Effect of Ribosome-Inactivating Proteins on Virus-Infected Cells. Inhibition of Virus Multiplication and of Protein Synthesis

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With 3 Figures

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Summary

HEp-2 cells were infected with herpes simplex virus-1 (HSV-1) or with poliovirus I in the presence of plant proteins which inactivate ribosomes in cell-free systems, while exerting scarce effect on whole cells. Ribosome-inactivating proteins used were gelonin, from the seeds of *Gelonium multiflorum*, an inhibitor from the seeds of *Momordica charantia*, dianthin 32, from the leaves of *Dianthus caryophyllus* (carnation), and PAP-S, from the seeds of *Phytolacca americana* (pokeweed). All proteins tested had the following effects: 1. They reduced viral yield; 2. They decreased HSV-1 plaque-forming efficiency; 3. They inhibited protein synthesis more in infected than in uninfected cells. These results strongly suggest that ribosome-inactivating proteins impair viral replication by inhibiting protein synthesis in virus-infected cells, in which presumably they enter more easily than in uninfected cells.

Introduction

A number of proteins isolated from plant materials inhibit protein synthesis by inactivating ribosomes in cell-free systems, whilst being much less effective on whole cells, in which they do not enter easily. These proteins resemble the A-chains of ricin and related toxins (reviewed in 12) and include the pokeweed antiviral protein (PAP) (10, 11), the wheat germ inhibitor (14), the *Momordica charantia* inhibitor (3), gelonin (16) and dianthins (17).

The pokeweed antiviral protein inhibits the transmission of plant viruses (18), thus accounting for the antiviral effect of the extracts of *Phytolacca americana* (pokeweed) leaves (20) and reduces also the multiplication of influenza virus (18),
of poliovirus (19) and of herpes simplex virus (HSV-1) (1) in cell cultures. These
effects were attributed to increased penetration of PAP into virus-infected cells,
with subsequent inhibition of protein synthesis (13, 19). The other known ribosome-
inactivating proteins also inhibit infection by tobacco mosaic virus, presumably
acting in the same manner as PAP (15, 17).

We report now that gelonin, the *Momordica charantia* inhibitor, and dianthin 32,
like PAP, all inhibit multiplication of HSV-1 and of poliovirus I in HEP-2 cells,
and have a greater inhibitory effect by protein synthesis of cells infected with
these viruses than by uninfected cells.

**Materials and Methods**

**Ribosome-Inactivating Proteins**

*Momordica charantia* inhibitor was purified as described by Bardelli *et al.* (3)
and gelonin and dianthin 32 as described by Stirpe *et al.* (16, 17). The pokeweed
antiviral protein from seeds (PAP-S), similar but not identical with PAP, was purified
from the seeds of *Phytolacca americana* as described by Bardelli *et al.* (2).

**Cells and Viruses**

HEP-2 cells were grown in Eagle's minimum essential medium (MEM) containing
10 per cent of newborn calf serum (NCS) (Flow Laboratories, Irvine, Scotland).
Maintenance medium for infected cells consisted of MEM containing 1 per cent NCS.
Viruses used were HSV-1 (F) (5), HSV-1 (MP) (9) and poliovirus I.

**Determination of Virus Yield**

Cell cultures, 24 hours-old, in 16 mm multiwell trays (Nunc, Roskilde, Denmark)
containing 3.5—5 × 10^5 cells per well, were infected at an input MOI of 1 PFU/cell,
in the presence of the indicated concentrations of ribosome-inactivating proteins,
with quadruplicate cultures for each concentration. The inoculum was removed after
a 90- or 60-minutes adsorption period for HSV-1 (F) or poliovirus I, respectively.
Cells were rinsed three times with phosphate-buffered saline (PBS) and overlaid
with maintenance medium containing, when appropriate, the ribosome-inactivating
proteins. After incubation at 37° C in a humidified atmosphere with 5 per cent CO_2
for 48 hours [for HSV-1 (F)] or for 24 hours (for poliovirus I), infected cells were frozen
and thawed three times. The media from quadruplicate cultures were pooled and
viruses were titrated by plaque assay in HEP-2 cells.

**Efficiency of Plaques**

Duplicate cultures of 24 hours-old HEP-2 cells in 35 mm Falcon dishes were
infected with 150 or 700 PFU of HSV-1 (F) or of HSV-1 (MP), in the presence of
ribosome-inactivating proteins. After virus adsorption the inoculum was removed and
cells were overlaid with maintenance medium containing the ribosome-inactivating
proteins and 0.2 per cent human gamma globulin. After 56—60 hours monolayers
were rinsed, fixed with methanol and stained with Giemsa for plaque scoring.

**Effect of Ribosome-Inactivating Proteins on Virion Infectivity**

Aliquots of 1.5 ml of HSV-1 (F) or of poliovirus I were incubated with ribosome-
inactivating proteins (200 µg/ml) at 37° C in a shaking incubator. At the indicated
times duplicate samples of 0.1 ml each were taken and immediately frozen. Infectious
virus was estimated in each sample by plaque assay.