Protease Activation of Sendai Virus Infectivity; Studies in Non-Permissive and Permissive Cells

(Accepted 20 July 1978)

SUMMARY

Non-infectious virus particles are produced by BSC-1 cells after infection with Sendai virus. Trypsin treatment of these particles activates their infectivity. The studies reported here show that such non-infectious virus particles adsorb normally to cells but cannot initiate infection even after very long adsorption periods. Secondary Rhesus monkey kidney cells support the growth of Sendai virus but cannot activate the infectivity of virus grown in BSC-1 cells. The significance of these results is discussed.

Recent studies on the growth of Sendai virus in various host cells have indicated that proteolytic cleavage of a virion component is a necessary step in the production of infectious virus particles. Non-infectious virions produced by certain host cells contain a precursor glycoprotein, termed Fo (Scheid & Choppin, 1974). Treatment of such virions with trypsin cleaves the Fo precursor polypeptide into two smaller components, F1 and F2, and renders the virions infectious; virions also acquire cell fusing and haemolytic properties (Homma & Ohuchi, 1973; Homma & Tamagawa, 1973; Scheid & Choppin, 1974, 1977). The infectious virions produced by permissive host cells contain polypeptides F1 and F2, cleavage of the Fo polypeptide having occurred in vivo.

As non-infectious virus particles containing the uncleaved polypeptide Fo possess fully active haemagglutinating and neuraminidase activities and as protease treatment of such virus leads to the simultaneous acquisition of infectivity and cell fusing ability, it has been proposed that the cleavage products of polypeptide Fo are involved in virus penetration, this process involving fusion of the viron envelope with the cell membrane (Scheid & Choppin, 1974).

This paper describes the results of experiments which examined the ability of non-infectious virions which can be activated by protease to adsorb to cells before and after the activation of infectivity by trypsin treatment and which investigated the possibility that permissive cells might be able to activate this type of virus after adsorption.

Sendai virus was grown in 10-day-old fertile hens' eggs. The allantoic cavity of each egg was inoculated with about 10⁴ p.f.u. of virus; after 48 h incubation at 31 °C the eggs were chilled and the allantoic fluid harvested. Virus was also grown in BSC-1 cell monolayers in Petri dishes, which were infected at an input multiplicity of 20 p.f.u./cell. After 24 h incubation at 31 °C the medium was harvested.

BSC-1 cells were propagated in rotating Winchester bottles using Eagle's medium with 10% foetal bovine serum. Secondary cultures of Rhesus monkey kidney cells were obtained from Dr E. J. Bell, Regional Virus Laboratory, Ruchill Hospital, Glasgow. Soy bean trypsin inhibitor was obtained from Calbiochem.

Virus infectivity was assayed using a haemadsorption technique. Virus samples were allowed to absorb to BSC-1 cell monolayers for 1 h at 31 °C. Eagle's medium was then added and the cells were incubated for 24 h at 31 °C. The medium was then removed and a 1%
Table 1. Effect of trypsin treatment of Sendai virus grown in BSC-1 cells and in cells of the chick chorioallantoic membrane

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Treatment</th>
<th>Titre (HAD units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSC-1</td>
<td>PBS</td>
<td>5</td>
</tr>
<tr>
<td>BSC-1</td>
<td>Trypsin before adsorption</td>
<td>2.35 × 10⁶</td>
</tr>
<tr>
<td>BSC-1</td>
<td>Trypsin after adsorption</td>
<td>1.20 × 10⁴</td>
</tr>
<tr>
<td>Chorioallantoic</td>
<td>None</td>
<td>3.35 × 10⁶</td>
</tr>
<tr>
<td>membrane</td>
<td>Trypsin after adsorption</td>
<td>4.00 × 10⁸</td>
</tr>
</tbody>
</table>

* Virus was treated with an equal volume of trypsin (Difco trypsin 1:250) in Dulbecco’s phosphate buffered saline (PBS) [final concentration of trypsin 5 μg/ml] or with an equal volume of PBS for 15 min at 31 °C. Tenfold dilutions of these virus preparations were made and were allowed to adsorb to BSC-1 cell monolayers for 1 h at 4 °C. The inoculum was then removed and the cells washed with PBS. Some monolayers were treated with trypsin (5 μg/ml in PBS) for 15 min at 31 °C followed by soybean trypsin inhibitor (100 μg/ml in PBS) for 5 min at 31 °C. This was removed and Eagle’s medium was added. Haemadsorbing cells were counted after incubation for 24 h at 31 °C.

Table 2. Interaction between secondary rhesus monkey kidney cells and Sendai virus grown in either BSC-1 cells or in cells of the chick chorioallantoic membrane*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Treatment</th>
<th>Titre (HAD units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSC-1</td>
<td>None</td>
<td>5</td>
</tr>
<tr>
<td>BSC-1</td>
<td>Trypsin before adsorption</td>
<td>1.0 × 10⁴</td>
</tr>
<tr>
<td>BSC-1</td>
<td>Trypsin after adsorption</td>
<td>0.5 × 10⁴</td>
</tr>
<tr>
<td>Chorioallantoic</td>
<td>None</td>
<td>1.6 × 10⁸</td>
</tr>
<tr>
<td>membrane</td>
<td>Trypsin after adsorption</td>
<td>1.75 × 10⁸</td>
</tr>
</tbody>
</table>

* Experimental conditions were as described in the legend to Table 1 except that cell monolayers were not treated with soybean trypsin inhibitor.

