Artificial Assembly of Envelope Particles of HVJ (Sendai Virus). Fusion Activity of Envelope Particles

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SUMMARY

An assay system for fusion activity of envelope particles of Sendai virus, re-assembled from NP40-solubilized envelopes, was established and conditions for the artificial assembly of NP40-solubilized Sendai virus envelope particles with haemolytic and fusion activities were investigated. Large (GP1) and small (GP2) glycoproteins and lipids seemed to be required for the expression of haemolytic and fusion activities of envelope particles. Potential haemolysin activity was associated with GP2. A relatively high proportion of GP1 was required for formation of envelope particles with a high fusion activity.

When the top lipid fraction (Hosaka & Shimizu, 1972a) was used for reassembly, the envelope particles usually exhibited both fusion and haemolytic activities but the optimal concentrations of the lipid for the two activities were different. An unidentified factor extractable with NP40 seemed to be necessary for fusion activity but not for haemolytic activity.

INTRODUCTION

Previously, we reported the in vitro assembly of active envelope particles of Sendai virus (HVJ), with haemolytic and fusion activities, from envelopes solubilized by the neutral detergent Nonidet P40 (NP40) (Hosaka & Shimizu, 1972a). We used the term ‘artificial assembly’ of envelope particles for this type of assembly in vitro, because the assembled particles differed from the original envelopes in having spikes on both sides of the membranes. In the preceding papers of this series (Hosaka & Shimizu, 1972a, b), the authors were concerned mainly with the assembly of Sendai virus envelope particles with haemolytic activity.

This paper deals with the assembly of envelope particles with fusion activity and describes: (1) the virus nature of fusion activity; (2) a comparison of fusion activity for KB cells in suspension and monolayer between assembled particles and virus particles; (3) the ineffectiveness of isolated lipid components for assembly of envelope particles with fusion activity and (4) a comparative study of the polypeptides required for assembly of envelope particles with fusion and haemolytic activities. An assay system for the fusion activity of assembled particles was established during these studies.

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METHODS

Virus. Sendai virus, Z strain, was used. The virus was inoculated into the chorioallantoic cavity of 10-day-old chick embryos and incubated for 3 days. The virus particles were then partially purified by differential sedimentation of infected chorioallantoic fluids and suspended in SSC (0.15 M-NaCl + 0.015 M-sodium citrate) (Hosaka, Kitano & Ikeguchi, 1966).

Preparation of envelope protein bands and top lipids. The upper and lower bands of envelope protein and the top lipids (see below) were prepared as described previously (Hosaka & Shimizu, 1972a). Briefly, the method used was as follows: partially purified Sendai virus particles were sedimented and the resulting pellets resuspended in 0.005 M-tris, pH 7.4. After clarification by low-speed sedimentation the virus suspension (40,000 H.A.U./ml) was treated with 0.25 % NP40. The treated virus particles were centrifuged at 100,000 g for 30 min. The resulting supernatant fluids, designated the 'solubilized envelopes', were subjected to equilibrium sedimentation in caesium chloride (CsCl). Two visible bands of envelope proteins were formed at density levels of 1.26 and 1.28 g/ml, whereas the lipids and NP40 were confined to the top of the tube and designated 'top lipids'. Sometimes, depending on the particular lot of CsCl, the envelope protein thus obtained was inactive for the reassembly of active envelope particles; we found that the addition of trace amounts of gelatine (Difco) to a final concentration of about 10 μg/ml kept the envelope protein active during the equilibrium sedimentation.

Preparation of virus lipids and lipid components. Concentrated Sendai virus (400,000 H.A.U./ml) in tris was extracted with 20 vol. of chloroform-methanol (2:1, v/v) at 40 °C. The extract was washed once with a quarter vol. of water. The chloroform layer was removed, the solvent was evaporated under an N2 stream, the residue was dissolved in 0.2 % NP40 and insoluble materials were removed by sedimentation at 2000 rev/min for 5 min. Sometimes, NP40 was added during chloroform–methanol extraction and then the final residue was dissolved in tris.

Lipid components of the virus were isolated from the extract by thin layer chromatography, as described previously (Hosaka & Shimizu, 1972b).

