Fusion of influenza virus particles with liposomes: requirement for cholesterol and virus receptors to allow fusion with and lysis of neutral but not of negatively charged liposomes

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Influenza virus particles are able to fuse with liposomes composed of negatively charged or neutral phospholipids, as shown by using fluorochrome-labelled virions and fluorescence dequenching methods. Fusion with liposomes composed of only phosphatidylcholine (PC) was dependent on the presence of cholesterol (Chol), whereas fusion with liposomes containing negatively charged phospholipids, such as phosphatidylserine (PS), or of PC and phosphatidylethanolamine (PE) occurred in the absence of Chol. Fusion of influenza virions with PC:Chol liposomes was observed at pH 5.0, but not at pH 7.4, whereas a low degree of fusion with negatively charged liposomes or those containing PE was observed at pH 7.4. In addition, non-fusogenic influenza virions or HA0 influenza virions fused with PS- or PE-containing liposomes, especially at pH 5.0. Influenza virus particles were also able to induce the release of the fluorochrome calcine from negatively charged calcine-loaded liposomes at pH 5.0, as well as at pH 7.4, but failed to do so with PC:Chol liposomes. Lysis of PC:Chol by influenza virions was dependent on the presence of virus receptors, namely gangliosides (sialoglycolipids), and was observed only at pH 5.0. The results show that fusion of influenza virions with negatively charged or PE-containing liposomes does not reflect the biological activity of the virus needed for penetration and infection of living cells. On the other hand, fusion with PC:Chol liposomes is probably due to the activity of the viral fusion protein, the haemagglutinin glycoprotein.

Introduction

Penetration into and infection of living cells by influenza virions occurs in two steps (White et al., 1985). Initial binding of virus particles to membrane receptors, composed of sialic acid residues linked mainly to glycolipids, is followed by receptor-mediated endocytosis. Subsequently, fusion of the viral envelope with the membrane of endocytic vesicles is induced by the intraendosomal low pH environment (Sato et al., 1983; White et al., 1985). The viral haemagglutinin (HA) is composed of the HA1 and HA2 subunits, which are responsible for binding of virus particles to recipient cells and for endosomal fusion, respectively (Wilson et al., 1981; White et al., 1985).

An excellent tool for study of early stages of the fusion process is the use of liposomes as a recipient membrane. Numerous studies have demonstrated fusion between various enveloped viruses and liposomes of differing compositions (Hoekstra et al., 1984; Hsu et al., 1981; Klappe et al., 1988). Liposomes have also been used to show preferential insertion of the influenza and Sendai virus fusion polypeptides into target membranes (Novick & Hoekstra, 1988; Hurter et al., 1989). This was studied by the insertion of hydrophobic photoactive probes into liposomes composed of either phosphatidylcholine (PC), or negatively charged phospholipids such as phosphatidylserine (PS) or cardiolipin. The results show that viral fusion polypeptides are labelled first after interaction with these phospholipid vesicles.

Whether such fusion reflects the viral fusogenic activity needed for penetration and infection is not known. This is especially important in the light of experiments (Chejanovsky et al., 1986) showing that the non-fusogenic binding protein of Sendai virus, haemagglutinin–neuraminidase, exhibits fusogenic activity on interaction with negatively charged phospholipids, especially at low pH. These (Amsalem et al., 1985; Chejanovsky et al., 1986) and other (Citovsky & Loyter, 1985) observations raise the possibility that viral
glycoproteins may exhibit non-specific fusogenic activity with liposomes which is not necessarily related to that required for fusion with biological membranes.

In the present study an effort was made to study whether the fusion of influenza virions with liposomes was dependent on (i) phospholipid composition, (ii) the presence of virus receptors and (iii) the biological activity of the viral HA subunit.

Using fluorescence dequenching methods (DQ) (Hoekstra et al., 1984; Citovsky & Loyter, 1985; Loyter et al., 1988), we have shown that fusion with liposomes composed of PC and cholesterol (Chol) and lysis of PC:Chol:ganglioside (gang) liposomes, but not with those composed of negatively charged phospholipids, reflects the viral fusogenic activity needed for penetration and infection.

