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ELECTRON MICROSCOPIC STUDY ON THE INTERACTION OF SENDAI VIRUS WITH LIPOSOMES CONTAINING GLYCOPHORIN

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The interaction of liposomes containing glycophorin, a major sialoglycoprotein of human erythrocytes, with Sendai virus was studied by freeze-fracture and negative staining electron-microscopy. Viral envelopes were absorbed on liposomal membranes at 0°C. When the temperature was shifted up to 37°C, the viral envelopes fused with the liposomal membranes (envelope fusion). Particles representing viral membrane components formed clusters on liposomal membranes after incubation for more than 1 h at 37°C.

Introduction

Previously we found that liposomes containing glycophorin were agglutinated by Sendai virus and that some virus particles attached to the liposomes were released on break-down of the binding site by viral neuraminidase [1]. We also found that liposomes were damaged by Sendai virus at 37°C, releasing markers trapped in their aqueous compartment [2]. Trypsinization of intact virus, which was shown to hydrolyze F protein exclusively, inactivated the activity to damage liposomes, indicating that the virus-induced permeability change is dependent on F protein. It is likely that viral envelopes fuse with liposomal membranes at 37°C, since the permeability change observed in liposomes was similar to virus-induced hemolysis, which is generally thought to be caused by fusion of viral envelopes with the erythrocyte membrane (i.e., envelope fusion). To obtain further information, we studied the interaction between liposomes and virus by electron microscopy.

Methods and Materials

Cholesterol, dicetyl phosphate and trypsin inhibitor were from Sigma Chemical Co., St. Louis, MO, U.S.A. Trypsin was from Miles Laboratories Ltd., Berkshire, U.K. Egg yolk phosphatidylcholine was prepared by chromatography on Aluminum Oxide Nertral and Unicil, and gave a single spot on silica-gel thin layer chromatography. Glycophorin was prepared as described previously [3]. The Z strain of Sendai virus was used after purification by differential centrifugation. The virus was inactivated by treatment with trypsin (20 μg/ml) at 37°C for 20 min in 10 mM phosphate buffer (pH 7.2), by the method of Shimizu and Ishida [4]; the hydrolysis of almost all the F protein without appreciable changes in other proteins was confirmed by sodium dodecyl sulfate polyacrylamine gel electrophoresis. The hemagglutination titer and hemolytic activity, was measured as described previously [2]. Under the experimental conditions, 3.3 hemagglutination units of Sendai virus was required for 50% hemolysis, while trypsinized Sendai virus did not induce any hemolysis at the highest dose tested (10000 hemag-

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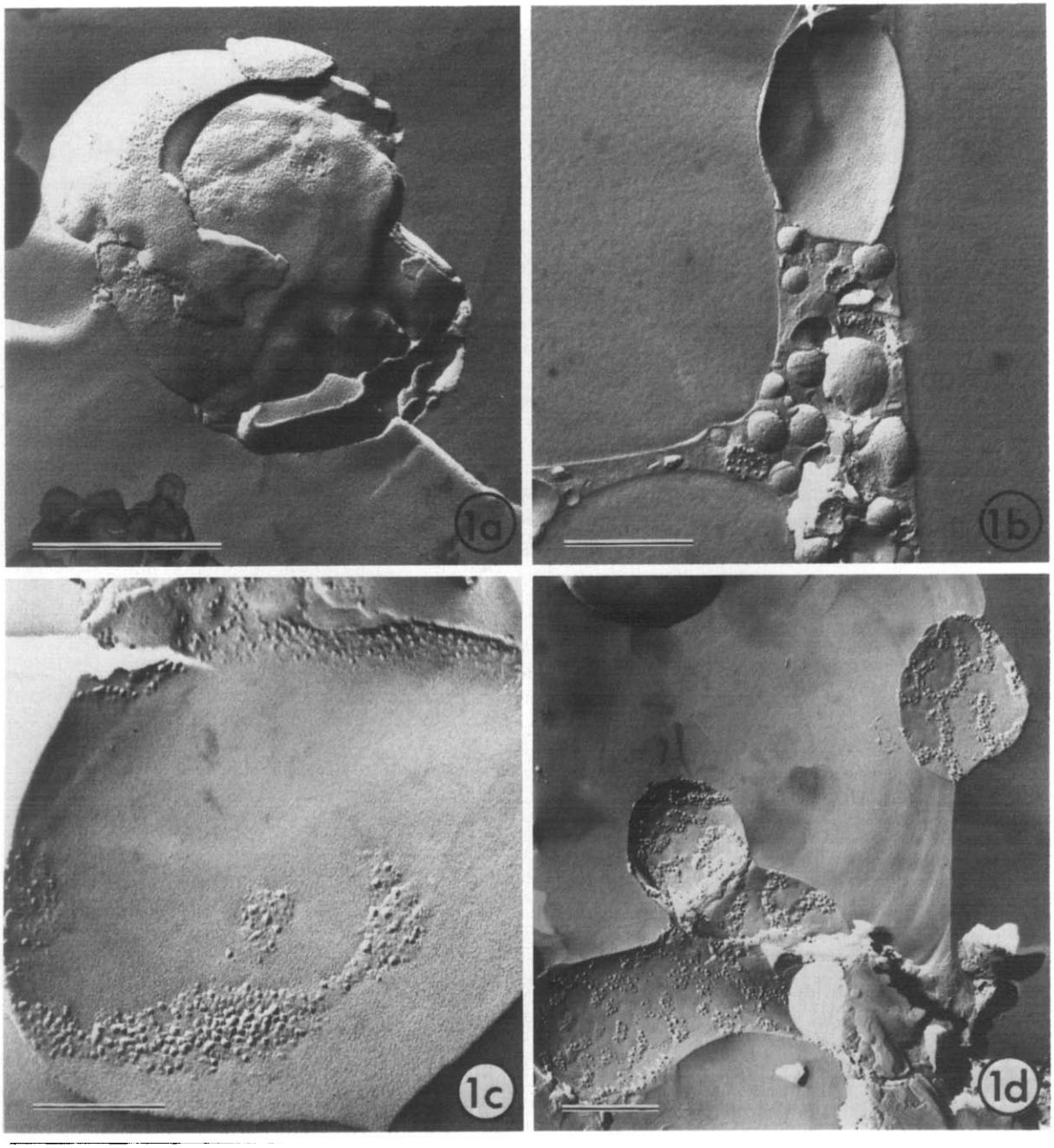