Artificial assembly of envelope particles. The top lipids, isolated lipid components, or mixtures of lipid components were mixed with the combined band of envelope protein (Hosaka & Shimizu, 1972a), separated by equilibrium sedimentation, or mixtures of the two bands (lower, L and upper, U) in various proportions and dialysed against phosphate-buffered saline (PBS) in the cold for 3 or 4 days, using fresh PBS each day. Usually dialysates were centrifuged at 27,000 rev/min for 30 min (Beckman no. 40) and the resulting pellets, referred to as 'assembled particles', were suspended in Eagle's minimal essential medium (MEM) and assayed.

Assay of fusion activity. One ml of virus sample, supplemented with 10 % calf serum, was inoculated onto KB cells in monolayers on coverslips and incubated at 37 °C for 3 h. The cells were then fixed, stained with haematoxylin and eosin, and observed. It was difficult to quantitatively measure the fusion of KB cells in monolayers, particularly when the fusion index (Okada & Tadokoro, 1962) was more than ten, and therefore fusion was assessed semi-quantitatively, as shown in Table 1.

Fusion of cells in suspension was expressed by a decrease of cell numbers. KB cells in monolayers were detached with 0.25 % trypsin and 0.02 % EDTA treatment and suspended in Eagle's MEM with 20 % calf serum. Virus samples in MEM were mixed with an equal vol. of this cell suspension in the cold for 15 min and then incubated at 37 °C for 60 min
Table 1. Semi-quantitative assessment of extent of fusion of KB monolayer cells

<table>
<thead>
<tr>
<th>Degree</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formation of a number of islands of fused cells, each consisting of several cells</td>
</tr>
<tr>
<td>2</td>
<td>Formation of many islands of fused cells, each consisting of ten or more cells</td>
</tr>
<tr>
<td>3</td>
<td>Intermediate between 2 and 4</td>
</tr>
<tr>
<td>4</td>
<td>Whole monolayer fused into irregular-shaped giant cells</td>
</tr>
<tr>
<td>5</td>
<td>Whole monolayer completely fused into a single giant cell</td>
</tr>
</tbody>
</table>

without shaking. Cell numbers were counted in haemocytometers. Cell degeneration was observed under a phase microscope.

Assay of other virus activities and chemical analyses. Assay methodology was as described previously (Hosaka & Shimizu, 1972a). Briefly, haemolytic activity was measured as follows: 0.1 ml of virus samples was mixed with 2 ml of 2% chicken red blood cells (RBC) in the cold for 15 min and then incubated at 37°C for 60 min, to determine liberated haemoglobin, unless otherwise mentioned.

Polyacylamide gel electrophoresis. This was carried out, using 7.5 % gel and following the method of Maizel (1970). Glycoprotein was identified with the periodic acid–Schiff’s technique (Zacharius et al. 1969).

Velocity sedimentation in sucrose gradients. Discontinuous sucrose gradients (5 to 20%, w/w) were made in PBS and allowed to stand a few hours at room temperature. Samples of 2 ml each were layered on the gradients and centrifuged in a Beckman SW 25.1 rotor at 20000 rev/min for 60 min in the cold. Then fractions were collected dropwise from the bottom.

RESULTS

Virus nature of biological activities of assembled particles

Although fusion of KB cells in monolayers could be induced with dialysed samples (6400 H.A.U./ml) of NP40-solubilized envelopes (2 or 3 h after inoculation, in MEM supplemented with more than 15% calf serum), usually cell fusion was associated with cell degeneration (Fig. 1 a). When the MEM contained less than 10% calf serum, cell degeneration was dominant after the same incubation time and most of the cells were detached during staining. After dialysis, either 0.25% NP40 alone or top lipids containing NP40 did not cause haemolysis or fusion but did cause cell degeneration in monolayer cells under the condition used. The dialysed top lipids had a haemagglutinating (HA) activity. Both cytopathogenicity and HA were heat-resistant (Table 2) and were not inhibited with a tenfold dilution of anti-Sendai rabbit serum; they were therefore considered to be non-virus in nature.