**Methods**

**Reagents.** Calcein, phosphatidylcholine (PC) (type V-E), phosphatidylethanolamine (PE), PS, Chol, gang (bovine brain, type II) and octylglucoside were purchased from Sigma. SM-2 Bio-Beads (20 to 50 mesh) were obtained from Bio-Rad; Triton X-100 (scintillation grade) was from Koch Light Laboratories and octadecylrhodamine B chloride (R18) was obtained from Molecular Probes.

**Virus.** Influenza virus (A/PR8 strain) was isolated from the allantoic fluid of fertilized chicken eggs (Chuchalowin & Rott, 1972). Influenza A virus containing uncleaved HA (HA0) (virus H107N) was obtained as described previously (Klenk et al., 1975). Trypsinization of the HA0 virus was performed by incubating 200 μg virus with 3 μg trypsin in a final volume of 200 μl PBS pH 7-4 for 20 min at 37 °C, essentially as described (Klenk et al., 1975). Viral haemagglutinating units and haemolytic activity were determined essentially as described previously (Chuchalowin & Rott, 1972).

For inactivation of the influenza viral fusogenic activity, 400 μg of viral protein in a volume of 200 μl was treated as follows. For heat and glutaraldehyde (GA) inactivation, a virus suspension in PBS was incubated at 85 °C for 30 min or with 0-1% GA for 30 min at 37 °C. Inactivation by low pH was performed essentially as described (Sato et al., 1983) by incubating a virus suspension in PBS–sodium acetate pH 5-0 for 30 min at 37 °C. For inactivation by NH2OH, a virus suspension was incubated in 1 % NH2OH pH 6-5 for 30 min at 37 °C essentially as described (Schmidt & Lambrecht, 1985). At the end of the incubation period, the various virus preparations were washed twice with 10 volumes of PBS and then resuspended in 200 μl PBS.

**Preparation of liposomes.** The liposomes used were of the following composition and molar ratio (mol: mol): PC: PS: Chol: gang (1:0:5:0:3), PS: PS: gang (1:0:3), PS: Chol: gang (1:0:5:0:3) and PS: Chol (1:0:5). The phospholipid vesicles were prepared as described (Citovsky & Loyter, 1985; Citovsky et al., 1986). Briefly, the lipids were mixed in the above proportions, dried from their chloroform solution under nitrogen at 4 °C and solubilized in octylglucoside (10% in PBS) to give a molar detergent: lipid ratio of 10:1. For preparation of glycophorin (GP)-bearing liposomes (PC: Chol: GP (1:0:5:0:001), PC: Chol: gang: GP (1:0:5:0:3:0:001), the desired amount of protein (GP) was added to the octylglucoside solution. GP was extracted from human erythrocyte membranes (Hamaguchi & Cleve, 1972) and octylglucoside was removed from solutions containing lipids as described (Citovsky & Loyter, 1985; Citovsky et al., 1986). The liposomes thus formed were washed by centrifugation (80000 g, 30 min at 4 °C) in 20 volumes of PBS, and resuspended in PBS, usually to give a concentration of 2 mg/ml phospholipid (25 mm-phospholipids).

For preparation of liposomes loaded with the fluorochrome calcein, 80 nm of calcein was added to the octylglucoside solution of lipids. Calcein-loaded liposomes (200 μl of 2 mg/ml phospholipids) were placed on top of a syringe containing 5 ml of dried Sephadex G-25 (fine; Pharmacia). The loaded vesicles were then separated from excess free calcein by centrifugation of the Sephadex G-25 columns (2000 g, 10 min at 4 °C) (Citovsky & Loyter, 1985).