Sendai virus was grown in BSC-1 cells, a continuous line of African green monkey kidney cells. The effect of treating this virus with trypsin is shown in Table 1, which demonstrates that these cells release virus which requires trypsin treatment for the expression of infectivity. This virus thus differs from that produced in the embryonated egg, the titre of the latter virus being unaffected by trypsin treatment.

Non-infectious Sendai virus which can be activated with proteases possesses a fully active haemagglutinin and therefore can, presumably, adsorb to susceptible cells. This possibility was tested directly by treating virus grown in BSC-1 cells with trypsin before and after adsorption and thereafter estimating its infectivity titre. It was assumed that if adsorption was significantly enhanced by trypsin treatment then the titre of virus treated with trypsin before adsorption would be considerably greater than that of virus treated
after adsorption. Such a result was not found, the difference being only twofold (Table 1), indicating that non-infectious protease-activable virus adsorbs to cells nearly as efficiently as infectious virus. The possibility that treatment of cells with trypsin directly enhances virus infectivity was ruled out in control experiments which showed that trypsin treatment of cells with adsorbed infectious virus did not result in enhancement of infectivity titres (Table 1).

The effect of delaying the addition of trypsin to virus adsorbed on cell monolayers was also examined. The amount of virus reactivable by protease treatment declined steadily over a 24 h period, indicating that even a prolonged adsorption period was not sufficient to initiate infection. Virtually identical titres were obtained when monolayers infected with infectious egg-grown virus were treated with trypsin at various times after infection.

Secondary Rhesus monkey kidney cells release large amounts of infectious Sendai virus and therefore contain or release enzymes capable of cleaving polypeptide Fo. The possibility that such enzyme activity might activate non-infectious virus adsorbed to the cell surface was therefore tested. The results in Table 2 clearly demonstrate that protease-activable virus is not rendered infectious after adsorption, virus infectivity titres before and after protease treatment closely resembling those obtained in non-permissive BSC-I cells (Table 1). As in BSC-I cells, protease treatment did not affect the titre of infectious egg-grown virus in secondary Rhesus monkey kidney cells.

The results obtained in these experiments have shown that Sendai virus propagated in BSC-I cells is released in a non-infectious form. As in the case of the same virus grown in MDBK cells (Scheid & Choppin, 1974) and mouse L cells (Homma, 1971) the virus can be rendered infectious by trypsin treatment.

As such non-infectious virus has a fully active haemagglutinin, the assumption has been made that adsorption to cells takes place normally, the lack of infectivity of the virus being accounted for by a defect in a subsequent stage in the virus growth cycle. In view of the observation that trypsin treatment renders virus infectious and, at the same time, capable of cell fusion and haemolysis, it seems probable that the defective stage is that of entry into the cell and that a fusion event takes place during this process. In the present study it has been shown that non-infectious virus released from BSC-I cells does adsorb normally and that subsequent trypsin treatment renders this virus infectious, thus lending support to the hypothesis just outlined.

The failure of protease-activable virus to initiate infection even after prolonged exposure to cells lends support to hypotheses invoking specific mechanisms of virus entry, and argues against the possibility that only a non-specific phagocytic mechanism is required for initiation of infectivity. The decline in protease titre on prolonged incubation of virus with cells probably reflects a combination of thermal inactivation and neuraminidase-mediated elution of adsorbed virus.

The nature and location of the protease(s) responsible for the cleavage of polypeptide Fo in permissive cells are of considerable interest, the presence or absence of such enzymes obviously playing an important role in determining the extent to which Sendai virus multiplies in different cell types. The experiments of Scheid & Choppin (1976) have shown that only proteases with a trypsin-like specificity are capable of activating wild-type Sendai virus. Little, however, is known about the location of these enzymes. The experiments reported here show that the enzyme activity present in secondary Rhesus monkey kidney cells cannot activate adsorbed virus. It is therefore entirely possible that this enzyme is intracellular and that cleavage of polypeptide Fo takes place within the cell. On the basis of results of pulse-chase experiments in secondary Rhesus monkey kidney cells in which
no significant degree of intracellular conversion of polypeptide Fo occurred during a 4 h chase period it has been suggested that cleavage of polypeptide Fo occurs either on the plasma membrane or after the virus has been released (Lamb et al. 1976).

The results of the present experiments make the latter possibility unlikely. In addition, as we do not know how many Fo polypeptide molecules in a virion have to be cleaved in order to render that virion infectious, it is difficult to interpret the biochemical data; it is possible, for example, that cleavage of only a small proportion of the total number of Fo molecules might render virions infectious and that this amount of processing might not be readily detectable by biochemical techniques.

I thank Miss Mary Braidwood for excellent technical assistance.

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REFERENCES


(Received 21 April 1978)