As shown in Table 2, if the dialysed sample of solubilized envelopes was centrifuged at 27000 rev/min for 30 min (Beckman Rotor no. 40), the resulting pellet fraction induced fusion of KB cells in monolayer very efficiently (Fig. 1 b). It contained heat-labile HA, haemolytic (HL) and fusion activities, and was free of heat-resistant cytopathogenicity and HA activity (Table 2).

HA, HL and fusion activities of the above pellet fraction were specifically inhibited by anti-Sendai virus rabbit serum and not by anti-influenza virus rabbit serum or foetal calf serum (Table 3). The heat-resistant HA was found to be strongly inhibited with foetal calf serum. These biological activities of the pellet fraction were, therefore, considered to be virus in nature. In subsequent experiments, we used this pellet fraction as assembled particles from NP40-solubilized envelopes of Sendai virus.
Fig. 1. Cell fusion of KB cells in monolayers induced by dialysed preparation (6400 H.A.U./ml) of solubilized envelopes with 30% foetal calf serum (a), pellet fraction (3200 H.A.U./ml) with 10% foetal calf serum (b), and control cells (c). The cells were incubated 3 h after inoculation and stained with haematoxylin–eosin. The samples were the same as described in Table 2.

The nature of HA in the top lipid dialysate is still uncertain but this HA is considered not to be related to the heat-resistant cytopathogenicity, because, in spite of being devoid of HA activity, NP40 dialysate itself caused a similar effect. The heat-resistant cytopathogenicity of various kinds of dialysates described in Table 2 was not removed after 5 days dialysis through Neflex tubes and was probably due to some undialysable substance(s), which was originally contained in NP40.
Table 2. Heat stability of biological properties* of dialysates of solubilized envelopes and top lipids

<table>
<thead>
<tr>
<th>Samples</th>
<th>Haemagglutination H.A.U./ml</th>
<th>Haemolytic activity $E_{540}$</th>
<th>Fusion activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unheated</td>
<td>Heated</td>
<td>Unheated</td>
</tr>
<tr>
<td>Dialysate of solubilized envelopes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>6000</td>
<td>400</td>
<td>2.8</td>
</tr>
<tr>
<td>Supernatant fluid by sedimentation (27000 rev/min 30 min)</td>
<td>3200</td>
<td>400</td>
<td>2.4</td>
</tr>
<tr>
<td>Pellet by sedimentation (suspended in the original vol.)</td>
<td>2000</td>
<td>&lt; 20</td>
<td>2.2</td>
</tr>
<tr>
<td>Sendai virus particles</td>
<td>6400</td>
<td>&lt; 20</td>
<td>1.9</td>
</tr>
<tr>
<td>Dialysate of top lipids †</td>
<td>6400</td>
<td>3200</td>
<td>0.0</td>
</tr>
<tr>
<td>Dialysate of 0.25 % NP40 alone</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Original dialysate was one dialysed against PBS for 3 days and further against MEM an additional day and then assayed. Its fusion activity was tested after it was supplemented with 30 % foetal calf serum. The supernatant fluid was separated from 3 days dialysate and dialysed against MEM for one day and then similarly assayed. Top lipids and 0.25 % NP40 were similarly dialysed and assayed. The other samples were suspended in MEM and assayed, except that their fusion activity was examined after supplementation with 10 % foetal calf serum. Fusion activity of heated samples was examined after heating at 90 °C for 10 min, followed by supplementation of serum.

† Cell degeneration.
‡ Top lipids (phosphorus (P): about 200 nmol/ml) were 10 times diluted and dialysed against PBS and assayed as described above.

Fig. 2. Velocity sedimentation pattern of assembled particles. A 2 ml sample (25 600 H.A.U./ml) was layered on the sucrose gradients and centrifuged as described in the Methods. One ml samples of fractions were dialysed against MEM for 1 day, followed by supplementation with 10 % calf serum, then assayed for their fusion activity (□□□□□□). 0.1 ml samples of fractions were assayed for their haemolytic activity (HL, ○○○○○○). ●●●●●●, H.A.U./ml.