glutination units). Liposomes containing glycophorin were prepared by the method of MacDonald and MacDonald [5]. In freeze-fracture experiments, samples were rapidly frozen in freshly melted Freon 22 at liquid nitrogen temperature, transferred to liquid nitrogen, and fractured at −110°C in a Hitachi HFZ-1 apparatus. Replicas were made by platinum-carbon shadowing at a shadowing angel of 45°C. Replicas were cleaned with NaClO, washed with distilled water and examined at 80 kV in a JEM-100 U electron microscope. For negative staining, drops of lipid suspen-

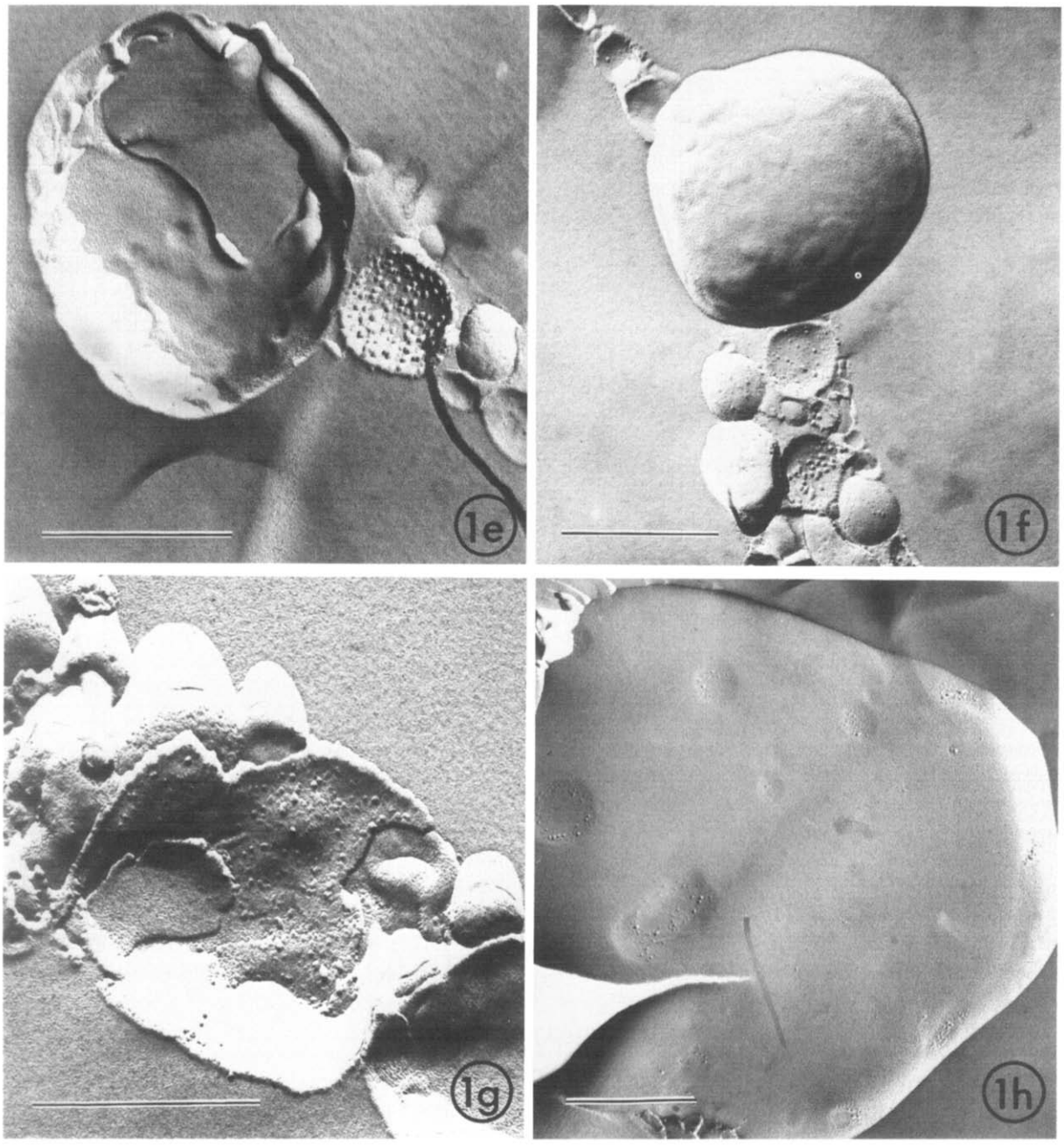

Fig. 1. Freeze-fracture replicas of liposomes and of liposomes interacting with Sendai virus. Liposomes prepared from egg yolk phosphatidylcholine, dicetyl phosphate, cholesterol with incorporated glycophorin (molar ratio about 250 : 25 : 125 : 2) (a). Liposomes containing glycophorin (1 μmol as egg yolk phosphatidylcholine) agglutinated with 2500 hemagglutination units of the virus in 200 μl of phosphate-buffered saline (pH 7.2) at 0°C. Fractured virus particles reveal concave E fracture faces covered with intramembrane particles and convex P faces with a complementary arrangement of pits (b). Liposomes which had been incubated with virus for 30 min at 37°C, after preincubation for 30 min at 0°C (c, d, g, h). Liposomes which had been incubated with trypsinized Sendai virus for 30 min at 37°C, after preincubation for 30 min at 0°C (e, f). Bar, 0.5 μm.

sion were placed on 180-mesh copper grids previously coated with carbon film. Excess sample was removed with filter paper and 2% sodium phosphotungstate (pH 6.5) was applied to the grids. Excess staining solution was removed with filter paper after 10 to 15 s and the grinds were promptly examined under an electron microscope (Hitachi HU 11-B) at 75 kV.

Results and Discussion

