Specific binding of herpes simplex virus type 1 (HSV-1) strain HSZP to host cell surface can provoke interference with early shutoff of host protein synthesis induced by HSV-1 strain KOS

J. Matis* and M. Krivjanská

Institute of Virology, Slovak Academy of Sciences, 84246 Bratislava, Slovakia

The HSZP strain of herpes simplex virus type 1 (HSV-1) is defective with respect to the early shutoff of host protein synthesis but is effective at interfering with the early shutoff function of the HSV-1 KOS strain, even when heat-inactivated or neutralized by antibody. The interference was not due to exclusion of strain KOS by HSZP at the level of adsorption or penetration. The component responsible for the interference is an integral part of HSZP virions. Strain HSZP inactivated with zinc ions failed to interfere with the early shutoff function of the superinfecting strain KOS. The same effect was also found with strain HSZP purified from cells treated with 2-deoxy-D-glucose. This finding supports the idea that a specific interaction between HSZP virions and the cell surface can be responsible for the interference phenomenon.

Infection of susceptible tissue culture cells with herpes simplex virus (HSV) results in the overall inhibition of host protein synthesis and in expression of at least 50 virus-induced infected cell proteins (Roizman et al., 1965; Sydiskis & Roizman, 1966). However, experiments involving u.v.-irradiated virus or the presence of drugs precluding expression of infecting virions revealed that some strains of HSV-1 possessed the ability to suppress cellular protein synthesis even in the absence of viral protein synthesis. This so-called early shutoff or virion-associated host shutoff mechanism is mediated by a component of the virion (Fenwick & Walker, 1978; Fenwick & McMenamin, 1984; Kwong et al., 1988). The component is a product of gene UL41 and is not essential for viral replication (Read & Frenkel, 1983; Everett & Fenwick, 1990).

In this study we present evidence that the component responsible for the interference phenomenon is present in the HSZP inoculum in a form that can be sedimented by high-speed centrifugation and seems to be an inseparable part of the virion and that zinc-inactivated strain HSZP, as well as strain HSZP grown in the presence of 2-deoxy-D-glucose (2-DOG), failed to interfere with the early shutoff function of superinfecting strain KOS. The effects of heat inactivation, antibody neutralization and zinc ions on the ability of strain HSZP to adsorb and penetrate host cells were also investigated.

To determine whether the component (factor) of strain HSZP responsible for the interference phenomenon was present in the supernatant fraction of the virus inoculum after high-speed centrifugation, the HSZP inoculum was overlaid onto a 30% (w/v) sucrose cushion in PBS and centrifuged at 80000 g for 60 min. After centrifugation, the supernatant fraction and the pelleted fraction, resuspended in PBS, were used to infect Vero cells. Vero cells were also mock-infected and infected with strain HSZP or KOS. Cell cultures were then labelled for 4 h and subjected to electrophoresis. Part of the cultures were infected with HSZP inoculum as well as the supernatant or pelleted fraction, superinfected with KOS after 2 h and, before electrophoresis, were incubated and labelled for 4 h. The procedures were carried out in the presence or absence of actinomycin D. The results are shown in Fig. 1. No synthesis of viral polypeptides was
Table 1. Effect of m.o.i. of strain HSZP on its ability to evoke interference with early shutoff function induced by strain KOS

<table>
<thead>
<tr>
<th>Strain HSZP m.o.i</th>
<th>TCA-precipitable counts (% of control count*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>96</td>
</tr>
<tr>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>84</td>
</tr>
<tr>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

* Control counts were obtained with mock-infected cells, labelled for 5 h.

