SELECTIVE EXTRACTION OF BIOLOGICALLY ACTIVE F-GLYCOPROTEIN FROM DITHIOTHREITOL REDUCED SENDAI VIRUS PARTICLES

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1. Introduction

Membrane vesicles made of either pure phospholipids (liposomes) or phospholipids containing inserted glycolipids or glycoproteins, are used to introduce macromolecules into cells in culture [1] or whole animals [2]. However, since liposomes are mainly phagocytozed and not fused by living cells [3], they are very poor carriers. It appears that empty envelopes of fusogenic viruses such as reconstituted envelopes of Sendai virus may be superior to liposomes as an efficient vehicle for macromolecules. Reconstituted Sendai virus envelopes (RSVE) fuse efficiently with plasma membranes of eucaryotic cells, thus delivering their content directly into the intracellular space of the cells [4,5]. As the Sendai virions interact with exposed sialic acid residues, the vesicles are able to fuse with a wide range of cells [6]. This ability of Sendai virus is due to its F-glycoprotein [7]. It was therefore, of interest to purify this glycoprotein and to insert it into bilayers of phospholipids, with the hope that the vesicles formed would exhibit high fusogenic activity, the specificity of which may be dependent upon the addition of suitable binding proteins. Affinity chromatography or a combination of sieve chromatography and ion exchange columns have been used to purify Sendai virus F-glycoprotein from detergent-solubilized virus solution [8,9]. These methods, however, yield a low amount of biologically active glycoprotein due to many purification steps.

Here we describe an efficient, simple and quick new method for obtaining high quantities of Sendai virus F-glycoprotein. The method involves reduction of intact Sendai virus particles with dithiothreitol (DTT) and solubilization of the reduced virus with Triton X-100. Centrifugation of the detergent-solubilized virus yields a supernatant containing the F-glycoprotein as its main protein component. After removing the detergent from the supernatant, F-glycoprotein containing vesicles were formed, which hemolyse human erythrocytes in the presence of the lectin wheat germ agglutinin (WGA).

2. Materials and methods

Sendai virus was isolated and its hemagglutination titer was determined as in [10]. F-glycoprotein was extracted from Sendai virus particles and membrane vesicles containing the F-glycoprotein were formed as in fig.1 and [11]. RSVE were prepared as in [11]. Human erythrocyte (type O^+, aged 3–6 weeks) were washed with solution A (150 mM NaCl, 20 mM Tris–HCl, pH 7.4) as in [10]. The erythrocytes (0.5 ml, 2.5%, v/v) were incubated for 15 min at 4°C with 50 μl of either RSVE (40–80 μg protein) or with F-glycoprotein containing vesicles (10–70 μg protein; see legend to fig.4 or table 1). After incubation in the cold, the reaction mixtures were transferred to 37°C. Wheat germ agglutinin was used to promote binding of F-glycoprotein containing vesicles to the erythrocytes [8,9]: After 5 min of incubation at 37°C with gentle shaking, WGA (0.5–5 μg/incubation mixture) was added and the suspension was allowed to incubate for another 25 min at 37°C.

Vesicle–cell fusion was terminated by the addition of 1.45 ml cold solution A. Hemolysis was determined by evaluating the absorbance of the supernatant at 540 nm, as before [10].

