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Selective extraction of haemagglutinin and matrix protein from Sendai virions by employing trifluoperazine as a detergent

Marta Baiocchi, Marina Pescarmona, Maria Letizia Bruschi, Cesare Montecucco*, Teodoro Squatriti and Maurizio Tomasi

Laboratorio di Biologia Cellulare, Istituto Superiore di Sanita, V. le Regina Elena 299, 00161 Roma and **Istituto di Patologia Generale, Università degli studi di Padova, Via Loredan 56, 3500 Padova, Italy*

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Incubation of trifluoperazine, a local anaesthetic, at concentrations higher than the cmc with Sendai virus particles produces the selective solubilization of the haemagglutinin neuraminidase (HN) and matrix (M) proteins. This phenomenon involves aggregation of the Sendai virions and therefore the separation of HN and M from the rest of the particle can be performed by bench centrifugation. The supernatant contains the HN and M proteins and HN, once inserted into liposomes, elicits its own biological activities. Therefore, the method seems suitable for purifying large amounts of HN.

Sendai virus; Fusion; Membrane protein; Neuraminidase; Phenothiazine; Surfactant

1. INTRODUCTION

The purification of viral envelope proteins is of considerable interest because they play a key role in viral infectivity, host immunological responses and in studies of fusion. At present, increasing attention is being focused on the glycoproteins of Sendai virus since, in addition to the implications mentioned above, they can be obtained in large amounts as Sendai virus propagates in embryonal eggs at high multiplicity [1].

The structure of Sendai virus (SV) envelope consists of a lipid membrane wherein two glycoproteins are inserted [2]. The envelope binds the viral particle to the cell surface through the recognition of receptors containing sialic acid [3]. It then fuses with the plasma membrane in a pH-independent manner [4]. The binding is mediated by the haemagglutinin neuraminidase (HN) [5] while fusion involves a specific glycoprotein named F [2,3,6]. Although several reports clarified many aspects of SV-induced fusion, the role of the single glycoprotein and of the domains in this process is poorly understood.

In order to elucidate the fusion mechanism, the reconstitution of SV envelope has been shown to be a powerful tool [7], particularly when the modification of HN or F can be performed separately. So far, a rapid and simple method exists only for the purification of F; this consists of a direct and selective Triton X-100 extraction using dithiothreitol-treated virus [8]. The purification of HN, instead, involves a combination of detergent extraction with column chromatography [9].

Here, we describe a method for obtaining HN separated from F. The procedure takes advantage of the surfactant properties of the local anaesthetic trifluoperazine (TFP). This substance, belonging to the phenothiazine group, is able to form micelles in aqueous solvents at concentrations above 0.3 mM because of its amphiphatic structure [10]. When injected into animals at micromolar concen-

Correspondence address: M. Tomasi, Laboratorio di Biologia Cellulare, Istituto Superiore di Sanita, V.le Regina Elena 299, 00161 Roma, Italy

Abbreviations: HN, haemagglutinin neuraminidase glycoprotein; F, fusogenic glycoprotein; M, matrix protein; SV, Sendai virus; TFP, trifluoperazine; PAGE, polyacrylamide gel electrophoresis

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trations (below the cmc) TFP induces local anaesthesia, whereas above the cmc it is toxic. At this concentration it has been observed that phenothiazines induce the formation of pores in biological membranes [11].

The present paper reports that TFP may be considered as a very selective detergent, since it solubilizes only HN and M among the six proteins of Sendai virus.

2. MATERIALS AND METHODS

Sendai virus, z strain, was propagated for 72 h in 10-day-old chicken embryos. The viral particles were purified by different centrifugation steps [7]. TFP was obtained from Fluka (Switzerland), dithiothreitol from Calbiochem (USA), Triton X-100 from Pierce Eurochemie (The Netherlands) and fetuin and neuraminic acid from Sigma (USA). All other reagents were purchased locally and were of the highest purity available.

Neuraminidase was assayed as described by Tozawa et al. [14] and the amount of sialic acid was determined by the thiobarbituric acid colorimetric assay [15]. Under the above conditions, the neuraminidase unit (NAU) is defined as the amount of protein sample able to remove 1 mol sialic acid/min. Protein content was determined as in [16]. Haemagglutination and haemolysis were assayed according to Moscufo et al. [17]. The reconstitution procedure was performed as in [12]. Hybrid reconstituted envelopes were obtained by mixing Triton X-100-solubilized F (0.5 mg F plus 10 mg Triton X-100 in a final volume of 100 μl) [8] with TFP-extracted HN and M, and after dialysis to remove the drug, the protein concentration was brought to 4 mg/ml and brief incubation with Triton X-100 (8 mg/ml) performed in a final volume of 350 μl. The final mixture contained 0.5 mg F, 0.5 mg HN and 20 mg Triton X-100 in a final volume of 450 μl. After brief incubation at 25°C, reconstitution was performed as in [12]. Polyacrylamide gel electrophoresis (PAGE) was carried on vertical slab gels (12% polyacrylamide) in SDS and β-mercaptoethanol using a discontinuous buffer system as described by Laemmli [13].

