Characterization of Visna Virus Envelope Neuraminic Acid

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Visna virus particles inhibit influenza virus hemagglutination in an assay for neuraminic acid-containing viruses. Pretreatment of visna virus with neuraminidase abolished hemagglutination inhibition activity but did not significantly affect attachment, infectivity, or virus-induced cell fusion in sheep choroid plexus cell monolayers.

The lipid composition and carbohydrate content of the viral envelope are determined in large part by the host cell with insertion of virus-specific proteins into the lipid bilayer (2, 6, 8). This is true for members of the myxo- and paramyxovirus groups; however, neuraminic acid, a constituent of the plasma membrane of vertebrate cells, is excluded by the localized action of viral neuraminidase (7). Visna virus, the agent of a progressive neurological disease of sheep, matures by the release of buds from the cell surface (4, 12) and shares many properties of other enveloped viruses but does not possess neuraminidase activity (13, 14). Other enveloped viruses including vesicular stomatitis virus (VSV), Sindbis, and Rauscher leukemia virus also lack neuraminidase and contain neuraminic acid (3).

The hemagglutinin protein of influenza virions specifically binds to neuraminic acid residues on cell surfaces and on erythrocytes, causing hemagglutination; it may also bind to heterologous virions that contain neuraminic acid, thus causing virus aggregation. These activities have been used in a rapid hemagglutination inhibition (HI) assay for a variety of enveloped viruses including VSV, Sindbis, Rauscher leukemia virus, mouse mammary tumor virus, and Mason-Pfizer monkey virus (3, 10; M. Yagi and R. Compans, Virology, in press). Treatment of these viruses with neuraminidase prior to assay prevented HI of influenza virus.

We have examined the ability of visna virus to inhibit influenza virus hemagglutination. Based on successful demonstration of HI activity, visna virus was further studied to determine the biological function of envelope neuraminic acid.

Visna virus strain K485 was propagated in sheep choroid plexus (SCP) cells derived from primary explants as previously described (5). Viruses were harvested from clarified tissue culture fluid by ammonium sulfate precipitation, twice purified on linear potassium tartrate gradients (5 to 40%, wt/wt) (9), and dialyzed against phosphate-buffered saline (PBS).

In the hemagglutination inhibition assay, purified visna virus was serially diluted in twofold steps in microtiter plates (Linbro). To each dilution, purified WSN strain influenza virus at 12 hemagglutination units per ml was added, and the titration was carried out as reported previously (3). The binding of visna virus to the surface of influenza virions prevented the characteristic influenza hemagglutination. As shown in Table 1, HI of influenza by purified visna virus was abolished by neuraminidase treatment but not by pretreatment with neuraminidase. The HI of visna virus was diminished to a level not detectable at 1:8 dilution (HI units/ml).

TABLE 1. Inhibition of influenza virus hemagglutination by visna virus

<table>
<thead>
<tr>
<th>Treatment of visna virus</th>
<th>HI units/ml</th>
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<tbody>
<tr>
<td>(A) None</td>
<td>&gt;16,384</td>
</tr>
<tr>
<td>(B) 1. Neuraminidase-</td>
<td>&lt;2</td>
</tr>
<tr>
<td>treated visna virus, 2</td>
<td>h at 37°C</td>
</tr>
<tr>
<td>2. Visna virus, 2 h at 37°C</td>
<td>4,096</td>
</tr>
</tbody>
</table>

* To serial twofold dilutions of visna virus in 50-μl portions in a microtiter plate (Linbro) was added 50 μl of purified WSN strain influenza virus diluted to 12 hemagglutination units per ml in PBS. After 1 h at room temperature, 100 μl of a 0.5% suspension of chicken erythrocytes was added. Titters were read after 1 h, and the highest dilution showing inhibition of agglutination was considered the end point. In experiment (B), 100 μl of purified virus was treated with 25 μl (500 U/ml) of neuraminidase from Vibrio cholerae (Behring Diagnostics) for 2 h at 37°C. Controls received PBS. Serial dilutions of treated virus were assayed as described above with incubation at 4°C. Titters were read after 0.5 h.
visna virus reached a titer greater than 16,384 HI units per ml when virus suspensions containing approximately 5 mg of protein per ml were used. To determine whether neuraminic acid residues on the surface of visna virions were essential for HI activity, virions were pre-treated with neuraminidase. Such treatment completely abolished the HI activity of visna virions (Table 1). Similar findings were reported for HI activity of other neuraminic-acid-containing viruses (3, 10). The presence of neuraminic acid in visna virions was also demonstrated by direct assay by the procedure of Aminoff (1), and 0.39 μg of neuraminic acid was detected per 100 μg of virion protein.

It has recently been shown for VSV that infectivity was lost after enzymatic removal of surface neuraminic acid (11). Furthermore, resialylation in vitro with sialyl transferase restored viral infectivity. It was therefore of interest to determine whether neuraminidase treatment of visna virions had any effect on the biological activities. Visna virus at 2 × 10⁸ mean tissue culture infective doses (TCID₅₀) per ml was treated with 5 or 1 U of neuraminidase (Behring Diagnostics) for 2 h at 37°C before inoculation. Visna virus incubated in PBS was used as a control. Infectivity was measured by end point dilution on SCP monolayers as described previously (5). A titer of 6.3 × 10⁷ TCID₅₀/ml was obtained for the control; virions treated with neuraminidase had titers of 7.3 × 10⁷ to 9.9 × 10⁷ TCID₅₀/ml.

To test the ability of neuraminidase-treated virus to induce rapid cell fusion, purified enzyme-treated and control visna virions were used for inoculation of SCP monolayers grown on glass cover slips. Examination after 7 to 8 h revealed scattered cell fusion, and by 24 h the entire monolayer was fused in both neuraminidase-treated and control virus preparations. Cells without virus appeared normal. The onset and the extent of cell fusion appeared identical in the two virus preparations.

In a kinetic study of visna virus adsorption, the rate and efficiency of attachment of virus to SCP cell monolayers was determined. L-[¹⁴C]leucine-labeled virus was prepared, and a portion was treated with neuraminidase. Control and neuraminidase-treated virus was incubated at 37°C with SCP cells on glass cover slips, and at various times slips were harvested and assayed for adsorbed radioactivity. As demonstrated by Valentine and Allison (14), the theory of Brownian motion may be applied to virus-cell interactions to predict the rate at which particles arrive at the surface of the monolayer; a straight line is determined when the percentage of attached virus is plotted against the square root of time. The graph in Fig. 1 illustrates the data presented in this manner for the adsorption kinetics of neuraminidase-treated and control virus preparations. The slopes of these lines reflect the relative attachment rates, indicating a slight enhancement with enzyme-treated virus, 1.1 times the rate of control virus attachment. Statistical analysis of these results showed correlation coefficients of 0.94 and 0.83 from untreated and enzyme-treated samples. Use of t-tests indicated that results were significant at better than 95% confidence. The slight increase in rate and efficiency of attachment of enzyme-treated visna virus suggested some enhancement by neuraminidase release of sialic acid, perhaps exposing additional components inaccessible for interaction with receptors on the cell surface.

The retention of full biological activities of neuraminidase-treated visna virus is in contrast to the results obtained with VSV (11), indicating that the biological function of envelope sialic acid varies with different enveloped viruses.

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LITERATURE CITED