observed in cells treated with the supernatant fraction of the HSZP inoculum (compare lanes 1 and 4). Infection of cells with strain KOS in the presence of actinomycin D caused the inhibition of host protein synthesis, reflecting its early shutoff function (lane 10). In cells treated with the pelleted fraction of the HSZP inoculum and superinfected with strain KOS in the presence of actinomycin D, the inhibition of early shutoff of host protein synthesis was observed (lane 8), but in cells treated with the supernatant fraction in the presence of actinomycin D, early shutoff was observed (lane 7). These findings indicate that the component responsible for the suppression of early shutoff function of the superinfecting strain KOS was not present in the HSZP inoculum in a free (soluble) form but was capable of penetrating a 30% (w/v) sucrose cushion under high-speed centrifugation conditions. The component seems to be a part of the virion, as HSZP virus particles (8 × 10⁹ p.f.u./mg protein), purified according to Spear & Roizman (1972), can interfere. To investigate the amounts of HSZP needed to interfere with shutoff by strain KOS, Vero cells were infected with purified HSZP virions (8 × 10⁹ p.f.u./mg protein) at different multiplicities. Cells were washed with PBS 1 h later, incubated for 3 h and superinfected with strain KOS (20 p.f.u./cell). After an incubation period of 4 h, the cells were labelled with ³⁵S-amino acids (5 μCi/ml) for 5 h and washed with PBS. The procedures were carried out at 37 °C in the presence of actinomycin D (5 μg/ml). The TCA-precipitable radioactivity was determined by scintillation counting. As shown in Table 1, amounts of purified HSZP corresponding to less than 1 p.f.u./cell were not sufficient to provoke interference with the early shutoff function induced by strain KOS.

We confirmed the finding that neutralized HSV particles do not penetrate host cells (Fuller & Spear, 1987; Highlander et al., 1988; Fuller et al., 1989). In comparison with native virus, only the genome of heat-inactivated virus particles exhibited any tendency to enter and/or associate with the nuclei of host cells (Fig. 2). Therefore, the effect of ZnSO₄ and 2-DOG on the virus–cell interaction was investigated. In order to explain the antiviral mechanism of zinc ions, the effect of ZnSO₄ on HSV replication, adsorption, penetration, viral DNA synthesis and virus egress was investigated by Kümel et al. (1990). The long-standing hypothesis that zinc ions block multiplication of HSV by selective intracellular inhibition of the viral DNA polymerase (Friedlender et al., 1978) is no longer valid. Inhibition of HSV replication in cultured cells is due to the cytotoxicity of the selective zinc concentration. Kümel et al. (1990)
Fig. 2. Effects of antibody neutralization, heat- or zinc-inactivation of strain HSZP on its ability to adsorb and penetrate Vero cells. Aliquots of a \(^{3}H\)thymidine-labelled (25 μCi/ml from 3 to 24 h post-infection) preparation of purified HSZP virions were used to prepare antibody-neutralized (N), heat-inactivated (H) or zinc-inactivated (Z) virus. The virus was heat-inactivated (20 min at 52 °C) or neutralized by a rabbit anti-HSV-1 hyperimmune serum as described previously (Matis & Krivjanská, 1988; Matis et al., 1992). Zinc inactivation was performed using 15 mM-ZnSO\(_4\) in PBS and the virus was incubated for 2 h at 37 °C. Adsorption was carried out at 4 °C for 2 h with an amount of inactivated virus corresponding to 20 p.f.u./cell (before inactivation procedures) or with native virus (N). After washing with ice-cold PBS, the cells were either immediately harvested for the measurement of adsorption (a), indicated by TCA-precipitable radioactivity or overlaid with culture medium and incubated at 37 °C. At different intervals the nuclear fractions of cell cultures were prepared (Braun et al., 1983). Radioactivity in TCA-precipitable material from cell nuclei (b) was taken as a measure of viral DNA transport into the cell nucleus after penetration.

Also studied in detail the effect of zinc ions on free virus particles. They presented evidence that zinc is deposited onto the virion but the integrity of the virus particle is not affected. It was suggested that zinc ions inactivate HSV by interference with glycoproteins involved in virus penetration.