**Virus-induced release of calcein from liposomes.** Calcein-loaded liposomes (0-5 μg in phospholipids) were incubated with virus preparations [intact, HA0 virus or reconstituted influenza virus envelopes (RIVE); 25 μg viral protein each] in a final volume of 200 μl in PBS for 10 min at 4 °C. The pH of this medium was either maintained at 7-4 or adjusted to 5-0 by the addition of 50 μl 0-5 M-phosphate buffer (Na2HPO4/NaH2PO4), and the suspension obtained was then incubated for 20 min at 37 °C. At the end of the incubation period, 1 ml PBS was added to the reaction mixture and the degree of fluorescence was estimated before and after addition of Triton X-100 (0-1% final concentration). The level of fluorescence obtained in the presence of the detergent was considered as 100% calcein release. Fluorescence measurements were performed with an MFP-4 Perkin-Elmer spectrofluorometer (calcein excitation at 495 nm and emission at 525 nm) (Citovsky & Loyter, 1985).

**Measurements of virus–human erythrocyte ghost (HEG) and virus–liposome fusion.** Fluorochrome-labelled, intact HA0 virus or RIVE (5 μg of each) were incubated with HEG, prepared as described (Fairbanks et al., 1971), or liposomes in a final volume of 200 μl of PBS. Following 10 min of incubation at 4 °C, the pH of the medium was adjusted to that desired by the addition of 50 μl sodium acetate (0-5 M), and the suspension obtained was then incubated at 37 °C. At the end of the incubation period, a volume of 1 ml PBS was added to the reaction mixture, and the degree of fluorescence (excitation at 560 nm, emission at 590 nm) of each sample was estimated before and after solubilization with 0-1% Triton X-100 (Hoekstra et al., 1984; Citovsky & Loyter, 1985; Nussbaum et al., 1987). The percentage fluorescence DQ was calculated as described (Nussbaum et al., 1987).

**Results**

**Fusion of influenza virions with neutral phospholipids: requirement for Chol**

Incubation of R18-labelled influenza virions with liposomes composed of PC at pH 4-5 to 7-5 resulted in a low level of DQ (Fig. 1a). However, a steep increase was observed at pH <4-5, reaching about 65% DQ at pH 3-5 (Fig. 1). A different pattern of DQ was obtained when influenza virions were incubated with liposomes composed of PC and Chol (PC: Chol) or of PC: Chol liposomes bearing the virus receptor, PC: Chol: gang (Fig. 1a). Incubation of such liposomes at pH <6-5 resulted in a gradual but significant increase in fluorescence.

Incubation of the non-fusogenic HA0 influenza virions with PC: Chol or PC: Chol: gang liposomes at
Fig. 1. Fusion of influenza virions with phospholipid vesicles. Preparation of liposomes, labelling of influenza virions with R$_{18}$ and estimation of DQ were performed as described in Methods. (a) Fluorescein-labelled influenza virions (5 µg of viral protein) were incubated with liposomes composed of PC (Δ), PC : Chol (○) or PC : Chol : gang (●) (200 µg of PC each) in a final volume of 200 µl PBS for 10 min at 4 °C. The pH of the incubation solution was adjusted to the values indicated by addition of 50 µl of sodium acetate (0.5 M) of the appropriate pH. After 30 min incubation at 37 °C, the extent of DQ was estimated as described in Methods. (b) HA$_0$ influenza virions were trypsinized and fluorescein-labelled with R$_{18}$. HA$_0$ (Δ, ○) or trypsinized HA$_0$ virions (▲, ○) (5 µg of viral protein each) were incubated with liposomes composed of PC : Chol (●, ○) or PC : Chol : gang (▲, ▲) as described for (a).

pH 4-5 to 5-2 resulted in a low degree of DQ. However a high degree of DQ was observed at pH < 4-5 (Fig. 1b). This indicated that the increase in DQ obtained below pH 4-5 is due to lipid–lipid exchange (McDonald, 1987) and not to virus–membrane fusion. The results in Fig. 1(b) also show that a relatively high degree of DQ was observed at pH 4-5 to 5-2 following the incubation of activated fusogenic, namely trypsinized HA$_0$, virus particles (Klenk et al., 1975) with either PC : Chol or PC : Chol : gang liposomes. The degree of DQ obtained following incubation of these virions at pH 5-0 with liposomes composed of either PC : Chol : gang (36%) or of these liposomes containing erythrocytic GP (43%, data not shown) was close to that obtained following incubation with HEGs (45%; data not shown). A relatively high degree of DQ (23%) was obtained also when trypsinized HA$_0$ influenza virions were incubated with liposomes lacking virus receptor, namely PC : Chol (Fig. 1).