3. RESULTS

Incubation of SV particles with TFP (10 mg viral proteins mixed in 1 ml of Mes buffer (pH 6.0) with 7 μmol TFP) produces evident aggregation of particles. The aggregate tends to sediment in 2 h at $1 \times g$ and in 10 min at $450 \times g$. Fig.1 condenses the essential steps of the experimental procedure which comprises incubation and bench centrifugation. Altogether, the method takes about 20 min.

Fig.2 shows the Coomassie blue staining pattern of PAGE in SDS and β-mercaptoethanol of SV samples before and after treatment with TFP and centrifugation. It appears that the supernatant (S) contains only HN and M whereas the pellet (P) contains the nucleocapsid proteins P and NP, plus the two subunits of F, F_1 and F_2, with the residual HN. The percentage distributions are calculated from the densitometric profiles of I, S and P line reported in fig.3. The data indicate that HN and M partition in the supernatant to an extent of 80 and 50%, respectively. It may be pointed out that glycoprotein F, despite being solubilized by all other detergents, is not extracted by TFP and becomes irreversibly insoluble with respect to Triton X-100 even after removing TFP by extensive dialysis.

The lipids remain mainly associated with the pellet (fig.1). In fact, 20% of the total lipid follows

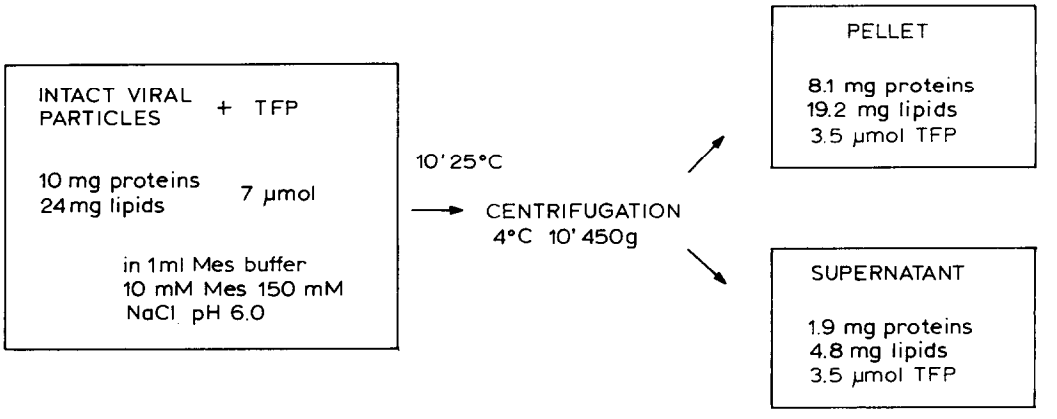

Fig.1. Schematic experimental design for selective extraction of haemagglutinin neuraminidase glycoprotein and of matrix protein of Sendai virus by trifluoperazine.

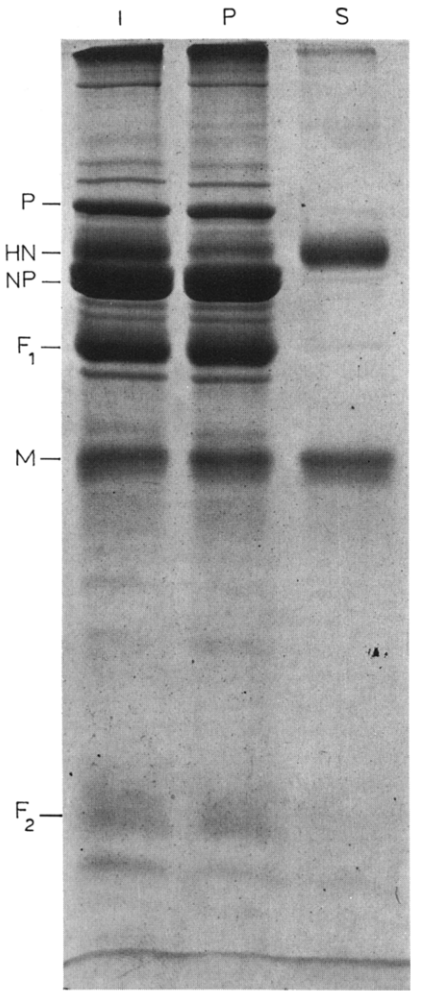

Fig.2. Coomassie blue staining pattern of Sendai virus proteins (I) and after trifluoperazine treatment and separation of the pellet (P) and supernatant (S) by centrifugation and following of the procedure described in section 2.

the supernatant, whereas the TFP partitions at 50%.

