AN EFFICIENT METHOD FOR REASSEMBLY OF FUSOGENIC SENDAI VIRUS ENVELOPES AFTER SOLUBILIZATION OF INTACT VIRIONS WITH TRITON X-100

D. J. VOLSKY and A. LOYTER
The Hebrew University of Jerusalem, Department of Biological Chemistry, Jerusalem, Israel

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1. Introduction

The agglutination, hemolysis, and fusion activities of intact Sendai virus particles are attributed mainly to two viral glycoproteins that are located on the viral envelope [1]. Active reassembled viral envelopes can be obtained if intact virus particles are solubilized with the detergent NP-40 and then the detergent is removed by slow dialysis [2]. During the reconstitution process, the detergent-insoluble viral nucleocapsid can be removed and substitued by exogenous proteins which, if present in the reconstitution medium, are trapped within the reassembled viral envelopes [3]. As shown in [3]. the trapped proteins can be then 'injected' into recipient cells during viral envelope–cell fusion. The use of the reassembled viral envelopes as a vehicle for microinjection of macromolecules into animal cells, evidently has a high potential in the field of enzyme therapy and gene engineering. It appears, however, that the practical use of the above method is as yet limited by the low efficiency of the reconstitution process. According to the available data, only about 4% of the original viral hemagglutinin activity can be recovered in the reassembled envelopes [3]. This might be due to partial denaturation of the viral glycoproteins during the long dialysis (72–100 h) needed for the removal of the detergent [2,3]. It is therefore important to find out whether other detergents, besides NP-40, can be used for the solubilization of Sendai virus.

Recently, the detergent Triton X-100 was used for the solubilization of human erythrocyte membranes and the resolution of their anion exchange system [4] as well as for the reconstitution of the glucose transport system [5]. Triton X-100 was used for solubilization of *Semliki Forest* virus membrane [6]. The main advantage of using Triton X-100 for the solubilization of biological membranes is its easy and rapid way of removal [7] and the fact that it does not cause denaturation of membrane proteins [8]. In addition, the availability of [^{3}H]Triton X-100 makes it possible to study, on a quantitative basis, the interaction between the detergent and the viral membranes as well as the kinetics of its removal.

In the present work we have used Triton X-100 for solubilization of Sendai virus. Removal of the detergen by dialysis against a buffer containing the Bio-beads SM-2 [7] resulted in reassembly of active virus envelopes. The properties of the reassembled virus envelopes are discussed.

2. Materials and methods

Blood was collected from the necks of decapitated chickens into an Erlenmeyer flask containing heparin (100 U/ml), and washed with a buffer containing 160 mM NaCl and 10 mM Tris–HCl, pH 7.4 (solution Na^+), as in [9].

HeLa cells were grown in Swim's-77 medium plus 2 mM glutamine, 100 μg/ml $CaCl_2$, and 10% calf serum. Before the experiment, confluent monolayers of the cells were prepared on cover slips, washed, and then transferred to solution Na^+.

Sendai virus was isolated and its hemagglutinin titre (HAU) determined as in [9]. The procedure for the solubilization of intact *Sendai* virus by Triton X-100 and the reassembly of active viral envelopes,

upon removal of the detergent by dialysis in the presence of Bio-beads SM-2 [7], is given in fig.1.

Chicken erythrocytes (2% v/v), suspended in solution Na^+ which contained 10 mM Ca^{2+}, were fused and the extent of fusion was calculated as in [9]. In these experiments 2000 HAU/ml of both intact Sendai virus and reconstituted viral envelopes were used.

Monolayers of HeLa cells, washed in solution Na^+, were first cooled on ice and then intact Sendai virus or reassembled viral envelopes (2000 HUA/cover slip) were added to the cells which were then incubated in the cold for 10 min. Solution Na^+, containing 10 mM $CaCl_2$, was then added, and the cells were incubated

