THE MEMBRANE PROTEINS OF SEMLIKI FOREST VIRUS HAVE A HYDROPHOBIC PART ATTACHED TO THE VIRAL MEMBRANE

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1. Introduction

We are studying the envelope of Semliki Forest virus (SFV) as a membrane model for structural and assembly studies. Semliki Forest virus and other group A arboviruses contain a nucleocapsid core surrounded by a lipid-containing envelope [1, 2], which the nucleocapsid acquires as it leaves the host cell by budding through the plasma membrane [3]. The lipids of the viral envelope resemble those of the host cell plasma membrane [4] and these are evidently arranged into a bilayered structure [5]. The viral envelope has a simple protein composition. One polypeptide with an apparent molecular weight of about 50,000 and a carbohydrate content of 14% has been found in the membrane [6, 7]. This protein has recently been split into two bands using discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis [8]. Most of the protein of the viral envelope is located outside the lipid bilayer [9] as spikes which can be removed by proteolytic enzymes [10–12].

We have earlier found that the delipidated envelope proteins of SFV bind substantial amounts of the mild detergent sodium deoxycholate and Triton X-100 [13, 14]. This property they have in common with other membrane proteins, whereas standard hydrophilic proteins bind little or none of these detergents [13]. Integral membrane proteins [15] contain a relatively large proportion of hydrophobic amino acids [16]. We assume that the detergents bind to hydrophobic regions of the proteins which in the intact membrane interact with lipids. In the present investigation we have used the proteolytic enzyme thermolysin to digest intact SFV and have found that part of the envelope protein is left in the spikeless particle. This fraction is enriched in hydrophobic amino acids.

2. Materials and methods

A prototype strain of SFV was grown in BHK21 cells and purified as described previously [2]. SFV was labelled with a mixture of ^{14}C-amino acids (New England Nuclear) (8 μCi/ml medium), with [^{3}H]leucine [^{3}H]isoleucine and [^{3}H]valine (New England Nuclear) (33 μCi of each/ml) or with carrier-free [^{32}P]orthophosphate (Institutt for Atomenergi, Kjeller, Norway) and purified as described previously [17]. Hemagglutination was performed as described [18]. Enzymatic digestion of SFV was performed at 37° for 90 min with thermolysin (Calbiochem) in 0.1 M NaCl, 0.05 M Tris-HCl, 5 mM $CaCl_2$, pH 7.4. The virus(protein)/enzyme ratio was varied to establish optimal conditions. Spikeless particles were obtained in the ratio of 2:1 (w/w) and this ratio was used in the studies described here. The control was incubated similarly, without the enzyme. After digestion the virions were centrifugated to isopycnic density in a 20–50% phosphate buffered linear sucrose gradient in the Spinco SW50 rotor at 45,000 rpm for 3.5 hr. Fractions were collected from below and aliquots counted in Bray's solution [19]. Polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) was performed according to

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Weber and Osborn [20] with 7.5% acrylamide gels, the slices being counted after NCS (Amersham/Searle) solubilization [21]. Lipid-bound and total phosphorus was determined as described [4]. Electron microscopy was performed after negative staining with potassium phosphotungstate, pH 7.0 [2].

3. Results and discussion

When SFV was digested with thermolysin electron micrographs of the particles revealed that they had lost their surface spikes (fig. 1A and B). The hemagglutinating activity of the virus was also lost. The spikeless particles were purified by isopycnic sucrose density gradient centrifugation and had a peak density of 1.14 g/ml compared to 1.19 g/ml for the control virus. When both the RNA and phospholipids were labelled with ^{32}P, the purified spikeless particles were found to have a ratio of lipid-bound phosphorus to total phosphorus of 0.59, almost identical to that of the control 0.60. No ^{32}P-label was lost from the spikeless particle fraction. This is in agreement with the findings of Compans, who removed the spikes from Sindbis virus with bromelain [12].

The SDS-PAGE of purified intact and spikeless SFV is shown in fig. 2A and B. The virus was doubly labelled with a mixture of ^{14}C-amino acids and ^{3}H-labelled hydrophobic amino acids (valine, leucine, isoleucine). No intact envelope protein is seen in the spikeless SFV (fig. 2B). Instead a low molecular weight degradation product marked HF migrates with the marker dye. 7–12% of the ^{14}C-amino acid label in the envelope protein is found in the hydrophobic fragment(s). This must be derived from the envelope proteins as no degradation of the nucleocapsid protein coud be detected after thermolysin digestion (table 1). The amounts of virus applied to the gels were quantitated by determining the lipid-bound phosphorus in the virus preparations prior to electrophoresis. HF is clearly enriched in the ^{3}H-labelled hydrophobic amino acids (table 1).

Table 1
Comparison of control virus and thermolysin-treated purified spikeless SFV*.

	Control SFV (dpm × 10^{-4}/μg lipid P)			Thermolysin-treated SFV (dpm × 10^{-4}/μg lipid P)		
	[^{3}H]	[^{14}C]	[^{3}H]/[^{14}C]	[^{3}H]	[^{14}C]	[^{3}H]/[^{14}C]
Envelope proteins	64.4	2.71	23.8	0	0	–
Core protein	15.6	0.71	19.7	15.1	0.87	17.4
HF	0	0	–	15.5	0.25	62.0

* SFV was double labelled with [^{3}H]leucine, [^{3}H]isoleucine and [^{3}H]valine, and with ^{14}C-amino acid mixture. Electrophoresis was performed in polyacrylamide gels in the presence of sodium dodecyl sulphate. The amount of virus applied to the gels was quantitated by its lipid phosphorus content. Data from one typical experiment are shown.