In order to determine whether a high concentration of TFP, such as 7 mM, denatures the HN protein, we performed the HN reconstitution in vesicles with and without F. The F added to the HN, prepared using TFP, was purified by Triton X-100 extraction of dithiothreitol-treated SV as in [8]. The hybrid reconstituted vesicles were assayed for haemagglutination, neuraminidase and haemolysis activities. The results reported in table 1 indicate that HN, extracted by TFP, is able either to

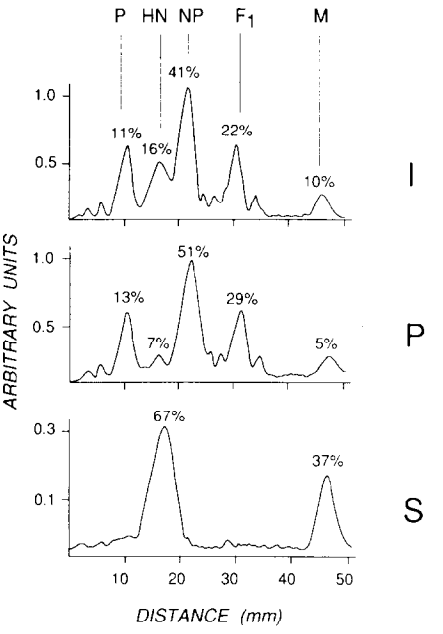

Fig.3. Densitometric profile of Coomassie blue staining pattern of intact virus (I), pellet (P) and supernatant (S) as reported in fig.2.

agglutinate human erythrocytes, to retain neuraminidase activity or to cooperate with F in inducing haemolysis.

Since the presence of M may disturb structural

Table 1

Biological activities of trifluoperazine-extracted Sendai virus haemagglutinin neuraminidase glycoprotein

	HAU/mg	NAU/mg	LAU/mg
Intact virions[a]	6400	5	1600
Pellet	246	-	1
Supernatant	134736	20	1
Glycoprotein F[b]	1	-	1
Hybrid reconstituted viral envelopes[c]	51200	-	2500

[a] Virions (10 mg protein) were incubated with 7 μmol trifluoperazine in 10 mM Mes, 150 mM NaCl (pH 6.0); after incubation at 25°C for 10 min the mixture was centrifuged at 4°C for 10 min at 450 × g, pellet (containing F, P, NP) and supernatant (containing HN, M) were the samples analysed for biological activities and of which the preparation is described in figs 1,2

[b] F glycoprotein was purified from DTT-treated virus and extracted with Triton X-100 as reported in [8]

[c] Hybrid reconstituted envelopes were prepared by mixing in Triton X-100 the F glycoprotein obtained as described in b and HN and M prepared as in a

studies on HN we tried to obtain HN without M. We observed that in the reconstituted viral envelope the amount of M significantly decreased when 600 mM NaCl was present in Tris buffer (not shown). However, to obtain pure HN it was necessary to introduce a sieve chromatographic procedure.

4. DISCUSSION

The results clearly show that TFP can be used as a selective detergent to solubilize HN and not F of the SV envelope. TFP incubation with SV particles produces rapid extrusion of HN and M concomitant with the aggregation of virions. HN, extracted with TFP, maintains the neuraminidase and haemagglutinin activities and moreover correctly cooperates with F protein to promote fusion. In contrast, TFP modifies irreversibly the solubility of F in non-ionic detergents. This phenothiazine thus seems to affect dramatically the conformation of F, probably by inducing aggregation.

The mechanism by which the two glycoproteins react so differently to TFP treatment is unknown. However, it may be observed that TFP is an amphiphatic compound which at pH 6.0 is cationic and then preferentially interacts with negatively charged substances. In this respect, glycoprotein F seems to fulfill such a characteristic, since the p*I* of F was evaluated as 4.9 whereas the p*I* of HN is 6.5 [18]. From this point of view the selective solubilization of HN should be regarded as a selective insolubilization of F protein. It may be noted that upon TFP treatment the partitioning of lipids in the supernatant containing HN and M amounts to as much as 20 and 80% in pellet containing F and nucleocapsid. This might be interpreted as being due either to the formation of stable TFP-lipid mixed micelles being hindered or to a stronger interaction between lipids and F than between lipids and HN. In addition, the results show that M is coextracted with HN. TFP, therefore, shows unusual behaviour with respect to other detergents. In fact, non-ionic detergent (Triton X-100, octylglucoside, etc.) solubilizes HN and F but not M; cholate and deoxycholate solubilize almost all viral proteins even if the extraction of HN and F is complete while the other proteins are only partially solubilized; SDS is commonly used as solubilizer of all viral proteins. The reasons for the extraction of M appear, therefore, to be quite obscure and we tentatively speculate that TFP at concentrations such as 7 mM destroys the lipid bilayer organization by creating holes large enough to permit the passage of M. In this case the depolymerization of M appears to be a concomitant event. Another possibility is that M protein follows HN because of an interaction between the two proteins. In other words, M is removed from leaky virions by HN. In any case, we were unable to separate HN from M with the simple procedure and this is certainly due to a poor understanding of the phenomenon. We are presently carrying out a study on this subject.

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