**Involvement of Glycolipids in Myxovirus-induced Membrane Fusion (Haemolysis)**

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SUMMARY

Myxoviruses (influenza viruses and paramyxoviruses) penetrate their host cells by fusion of viral and cellular membranes. During this process, virus envelopes react with galactose-terminating glycolipids of cellular membranes. This was suggested by experiments showing the ability of these lipids to inhibit fusion (as measured by haemolysis) when reacted with the viruses, and to enhance it when added as enrichment to erythrocytes.

During the initial phase of infection, the interactions of myxoviruses with their host cells lead to several events such as attachment, membrane fusion and endocytosis (Dimmock, 1982). The attachment occurs through a specific binding of neuraminic acid-containing receptors of cellular membrane with the haemagglutinin of influenza virus or the haemagglutinin-neuraminidase complex of paramyxovirus (Rott & Klenk, 1977; Compans & Klenk, 1979). The membrane fusion has been proposed to occur at the cellular surface (Dourmashkin & Tyrrell, 1974; Huang et al., 1980b, 1981b) or solely in the lysosomal milieu after endocytosis (White et al., 1981). The fusion process seems to be mediated by a lipophilic peptide segment of virus glycoproteins present in the amino-terminal region of the HA2 polypeptide of the haemagglutinin of influenza virus or the F1 portion of the fusion protein of paramyxoviruses (Scheid et al., 1972; Klenk et al., 1975; Richardson et al., 1980). But the cellular counterparts involved in this fusion step have not as yet been characterized. I report here that there is a specific interaction of the virus envelope with certain glycolipids preceding fusion. This interaction may be fundamental to the fusion process.

Sendai virus and fowl plague virus were used as models of paramyxovirus and influenza virus. These viruses have been shown to cause extensive fusion with cellular membranes and when reacted with erythrocytes the resulting membrane fusion is accompanied by haemolysis which can be quantified (Bachi et al., 1977; Huang et al., 1981a). Evidence of the involvement of glycolipids in membrane fusion was obtained by their ability to inhibit membrane fusion as measured by inhibition of haemolysis. Haemolysis was carried out at pH 7 and pH 5.5, the optimal conditions for Sendai virus and influenza virus respectively (Maeda & Ohnishi, 1980; Huang et al., 1981a; Miller & Lenard, 1981). The results of such experiments are summarized in Table 1.

As is evident from the table, several neuraminic acid-free glycolipids greatly inhibited haemolysis. All active natural glycolipids tested contained galactose at terminal positions (Table 1, A, B and C). Globoside, which terminates in N-acetylgalactosamine (Table 1, D), inhibited haemolysis only at much higher concentrations. Three synthetic analogues of glycolipids (Table 1, E, F and G) containing either β- or α-terminal galactose also possessed high haemolysis-inhibiting activity. In addition to haemolysis-inhibition activity, all compounds at the concentrations indicated in Table 1 also completely inhibited virus-induced fusion of erythrocytes (light-microscopical observation, not shown).

None of the glycolipids tested impaired the adsorption process of myxoviruses, since the viruses retained their ability to agglutinate erythrocytes after interaction with these substances (not shown). The above haemolysis-inhibition results were interpreted to mean that lipids interfered with the fusion process by competitively reacting with the virus glycoproteins responsible for fusion. This assumption was supported by experiments showing that erythrocytes enriched with the galactose-terminating glycolipids listed in Table 1 could be more readily fused by the viruses. Fig. 1 illustrates the results obtained with oleyl lactoside. It can be...
Fig. 1. Enhancement of influenza virus (fowl plague virus)-induced haemolysis by preincubation of erythrocytes with oleyl lactoside (Table 1, E). Preincubation was carried out in a 1% erythrocyte suspension containing the indicated amounts of glycolipids or Tween-20 for 10 min at 37 °C. Pretreated erythrocytes were washed twice with physiological saline, resuspended as 1% suspension in saline and used for haemolysis as described in Table 1. 1, Control experiment: erythrocytes were pretreated with oleyl lactoside and incubated in the absence of the virus. 2, Control experiment: erythrocytes were pretreated with globoside, an N-acetylgalactosamine-terminating glycolipid, and incubated in the presence of the virus (10 μg protein/ml). 3, Control experiment: erythrocytes were pretreated with the detergent Tween-20 in the concentrations indicated and incubated in the presence of the virus (10 μg protein/ml). 4, Erythrocytes were pretreated with oleyl lactoside and incubated in the presence of the virus (10 μg protein/ml).