To inactivate our virus strains, purified virions were resuspended in PBS containing 15 mM-ZnSO\(_4\) and incubated at 37 °C. To remove ZnSO\(_4\) from inactivated virus preparations, gel filtration was used. The void volume of a Sephadex G-25 column prepared in PBS was checked by the use of Blue Dextran 2000. It corresponded with the elution volume of the virus preparation. Gel filtration of \(^{3}H\)thymidine-labelled and zinc-inactivated purified virus preparations revealed that up to 90% of the input radioactivity was regularly found in the void volume (data not shown). In cells treated with ZnSO\(_4\)-inactivated HSZP, no synthesis of viral polypeptides was observed (Fig. 3, lane 6). Inactivation by zinc ions also abolished the ability of strain KOS to inhibit host protein synthesis in the presence of actinomycin D (Fig. 4, lanes 5, 6, 9 and 10). Furthermore, the results confirmed that concentrations of ZnSO\(_4\) up to 100 μM are not toxic to the cells (lane 1). Our superinfection experiments revealed that the treatment of cells with zinc-inactivated strain HSZP at amounts corresponding to 20 to 40 p.f.u./cell (titre before inactivation) did not prevent the superinfecting virus from entering cells. In cells treated with zinc-inactivated strain HSZP, strain KOS was able to replicate, as well as to shut off the host protein synthesis (Fig. 5, lanes 2 and 4). The failure of zinc-inactivated strain HSZP to interfere effectively with the early shutoff function of strain KOS hints at the participation of virion glycoproteins in the interference phenomenon. To support this assumption, the effect of 2-DOG was investigated. It is known that 2-DOG affects the glycosylation of viral glycoproteins and thus prevents proper interaction of the virus with cell surface membrane, indicated by a reduction in virus penetration, infectivity and syncytium formation (Gallagher et al., 1973; Courtney, 1976; Spivack et al., 1982; Glorioso et al., 1983). We confirmed that virions grown...
Fig. 3. Gel filtration of ZnSO₄-inactivated HSZP strain. Purified virus was resuspended in PBS containing ZnSO₄ (15 mM) and incubated for 2 h at 37 °C. It was then subjected (0.5 ml; titre before inactivation 6 x 10⁶ p.f.u./ml) to gel filtration on a column of Sephadex G-25 (bed volume 2 ml). The material present in the void volume (1 ml) was plated on Vero cells, which were labelled 2 h later for 5 h and lysed for electrophoresis (lane 6). The same procedures were carried out with non-inactivated virus (lane 5), as well as with the ZnSO₄ solution (15 mM) alone in PBS (lane 2). Also, mock-infected cells were labelled for 5 h before lysis and electrophoresis (lane 1). Cells were also treated with unfiltered PBS containing ZnSO₄ (15 mM) and labelled (lane 3), or infected with unfiltered HSZP and labelled (lane 4). The positions of M₄ markers and the major capsid polypeptide ICP5 (●) are shown.

Fig. 4. The effect of ZnSO₄ on the ability of strain KOS to inhibit host cell protein synthesis. Vero cells treated with ZnSO₄-inactivated and gel-filtered strain KOS (incubated with ZnSO₄ at 37 °C for 2 h in lanes 5 and 9 and 5 h in lanes 6 and 10) in the absence (lanes 5 and 6) or presence (lanes 9 and 10) of actinomycin D were incubated for 4 h, labelled for 5 h and analysed by electrophoresis. Cells infected with strain KOS (20 p.f.u./cell) in the absence (lane 4) or presence (lane 8) of actinomycin D were incubated and labelled. Cells treated with medium containing 100 μM-ZnSO₄ (lane 1) were also incubated and labelled. Cells treated with gel-filtered (void volume) medium containing ZnSO₄ (15 mM) were incubated and labelled (lane 2). Mock-infected cells, labelled in the absence (lane 3) or presence (lane 7) of actinomycin D, were used as controls. The positions of M₄ markers are shown to the right. The position of ICP5 is marked (●).

in the presence of 2-DOG were able to adsorb (about 90% of the HSZP virions) but failed to penetrate the host cell, and contained glycoproteins that exhibited a shift in electrophoretic mobility. However, the presence of 2-DOG in infected cells has essentially no effect on the number of virions produced (Courtney et al., 1973) or on the incorporation of radioactive amino acids into the protein (Dix & Courtney, 1976). Therefore, it was anticipated that the protein content of the purified HSZP virions grown in the presence of 2-DOG would not significantly differ from a preparation of purified virions grown in the absence of the drug. Also, it was regularly found that the preparations of purified HSZP virions grown in the presence of 2-DOG were about 1000-fold less infectious than the untreated ones. These observations were taken into consideration in the experiments outlined in Table 2. As shown, in comparison with the untreated counterpart, up to 20 times more drug-treated HSZP virions were needed to infect the cells. Despite this, the drug-treated HSZP virions failed to interfere with the early shutoff function of the superinfecting strain.