Association of haemolytic and fusion activity of assembled particles with their haemagglutinins

The association between HL and HA activities in the dialysed sample of NP40-solubilized envelopes was previously shown by fractionation using equilibrium sedimentation in CsCl (Hosaka & Shimizu, 1972a). The association of the three activities of assembled particles described above was demonstrated in this study by fractionation using velocity sedimentation in sucrose gradients (Fig. 2). The proportions of the activities varied depending on the
fraction; the upper fractions had a high HA and HL activity but low fusion activity and the lower fractions had a relatively high fusion activity. Association of HL and fusion activities in assembled particles with haemagglutinin, confirmed the virus nature of these activities. The relationships among the three activities and between the activity and particle sizes in these fractions has been described elsewhere (Hosaka, 1974).

Comparison of fusion activity of assembled particles and virus particles for cells in monolayer

Dose-response curves of fusion activity of assembled particles and virus particles for KB cells in monolayer are shown in Fig. 3. The fusion activity of virus particles was higher (about 4 times) than that of assembled particles on basis of equal HA titres. Usually, no degeneration of KB cells was observed under the conditions employed. To compare fusion activity of particles assembled under different conditions, particles of more than 1000 H.A.U./ml (the plateau region in Fig. 3) were usually used.

Comparison of fusion activity of assembled particles and virus particles for cells in suspension

Dose-response curves of fusion activity of assembled particles and virus particles for KB cells in suspension were compared (Fig. 4). With increasing concentrations of virus particles, the number of KB cells decreased and little cell lysis occurred. However, with assembled particles, the number of KB cells decreased to about a half and increased again at concentrations above $2^8$ H.A.U./ml. Apparently, when too much damage occurred on cell membranes with active virus envelopes, the fusion ability of the cell membranes was lost.

The above data indicated that it is easier to compare the extent of cell fusion induced by different preparations of assembled particles (i.e. formed under various conditions) using KB cells in monolayers, since fusion of KB cells in suspension was inhibited at high concentrations of assembled particles.
Fusion activity of assembled envelope particles

Fig. 4. Dose-response curves of fusion of KB cells in suspension and simultaneous cytolysis induced by assembled particles (○—○) and Sendai virus (●—●). +, ++, ++++ mark cell degeneration caused by assembled particles in about 25, 50, 100% of the whole KB cell population, respectively.

Fig. 5. Assembly of active envelope particles with the total envelope protein (250 μg) and various kinds of lipid fractions; top lipids of Sendai virus (○—○), chloroform–methanol extract of Sendai virus in the absence (●—●) or presence (▲—▲) of NP40. Assembled particles of 4 days dialysates were suspended in 1.5 ml of MEM and their haemolytic (HL) and fusion activity assayed.

Ineffectiveness of extracted lipid components for formation of particles with fusion activity

The second paper of this series (Hosaka & Shimizu, 1972b) reported the assembly of active haemolysins by dialysis of mixtures of the envelope protein (U, 25 to 400 μg) and phospholipid mixtures (0.75 to 120 nmol) or of mixture of the envelope protein (U, 200 μg), phosphatidyl ethanolamine (1 to 50 nmol) and cholesterol (5 to 40 μg). Under the present assay system, negligible fusion activity was found with assembled particles (the pellet
Fig. 6. Densitometer profiles of SDS-gel electrophoretograms of the fractions of the envelope protein, separated by equilibrium sedimentation in CsCl. Numbers correspond to the gradient fraction numbers in Fig. 7 (c). Each fraction was dialysed against 0.01 M-phosphate buffer and a 0.1 ml sample was treated with 1% SDS and 2-mercaptoethanol and then applied to gel (5.5 x 60 mm column of 7.5% gel) and run at 5 mA for about 6 h. Gels were stained with Coomassie blue and scanned with a densitometer (Gilford) at 570 nm. The migration is from left to right. GP1, GP2 and a slower small peak, indicated by an arrow, were glycoproteins, stained with periodate-Schiff’s reagent. The total distance moved is 6 cm.

