PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 455-458 (1976)

Effect of Zinc Ions on Synthesis of Herpes Simplex Virus Type 2-Induced Polypeptides[1] (39417)

PHALGUNI GUPTA AND FRED RAPP

Department of Microbiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, College of Medicine, Hershey, Pennsylvania 17033

Zinc ions at a concentration of 0.2 and 0.3 m*M* inhibit herpes simplex virus type 1 (HSV-1) DNA synthesis by inhibiting virus DNA polymerase (1). However, at a zinc ion concentration of 0.1 m*M*, although virus DNA synthesis was affected very little, only 5% of the virus was produced compared to the untreated controls (2). This indicates that at low concentration (0.1 m*M* or less), zinc ions may affect other biological processes involved in virus replication and may thereby inhibit virus progeny formation. Zinc ions have been shown to inhibit post-translational processing of the precursor polypeptides of rhinovirus and other picornavirus structural proteins (3, 4). Thus, it was of interest to examine the effect of zinc ions on herpesvirus-induced polypeptides in infected cells. Herpes simplex virus type 2 (HSV-2) was selected for this study since host cell protein synthesis is depressed much earlier in HSV-2-infected than in HSV-1-infected cells (5). The present study demonstrates that zinc ions at a concentration of 0.1 m*M* inhibit the synthesis of virus-induced polypeptides with little effect on virus DNA synthesis.

Materials and methods. Cells and viruses. Vero cells were propagated as monolayer cultures at 37° in medium 199 supplemented with 10% fetal calf serum (FCS). HSV-2 strain 333 was grown in Vero cells and used throughout this investigation.

DNA synthesis. Vero cells were infected with HSV-2 strain 333 at a multiplicity of infection (m.o.i.) of 3-5. One hour postinfection, cells were incubated with medium 199 with 5% FCS in the absence or presence of 0.05, 0.1, or 0.2 m*M* $ZnSO_4$. At 4 hr postinfection, [^{3}H]thymidine was added to the medium to a final concentration of 10 μCi/ml. The infected cultures were incubated for 10 hr at 37°, harvested by scraping the cells into the medium, and centrifuged at 750 *g* for 10 min. The cell pellet was then suspended in 0.1 × SSC (0.15 *M* NaCl, 0.015 *M* Na-citrate) containing 1% (w/v) sarkosyl and 1 mg/ml of pronase, and incubated at 37° for 12-14 hr. The digested sample was then analyzed by CsCl density gradient centrifugation at 32,000 rpm for 60 hr in a 40.3 rotor. Drops were collected from the bottom and TCA-precipitable radioactivity was determined. Densities were determined by measuring refractive index.

Protein synthesis. Labeling of virus-induced polypeptides was accomplished using the procedure described above, except that at 3 hr postinfection, medium was replaced with the labeling medium containing [^{35}S]methionine in the absence or presence of $ZnSO_4$. Labeling medium consisted of medium 199 containing one-half the normal concentration of essential amino acids, 5% FCS, and 5 μCi/ml of [^{35}S]methionine (sp act 360 Ci/mmole, Amersham Searles, Inc.). Infected cells were harvested 18 hr after infection and lysed with a buffer containing 0.0625 *M* Tris (pH 6.8), 0.4% Triton X-100, 10 m*M* $MgCl_2$, 50 μg/ml each of DNase and RNase, and 300 μg of methyl phenyl sulfonyl fluoride. The mixture was incubated for 10 min at room temperature, after which sodium dodecyl sulfate (SDS) and mercaptoethanol were added to a final concentration of 2 and 5%, respectively. After heating at 100° for 2 min, 50 μl of each mixture was analyzed by 5-15% gradient polyacrylamide slab gel electrophoresis in the presence of SDS, as described by Baum *et al.* (6). Following electrophoresis, the gel was dried and an autoradiogram was prepared using sarkosyl and pronase and analyzed by CsCl gradient centrifugation.