The extent of DQ was dependent on the liposome composition, the amount of phospholipid used and the time of incubation (Fig. 2a, b). The requirement for Chol to effect an increase in the degree of fluorescence is evident from the results in Fig. 2(c) which show that a low degree of DQ was observed with liposomes lacking Chol whereas maximum DQ was obtained with liposomes containing about 30% Chol (Chol : PC, 0-3 mol : mol). The results in Fig. 2(c) also show that a low degree of DQ was obtained at pH 7-4 with liposomes containing the optimal concentration of Chol.

Table 1. Virus–liposome fusion: requirement for an active HA glycoprotein

<table>
<thead>
<tr>
<th>Virus inactivated by</th>
<th>PC : Chol</th>
<th>PC : Chol : gang</th>
<th>HEG</th>
<th>HE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DQ (%)</td>
<td>Calcein release (%)</td>
<td>DQ (%)</td>
<td>Haemolysis (%)</td>
</tr>
<tr>
<td>Nothing</td>
<td>28</td>
<td>43</td>
<td>42</td>
<td>90</td>
</tr>
<tr>
<td>85 °C</td>
<td>12</td>
<td>15</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>GA</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Low pH</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>NH$_3$OH</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

* R$_{18}$-labelled virus preparations (5 µg viral protein each) were incubated with the liposome preparations (200 µg of PC each) or with HEG (200 µg of protein) in 200 µl of PBS pH 7-4, first for 10 min at 4 °C and then for 30 min at 37 °C at pH 5-0.
† The extent of DQ was estimated as described in Methods.
‡ For the determination of calcein release, untreated or treated influenza virions (25 µg viral protein) were incubated with liposomes composed of PC : Chol : gang (5 µg of PC) in 200 µl PBS, and the extent of calcein release was estimated as described in Methods.
§ For determination of haemolysis, untreated or treated influenza virions (5 µg viral protein) were incubated with human erythrocytes (HE) in PBS pH 7-4 with or without glutaraldehyde (Nussbaum & Loyter, 1985), as well as after incubation at 85 °C (Nussbaum & Loyter, 1985) or at low pH (Sato et al., 1983). Incubation of non-fusogenic virions with HEGs also resulted in a low degree of DQ. This is in contrast to the relatively high degree of DQ (28 to 42%) obtained upon incubation of fusogenic influenza virions with PC : Chol or PC : Chol : gang liposomes, or HEGs (Table 1).

The results shown in Table 1 suggest that most of the DQ observed reflects a process of virus–liposome fusion. A low degree of DQ, about 6 to 12%, was observed following incubation of non-fusogenic influenza virions with either PC : Chol or PC : Chol : gang liposomes (Table 1). Influenza virions were rendered non-fusogenic by treatment with either glutaraldehyde (Nussbaum & Loyter, 1987) or hydroxylamine (Schmidt & Lambrecht, 1985), as well as after incubation at 85 °C (Nussbaum & Loyter, 1987) or at low pH (Sato et al., 1983). Incubation of non-fusogenic virions with HEGs also resulted in a low degree of DQ. This is in contrast to the relatively high degree of DQ (28 to 42%) obtained upon incubation of fusogenic influenza virions with PC : Chol or PC : Chol : gang liposomes, or HEGs (Table 1).