obtained by sedimentation at 27000 rev/min/30 min) of these dialysates. To determine whether some component might have been missed during isolation of lipid components, we tested some crude lipid fractions for the assembly of envelope particles with fusion activity. For fusion activity, the top lipids of Sendai virus were the most effective, while the chloroform–methanol extract of Sendai virus lipids in the presence of NP40 was moderately effective and lipids in the absence of NP40 were ineffective (Fig. 5). All these fractions were very effective in the formation of active haemolysin (Fig. 5). All the lipid fractions obtained with the original NP40 treatment were effective and one lipid fraction without NP40 was ineffective, even though NP40 was added subsequently to the mixture of envelope protein and lipids. This led to two possible interpretations: one, that some component(s) extractable only in the presence of NP40 is (are) necessary for fusion activity; or two, that the presence of NP40 in the original treatment helps to conserve the configuration of envelope lipids that is favourable for cell fusion. At present, it is uncertain which interpretation is more probable.
Fusion activity of assembled envelope particles

The considerable difference in the optimal concentrations of lipids for HL and fusion activities of assembled particles is seen in Fig. 5. As reported elsewhere (Hosaka, 1974), after fractionation of these preparations by velocity sedimentation, active envelope particles were found to be heterogeneous in their HL and fusion activities, some fractions had HL activity alone and no fraction had fusion activity alone.

Polypeptides of envelope proteins separated by equilibrium sedimentation

The protein portion of envelopes of Sendai virus, which have been solubilized by NP40 treatment, consisted of two glycoproteins (GP1, mol. wt. 67000 and GP2, mol. wt. 51000) (Hosaka & Shimizu, 1972a; Shimizu, Hosaka & Shimizu, 1972). To examine the relationship between the two proteins and the expression of HL and fusion activities, we first studied the distribution of these glycoproteins in the envelope protein fractions separated by equilibrium sedimentation in CsCl (Hosaka & Shimizu, 1972a).

Fig. 6 shows the densitometric patterns of bands of polypeptides of envelope protein fractions, separated by SDS-gel electrophoresis. These bands were divided by mol. wt. and the resulting relative molar proportions of GP1 and GP2 are shown in Fig. 7(c), where fraction 6 and 8 to 11 corresponded approximately to the L and U bands, respectively. L and U contained predominantly GP1 and GP2, respectively. The previous finding (Hosaka & Shimizu, 1972a) that the two band fractions, L and U, had similar polypeptide compositions remains to be qualified for its environmental condition.

The distributions of GP1 and GP2 roughly corresponded to those of the HA and protein content, respectively, (Fig. 7b) of similar fractions from a parallel gradient. Previously, we reported (Hosaka & Shimizu, 1972a) that the distribution of neuraminidase activity after equilibrium banding in CsCl solution was similar to that of HA or HA antigen. These findings are consistent with those reported by Scheid et al. (1972), Scheid & Choppin (1973a, b, 1974) and Tozawa, Watanabe & Ishida (1973) that a large glycoprotein of paramyxoviruses is HA protein, associated with neuraminidase activity. Naturally, the correspondence of GP2 and protein content was the case particularly in those fractions in which the content of GP1 was low.

The small peak following GP1, seen in the gradient fractions no. 5 to 7 in Fig. 6, was a glycoprotein and was presumed from its mol. wt. to be a dimer of GP1. Since its band area was less than one tenth of GP1, it was neglected in the present experiments. Further, the small peak preceding GP2, seen in gradient fractions no. 8 to 24, which is considered to be a membrane protein, varied in its steepness, depending on the solubilized samples. Therefore, this peak was also dismissed, since it was not considered to be envelope-specific protein.

Polypeptides related to haemolytic activity

Fig. 7 (a and c) shows that the GP2 distribution roughly corresponded to that of the expression of HL activity after assembly with definite amounts of lipids. The lack of an exact correspondence between GP2 content and haemolysis was probably because the expression of HL activity varies with the concentration of lipids, the optimum of which depends on the envelope protein content (Hosaka & Shimizu, 1972b).

Fig. 8 (a) shows that on using a constant amount of total envelope protein and increasing concentrations of GP2, a higher HL activity occurs; the optimal amounts of the top lipids for HL activity, however, remain unchanged with GP2 concentrations. These findings indicate that GP2 is an essential factor required for HL activity and also that GP1 and GP2 can replace each other almost equally in the reaction with lipids.