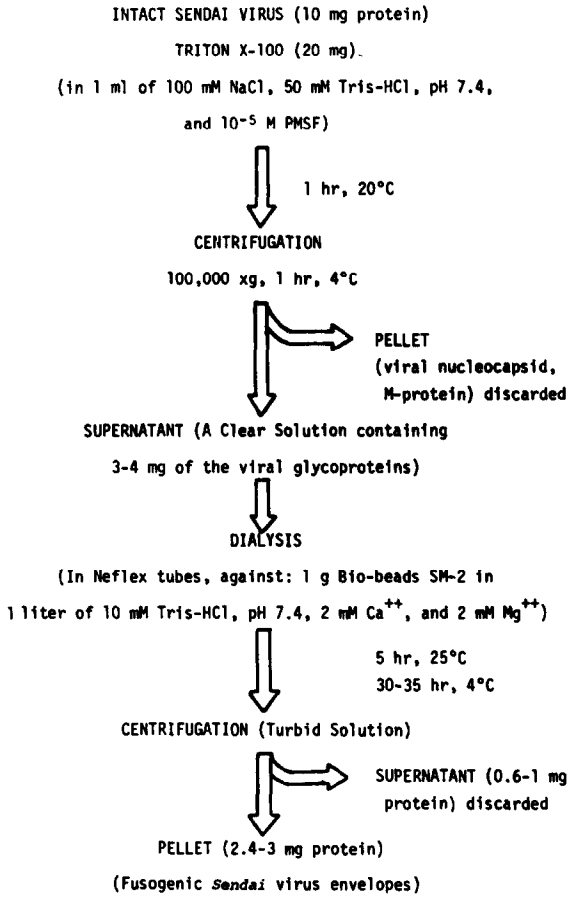

Fig.1. A procedure for solubilization of Sendai virus particles by Triton X-100 and reassembly of active virus envelopes upon removal of the detergent.

at 37°C for 1 h. The degree of hemolysis was determined at 540 nm as in [9].

Viral antigens were visualized by the use of ferritin-conjugated IgG. After interaction of reassembled viral envelopes with cells (fig.2D, E legend), the cells were fixed with 1% (v/v) glutaraldehyde for 1 h at 4°C. Antiserum obtained in rabbits against Sendai virus-envelope glycoproteins (prepared as in [10]) was then added, and after and additional 1 h at 4°C, ferritin-conjugated goat anti-rabbit IgG was added. After another 1 h at 4°C, a post-fixation with OsO_4 was performed (1% v/v). Between each step cells were extensively washed with solution Na^+ buffered with 10 mM sodium phosphate, pH 7.4. Thin sections were prepared, as in [9], and electron micrographs were obtained with a Philips EM 300 electron microscope.

Protein was determined by the Lowry method [11] with 0.1% sodium dodecyl sulfate (SDS) in the reaction mixture, using bovine albumin as standard. SDS–polyacrylamide gel electrophoresis (7.5% acrylamide) was performed as in [12].

3. Results

Treatment of Sendai virus with Triton X-100 at a ratio of detergent:virus 2:1 (w/w), caused complete solubilization of the viral envelope. The supernatant obtained after centrifugation of the solubilized virus appeared clear and contained the viral phospholipids (not shown) and the two viral envelope glycoproteins (fig.1, 2A′). Removal of the detergent by dialysis of the solubilized virus against a buffer containing the Bio-beads SM-2 [7], resulted in reassembly of virus envelopes (fig.1, 2A,C). Examination of the reassembled virus envelopes in the electron microscope after negative staining (fig.2A,C) or thin-sectioning (fig.2D,E), revealed that closed vesicles, ranging between 300–3000 Å in diameter, covered with small spikes (100 Å), were formed (fig.2A). Large envelopes of 2000 to 3000 Å in diameter and up to 10 000 Å in length were also detected among the reassembled virus envelopes (fig.2C).

The reassembled envelopes were active in causing agglutination, lysis, and fusion of cells (table 1). Interestingly, in order to obtain highly fusogenic