[1] This study was conducted under Contract NO1 CP 53516 within the Virus Cancer Program of the National Cancer Institute, NIH, PHS.

Densities were determined to distinguish virus DNA from cellular DNA. Labeling of virus-induced polypeptides was accomplished using the procedure described above, except that at 3 hr postinfection, medium was replaced with the labeling medium containing [^{35}S]methionine in the absence or presence of $ZnSO_4$. The cultures were harvested at the end of the virus growth cycle (18 hr postinfection) and lysed. The proteins were solubilized with SDS and mercaptoethanol and analyzed by 5–15% gradient polyacrylamide slab gel electrophoresis. Following electrophoresis, the gel was dried and an autoradiogram was prepared.

Results. Before attempting to determine the effect of zinc ions on virus replication, we examined their effect on host cells. Zinc ions at a concentration of 0.1 m*M* and higher were found to be toxic, resulting in rounding up of cells and detachment from the glass wall under conditions which slowed cell growth. This occurred with confluent cells and with serum-free or essential amino acid-free media. Hence, all experiments were carried out on subconfluent (70–80% confluent) cells in medium containing at least 5% FCS and one-half the normal concentration of all essential amino acids. Even under these conditions, however, zinc ions were found to be toxic at a concentration of 0.3 m*M*.

The effect of zinc ions on formation of HSV-2 virus progeny was measured. Zinc ions at a concentration of 0.05 m*M* and higher inhibited virion synthesis (Table I), a finding similar to that of Gordon *et al.* (2) with HSV-1. Removal of zinc ions 4 hr postinfection restored the virus yield to almost equal that of the untreated control.

The effect of various concentrations of zinc ions on the synthesis of HSV-2 and cellular DNA was determined. The infected untreated and zinc-treated cells were labeled for 10 hr (4–14 hr postinfection) with [^{3}H]thymidine. Labeled DNA was then analyzed by centrifugation in CsCl density gradients (Fig. 1). The amount of radioactivity banding at a density of 1.725 g/ml (virus DNA) and 1.698 g/ml (cellular DNA) was determined and plotted against various concentrations of zinc sulfate (Fig. 2). Zinc ions to a concentration of 0.1 m*M* had a slight inhibitory effect on the synthesis of virus DNA, while at a concentration of 0.2 m*M*, the synthesis of virus DNA was markedly inhibited. In the presence of 0.1 m*M* zinc ions, although DNA synthesis was inhibited only slightly, marked inhibition of virus yield was observed.

To determine the effect of zinc ions on HSV-2-induced polypeptides, infected cells were labeled with [^{35}S]methionine for 15 hr (3–18 hr postinfection) at various zinc ion concentrations. Figure 3 shows analysis by SDS-polyacrylamide gel electrophoresis of labeled virus-induced polypeptides. Zinc ions at a concentration of 0.2 m*M* had no obvious effect on host cell protein synthesis (lanes a and b). At a concentration of 0.05 m*M*, the zinc ions slightly inhibited the synthesis of virus-induced polypeptides, particularly polypeptide P_1 with an approximate

TABLE I. EFFECT OF ZINC IONS ON REPLICATION OF HSV-2.

$ZnSO_4$ (m*M*)	Virus yield[a] (% of control)
0	100
0.05	10
0.1	5
0.2	<0.1
0.1 for 4 hr, then washed	100

[a] $ZnSO_4$ was added 1 hr postinfection. Eighteen hours later, infected cells were scraped into the medium, frozen and thawed three times, and the resulting virus was assayed in rabbit kidney cells. Number of plaques found in untreated control cultures was regarded as 100%.