We notice that in Fig. 7, a small amount of GP1 occurs in all fractions expressing HL
activity. This GP1 may play a role in the adsorption step as a pre-requisite for the function of GP2. Our interpretation of the expression of HL activity is as follows: a potential haemolysin activity is associated with GP2. If GP2 is reacted with lipids to form membranous particles, then activation of the haemolysin occurs. When GP1 is involved in this reaction, the membranous particles can adsorb to cells and become fully active.
Table 3. Reactivity of haemagglutinating, haemolytic and fusion activities of assembled particles and top lipid HA to rabbit antiserum to Sendai virus and foetal calf serum

<table>
<thead>
<tr>
<th></th>
<th>Rabbit antiserum to Sendai virus</th>
<th>Rabbit antiserum to influenza virus (Adachi strain, H2N2)</th>
<th>Foetal calf serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemagglutination inhibition*</td>
<td>For assembled particles</td>
<td>For top lipids</td>
<td>Haemolytic† activity ($E_{	ext{max}}$)</td>
</tr>
<tr>
<td>Rabbit antiserum to Sendai virus</td>
<td>640</td>
<td>10</td>
<td>0·1</td>
</tr>
<tr>
<td>Rabbit antiserum to influenza virus (Adachi strain, H2N2)</td>
<td>10</td>
<td>10</td>
<td>1·8</td>
</tr>
<tr>
<td>Foetal calf serum</td>
<td>10</td>
<td>640</td>
<td>1·9</td>
</tr>
</tbody>
</table>

* Titre represents the maximum dilution of serum to inhibit haemagglutination by 4 H.A.U. sample.
† Assembled particles (2000 H.A.U./ml) were mixed with an equal vol. of serum 5 times diluted with MEM, kept in the cold for 30 min and then 0·1 ml samples were assayed for their haemolytic activity with 2 ml of 2% chicken red cells and 0·25 ml samples for their fusion activity on KB cells in monolayers.

Fig. 8. Haemolytic (HL), fusion and haemagglutinating (HA) activities of assembled particles from mixtures (total protein: 150 µg) of L (mainly GPI) and U (mainly GP2) bands in different proportions and the top lipids; U band alone ( ), U + L bands 2/3 + 1/3 ( ), 1/3 + 2/3 ( ), L band alone ( ). Assembled particles of 4 days dialysates were suspended in 1 ml of MEM and 0·1 ml samples were assayed for HL activity and the remainder for fusion activity.

Fig. 8(c) shows that although higher HA activity was found with increasing amounts of GPI, the HA activity of assembled particles tends to decrease when the amounts of lipids are excessive. The reason for this phenomenon is still unknown.

Polypeptides related to fusion activity

A high degree of fusion was observed with the gradient fractions no. 10 to 12 of Fig. 7(a) and low fusion activity with fractions 13 and 14. We wanted to determine whether a good expression of fusion activity needs the participation of more GPI than did HL activity. The amount of GPI necessary for optimum fusion activity is greater than that for haemolytic activity (Fig. 8b), which depends on amounts of GP2 (Fig. 8a). Under the condition employed, $L:U = 1:2$ ratio was best for the expression of fusion activity, which corresponded to GPI:GP2 = 1:2·5.

The requirement of a higher content of GPI for the expression of high fusion activity may be explained in two ways: one is that this higher quantity assures the stable attachment of assembled particles to cells favourable for fusion and this stable attachment is assumed to depend on the content of GPI; another possibility is that the higher GPI content assures
Table 4. Chemical constituents required for the four activities associated with artificially assembled Sendai envelopes

<table>
<thead>
<tr>
<th>Activity</th>
<th>Glycoprotein</th>
<th>Lipids</th>
<th>Unidentified factor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemagglutinating antigen</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Haemagglutinating activity</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Haemolytic activity</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fusion activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

the formation of a stable membrane favourable for fusion that may somewhat neutralize a lytic action by GP2. Other possibilities cannot be excluded at present.

