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HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) HSZP INTERFERES ALSO HEAT INACTIVATED WITH EARLY SHUTOFF OF HOST PROTEIN SYNTHESIS INDUCED BY HSV-1 KOS

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Summary. — The ability of two strains of herpes simplex virus type 1 (HSZP and KOS) to shut off the host protein synthesis in the presence of Actinomycin D was investigated. The HSZP strain proved to be defective with respect to the so-called early shutoff function. In superinfection experiments, the HSZP was effective at interfering with the early shutoff function of the KOS strain provided that the HSZP infection preceded KOS superinfection. Heat inactivation of the HSZP did not lead to the loss of its interfering ability. Evidence was given that this interference was neither due to the hindrance of the KOS by HSZP at adsorption nor due its exclusion during penetration.

Key words: herpes simplex virus type 1; heat inactivation; host shutoff; interference

Introduction

Mammalian cells permissive to herpes simplex virus (HSV) infection express approximately 50 virus induced infected cell polypeptides. It is well known that such productive infection is accompanied by the inhibition of host cell protein synthesis (Roizman *et al.*, 1965; Sydiskis and Roizman, 1966; 1967). However, it has been found later that some strains of HSV are capable of suppressing the cellular protein synthesis in the absence of viral protein synthesis. This process is known as early or also virion-associated shutoff in order to emphasize that it is caused by a component(s) of infecting virions. The fact that the synthesis of viral polypeptides is not involved in the early shutoff of host protein synthesis follows from the experiments done with UV-irradiated virus or in the presence of drugs precluding the expression of infecting virions (Fenwick and Walker, 1978; Nishioka and Silverstein, 1978; Read and Frenkel, 1983; Fenwick and McMenamin, 1984).

As described (Matis and Szántó, 1985), the HSZP strain of HSV-1 is defective with regard to the early shutoff of host protein synthesis. In this communication we show that also the heat inactivated HSZP interferes with the early shutoff induced by the KOS strain of HSV-1. Evidence is given that the KOS strain is capable of penetrating the HSZP-infected cells.

Materials and Methods

Cells. African green monkey kidney (Vero) cells were grown to confluency in Müller flasks in Eagle's basal medium (BEM) supplemented with 10% inactivated calf serum (ICS).

Viruses. HSV-1 strain HSZP (syn strain) passaged in ZP (rabbit lung) cell line (Szántó, 1960; Szántó *et al.*, 1972) and the prototype strain KOS (a non-syn strain passaged in Vero cells) were used throughout.

Assay of infectious virus. Viruses were titrated by plaque assay in Vero cells under methylcellulose overlay; virus titres were expressed as PFU/ml.

Metabolic inhibitor and radioisotope. Actinomycin D (Act D; BDH Chemical Ltd, England) was used at a final concentration of 5 μg/ml of medium and was present throughout the experiment. Radioisotope ^{14}C-amino acid hydrolysate (specific activity 40 × 10^3 MB/g, Prague, Czechoslovakia) was administered to the cell culture in medium BEM/10 (ten times reduced amino acids except of arginine) supplemented with 1% ICS.

Infection and radioactive labelling of cells. Cell monolayers in Müller flasks (approximately 1× 10^6 cells per flask) were exposed unless otherwise stated to 20—40 PFU of virus per cell in 1.5—2.0 ml of medium in the presence or absence of Act D, incubated at 37 °C for various periods of time and labelled for 2 hr (0.4 MBq/ml). In some experiments the viruses were heated before infection. In this case the Act D was added to the virus after heat treatment. Specific conditions of adsorption and superinfection varied with the experiment and are described in the legend to Figures.

Polyacrylamide gel electrophoresis (PAGE). At the end of the labelling period, the cells were rinsed with ice-cold phosphate buffered saline (PBS), treated with nucleases (Marsden *et al.*, 1970) in the presence of protease inhibitors (phenylmethylsulphonyl fluoride and tolylsulphonyl-lysylchloromethyl ketone purchased from Sigma, U.S.A.) and heated in lysis buffer (Spear and Roizman, 1972) to 100 °C for 5 min. Portions were either precipitated with trichloroacetic acid (TCA) and measured on a Packard liquid scintilation counter or loaded onto SDS-polyacrylamide gels (Matis and Rajčáni, 1980). After electrophoresis the gels were fixed, treated with 1 mol/l sodium salicylate to provide fluorographic enhancement and analysed by contact autoradiography using Medix Rapid X-ray film (Hradec Králové, Czechoslovakia).