The low degree of DQ (5 to 15%) obtained following incubation of non-fusogenic influenza virions with the Chol-containing liposomes at pH 5-0 (or at pH 7-4 with fusogenic influenza virions; Fig. 1) may be due to either lipid–lipid exchange processes or release of the virus-associated R$_{18}$ during the incubation period.

**Virus-induced release of loaded liposomes: requirement for sialoglycolipids**

Fusion of influenza virions with either human erythrocytes or cultured animal cells is always accompanied by
Fig. 2. Virus-liposome fusion: dependence on lipid composition, and kinetic studies. (a) Fluorochrome-labelled influenza virions (5 μg viral protein) were incubated with the indicated amounts of liposomes: PC (○), PC: Chol (△) or PC: Chol: gang (○). (b) All conditions and symbols as in (a). Following adjustment of the pH to 5.0, the samples were incubated for different times at 37 °C. (c) Inactivation of influenza virions with NH₂OH. Untreated (○, ○) or NH₂OH-treated influenza virions (△) (5 μg viral protein) were incubated with liposomes composed of PC and Chol at the indicated ratios (200 μg of PC) in a final volume of 200 μl PBS for 10 min at 4 °C. The pH of the medium was either 7.4 (○) or adjusted to 5.0 (△, ○). After 30 min incubation at 37 °C the extent of DQ was estimated.

Fig. 3. Virus-induced leakage of loaded liposomes: dependence on gang. (a) Influenza virions (25 μg viral protein) were incubated with calcein-loaded liposomes composed of PC, PC: Chol or PC: Chol: gang (0.5 μg PC each) in 200 μl PBS for 10 min at 4 °C. The pH of the medium was either 7.4 (□) or was adjusted to 5.0 (□), and after 30 min at 37 °C the extent of calcein release was estimated as described in Methods. (b) Different amounts of influenza virions were incubated with calcein-loaded liposomes composed of PC: Chol (△) or PC: Chol: gang (○, ○) (0.5 μg PC each) in 200 μl PBS for 10 min at 4 °C and for 30 min at 37 °C at pH 7.4 (□) or 5.0 (△, ○). (c) HA₀ (□) or trypsinized HA₀ influenza virions (●) (25 μg viral protein) were incubated with calcein-loaded liposomes composed of PC: Chol or PC: Chol: gang (0.5 μg PC each) in 200 μl PBS.

induction of haemolysis and cell lysis, respectively (Pasternak et al., 1985; White et al., 1985). The results in Fig. 3 show that influenza virions failed to induce release of calcein from liposomes composed of PC: Chol under conditions that allow virus–liposome fusion. Lysis was observed only in liposomes bearing virus receptors, namely gang (Fig. 3, Table 1).

The correlation between the viral fusogenic and lytic activities is evident from the results showing that only trypsinized HA₀ influenza virions were able to induce calcein release from PC: Chol: gang liposomes at pH 5.0 (Fig. 3c). This correlation is further substantiated by the results in Table 1 showing that non-fusogenic virions caused neither lysis of liposomes nor haemolysis of human erythrocytes.

RIVEs are reconstituted envelopes containing viral HA and neuraminidase polypeptides (Nussbaum et al., 1987). Upon incubation with liposomes composed of PC, Chol and gang, RIVEs showed the same features observed for intact virions. A measurable DQ above a background of about 10% was observed only with liposomes containing Chol (Fig. 4) and at pH 5-0 (not shown). RIVE-induced leakage of loaded liposomes was observed only following incubation with liposomes
Influenza virus–liposome fusion

Fig. 4. Interaction of RIVEs with liposomes. Fluorochrome-labelled (i), or NH₂OH- (ii) or GA-treated (iii) RIVEs (5 µg viral protein each) were incubated with liposomes composed of PC ( ), PC : Chol ( ), or PC : Chol : gang ( ) (200 µg PC each). The extent of DQ (a) and calcein release (b) were estimated as described in Methods.