Antiserum against the Sendai virus envelope glyco-

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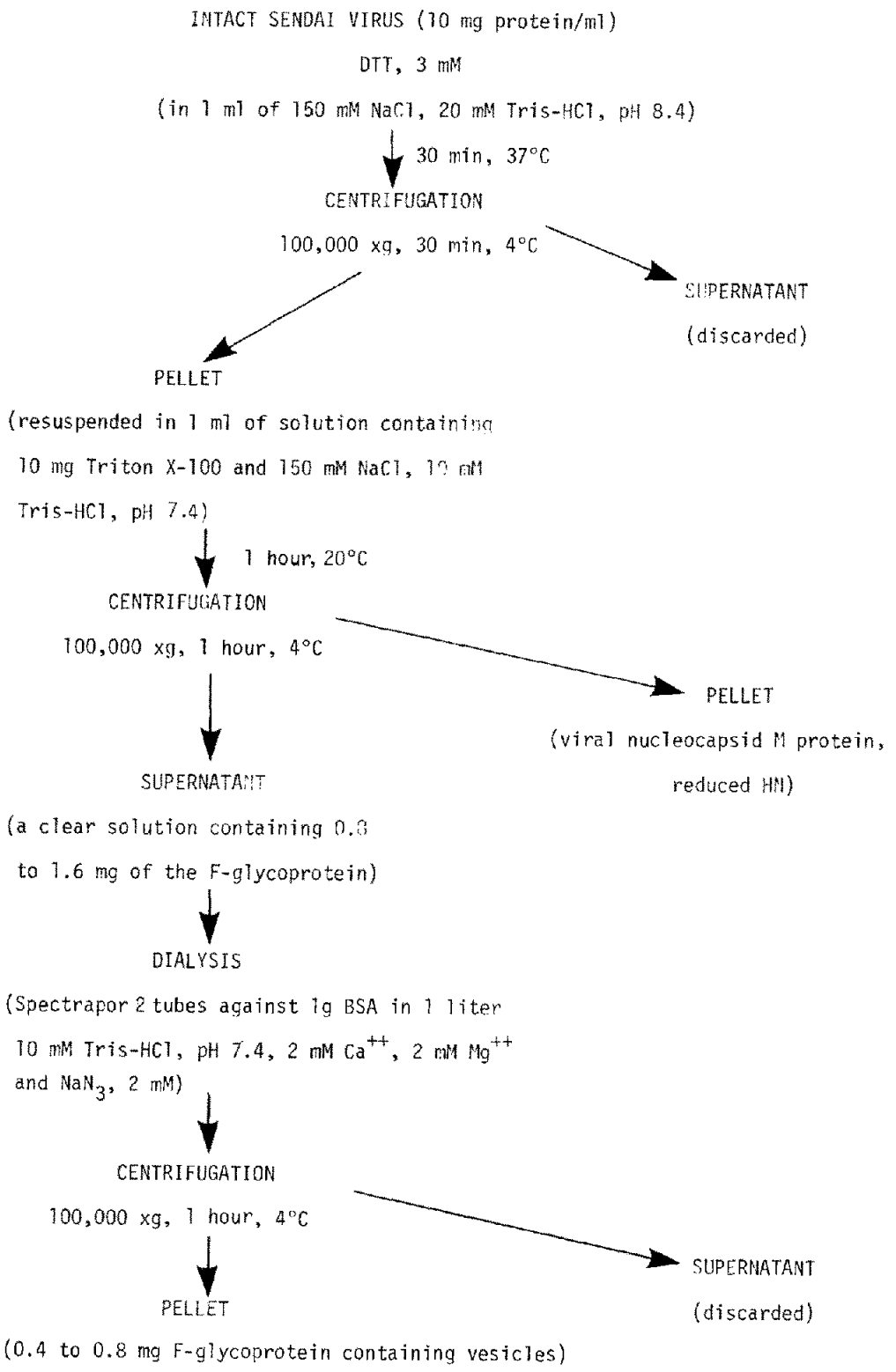

Fig. 1. A scheme summarising the method for obtaining highly purified Sendai virus F-glycoprotein (adapted with modifications from [11]).

proteins was obtained from rabbit and used as in [12].

Gel electrophoresis was performed in 5–15% (w/v) of polyacrylamide linear gradient gels with 2.5% acrylamide as stacking gel, using the discontinuous buffer system in [13]. At the end of the electrophoresis, the gels were stained by Coomassie blue and destained as in [14]. Protein was determined as in [15].

3. Results

Detergent treatment of Sendai virus particles resulted in solubilization of the two viral envelope glycoproteins, the hemagglutinin/neuraminidase (HN) and the fusion (F) protein [11]. These results show that if Sendai virus particles are reduced with DTT before solubilization with Triton X-100, only the F-glycoprotein is extracted into solution. On the other hand, the HN glycoprotein remains in the pellet as detergent-insoluble material. Fig.1 summarizes the experimental details for obtaining a detergent-solubilized Sendai virus F-glycoprotein. A highly purified Sendai virus F-glycoprotein is obtained only when the conditions detailed in fig.1 are followed. When lower concentrations of DTT or shorter incubation periods are used, different results are obtained, namely, both the HN and the F-glycoproteins are extracted into detergent–soluble fraction (fig.2A) [11].