Results

Ability of the HSZP and KOS to induce the early shutoff of host protein synthesis

Cells were mock-infected or infected in the presence of Act D with either HSZP or KOS strain of HSV-1, incubated for different intervals and after labelling subjected to electrophoresis. The results showed the following:

1. The HSZP strain failed to suppress the host protein synthesis in the presence of Act D. The polypeptide synthesis in HSZP-infected cells was not significantly reduced after incubation intervals of 2, 4, and 6 hr. In comparison with mock-infected cells, the TCA precipitable counts of the HSZP infected cells represent more than 90%. (Fig. 1, lanes 2, 5 and 8).

2. The KOS strain was capable of suppressing the host protein synthesis in the presence of Act D. The polypeptide synthesis in the KOS-infected cells after incubation interval of 2 hr was reduced approximately to the half. The prolongation of the incubation interval up to 6 hr further significantly reduced the polypeptide synthesis in KOS-infected cells (Fig. 1, lanes 3, 6 and 9).

3. Under given experimental conditions, no synthesis of viral polypeptides was observed (Fig. 1 compares the polypeptide profiles of mock-infected and infected cells).

To determine the amount of the KOS strain needed for the early shutoff of host protein synthesis, the cells were infected in the presence of Act D at various multiplicity (PFU per cell). Incubation interval of 5 hr, and inoculation of at least 10 PFU of KOS per cell were necessary for a significant suppression of host polypeptide synthesis (Fig. 2, lanes 8 to 13). When shortening the incubation period up to 2 hr, higher multiplicities of infection were needed (Fig. 2, lanes 1 to 7).

To gain insight into the thermostability of the virion component(s) responsible for the induction of early shutoff of host protein synthesis, the cells were infected with heat treated KOS. The KOS strain was prewarmed for 20 min at various temperatures and cells were infected in the presence or absence of Act D. The results were as follows:

1. The heat treatment of virus KOS up to 43 °C did not affect its ability to suppress the host polypeptide synthesis in the presence of Act D (Fig. 3, lanes 2 to 4). No differences in the viral polypeptide synthesis were observed in the absence of Act D (Fig. 3, lanes 8 to 10).
2. Heat treatment of the virus at temperatures above 43 °C (46 °C and 52 °C, respectively) caused its inactivation. No suppression of the host polypeptide synthesis as well as the production of the viral polypeptides was observed (Fig. 3, lanes 5, 6, 11 and 12).

Superinfection with KOS of the HSZP-infected cells

Experiments in which the HSZP-infected cells in the presence of Act D were superinfected with strain KOS were carried out. The following results were obtained:

1. In comparison with KOS-infected cells, no suppression of the early shutoff of host protein synthesis was observed in cells simultaneously infected with HSZP and KOS (Fig. 4, compare lanes 2 and 7; 5 and 12). However, when the HSZP infection preceded KOS superinfection strain HSZP interfered with the KOS-induced early shutoff function (Fig. 4, compare lanes 3 and 4; lanes 8 to 10 and 11).
2. Strain HSZP did not interfere with strain KOS induced early shutoff function when:

a) both virus strains were adsorbed onto the cells simultaneously (Fig. 5, lanes 1 to 4),

b) the KOS adsorption was allowed to take place after HSZP adsorption and vice versa (Fig. 5, lanes 6 and 7).

3. Removal of HSZP strain before superinfection of cells with strain KOS did not affect the degree of its interference (Fig. 6).
4. Heat inactivated HSZP did not lose the ability to interfere with early shutoff function induced by KOS (Fig. 7, lanes 5 to 7).
5. Amounts of HSZP corresponding to the PFU values of virus per cell less than 1 are not sufficient to evoke interference with the early shutoff function induced by KOS (Fig. 8).

Discussion

In this communication we describe the ability of two strains of HSV-1 (HSZP and KOS) to inhibit the protein synthesis in Vero cells under the conditions precluding the expression of infecting virions. The expression of the virus was blocked by the use of Act D which inhibits the transcription of viral genetic information. The results showed that the KOS strain was capable of suppressing the host protein synthesis in the presence of Act D. The HSZP strain has been found to be defective with respect to the so-called early shutoff function.

It was estimated that relatively high multiplicity of KOS infection must be used to evoke a marked early shutoff of host protein synthesis. To secure reproducible early shutoff effects, inoculation multiplicities from 20 to 40 PFU per cell and an incubation period of at least 4 hr were employed.