These results show that the SFV envelope proteins contain a fraction enriched in hydrophobic amino

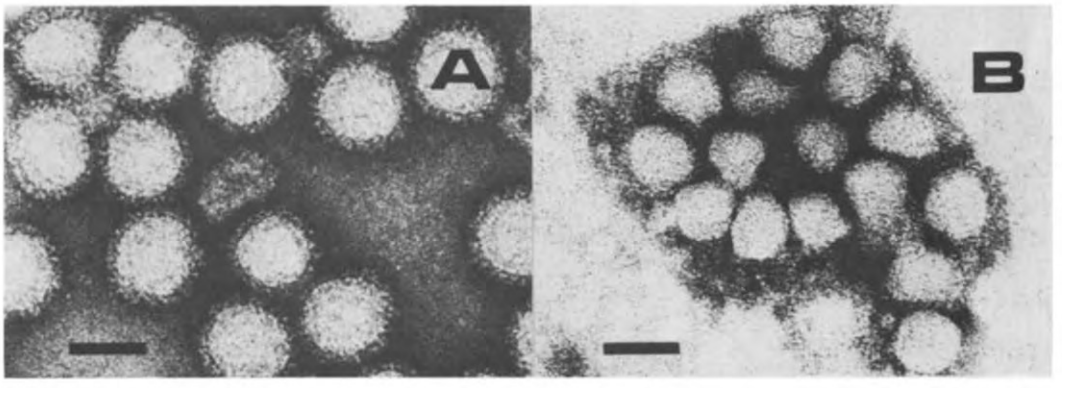

Fig. 1. Electron micrographs of intact (A) and thermolysin treated (B) SFV. Bar = 50 nm.

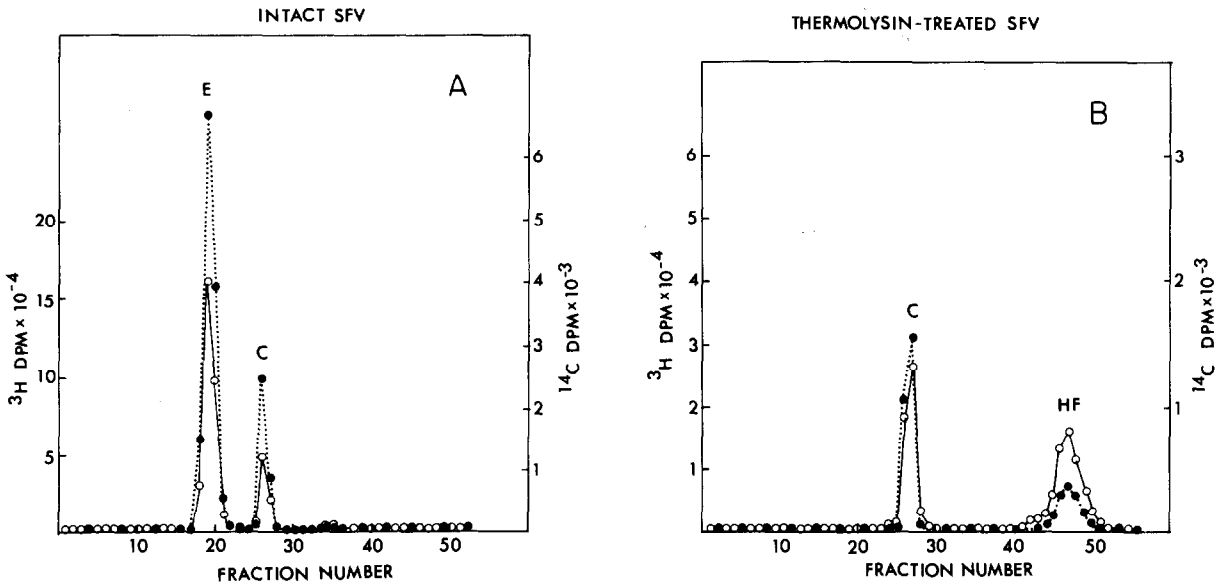

Fig. 2. SDS-PAGE of double-labelled control (A) and SFV digested with thermolysin (B). (•--•--•) = ^{14}C-amino acid mixture; (o—o—o) = [^{3}H]leucine, [^{3}H]isoleucine, [^{3}H]valine. E = envelope proteins (not separated in this system), C = nucleocapsid protein, HF = hydrophobic fragment(s), Bromphenol blue marker in fractions 46–48.

acids attached to the membrane. This finding is in keeping with other recent reports on the amphipathic nature of membrane proteins. The hydrophilic portion of the cytochrome b_5 molecule, including the catalytic site, can be cleaved from a hydrophobic "tail" which anchors the protein to the endoplasmic reticulum [22, 23]. And in the human erythrocyte membrane [24, 25], the major glycoprotein seems to have a hydrophobic segment in the membrane whereas the part of the protein carrying the carbohydrate is located outside the membrane.

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