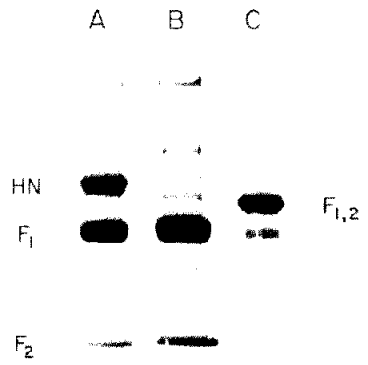

Fig.2. Gel electrophoretic analysis of Sendai virus F-glycoprotein in 5–15% (w/v) polyacrylamide gels: (A) 80 μg RSVE were loaded on the gel after boiling in 100 μl 5% SDS and 2% β-mercaptoethanol; (B) 60 μg F-glycoprotein – other experimental conditions as in (A) and section 2; (C) as in (B) but in the absence of β-mercaptoethanol (the 2% SDS solution did not contain β-mercaptoethanol).

The results of fig.2 also show that during removal of the detergent, reoxidation of the reduced SH-groups of the F-glycoprotein takes place. It can be seen that after dialysis, in the absence of β-mercaptoethanol, the F-glycoprotein migrates as a disulfide-linked complex $F_{1,2}$ (fig.2C). However, when this F-glycoprotein is treated with iodoacetamide, reoxidation is prevented and the mobility of the F-glycoprotein is the same in the presence (fig.2B) and in the absence of β-mercaptoethanol (not shown). In both cases the gels contain the two glycopeptides F_1 and F_2 in their reduced form (fig.2B).

Removal of Triton X-100 from the soluble fraction results in the appearance of a turbid suspension which apparently consists of resealed membrane vesicles containing the F-glycoprotein as their main component. Incubation of these vesicles with intact human erythrocytes causes neither agglutination nor significant hemolysis at 4°C or 37°C (table 1). The negligible hemolysis that is observed in the absence of agglutination, in the presence of these vesicles (table 1, fig.3), can be ascribed to the residual amount of Triton X-100 which probably present in these membrane preparations [16]. Hemolysis under these conditions was

Table 1
Induction of hemolysis in human erythrocytes by membrane vesicles containing biologically active F-glycoprotein

Treatment of F-containing vesicles	Addition of WGA	Hemolysis (% of total)[a]
None	–	2– 5
None	+	50–60
Antiviral antiserum	+	2– 5
Trypsin	+	2– 5
PMSF (10 mM)	+	0
DTT (10 mM)	+	2– 5
0°C, 6 h	+	2– 5

[a] The data are the mean of 5 expts

F-Glycoprotein containing vesicles (40 μg/system) were incubated with human erythrocytes in the presence or absence of WGA (1 μg/system) as in section 2. For trypsinization or for reduction, 100 μg in 95 μg of F-glycoprotein vesicles were incubated with either 5 μl trypsin (1 mg/ml) or 5 μl DTT (200 mM), respectively, in solution A, adjusted to pH 8.4, for 2.5 h at 37°C. Proteolysis was stopped by 30 μg soybean trypsin inhibitor and reduction was terminated by 15 μl 200 mM iodo-acetamide. Treatment with PMSF was performed essentially as described for DTT, except that it was performed in solution A at pH 7.4. After 2.5 h at 37°C, alkylation of the F-glycoprotein was stopped by the addition of 15 μl solution containing 100 mM serine

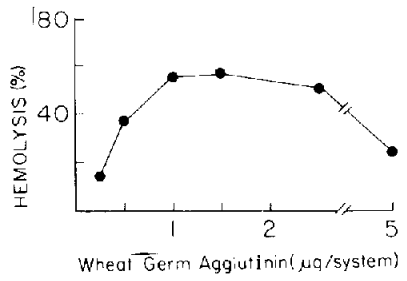

Fig.3. The effect of increasing concentrations of WGA on the degree of hemolysis of human erythrocytes in the presence of F-glycoprotein containing vesicles. Increasing amounts of WGA were added to a mixture containing 0.5 ml human erythrocytes (2.5%, v/v) and 50 μg F-glycoprotein containing vesicles. All other experimental conditions were as in section 2.