Fig. 5. Fusion of influenza virions with PS liposomes. HA₀ influenza virions were incubated with trypsin and labelled with R18 as described in Methods. Fluorochrome-labelled influenza A/PR8 virus ( ), or HA₀ ( ) or trypsinized HA₀ influenza virions ( ) (5 µg of viral protein) were incubated with PS liposomes (200 µg) in 200 µl PBS for 10 min at 4 °C. At the end of the incubation period the pH of the medium was adjusted to the values indicated by the addition of 50 µl sodium acetate (0-5 M). Following 30 min incubation at 37 °C, the extent of DQ was estimated.

Fusion of influenza virions with negatively charged liposomes

The results in Fig. 5 and Table 2 show that incubation of fluorochrome-labelled influenza virions with negatively charged PS liposomes caused increased DQ as well as release of the liposome content. The extent of DQ observed at pH 7.4 was low but increased significantly as the pH of the incubation medium was decreased (Fig. 5).

The DQ observed upon incubation with trypsinized HA₀ virions was slightly higher than that obtained with untreated HA₀ virus particles. However, the degree of DQ observed with the untrypsinized HA₀ influenza virions at pH 5 to 6.5 was relatively high (Fig. 5). It appears that the pH profile of DQ obtained following incubation with PS liposomes is different from that obtained with liposomes composed of PC : Chol (compare Fig. 5 to Fig. 1).

The results in Table 2 show that a high degree of DQ was observed upon incubation of inactivated influenza virions with PS liposomes. Only incubation with GA-treated virions resulted in a low degree of DQ (Table 2, exp. 1). Essentially the same results were obtained upon incubation of inactivated influenza virions with liposomes composed of PS : Chol, PS : gang or PS : Chol : gang (Table 2, exp. 1). Also, a relatively high degree of DQ was obtained upon incubation of HA₀ influenza virions with liposomes containing PS (Table 2, exp. 2). Incubation of influenza virions with calcein-loaded PS liposomes promoted a high degree of leakage, which was relatively unaffected by the pH (Table 2, exp. 1).

Table 2. Interaction of influenza virions with negatively charged liposomes

<table>
<thead>
<tr>
<th>Virus inactivated by</th>
<th>Virus incubated with DQ (%)</th>
<th>Calcein release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PS : Chol</td>
<td>PS : gang</td>
</tr>
<tr>
<td>Expt. 1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>7.4</td>
<td>18</td>
</tr>
<tr>
<td>5.0</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>85 °C</td>
<td>7.4</td>
<td>15</td>
</tr>
<tr>
<td>5.0</td>
<td>45</td>
<td>48</td>
</tr>
<tr>
<td>NH₂OH</td>
<td>7.4</td>
<td>10</td>
</tr>
<tr>
<td>5.0</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>GA</td>
<td>7.4</td>
<td>18</td>
</tr>
<tr>
<td>5.0</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>Expt. 2†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA₀ influenza virus treated with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>7.4</td>
<td>10</td>
</tr>
<tr>
<td>5.0</td>
<td>32</td>
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<tr>
<td>5.0</td>
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<td>49</td>
</tr>
</tbody>
</table>

* Preparation of liposomes and inactivation of influenza virus particles was as described in Methods. For determination of DQ, fluorochrome-labelled untreated and treated influenza virions (5 µg of viral protein) were incubated with liposomes composed of PS, PS : Chol, PS : gang, or PS : Chol : gang (200 µg of PS each) as described in Methods. For determination of calcein release, untreated and treated influenza virions (25 µg of viral protein) were incubated with calcein-loaded PS liposomes (0-5 µg PS) as described in Methods.
† HA₀ influenza virions were treated with trypsin as described in Methods. All other conditions were as described for exp. 1.
Furthermore, the non-fusogenic virions (Table 2, expt. 1 and 2) were able to induce a high degree of lysis in PS-loaded liposomes.