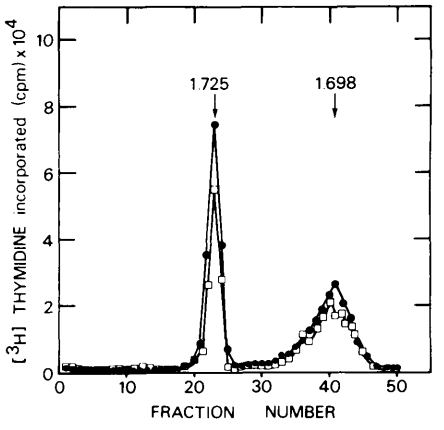

FIG. 1. Analysis of DNA from HSV-2-infected Vero cells in the presence of 0.1 m*M* $ZnSO_4$ (-●-●-) and in the absence of $ZnSO_4$ (-□-□-) by isopycnic centrifugation in CsCl density gradient.

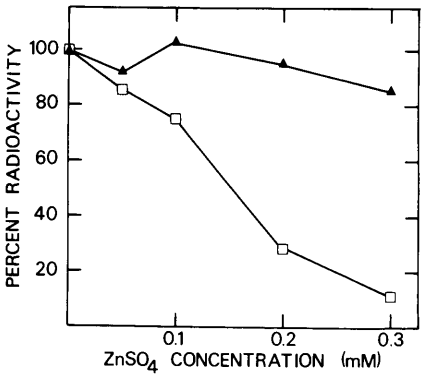

FIG. 2. Effect of zinc ions on the synthesis of virus and cellular DNA in HSV-infected cells. The infected cells were either untreated or treated with different concentrations of $ZnSO_4$. The cultures were labeled with [^{3}H]thymidine and the DNA was analyzed by CsCl density gradient centrifugation. The amount of radioactivity banding at a mean density of 1.725 g/ml (virus DNA) (-□-□-), and 1.698 g/ml (cellular DNA) (-▲-▲-), was determined and plotted against various concentrations of $ZnSO_4$ used. The DNA (virus or cellular) obtained from infected untreated cells (no $ZnSO_4$) was regarded as 100%, and virus and cellular DNA from the $ZnSO_4$-treated cells were compared to it.

molecular weight of 130,000 (lane d), when compared to untreated controls (lane c). The inhibition was more pronounced in the presence of 0.1 m*M* of $ZnSO_4$, when most of the polypeptides were synthesized in reduced amounts (lane e), particularly polypeptides P_1, P_2, P_3, and P_4 (P_2 = 88,000 mol wt; P_3 = 58,000 mol wt; P_4 = 38,000 mol wt). At a concentration of 0.2 m*M* $ZnSO_4$, very little virus-induced protein was synthesized (lane f). Unlike with picornaviruses, no accumulation of large virus polypeptides was noticed in zinc-treated infected cells. The inhibitory effect of zinc could be removed by washing the infected cells up to 4 hr postinfection, which resulted in the resumption in normal amounts of virus-induced polypeptide synthesis (lane g) and the production of normal amounts of virus progeny (Table I). Similar inhibition of virus-induced polypeptides was also observed in HSV-2 grown in human embryo lung cells (data not shown).