Fig. 1a shows a freeze-fracture elecron micrograph of liposomes prepared from egg yolk phosphatidylcholine, dicetyl phosphate, cholesterol and glycophorin. Fracture faces (bilayer hydrophobic region) are smooth with only a few particles sometimes visible on them. Grant and McConnell [6] observed particles of 4 nm diameter on the fracture face of liposomes containing glycophorin (molar ratio of lipid: glycoprotein, 120:1), and concluded that these particles were small clusters of glycophorin. Van Zoelen et al. [7] observed the similar particles with a mean diameter of 4–5 nm. They also proposed that glycophorin is present in a slightly aggregated form. Under the present conditions, we usually observed very few particles on liposomal fracture faces. This discrepancy may be due to differences in procedures for examining the liposomes, for example, differences in the shadowing angles.

These liposomes were heterogeneous in size, but most were about 500 nm–1 μm in diameter. Liposomes which had been incubated with Sendai virus at 0°C for 10 min are shown in Fig. 1b. Freeze-fracture faces of free viral envelopes reveal internal aspects of their structure. Concave E fracture faces (the inner sides of outer leaflets) are covered with intramembrane particles, 10–20 nm in diameter, while convex P faces (the outer sides of inner leaflets) have a complementary arrangement of pits, as found by Knutton [8] and by Kim et al. [9]. Most virions are roughly spherical and less than 300 nm in diameter. Since the fracture faces of liposomes are rather smooth, the E faces of liposomes are easily distinguishable from those of viral envelopes, which have numerous particles. The P faces of liposomes can also be distinguished from those of viral envelopes by the presence of pits on the latter only. As can be seen, some viruses become attached to liposomes. Incubation of virus particles with liposomes for more than 30 min at 0°C did not cause any change in the morphology or in the distribution of intramembrane particles on fracture faces of either liposomes or viral envelopes.

