Permeability to inhibitors of protein synthesis in virus infected cells

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Abstract

Infection of HeLa cells with different viruses induces permeabilization of the cell membrane to protein toxins such as α-sarcin. This phenomenon occurs with HeLa, KB, BHK-21 and L929 cells and EMC, SFV, VSV and Polio virus and is dependent on the ability of the virus to infect the cells. Inhibitors of endocytosis and lysosomotropic agents do not affect this process. Cells become sealed to the toxin approximately four hours after the infection. Sulfhydryl reagents impair cellular permeabilization to α-sarcin.

Introduction

The mechanisms underlying viral infection of cells are not very well understood. Following attachment to membrane receptors, viruses cluster in areas of the cell surface called patches and caps. Viral particles appear to enter the cells by different mechanisms: endocytosis, cell fusion or direct entrance. After viral infection changes in membrane permeability are produced, as well as a loss of the ionic barrier, a lowering of the membrane potential, and variations in membrane fluidity (8, 9).

Molecules of different sizes can enter infected cells with variable efficiency (2, 4). It has been suggested that viral enzymes might participate in cellular permeabilization and in the activation of membrane enzymes (7, 10). Epidermal growth factor and Pseudomonas toxin enter the cell in an endocytic vesicle and they are released into the cytosol of KB cells (6). In previous studies we showed that infection of HeLa cells with encephalomyocarditis virus make the cells permeable to the single-chain toxin α-sarcin (3, 4). This toxin (16 800 molecular weight) is an inhibitor of protein synthesis. It inactivates ribosomes enzymatically in a way similar to that of ricin and abrin A-chains (1, 5). The toxin entrance into the cell is an early process, being the protein moiety of the virus the one that induces permeabilization. The cell becomes again impermeable to the toxin 4 h after infection (4).

In the present paper we study the permeabilization to α-sarcin that follows infection of different cell types (HeLa, KB, BHK-21 and L929) with enveloped (VSV, SFV) or nonenveloped (EMC, Polio) viruses.

Materials and methods

Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% newborn calf serum. EMC virus was grown on L929 cells. Vesicular Stomatitis virus (VSV) and Semliki Forest virus (SFV) were grown on BHK-21 cells. Type I Poliovirus (attenuated Sabin) and Adenovirus type 5 were grown on HeLa cells. The fraction obtained after removal of the cell debris by centrifugation was used as a source of the corresponding virus.

Dulbecco’s modified Eagle’s medium E4, newborn calf serum and trypsin were purchased from GIFCO Biocult. α Sarcin was a gift from D. M. Shuurmans (Department of Public Health, Lan-
Figure 1-4. Inhibition of protein synthesis by α-sarcin in virus infected cells, in various systems. Cells were incubated for an hour with the virus at the m.o.i. (multiplicity of infection) indicated in the figure and with a concentration of α-sarcin of 15 μM. At the end of the incubation period the cells were washed and after the addition of fresh medium incubation was allowed to proceed for another 2 h, at which time a pulse of [35S]methionine was given for 1 h and protein synthesis was estimated. Results are expressed as percentage of [35S]-methionine incorporation in parallel control cultures without α-sarcin. (A) KB cells; (B) HeLa cells; (C) BHK-21 cells; and (D) L929 cells.

Fig. 1. EMC virus. Fig. 2. Poliovirus. Fig. 3. SFV. Fig. 4. VSV.