Discussion

The results presented show that influenza virions fuse efficiently with liposomes composed of either PC:Chol or of negatively charged phospholipids. Incubation of fluorochrome-labelled influenza virions with liposomes composed of PS, but not those composed of PC, resulted in a relatively high degree of DQ. The DQ obtained following incubation with PS liposomes at low pH was significantly higher than that observed at pH 7-4, suggesting that an active HA glycoprotein is required for this kind of virus–liposome fusion. However, it is doubtful whether fusion of influenza virions with negatively charged liposomes reflects the fusogenic activity utilized by the virus for penetration in vivo. This conclusion is based on experiments showing that incubation of non-fusogenic virions with these liposomes resulted in a high degree of DQ. Only GA-treated virions failed to promote DQ upon incubation with PS liposomes, indicating that the DQ observed was due to intermixing of the viral and liposome membranes (virus–liposome fusion), and not to lipid–lipid exchange processes (McDonald, 1987). Previous results have also demonstrated fusion of influenza virions with negatively charged liposomes (Hoekstra et al., 1984, White et al., 1985; Stegmann et al., 1986).

Fusion between influenza virions and PS liposomes may result from an electrostatic interaction between the negatively charged phospholipids and the viral glycoproteins which possess, especially at low pH, a high density of positive charges (Lear & De Grardo, 1987). The ability of basic polypeptides such as polylysine to promote fusion of negatively charged liposomes, even at pH 7-4, has been documented (Gad, 1983). Incubation of influenza virions with PS: gang liposomes at pH 7-4 also resulted in a high degree of DQ. The presence of gang, which serves as a virus receptor, should stimulate binding of influenza virions to PS liposomes and thus increase the probability of virus–liposome fusion. In fact, influenza virions were able to induce leakage of loaded PS liposomes bearing or lacking virus receptors. Induction of lysis from liposomes, similarly to virus-induced lysis of cultured cells, has been attributed to the viral fusogenic activity and has been claimed to represent the process of virus–membrane fusion (White et al., 1985). However, our results clearly show that PS liposomes are lysed at pH 7-4 and that lysis is also induced by non-fusogenic virus particles, supporting the view that fusion with and lysis of negatively charged liposomes does not reflect the biological activity of the virus.

It is noteworthy that fusion of virus particles with PE-containing liposomes did not require the presence of Chol, occurred at pH 7-4 and was observed with non-fusogenic virus particles. However, calcein release from these liposomes did require the presence of gang (not shown).

We suggest that only fusion of influenza virions with liposomes composed of PC and Chol (lacking or bearing gang) can be attributed to the biological activity of the viral HA glycoprotein. Such fusion is highly dependent on the ratio of PC:Chol, being maximal at 1:0.3 (mol: mol), and is obtained only with active fusogenic virions and at low pH. Thus, fusion with such liposomes possesses the same features as fusion with erythrocyte membranes or living cells (White et al., 1985; Hurter et al., 1989).

It should be mentioned that fusion of Semliki Forest virus or Sendai virions with liposomes composed of neutral phospholipids has also been shown to be dependent on the presence of Chol in the recipient phospholipid bilayer (Citovsky & Loyter, 1985; White et al., 1985). In addition, it has been reported that fusion of Sendai and influenza virions with biological membranes is dependent on the presence of Chol (Asano & Asano, 1988; Citovsky et al., 1988). However, our present and previous results (Citovsky & Loyter, 1985) clearly show that, unlike fusion with biological membranes, fusion with PC:Chol liposomes occurs in the absence of any specific virus receptors. This is probably because in liposomes the phospholipid molecules are ‘naked’ and susceptible to interaction with the viral envelope glycoproteins. It appears that the incorporation of virus receptor into PC: Chol liposomes renders them susceptible to the lytic activity of the virus. We propose that functional interaction of influenza virions with their receptors results in an increase in the recipient membrane’s permeability.

The results suggest that the mechanism by which influenza virus envelopes fuse with negatively charged liposomes is distinct from fusion with PC: Chol or with PC: Chol: gang liposomes. Only fusion with the latter is due to the activity of the viral HA fusion polypeptide and represents fusion with biological membranes.

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References


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