Phagocytosis* of Sendai Virus by Model Membranes

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SUMMARY

Sendai viruses were attached to liposomes (vesicular model membranes) at 0 to 4 °C, and were then incubated at 37 °C. Liposomes made of phosphatidylcholine, cholesterol and gangliosides enveloped the viruses at 37 °C to give a picture that resembles the ingestion step of phagocytosis. Virus particles were enveloped only by liposomes that contained gangliosides which serve as Sendai virus receptors.

INTRODUCTION

Phagocytosis is important in relation to viruses both because phagocytosis by blood and tissue phagocytes is one of the first lines of defence against virus infection (Silverstein, 1970) and because many viruses penetrate their host by phagocytosis (Dales, 1973). Stossel (1974), in reviewing phagocytosis by blood and tissue phagocytes, has attributed a part in the ingestion step to membrane receptors, active energy production and microtubules. In the whole cell, however, it is difficult to tell what is a primary and what is a secondary effect. Penetration of the host by paramyxoviruses appears to be by phagocytosis in some cell types (Silverstein & Marcus, 1964; Hosaka & Koshi, 1968), by fusion of cell and virus membranes in others (Dourmashkin & Tyrrell, 1970) or by both fusion and phagocytosis (Meiselman, Kohn & Danon, 1967; Morgan & Howe, 1968). These differences probably depend upon local conditions of the host membrane at the area of virus attachment.

In this work the properties which a membrane must have to promote ingestion of a paramyxovirus are studied with Sendai virus and liposomes. Liposomes are model membranes which can be made from any combination of lipids that can make bilayers. The resultant bilayers form closed vesicles (Bangham, Standish & Watkins, 1965; Bangham, Hill & Miller, 1974). Previous work (Haywood, 1974a, 1975) has shown that when gangliosides are included in the composition of liposomes, they serve as Sendai virus receptors, and that the virus binds to the liposomes at 0 to 4 °C. Paramyxoviruses attach to their hosts at 0 to 4 °C but can penetrate them only at higher temperatures. In this work it is shown that if the temperature is raised to 37 °C after Sendai viruses have attached at 0 to 4 °C to ganglioside-containing liposomes, the liposomes encircle the viruses.

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METHODS

Materials. Phosphatidylcholine was isolated from egg yolks by silicic acid chromatography as previously described (Bangham et al. 1974). Chromatography standard grade cholesterol was obtained from Sigma Chemical Company. Gangliosides from bovine brain were obtained from Koch-Light Laboratories Ltd. The phosphatidylcholine and cholesterol were stored in CHCl₃-CH₃OH, 1:1 (v/v), and the gangliosides were kept in CHCl₃-CH₃OH-H₂O, 1:1:0.1 (v/v/v).

Buffers. PBS is 137 mM-NaCl, 27 mM-KCl, 8 mM-Na₂HPO₄, and 1 mM-KH₂PO₄ (pH 7.3). NTE is 100 mM-NaCl, 1 mM-EDTA and 10 mM-tris (pH 7.4).

Virus. Sendai virus, strain ESW5, obtained from R. J. Johnson, was grown in eggs. The chorioallantoic fluid from the infected eggs was centrifuged at 9000 g to remove cellular debris, then layered above 1 ml of 60% sucrose and 17.5% potassium tartrate in NTE, and centrifuged for 1.5 h at 25000 rev/min at 4 °C in a Spinco 30 angle head rotor. The virus was diluted with NTE and again centrifuged at 25000 rev/min for 1.5 h. Purified virus in addition was layered onto a linear gradient of 15 to 60% sucrose in NTE, centrifuged 4.5 h at 22000 rev/min in a Spinco SW25 rotor, and the virus band was collected. Virus was dialysed against PBS.

Liposomes. Liposomes were made by the method of Bangham et al. (1974). In brief, the lipids were mixed, taken to dryness in vacuo, resuspended in CHCl₃-CH₃OH, 1:1 (v/v) and taken to dryness again to be sure that any water that was originally with the gangliosides was removed. The lipids were then resuspended in PBS. As the lipids form bilayers and close off, large vesicles often form around smaller ones, so that, as seen in many of the electron micrographs, liposomes frequently contain inner vesicles within the outer membranes. Liposomes containing gangliosides do not tend to form the many concentric lamellae often seen with liposomes without gangliosides (Bangham et al. 1965). During the course of the experiments the liposomes were kept under nitrogen.

Electron microscopy. At the end of each experiment, the samples were moved from the 37 °C bath to ice and samples were then removed for preparation for electron microscopy. For negative staining samples were diluted fivefold in water. A drop was put on a grid, a drop of 2% ammonium molybdate was added, and the excess fluid was removed after 30 s. The sample was examined in a Siemens electron microscope.

For sections the liposomes were fixed with an equal vol. of 4% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.2) and 2% sucrose for 60 min. The liposomes were centrifuged at 100000 g for 1 h. The pellet was rinsed with phosphate buffer, and 1% OsO₄ in 0.1 M-veronal acetate (pH 7.2) was added for 1 h. The pellet was washed with H₂O and left for 1 h in 2% uranyl acetate. The pellet was again washed with H₂O, and then dehydrated and embedded in Araldite. Sections were stained with saturated uranyl acetate in 50% ethanol and then with lead citrate. The sections were examined in an A.E.I. electron microscope 6B.