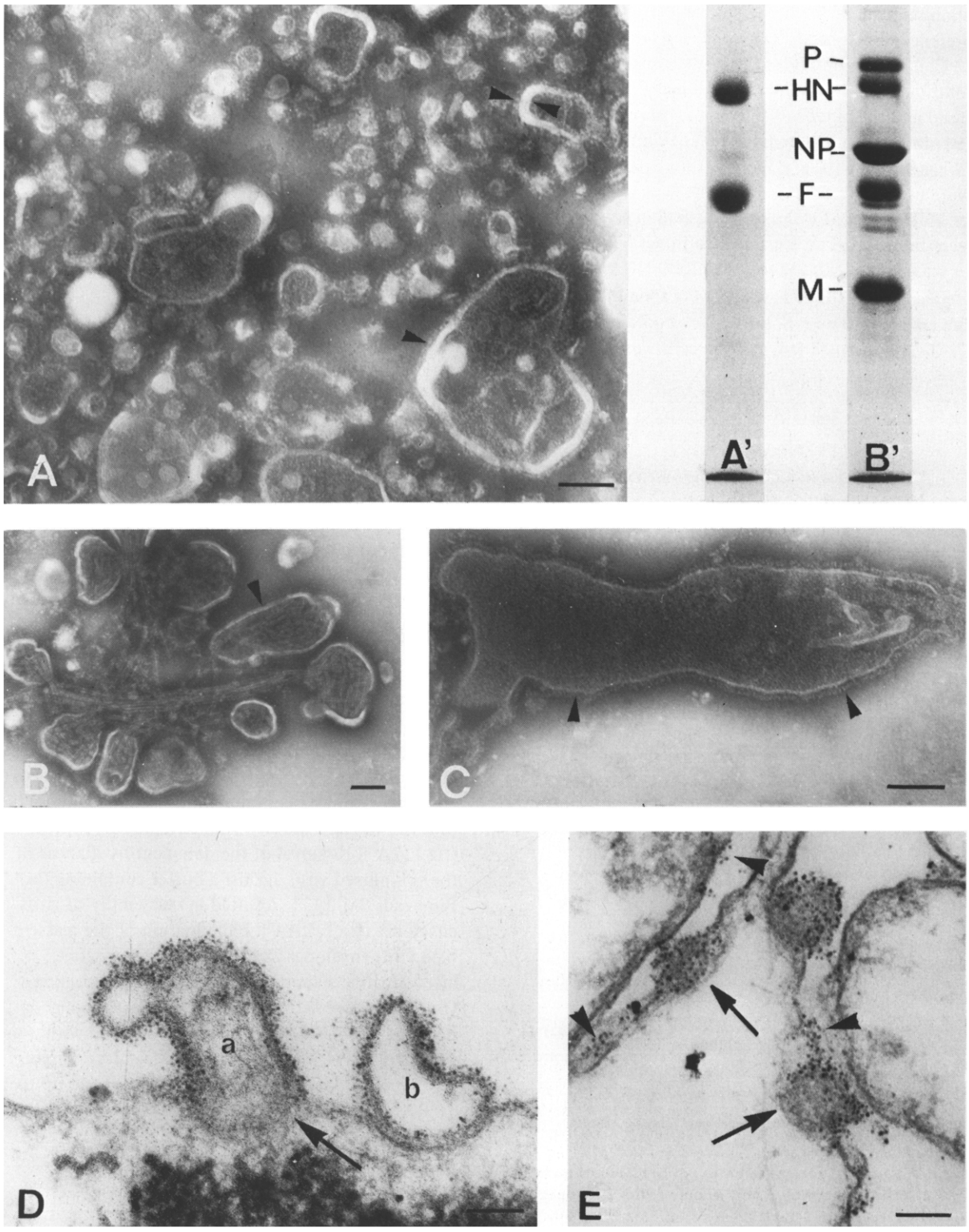
A
P
HN
NP
F
M
A'
B'
B
C
a
b
D
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Table 1
Fusion ability of *Sendai* virus envelopes reassembled under different conditions

System	Hemagglutination (HAU/mg viral protein)	Hemolysis (%/500 HAU)	Fusion	
			Chicken erythrocytes (% total)	HeLa cells in monolayer
Intact virus	10 000–20 000	30	70	+
Virus envelopes reassembled by dialysis at 25°C and 4°C	30 000–40 000	25	35	+
Virus envelopes reassembled by dialysis at 4°C only	65 000–80 000	5	1–5	+
Virus envelopes reassembled upon removal of detergent by Bio-beads SM-2 without dialysis	65 000–80 000	5	0	+

viral envelopes, it was essential to remove the detergent by two-step dialysis: first at 27°C for 5 h and then at 4°C for 30–35 h. As can be seen in table 1, vesicles formed under these conditions were able to cause a similar percentage of hemolysis as intact viral particles, and promoted fusion of nucleated cells in suspension and monolayer. When the detergent was removed by dialysis only in the cold (4°C) or by addition of the Bio-beads SM-2 directly to the solubilized virus solution (table 1), a turbid suspension containing reassembled viral envelopes was obtained. The envelopes' dimension and morphology were similar to those shown in fig.2A. However, these vesicles had high agglutination but poor hemolytic and fusion activities when incubated with nucleated cells such as chicken erythrocytes or Friend erythroleukemic cells (table 1).

All three envelope preparations (obtained under the different conditions summarized in table 1) were able to interact and fuse with plasma membranes of cells in suspension, irrespective of their ability to promote cell–cell fusion. This was revealed by the presence of viral antigens within the plasma membranes of chicken erythrocytes incubated at 37°C with the different preparations of the virus envelopes (fig.2D,E).