After incubation of the agglutinated liposome suspensions at 37°C for 30 min, we observed some morphological changes (Figs. 1c, 1d, 1g). Numerous intramembrane particles could be observed on the fracture face of large vesicles. No free virus could be seen on any of the fracture faces of liposomes. The fracture faces of vesicles with intramembrane particles were not always spherical (Fig. 1d) and vesicles of several micrometers in diameter could often be observed. Most intramembrane particles were about 15 nm in diameter, like those observed on the fracture face of viral envelopes. It is noteworthy that most of the particles appeared to be clustered on the fracture faces of large vesicles. These intramembrane particles could be observed only on the E fracture face.

The distinct morphological changes could be observed after 10 min incubation at 37°C, though the partial changes occurred even within 2 min (data not shown). The lack of lag period for the 'fusion' is consistent with our previous findings that F protein-dependent permeability change of liposomes could be observed immediately after mixing of liposomes with the virus [2]. The morphological change was depended on the incubation temperature, since the change could be seen above 25°C, whereas below 15°C no appreciable changes could be observed (data not shown).

Electron micrographs showed that when liposomes without glycophorin were incubated with Sendai virus, the liposomes and the virus were distributed independently. We could not observe any intramembrane particles on the fracture faces of larger vesicles (liposomes), even after incubation with virus at 37°C. This indicates that the morphological change may depend on the presence of glycophorin. Since we never observed large vesicles with intramembrane particles in virus preparations not incubated with liposomes, it is quite possible that the large vesicles are liposomes fused with viral envelopes. Fusion between liposomes and viral envelopes was also seen when liposomes

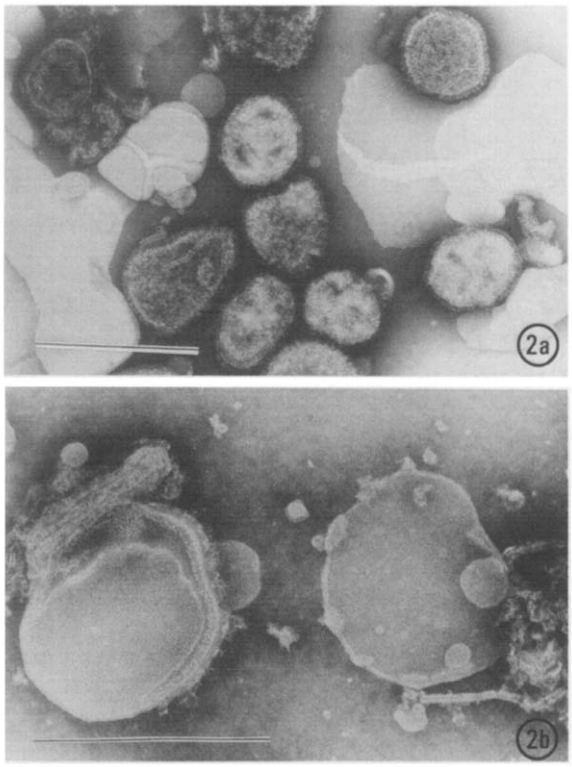

Fig. 2. Negatively stained image of liposomes containing glycophorin which had been incubated with Sendi virus at 0°C (a) or at 37°C (b). Bar, 0.5 μm.

were incubated with virus at 37°C and then negatively stained with phosphotungstate. As shown in Figs. 2a and 2b at 0°C to 4°C, Sendai virus was adsorbed to liposomes containing glycophorin but on raising the temperature to 37°C it fused with the liposomes.

The morphological change observed in liposomes incubated with virus at 37°C seems to be dependent on viral F protein, since trypsinized Sendai virus was adsorbed to liposomes but caused no morphological change (fusion) at 37°C (Figs. 1e, 1f). This finding is consistent with our previous finding that trypsinized virus showed agglutinating activity but not permeability-increasing activity toward liposomes [2]. As shown in Fig. 1g, the present liposome preparations contained some multilamellar vesicles. Particles could be observed only on the outermost layers of the fracture face, indicating that the outermost bilayers of liposome were affected by virus. This finding can explain our previous observation that only part of the markers trapped in liposomes could be released by the virus [2]. When liposomes were incubated with the virus at 37°C, we often observed depressions of the same size as Sendai virus particles on their P fracture face (Fig. 1h). The particles on the convex parts were apparently smaller in diameter than particles observed on the E face of the virus or of liposomes fused with virus. These particles might be clusters of glycophorin molecules binding with viral HN proteins that became visible under the present conditions. Knutton et al. observed similar depressions in the membrane of erythrocytes incubated with virus at 4°C [8]. They proposed that binding with virus deformed the erythrocyte membrane causing these depressions. When liposomes were incubated with virus at 0°C, no depressions could be observed on the lipsomal fracture face, suggesting that those observed in the present system were related with some early stage of virus-liposomal membrane interaction causing envelope fusion.