≤5%. Interestingly, addition at 37°C of the lectin WGA to a mixture of F-glycoprotein containing vesicles and human erythrocytes promptly induces agglutination. When the agglutinated erythrocytes are allowed to incubate at 37°C for a certain time, hemolysis is induced (table 1), the extent of which was dependent upon the time of incubation and the concentrations of both lectin (fig.3) and the F-glycoprotein (fig.4).

Optimal hemolysis is obtained when 1–3 μg WGA are added/system. At higher concentrations of WGA, agglutination is increased but, conversely, hemolysis is significantly reduced (fig.3). Hemolysis is absolutely dependent on the presence of the viral F-glycoprotein, as is apparent from the results summarized in table 1 and fig.4.

Antiviral antibody completely blocks hemolysis induced by the F-glycoprotein containing vesicles when added to the system before the WGA. Furthermore, treatment of the vesicles with trypsin, phenylmethyl sulfonyl fluoride (PMSF) (10 mM) and DTT (10 mM) causes inactivation of their hemolytic activity (table 1). Induction of hemolysis requires relatively high temperature (37°C). Incubation of the vesicles with erythrocytes in the cold, even for a long period (6 h), did not result in measurable hemolysis (table 1).

These findings clearly prove that the presence of biologically active F-glycoprotein is necessary for the induction of hemolysis under the present experimental conditions. The same treatment that blocks hemolysis by the F-glycoprotein containing vesicles, prevented induction of hemolysis but not of agglutination by intact Sendai virus particles or by RSVE [7] (M. T., A. L., unpublished).

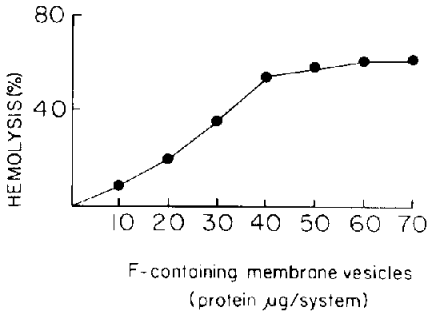

Fig.4. Hemolysis as a function of F-glycoprotein concentration. WGA (2 μg) was added to a mixture of human erythrocytes and increasing amounts of F-glycoprotein containing vesicles, as in section 2.

4. Discussion

This work describes a new and quick way, without the use of chromatography, to obtain biologically active F-glycoprotein of Sendai virus in a highly purified form. The method is based on the observation that solubilization with Triton X-100 of Sendai virus particles which were first reduced with DTT, leads to the extraction of the F-glycoprotein but not of the viral HN glycoprotein. Since without treatment with DTT an unreduced HN is recovered in the Triton-solubilized fraction [11], it may be inferred that reduction with DTT induces drastic conformational changes rendering this protein detergent-insoluble. The reduced HN glycoprotein probably forms large aggregates caused by protein–protein interactions prevented in the native unreduced glycoprotein. The view that reduction induces conformational changes in the HN protein, is strengthened by observations [17,18] showing that treatment with DTT completely blocked the biological activity of the HN, namely, agglutination of cells both at 4°C and at 37°C.

There are convincing indications that the biological function of the viral F-glycoprotein is to promote fusion between the viral envelope and the plasma membrane of recipient cells [6]. This assumption was confirmed by experiments where F-glycoprotein containing liposomes were shown to induce hemolysis of human erythrocytes at 37°C [8,9]. However, lysis was induced only in the presence of WGA which probably mediates binding of these vesicles to recipient cell surfaces [8,9]. This is understandable, as F-vesicles lack the viral native binding protein, i.e.,

the HN glycoprotein. Lysis by viral particles is assumed to reflect a process of virus–cell fusion [20].

Membrane vesicles containing the F-glycoprotein obtained by this method exhibit essentially the same characteristics as the F-glycoprotein obtained by either affinity or sieve chromatography methods [8,9].

Therefore, it may be assumed that in the presence of WGA, the F-containing vesicles readily fuse with plasma membranes. Further studies with F-containing vesicles are required to prove this point.

Acknowledgements

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