Fig.2. Electron microscope observations of reassembled Sendai virus envelopes and their fusion with chicken erythrocytes. (A–C) Negatively-stained (1% phosphotungstic acid) Sendai virus particles. (A) Sendai virus envelopes reassembled by the method summarized in fig.1. Note the appearance of large vesicles. (B) Intact Sendai virus. (C) Extra-large reassembled envelopes. Note the symmetric distribution of viral 'spikes' (small arrowheads) in some of the reassembled viral envelopes. (A',B') Electrophoretic pattern of viral proteins of reassembled viral envelopes and of intact Sendai virus, respectively. HN and F represent the two viral envelope glycoproteins, the hemagglutinin/neuraminidase (mol. wt 65 000) and the hemolysis/fusion (mol. wt 53 000) respectively. P, NP and M represent non-envelope Sendai virus proteins [1]. (D,E) Electron micrographs of thin sections showing the integration of reassembled Sendai virus envelopes into chicken erythrocyte membranes. Viral antigens were identified by ferritin conjugated antibodies (see section 2). Chicken erythrocytes (2% v/v) were incubated with reassembled envelopes (2000 HAU/ml) for 60 min at 37°C. At that temperature almost total lysis of cells occurred. In (D) two large reconstituted vesicles 'a' and 'b' are shown. Vesicle 'a' fused with plasma membrane and, therefore, probably contained some of the erythrocyte cytoplasm. Vesicle 'b' remained closely associated but did not fuse with the plasma membrane, and is completely empty. In (E) a few other reconstituted envelopes, that fused with the plasma membranes, are shown. Note that no membrane barriers can be distinguished between the reassembled envelope and the cell interiors (black arrows in (D,E)). Note also that some of the viral antigens are spread out on the plasma membranes (large arrowheads). Bar in fig.(A–E) 0.1 μm.

4. Discussion

The present work describes a simple and efficient method for the reassembly of active Sendai virus envelopes after solubilization of intact virus particles with the non-ionic detergent Triton X-100. From the quantitative measurement of protein concentration, it appears that the reassembled envelopes contain about 30–40% of the initial concentration of the intact virus proteins, thus indicating almost full recovery of the envelope glycoproteins [1].

It appears from the present studies that the temperature at which the initial steps of the reassembly process take place and its duration have a significant effect on the activity of the reassembled virus (table 1). Similar observations were reported in reconstitution experiments of the inner mtiochondrial membrane [13] and the endoplasmic membrane [14]. Reassembled envelopes obtained by removal of the detergent by dialysis only in the cold, or by addition of Biobeads directly to the solution of the solubilized virus, exhibit high agglutination/mg protein (table 1) and neuraminidase activity (not shown), although they have poor fusogenic activity when interacted with nucleated cells in suspension. It is conceivable that because of their high neuraminidase activity, these envelopes caused rapid disagglutination of virus–cell agglutinates at 37°C and, therefore, induction of cell–cell fusion was substantially prevented.

The envelope preparations that failed to induce fusion in cell suspension but fused with their membranes may be of extreme importance in microinjection experiments. For developing an efficient microinjection method and for preserving the viability of the injected cells, it is important to avoid cell–cell fusion but allow virus–cell fusion. This way the viral envelopes are injecting their content directly into the cytoplasm of single cells. The present work describes experimental conditions for the preparation of such virus envelopes.

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References

[1] Rott, R. and Klenk, H. D. (1977) in: Cell Surface Reviews (Poste, G. and Nicolson, G. L. eds) vol. 2, pp. 47–81, North-Holland, Amsterdam, New York.
[2] Hosaka, Y., Semba, T. and Fukai, K. (1974) J. Gen. Gen. Virol. 25, 391–404.
[3] Ushida, T., Yamaizumi, M. and Okada, Y. (1977) Nature 266, 839–840.
[4] Cabantchik, Z. I., Wolosin, J. M., Ginsburg, H. and Zemel, O. (1977) in: Biochemistry of Membrane Transport (Semenza, G. and Carafoli, E. eds) pp. 328–345, Springer-Verlag, New York.
[5] Kasahara, M. and Hinkle, P. C. (1977) J. Biol. Chem. 252, 7384–7390.
[6] Helenius, A. and Söderlund, H. (1973) Biochim. Biophys. Acta 307, 287–300.
[7] Holloway, P. W. (1973) Ann. Biochem. 53, 304–308.
[8] Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79.
[9] Toister, Z. and Loyter, A. (1973) J. Biol. Chem. 248, 248, 422–432.
[10] Sato, J. T., Becht, H. and Rott, R. (1974) Virology 61, 354–360.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Scheid, A., Caliquiri, Z. A., Compans, R. W. and Choppin, P. W. (1972) Virology 50, 640–652.
[13] Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487.
[14] Feigenbaum, A., De Groot, N. and Hochberg, A. A. (1978) personal communications.