The KOS heat treatment experiments revealed that temperatures which caused the inhibition of viral polypeptide production also inactivated the virion-associated function, responsible for the early shutoff of host protein synthesis (Fig. 3). It is known that the early shutoff function is mediated by a component(s) of the virus particle that enhances mRNA degradation, at least in Vero cells (Schek and Bachenheimer, 1985). Strom and Frenkel (1987) have discussed three possible mechanisms leading to RNA degradation. One mechanism suggests the penetration of an RNase into host cells as a part of the virion. Since the RNases are generally known to be thermostabile enzymes, it would be of interest to investigate in what extent the heat treatment of the virus could affect its ability to enter the host cells (see also below the use of heat inactivated virus in superinfection experiments).

The superinfection experiments revealed that the HSZP strain was effective at interfering with the early shutoff function of the KOS strain when a) the HSZP infection of the cells preceded the KOS superinfection, b) amounts of the HSZP corresponding to values higher than 1 of virus per cell were used. No interference was observed when the cells were infected with both strains simultaneously, as well as, when both strains were adsorbed on to the cells simultaneously and/or successively. It seems not likely, therefore, that the interference was due to exclusion of the KOS by HSZP at the level of adsorption or penetration. At least, the KOS component(s) responsible for the early shutoff function must be capable of penetrating the HSZP infected cells. The degree of interference was not diminished by the removal of HSZP from the cells at the point of the KOS superinfection (Fig. 6). This may indicate that the amount of HSZP penetrating into the cells prior to KOS superinfection was responsible predominantly for the observed interference.

The experiments further showed that also heat inactivated HSZP was able to interfere with the early shutoff function of the KOS. Superinfection experiments were carried out in the presence, as well as in the absence of Act D. In the absence of the drug, the viral polypeptides were detected in cells treated with heat inactivated HSZP and superinfected with KOS

(Fig. 7 and 8). This finding allowed to assume the ability of the superinfecting virus to enter the cells. We have found only recently that by using heat inactivated KOS instead of HSZP in superinfection experiments, the interference could also be demonstrated. Thus, the component(s) responsible for the early shutoff function, although inactivated, is capable of interfering with the action of its active counterpart.

Hill *et al.* (1985) demonstrated that a virion component from HSV-1 delayed shutoff strain could interfere with the early shutoff of host protein synthesis induced by a HSV-2 strain. They 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. The results presented here support such a postulation.

We have shown previously (Matis and Szántó, 1985) that the HSZP immediate early mRNAs exhibited a pronounced functional stability — a typical property of the HSV mutants defective in the virion-associated shutoff of host protein synthesis (Read and Frenkel, 1983; Strom and Frenkel, 1987). Moreover, we have shown that the superinfection of the HSZP-infected primary rabbit kidney cells with the KOS strain in the presence of Act D affected neither the stability of the HSZP immediate early mRNA translation nor the host polypeptide synthesis. It was reported recently that the virion-associated function could indiscriminately reduce the half-lives of both host and viral mRNAs (Kwong and Frenkel, 1987). In the light of these facts it is tempting to speculate that the interference phenomenon does not affect the synthesis of host proteins only. The interference with the action of superinfecting virus may be a consequence of the HSZP induced stability of infected cell mRNAs.

Although the early shutoff function is well documented, nothing is known about the precise mechanism leading to this phenomenon. Nevertheless, taking into the consideration that the early shutoff function can play a versatile role in viral replication, it may be of interest to use the cells treated with heat inactivated virus for the study of virus-cell interactions. This work and experiments with UV-irradiated viruses are currently under way.

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Explanation to Figures (Plates LII—LVIII):

Fig. 1. Polypeptides synthesized in HSZP or KOS-infected cells in the presence of Act D. Cells were mock-infected and infected in the presence of Act D and after incubation intervals labelled for 2 hr (0.4 MBq/ml). Lanes 1, 4, and 7 show mock-infected cells; lanes 2, 5, and 8 HSZP infected cells; lanes 3, 6, and 9 KOS-infected cells. Lanes 1 to 3 incubation for 2 hr; lanes 4 to 6 incubation for 4 hr; lanes 7 to 9 incubation for 6 hr. Below TCA precipitable counts expressed in percentage of the controls.

Fig. 2. Relationship between multiplicity of infection and the ability of the KOS to induce early shutoff of host protein synthesis. Cells were mock-infected (lanes 6 and 13) or infected in the presence of Act D with 40 (lanes 1 and 8), 20 (lanes 2 and 9), 10 (lanes 3 and 10), 1 (lanes 4 and 11) and 0.1 (lanes 5 and 12) PFU of KOS per cell, respectively. Lane 7 shows the polypeptide profile of cells infected with 40 PFU of HSZP per cell. Cells were incubated for 2 hr (lanes 1 to 7) or 5 hr (lanes 8 to 13), respectively and after labelling subjected to electrophoresis. For further explanations see Fig. 1.