Table 1. Inhibition of myxovirus-induced haemolysis by glycolipids*

<table>
<thead>
<tr>
<th>Concentration (μg/ml) for 50% inhibition</th>
<th>Sendai virus†</th>
<th>Fowl plague virus†</th>
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</thead>
<tbody>
<tr>
<td>A. Ceramide-glcβ-gal</td>
<td>40±</td>
<td>15</td>
</tr>
<tr>
<td>B. Ceramide-glc-gal-galNAcβ-gal</td>
<td>55</td>
<td>25</td>
</tr>
<tr>
<td>C. Ceramide-glc-galβ-gal</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>D. Ceramide-glc-galβ-galNAcβ-gal</td>
<td>250</td>
<td>200</td>
</tr>
<tr>
<td>E. Oleyl-glcβ-gal</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>F. Phytol-glcβ-gal</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>G. Phytol-glcα-gal</td>
<td>35</td>
<td>15</td>
</tr>
</tbody>
</table>

* To a series of 10 ml conical centrifuge tubes were given 400 μl of a sodium acetate buffer (0.5 M, pH 5.5) or a sodium phosphate buffer (0.5 M, pH 7) containing increasing amounts (25 μg gradient) of various glycolipids. Lipids were added from stock suspensions (2.5 mg/ml) which had been finely dispersed in the respective buffers by sonication. Then 0.1 ml of Sendai virus or fowl plague virus containing 100 μg protein was added to each tube and mixed. Haemolysis was carried out for 15 min at 37 °C after addition of 2 ml of a 1% suspension of chick erythrocytes in each tube. The tubes were centrifuged briefly and the supernatant was measured for haemoglobin using a Zeiss photometer (at 540 nm). The values indicate the concentrations of lipids in the mixture which caused 50% inhibition of haemolysis as compared to the control tubes which contained no inhibitors. Haemolysis in control tubes gave an absorbance of about 1.

† Sendai virus, strain Z, and fowl plague virus, strain Rostock (H7N1), were grown and purified according to Chucholowius & Rott (1972). Glycolipids A to D were prepared as described by Huang (1978). Synthetic glycolipids E to G were obtained by coupling the acetobromo derivatives of lactose (β-) or melibiose (α-) to the 1-OH group of oleyl alcohol or phytol by the Königs-Knorr reaction as described previously (Huang, 1978).

‡ Values are averaged from triplicate determinations.

seen that haemolysis steeply increased when elevating amounts of this glycolipid were used to pretreat erythrocytes (line 4). Preincubation of erythrocytes with the glycolipid alone did not lead to the same effect (line 1), nor did the detergent Tween-20 cause haemolysis in the presence of the virus (line 3). Another detergent, Triton X-100, was equally inactive under the same conditions (not shown). When globoside, an N-acetylgalactosamine-terminating glyco-
lipid (Table 1, D), was used to pretreat erythrocytes, there was even a slight suppression of haemolysis with increasing doses of this glycolipid (line 2). This shows that the virus interacted with the glycolipids in a highly specific manner and that the effect of glycolipids observed was not due to an unpecific detergent action. It remains to be established in which way glycolipids react with the virus envelope and if glycolipids are the only lipids involved in fusion, since it has been suggested that phospholipids in the form of liposomes may also directly interact with the virus envelope (Haywood, 1974; Maeda et al., 1981). It is possible that, as compared to phospholipids, glycolipids may be more accessible in natural membranes to the invading virus due to their long extending carbohydrate chains. Moreover, galactose-terminating glycolipids are generated from neuraminic acid-containing glycolipids after reaction with viral neuraminidases. Specific membrane interactions involving glycolipids have already been described in the case of intercellular adhesion (Huang, 1978).

From investigations of the past, it seems clear that neuraminic acid is the most probable attachment site of the myxovirus. The present study demonstrates that neutral glycolipids can also react with the virus. In natural membranes, these lipids are probably shielded from direct contact with the virus by masking proteins. Based on a recent observation that neuraminidase is needed during myxovirus-induced membrane fusion, we postulated the existence of a second receptor in the cellular membrane, which is essential for membrane fusion but is exposed only after the action of neuraminidase (Huang et al., 1980a). In this sense, the glycolipids described here may be such compounds, which can be unmasked by the action of neuraminidase before they participate in membrane fusion. It is possible that these lipids are the natural reaction partners of the F1 polypeptide of paramyxoviruses and the HA2 polypeptide of influenza virus, which contain similar hydrophobic N-terminal structures (Gething et al., 1978). The exposure of these hydrophobic peptides by proteolytic cleavage during the replication of myxoviruses is known to be essential for the fusion property of these viruses (Compans & Klenk, 1979; Huang et al., 1980b). Of late, evidence is mounting that several other enveloped viruses infect their host cells by membrane fusion (Väänänen & Kääriäinen, 1979; Helenius et al., 1980; Miller & Lenard, 1981) and it would be interesting for future investigation to see if a similar mechanism of membrane fusion operates in all these viruses.

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REFERENCES


Short communications


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