The mechanism of early shutoff of host protein synthesis by a virion-associated factor is still not understood. Hill et al. (1985) reported that a virion component from a HSV-1 strain that delayed shutoff could interfere with the early shutoff of host protein synthesis induced by a HSV-2 strain. We confirmed this finding using HSV-1 strains (Matis & Krivjanská, 1988). It was postulated that the observed inhibition of HSV-2-induced early shutoff by HSV-1 occurred by competition for target sites within the cell by both HSV-1 and HSV-2
virion-associated components. However, such a predominance of the weak shutoff function was not confirmed in experiments using a recombinant virus containing both weak and strong alleles of UL41 (Fenwick & Everett, 1990). This observation indicates that the spatial relationship of both UL41 gene products can play a role in the interference.

Considering that HSV neutralized by antibodies was arrested prior to initiation of a visible fusion bridge formation between the virus and host cell (Fuller & Lee, 1992) and our finding that neutralized strain HSZP is still capable of interfering with the early shutoff function of superinfecting virus (Matis et al., 1992) allows us to conclude that the HSZP particles exhibiting the interference phenomenon do not have to penetrate the host cell. The deposition of zinc in the envelope of strain HSZP must lead to inactivation of some components that play a role in the very early stages of virus–cell interaction. The observation that strain HSZP grown in the presence of 2-DOG failed to interfere provides evidence for specificity of the blocking reaction and supports the suggestion that glycoprotein-dependent functions may be involved in the interference phenomenon. In cells treated with heat-inactivated or neutralized HSZP virions and superinfected with KOS in the absence of actinomycin D, the synthesis of viral polypeptides was observed (Matis et al., 1992). Therefore, the interference phenomenon is not due to hindrance of strain KOS by strain HSZP at the level of adsorption and/or penetration. It is interesting to note that in neomycin-treated Hep-2 cells, the virion-associated shutoff of host protein synthesis does not occur (Garcin et al., 1990). Because neomycin is an inhibitor of the inositol phospholipid pathway (Reid & Gajjar, 1987), this finding suggests a possible contribution of the signal-transduction mechanism in the shutoff of host protein synthesis in HSV-1-infected Hep-2 cells. Furthermore, a mutant of HSV-1 (ts 1204) that is not capable of penetrating the plasma membrane is able to activate expression of a cellular gene by specific binding to the cell surface (Preston, 1990). These studies provide the groundwork for further investigations into the mechanism of the interference phenomenon. The next step will be to identify the component(s) of strain HSZP responsible for the observed interference.

This investigation was supported by a grant (number 2/999512/92) from the Slovak Academy of Sciences.

**References**


---

**Table 2. Ability of HSZP virions grown in the presence of 2-DOG to interfere with early shutoff function of superinfecting strain KOS**

<table>
<thead>
<tr>
<th>Infection with HSZP*</th>
<th>Superinfection with KOS†</th>
<th>TCA-precipitable counts (%) of control count‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.f.u./cell</td>
<td>Virus protein (μg)</td>
<td>P.f.u./cell</td>
</tr>
<tr>
<td>0.02</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>0.10</td>
<td>25</td>
<td>20.0</td>
</tr>
<tr>
<td>0.20</td>
<td>50</td>
<td>20.0</td>
</tr>
<tr>
<td>0.40</td>
<td>100</td>
<td>20.0</td>
</tr>
<tr>
<td>0.00</td>
<td>0</td>
<td>20.0</td>
</tr>
<tr>
<td>0.00</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>20.00§</td>
<td>5</td>
<td>20.0</td>
</tr>
</tbody>
</table>

* Purified virions, $9 \times 10^5$ p.f.u./mg of protein.
† Purified virions, $7 \times 10^5$ p.f.u./mg of protein.
‡ See text; control counts were obtained with mock-infected cells, labelled for 5 h.
§ Infection with purified HSZP virions grown in the absence of 2-DOG.

---

**Fig. 5.** Superinfection with strain KOS of cells treated with ZnSO₄-inactivated strain HSZP. Vero cells were treated with ZnSO₄-inactivated and gel-filtered strain HSZP (lanes 1 to 4). Cells were washed with PBS 2 h later and either mock infected (lanes 1 and 3) or superinfected with strain KOS (lanes 2 and 4) in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of actinomycin D. Cells were labelled 4 h later for 5 h and subjected to electrophoresis. Mock-infected, labelled cells (lane 5) were included as a control. The positions of Mr markers are shown. The position of ICP5 is marked (●).


(Received 20 December 1993; Accepted 7 February 1994)