained. We conclude that the reversal of inhibition permitted the capping of preexisting viral mRNA in the first place.

DISCUSSION

We report on the effect of SIBA, an analogue of SAH, on HSV multiplication in HEp2 cells which provided information concerning the requirement for methylation for mRNA for virus synthesis. The effect of SIBA on RNA methylation has been demonstrated in the case of tRNA in vitro where a competitive inhibition was observed (7). The irreversible inhibition of Rous sarcoma virus in chicken embryo cells treated with SIBA has been attributed to a block of virus RNA methylation (17).

We have seen that SIBA inhibits HSV multiplication in HEp2 cells. However, the inhibition was released by removing the drug. Virus production could start again even when the time of contact was long enough to block irreversibly HEp2 cell proliferation.

The observed inhibition of HSV production correlated best with the inhibition of virus protein synthesis. Pulse-labeling of infected cells with amino acids has shown that the rate of protein synthesis decreases progressively. It should be remembered that HSV provokes a very efficient shut off of cellular protein synthesis and therefore most of the postinfectional synthetic events can be considered as virus related.

The release from SIBA inhibition was rapidly (within 30 min) followed by an increase in amino acid incorporation, primarily into the α group of polypeptides characteristic of the first step of HSV infection. The switching back to protein synthesis after release from inhibition was similar to that observed by Honess and Roizman after reversal of the cycloheximide-induced block of HSV production (8). However, cycloheximide stops protein synthesis rapidly, whereas SIBA does it slowly. Therefore, some β polypeptides like number 6 and γ polypeptides like number 5 can be founded in the electrophoretic map obtained after removal of SIBA.

Interference with the transcription of viral mRNA is unlikely to be responsible for inhibition of protein synthesis according to the results of hybridization experiments and also those using actinomycin D. Therefore, changes in methylation appeared the most likely cause of the observed inhibition. When we investigated the amount of radioactive methyl groups fixed on mRNA we found that SIBA decreased the total methylation of RNA by about 75% by 7 h, a time when methylation was maximal in controls. Furthermore, the different sites of methyl fixation on an RNA molecule were not affected to the same extent. The methylation of proteins was only slightly inhibited (6%).

It is tempting to correlate the progressive inhibition of protein synthesis to the decreased methylation of the 5′ end of mRNA. We suggest that protein synthesis continues in the presence of SIBA as long as correctly methylated mRNA is available. When the mRNA decays, or eventually the cap at its 5′ end decays, the synthesis stops because the unmethylated RNA cannot be translated (3). The removal of SIBA permits the rapid methylation of accumulated RNA. Results of the experiments showing the fast reappearance of a cap after release from SIBA inhibition even in the presence of actinomycin

D are much in favor of this hypothesis. However, as some of the effects of SIBA on cellular metabolism are not yet well characterized (for instance the change in permeability for nucleotides), it is difficult to say that other explanations could not be found.

Another implication of our results concerns cellular methylases. The various methylations were not affected by SIBA to the same extent. This indicates the existence in the same cell of various methylases with different K_m's for the methyl donor and for the analogue.

The proliferation of HEp2 cells and also HeLa cells was irreversibly arrested after about 12 h of contact with SIBA in contrast with chicken embryo cells (17) and normal human fibroblasts (MRC5 cell line) (unpublished data) which are reversibly inhibited up to 72 h. This difference may be related to the reported differences in methylase activities between normal and cancer cells (4, 10).

A discrepancy exists between the irreversible inhibition by SIBA of Rous virus production (17) and our results with HSV and also with adenovirus, encephalomyocarditis virus, Newcastle disease virus, and fowl plaque virus (unpublished data) where virus multiplication always resumes after the removal of the drug. It could be that in the case of RSV the virus-producing cells are transformed and have changed methylases that are less stable or perhaps irreversibly inhibited by SIBA during the 72-h contact used.

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