Discussion. The present study demonstrates that $ZnSO_4$ at a concentration of

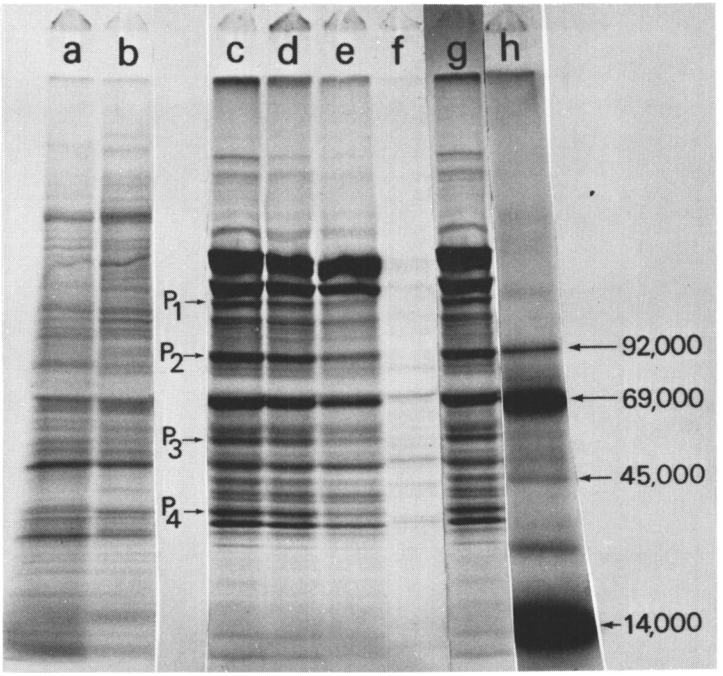

FIG. 3. Polyacrylamide gel electrophoresis analysis of the polypeptides synthesized in infected untreated and zinc-treated cells. Lane a, uninfected culture; b, uninfected culture treated with 0.2 m*M* $ZnSO_4$; c, infected culture (no $ZnSO_4$); d–f, infected cultures treated with 0.05, 0.1, and 0.2 m*M* $ZnSO_4$; g, infected culture exposed to 0.1 m*M* of $ZnSO_4$ for 4 hr, after which it was removed; h, standard proteins of known molecular weight indicated by arrows.

0.05 and 0.1 m*M* prevents the synthesis of virus-induced polypeptides, especially polypeptides P_1, P_2, P_3, and P_4, and also prevents virus progeny formation. In contrast, 0.1 m*M* or less $ZnSO_4$ has little effect on virus DNA synthesis. In the presence of 0.2 m*M* $ZnSO_4$, synthesis of both virus DNA and protein is markedly inhibited. It has been shown that $ZnSO_4$ at a concentration of 0.2 m*M* prevents the synthesis of HSV DNA by inhibiting virus DNA polymerase activity. Zinc ions have no effect on the synthesis of virus DNA polymerase (1). Thus, at 0.2 m*M* $ZnSO_4$, in addition to an inhibitory effect on synthesis of specific virus-induced polypeptides, drastic reduction in virus DNA synthesis probably results in a marked inhibition of virus-specific transcription and, hence, of synthesis of total virus-induced polypeptides. The inhibitory effect of zinc ions could be reversed by removing zinc ions up to 4 hr postinfection, a finding contradictory to that of Gordon *et al.* (2), who reported the inhibitory effect to be irreversible with HSV-1 and BSC-1 cells. This contradiction could be due to the difference in cells and virus used. Since certain virus-induced polypeptides are virion structural proteins, it is conceivable that inhibition of synthesis of one or more of the polypeptides will also inhibit virus progeny formation. The mechanism by which zinc ions inhibit herpesvirus-induced polypeptides has not yet been determined.

Summary. Treatment of herpes simplex virus-infected cells with zinc sulfate ($ZnSO_4$) inhibited formation of virus progeny, but had little effect on virus DNA synthesis. Analysis of polypeptides synthesized in infected cells in the presence of $ZnSO_4$ revealed a markedly reduced synthesis of virus-induced polypeptides.

1. Shlomai, J., Asher, Y., Gordon, Y. J., Olshevsky, U., and Becker, Y., Virology **66,** 330 (1975).
2. Gordon, Y. J., Asher, Y., and Becker, Y., Antimicrob. Ag. Chemother. **8,** 337 (1975).
3. Butterworth, B. E., and Korant, B. D., J. Virol. **14,** 282 (1975).
4. Korant, B. D., Kaher, J. C., and Butterworth, B. E., Nature (London) **248,** 588 (1974).
5. Powell, K. L., and Courtney, R. J., Virology **66,** 217 (1975).
6. Baum, S. G., Horwitz, M. S., and Maizel, J. V., Jr., J. Virol. **10,** 211 (1972).

Received March 12, 1976. P.S.E.B.M. 1976, Vol. 152.