Fig. 3. Ability of the heat treated KOS strain to induce early shutoff of host protein synthesis. Cells infected in the presence of Act D with heat treated KOS — for 20 min at 24, 36, 43, 46, and 52 °C, respectively, were incubated for 6 hr and labelled (lanes 2 to 6). In lanes 8 to 12 are the polypeptide profiles of the same experiment done in the absence of Act D. Mock-infected cells incubated for 6 hr and labelled in the presence (lane 1) or absence (lane 7) of Act D.

Fig. 4. Effect of the KOS superinfection on the early shutoff of host protein synthesis in the HSZP-infected cells. Cells were infected or mock-infected in the vol of 1.5 ml. In superinfection experiments, the KOS was added in the volume of 0.5 ml. Lanes 1 and 6 mock-infected cells; lanes 2 and 7 cells infected with HSZP and KOS simultaneously; cells superinfected with KOS 1 hr after HSZP infection (lane 3); cells superinfected with KOS 1, 2, and 3 hr after HSZP infection, respectively (lanes 8 to 10); HSZP-infected cells (lanes 4 and 11); KOS-infected cells (lanes 5 and 12); labelling 2 hr (lanes 1 to 5) and 5 hr (lanes 6 to 12) after the first infection.

respectively. All procedures were done in the presence of Act D. For further explanations see Fig. 1.

Fig. 5. Role of adsorption in the superinfection experiments. Viruses in a volume of 0.5 ml were adsorbed (1 hr at 4 °C) onto the cell monolayer. After adsorption, the cells were washed with PBS at 4 °C, incubated for 5 hr at 37 °C in a water bath and labelled. All experiments were done in the presence of Act D. 1. Mock adsorption (ads). 2. HSZP ads. 3. KOS ads. 4. ads with HSZP and KOS simultaneously. 5. Mock ads and mock ads. 6. HSZP ads and KOS ads. 7. KOS ads and HSZP ads. For further explanations see Fig. 1.

Fig. 6. Superinfection with KOS of HSZP-infected cells in the presence of Act D. Cells were infected with HSZP (1.5 ml). Superinfection with KOS was done by adding the virus in volume of 0.5 ml at 0, 1, 2, and 3 hr after HSZP infection (lanes 1 to 4) or, after removal of HSZP, by adding in volume of 2.0 ml at 1, 2, and 3 hr after HSZP infection (lanes 6 to 8), respectively. After superinfection the cells were incubated for 5 hr and labelled. Cells infected with KOS (lane 5) or mock-infected (lane 9) were incubated for 5 hr and labelled. For further explanations see Fig. 1.

Fig. 7. Superinfection with KOS of cells infected with heat inactivated HSZP. Cells infected with heat inactivated (for 20 min at 52 °C) HSZP (1.5 ml) were superinfected with KOS (0.5 ml) at 0, 1, and 3 hr after HSZP infection, respectively. After superinfection the cells were incubated for 4 hr and labelled (lanes 2 to 4). In lanes 5 to 7 are the polypeptide profiles of the same experiment in which the superinfection was done in the presence of Act D. Cells infected with heat inactivated HSZP were incubated for 4 hr and labelled (lane 1). Cells infected with KOS (1.5 ml) in the absence (lane 8) or presence (lane 9) of Act D were incubated for 4 hr and labelled. Several host polypeptides are marked (●) and infected cell polypeptides numbered according to Morse et al. (1978).

Fig. 8. The relationship between the HSZP concentration and its ability to evoke the interference with the early shutoff induced by KOS. Cells were infected with HSZP in the presence of Act D (lanes 1 to 3), with heat inactivated (for 20 min at 52 °C) HSZP in the absence of Act D (lanes 4 to 9 and 12) and mock-infected in the absence of Act D (lanes 10 and 11). Cells were then incubated for 4 hr, superinfected with KOS in the presence (lanes 1 to 6 and 10) or absence (lanes 7 to 9 and 11) of Act D, respectively, and before labelling further incubated for 4 hr. Cells in lane 12 were incubated for 8 hr and labelled in the absence of Act D. Amount of HSZP corresponding to 20 PFU of virus per cell (lanes 1, 4, 7, and 12); amount of HSZP corresponding to 2 PFU of virus per cell (lanes 2, 5, and 8); amount of HSZP corresponding to 0.2 PFU of virus per cell (lanes 3, 6, and 9). For further explanations see Fig. 7.