A diagram of the events supposed to take place judging from freeze-fracture data is shown in Fig. 3. The molecular organization of the virus envelope has been proposed by Knutton [8]. Fig. 3A may explain the image shown in Fig. 1h. The most interesting observation is that the particles appear to be mostly clustered on the fracture surface of liposomes fused with Sendai virus. The distribution of particles did not change even on incubation for more than 1 h at 37°C, suggesting that free lateral diffusion of viral envelope components within the plane of the liposomal membrane is suppressed by association with some other virus components, such as M protein (Fig. 3B). It is interesting that patching of viral proteins in liposomes was previously observed in negatively stained liposome preparations fused with virus [10, 11]. Wyke et al. [12] recently proposed that the change in cell permeability might result from cluster of viral glycoproteins forming a hydrophilic channel when the virus fused with the cells. The observed clustering of viral glycoproteins in liposomes may be related with the permeability change of liposomes.

The possibility that the particles observed in liposomes were clustered glycophorin molecules

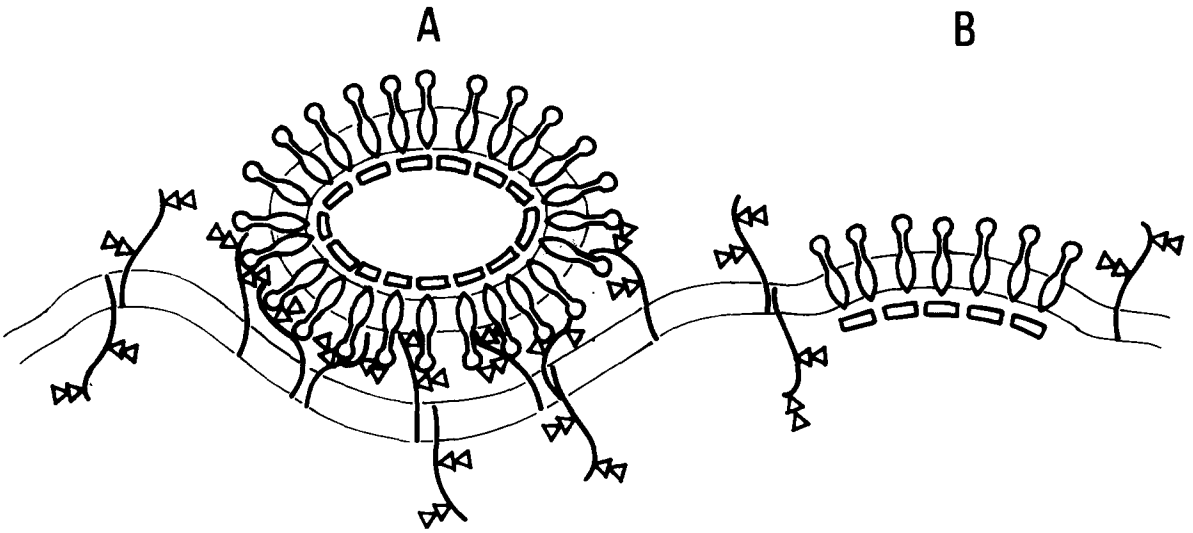

Fig. 3. Diagrammatic representation of the interaction between the viral envelope and liposomal membrane.

rather than viral proteins is excluded by the fact these particles were never observed on P fracture faces of liposome, because supposing that glycophorins exposing their sugar residue outside the liposome are exclusively affected by HN protein, particles due to aggregated glycophorin should be seen only on the P fracture face. Another possibility is that some of the observed particles represented inverted lipid micelles. Verkleij et al. [13] suggested that fusion may proceed via formation of intermediary inverted micellar structures at the fusion interface. In fact, the viral envelopes contain much phosphatidylethanolamine, which is reported to tend to form non-bilayer structures [14]. At present this possibility cannot be neglected, because the reported size of inverted micelles is rather close to that of the present particles.

Acknowledgements

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