RESULTS

In previous work electron micrographs showed that after adsorption at 0 to 4 °C Sendai virus particles contact the surface of liposomes containing gangliosides by multiple spikes on one side of the virus (Haywood, 1974a). In this work, Sendai viruses, which have been adsorbed at 0 to 4 °C to liposomes made of phosphatidylcholine, cholesterol and gangliosides, were incubated at 37 °C for 2 h. At 37 °C liposomes were observed to flow around the virus particles to surround much or often nearly all of their circumferences.
Fig. 1. Liposomes made from 1 μmol phosphatidylcholine, 0.5 μmol cholesterol and 0.22 mg gangliosides, containing 0.1 μmol N-acetyl-neuraminic acid (NANA), were incubated with 3400 haemagglutinating units (H.A.U.) of Sendai virus.

Fig. 2. Liposomes made from 1 μmol phosphatidylcholine, 0.5 μmol cholesterol and 0.44 mg gangliosides, containing 0.2 μmol NANA, were incubated with 3400 H.A.U. of Sendai virus.

Fig. 3 and 4. Liposomes were made from 1 μmol phosphatidylcholine, 0.5 μmol cholesterol and 0.22 mg gangliosides, containing 0.1 μmol NANA, and were incubated with 3400 H.A.U. of Sendai virus.
Fig. 5 and 6. Liposomes made from 4 \( \mu \)mol phosphatidylcholine, 2 \( \mu \)mol cholesterol, and 0.84 mg gangliosides, containing 0.3 \( \mu \)mol NANA, were incubated with 512 H.A.U. of purified Sendai virus. The electron micrographs were made by Dr Peter Wooding.

Fig. 7. Liposomes made from 1.5 \( \mu \)mol phosphatidylcholine and 0.75 \( \mu \)mol cholesterol were incubated with 3100 H.A.U. of Sendai virus.

Fig. 1 shows an extremely large virus particle which has small liposomes attached to it and a large liposome flowing around it on both sides. Fig. 2 shows a smaller virus particle almost completely surrounded by a liposome. Fig. 3 shows an aggregate of several virus particles completely encircled by a liposome except for a channel to the outside. Channels such as this were often seen to extend a considerable distance into the liposome. The
channels were never observed to close off which would, of course, necessitate a fusion step. One of the virus particles in the aggregate in Fig. 3 has released a small fragment of RNA. This virus particle is probably one of the disrupted viruses seen occasionally in the inoculum. The possibility cannot be absolutely eliminated, however, that virus membranes are occasionally altered as a result of envelopment by these liposomes. In contrast to Fig. 1 to 3 which show virus particles encircled by single liposomes, an aggregate of liposomes surrounding a virus particle is seen in Fig. 4.

To discover if sections of these preparations of liposomes and viruses would give results consistent with those seen with negative staining, sections from the material from one such experiment were kindly made by Dr Peter Wooding. These sections showed liposomes which had virus particles inside, although usually the membrane immediately surrounding the virus particles was not visible (Fig. 5). Fig. 6 shows a virus particle inside a channel from the liposome surface.

To determine whether the encirclement of virus particles by liposomes was affected by ganglioside concentration, liposomes containing molar ratios of N-acetyl-neuraminic acid to phosphatidylcholine of 0.05, 0.1 and 0.2 were incubated at 37 °C with the same number of virus particles. All three preparations encircled the virus particles, but liposomes containing the highest concentration of gangliosides did so much less frequently. The lower frequency at high ganglioside concentration may result from repulsion between the negative charge of the gangliosides and the negative charge of the virus particles.

To see whether lack of receptors affected the way liposomes enveloped viruses a search was made for the rare particles which lay next to liposomes which contain no ganglioside. Fig. 7 shows such particles on top of and alongside ganglioside-free liposomes which do not encircle the particles. Similarly in a section from an experiment with ganglioside-free liposomes, no viruses were seen inside the liposomes.

**DISCUSSION**

This work shows that if a fluid vesicular lipid bilayer contains receptors for a virus particle that has ligand (haemagglutinin) over its complete surface, the bilayer has the capacity to surround that virus particle as in the ingestion step of phagocytosis. That a haemagglutinin-receptor interaction is important in virus penetration was also suggested by Silverstein & Marcus (1964), who showed that penetration by another paramyxovirus, Newcastle disease virus, is decreased when virus antibody is added after adsorption at 2 °C but before incubation at 37 °C.

How the haemagglutinin-receptor binding could cause a cell membrane to envelope a virus particle is easy to visualize. A bilayer of the composition of these liposomes should be completely fluid at 37 °C, and without constraints would be expected to flow randomly. Attachment of the liposome to a virus particle, however, would increase the probability that those parts of the liposome neighbouring the area of binding extend contact with and become bound to neighbouring areas of virus until finally all the haemagglutinin of the virus particle is bound to the liposome receptors and the virus particle is completely enclosed by the liposome. The virus particle polymerically bound to receptors in the membrane should make a rigid complex past which the membrane could flow to produce channels.

If in addition to the lipids used in these experiments phosphatidylethanolamine (and sphingomyelin) are used to make liposomes, the virus membrane fuses with the liposomes (Haywood, 1974b). The liposomes containing phosphatidylethanolamine also can be seen to enclose virus, but fusion mainly appears to take precedence over enclosure. The
fact that the lipid composition of a liposome determines whether it has the capacity to fuse
with the virus membrane suggests that whether a paramyxovirus penetrates its host by fusion
or by phagocytosis depends upon the composition of the host membrane at the site of attach-
ment. Within cells there is exchange of phospholipids between cell membranes (Dawson,
1973; Wirtz, 1974). This raises the possibility that if a myxovirus or paramyxovirus attaches
to a segment of plasma membrane lacking the lipids necessary for fusion, it stimulates the
host to enclose it in a phagocytic vacuole, the composition of which is altered within the
cell to allow fusion of the virus and vacuole membranes. Fusion of the membrane of a
phagocytic vacuole with a virus membrane has been described for influenza virus (Dour-
mashkin & Tyrrell, 1974).

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