Again, in this experiment, the optimal concentration of the top lipids for fusion activity was higher than that for HL activity. Generally, these higher concentrations of lipids produced larger membranous particles (Hosaka & Shimizu, 1972b; Hosaka, 1974). At present, we are not sure whether the physical size of the particle is solely responsible for the higher fusion activity. It is interesting, however, that those conditions giving assembled particles maximal HL activity are not as favourable for the fusion activity of assembled particles.

DISCUSSION

In the present paper an assay system was established for the fusion activity of envelope particles of Sendai virus assembled from NP40-solubilized envelopes. These assembled particles had a high fusion activity comparable to that of virus particles for cells in monolayers. Papahadjopoulos, Post & Schaeffer (1973) recently reported fusion of mammalian cells by artificial lipid vesicles, in which the fusion was measured as the ratio of nuclear number in fused cells to total nuclear number. The fusion efficiency of Sendai virus as well as that of lipid vesicles in their system was very low compared with ours. According to their measurements and even though the cells used by them were different from ours, the amount of fusion in our semi-quantitative system would be 90 to 100% even in grade 3 although detailed comparisons have not yet been done.

Based on the foregoing results, the chemical constituents required for the four activities of assembled particles of Sendai virus are summarized in Table 4. We have identified the biological significance of GP2 of fully active, egg-grown Sendai virus, as having the potential to induce haemolysis since, on assembly of membranous particles with GP1 and lipids, GP2 can express the HL activity. Although fusion is a more complex phenomenon than haemolysis, a biologically active GP2 is assumed to work both in fusion and haemolysis. Probably, additional requirements for fusion activity mean conditions of membranous particles favourable for the connexion or repair of damaged cell membranes.

Shimizu et al. (1972) first showed the polypeptide pattern of egg-grown Sendai virus, identifying two kinds of glycoproteins; K. Shimizu and his co-workers (personal communication) have also separated HA-neuraminidase and non-active fractions by electrofocusing into large and small glycoprotein, respectively, from alkali-Emasol solubilized envelopes. Many workers (Content & Duesberg, 1970; Mountcastle et al. 1970; Mountcastle, Compans & Choppin, 1971; Stone, Kingsbury & Darlington, 1972; Tozawa et al. 1973) studied polypeptide patterns of tissue culture-grown Sendai virus and all except Mountcastle et al. (1970) identified three kinds of glycoproteins. Tissue culture-grown Sendai virus usually
Fusion activity of assembled envelope particles exhibited low infectivity for cultured cells and had a low or negligible HL and fusion activity (Ishida & Homma, 1961; Hosaka, 1962; Matsumoto & Maeno, 1962; Homma, 1971, 1972; Homma & Tamagawa, 1973), although Tozawa et al. (1973) and Stone et al. (1972) obtained high yield of infectious Sendai virus in calf kidney and chicken lung cells, respectively.

Homma (1971, 1972) first reported the activation of infectivity and HL activity of tissue culture-grown Sendai virus by trypsin treatment. Subsequently, at the conference of Negative Strand Viruses (1973), Homma and Scheid & Choppin independently reported the change of polypeptide pattern of tissue culture-grown Sendai virus by trypsin treatment, accompanying activation of infectivity, HL and fusion activities. However, these two phenomena cannot be directly related to each other, as Homma & Ohuchi (1973) pointed out. In fact, infectious Sendai virus grown in calf kidney cells (Tozawa et al. 1973) contained a considerable amount of the precursor polypeptide (GP2 in Homma & Ohuchi's designation). The altered polypeptide patterns of Sendai virus are to be interpreted correctly on the basis of the data here presented of the polypeptides of fully active egg-grown Sendai virus. Thus, the findings by Homma & Ohuchi (1973) and Scheid & Choppin (1973b, 1974) are consistent with the present work in that the small glycoprotein (mol. wt. 51,000) is mainly involved in the expression of HL and fusion activities of virus particles with HA activity. The problem of growth of infectious Sendai virus in some tissue culture cells (Stone et al. 1972; Tozawa et al. 1973) remains to be solved.

It is easily understood from Table 4 that envelope particles with HL activity free from fusion activities could be assembled but not vice versa. In Sendai virus particles, all the chemical constituents shown in Table 4 are present on their envelopes and so their HL and fusion activity could not be easily separated from each other.

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