Purification and Characterization of the Major Envelope Glycoprotein of Simian Foamy Virus Type 1

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SUMMARY

Simian foamy virus type 1 (SFV-1), the prototype of the Spumavirinae, was subjected to disruption and serial purification procedures. Separation of SFV-1 envelope components from viral cores was verified by electron microscopy, density gradient centrifugation and polyacrylamide gel electrophoresis. After affinity chromatography of the envelope polypeptides on a concanavalin A-Sepharose column, a highly purified 70000 mol. wt. protein was recovered. Glycosylation of this gp70 was confirmed by glucosamine labelling. Immunological studies with anti-SFV antisera confirmed the type-specificity of this envelope gp70.

INTRODUCTION

Foamy viruses belong to the subfamily Spumavirinae of the Retroviridae. They have been isolated from various mammalian species and are known for their latency in vivo and their characteristic c.p.e. in vitro. Ten different serotypes of simian foamy virus (SFV) have been isolated from tissues of healthy or diseased New World and Old World monkeys (Hooks & Gibbs, 1975; Hooks & Hooks, 1981; Rhodes-Feuillette et al., 1979). An isolate obtained from human nasopharyngeal carcinoma tissue has been shown to be related to the SFV types 6 and 7 previously found in chimpanzees (Achong et al., 1971; Epstein et al., 1974; Brown et al., 1978).

We thought it of interest to investigate the biochemical structure of Spumavirinae since very little is known about this. Having identified the viral 70S RNA genome and the main viral polypeptides of SFV type 1 (Cavaliere et al., 1981) we have pursued our studies by separation of viral cores from envelope components. We have previously reported the purification and characterization of the viral reverse transcriptase (Benazir et al., 1982) and its associated RNase H (Benazir et al., 1983), and have also investigated the structural proteins of the core (A. B. Benazir et al., unpublished). This report concerns the separation and characterization of the highly purified glycosylated envelope polypeptide which reacts type-specifically in immunological tests.

METHODS

Virus and sera. SFV-1 was provided by courtesy of the National Institutes of Health (Bethesda, Md., U.S.A.) and propagated in canine thymus C12Th cells. Virus growth and purification were carried out as previously described (Benazir et al., 1982). Uninfected C12Th cells were processed as controls.

Electron microscopy (EM) of infected and uninfected C12Th cultures was carried out 6 days post-infection, immediately before harvesting the virus. Samples were fixed in glutaraldehyde and stained with uranyl acetate and lead citrate. Viral pellets and viral cores were examined after negative staining with phosphotungstic acid (Brenner & Horne, 1959).

Horse antibodies against SFV-1 (8CDEL010) and SFV-2 (2VEFG1100) were supplied by the NIH. Rabbit antisera against SFV-1 and SFV-10, and mouse (BALB/c) antibodies against the purified envelope glycoprotein (gp70) of SFV-1, were prepared according to previously described immunization protocols (Johnston, 1971).
**Purification.** The envelope polypeptides were extracted by a modification of the two-step procedure of Strand & August (1976). Briefly, 12 mg of purified virus diluted in TNE buffer (100 mM-Tris-HCl pH 7.4, 10 mM-NaCl, 1 mM-EDTA) at 2 mg/ml was disrupted by three cycles of freezing at −80 °C and thawing (D1). A first supernatant (S1) and a pellet (P1) were obtained by centrifugation (C1) at 100000 g for 90 min at 4 °C in a Sorvall R30 rotor. The disrupted virus was purified by isopycnic banding in a 20 to 70% sucrose gradient, and analysed by EM and SDS-PAGE according to Laemmli (1970). Supernatant S1 was similarly analysed and shown to contain envelope components. Following disruption of pellet P1 in TNE buffer, a second centrifugation (C2) at 100000 g yielded supernatant S2 and pellet P2. After analysis by EM and PAGE, it was found that S1 and S2 could be combined and that P2 contained the core particles. The pooled G1 sample obtained by combination of S1 and S2 was dialysed twice for 12 h against 600 ml of PIPES-EDTA (10 mM-PI Pes pH 6.5, 1 mM-EDTA).

**Purification of envelope polypeptides.**

**Phosphocellulose chromatography.** G1 was pumped onto a 1 × 3 cm phosphocellulose column equilibrated with PIPES-EDTA buffer. The column was washed with the buffer until the fluid collected had an A_{280} below 0.02. This was followed by a 60 ml linear gradient of KCl (0.1 to 500 mM) in PIPES-EDTA buffer. One ml fractions were collected at 15 ml/h, and the eluted proteins were detected by A_{280}. The appropriate fractions were pooled (pool G2), analysed by PAGE, and chromatographed on a concanavalin A-Sepharose column (Con A-Sepharose).

**Con A affinity chromatography.** Pool G2 was dialysed three times (1 h, 4 h, 12 h) against 300 ml of buffer A (0.10 M-sodium acetate pH 6.0, 0.15 M-NaCl, 1 mM-CaCl₂, 1 mM-MgCl₂, 1 mM-MnCl₂). G2 was then pumped on to a 1 × 2 cm Con A-Sepharose 6MB column and equilibrated with 10 volumes of buffer A. The column was washed with a further three volumes of buffer A, followed by a 50 ml linear gradient of NaCl (0.15 to 1.5 M) in buffer A. Fractions of 0.5 ml were collected at 10 ml/h and eluted polypeptides were detected by A_{280}. The appropriate fractions were pooled (pool G3) before PAGE analysis and immunological characterization by the method of Ouchterlony (1962).

**Radioactive labelling and specific immunoprecipitation.** Cf2Th cells infected by SFV-1 were used for radioactive labelling; uninfected cells were used as controls. At 48 h post-infection, the culture medium was replaced by 20 ml of Dulbecco’s medium supplemented by 5% foetal bovine serum (FBS). After another 72 h at 37 °C, a preincubation of 5 h was begun with either 15 ml of Eagle’s minimum essential medium (MEM) per flask (low glucose) supplemented by 5% FBS, or 15 ml of methionine-free MEM supplemented by 5% FBS, depending on the labelled amino acid to be used. Both infected and uninfected cultures were labelled with either 50 μCi/ml [3H]glucosamine for 5 h in the low glucose medium or with 50 μCi/ml [35S]methionine for 10 min in the methionine-free one. Cells were harvested by washing three times with cold phosphate-buffered saline (PBS) and twice with 0.15 M-NaCl, 50 mM-Tris–HCl, 1 mM-EDTA at pH 7.4. The cells were scraped off with a rubber policeman and lysed in 0.5 ml of lysis buffer (0.1 M-NaCl, 10 mM-Tris–HCl, 2 mM-EDTA, 1% NP40, 0.5% deoxycholate). The lysate was centrifuged for 10 min at 2500 g and, after addition of 1% phenylmethylsulphonyl fluoride (PMSF), re-centrifuged for 10 min at 10000 g. After an overnight incubation at 4 °C with 10 μl normal serum and 100 μl of *Staphylococcus aureus* Protein A preparation (Staph A) per 1 ml of cytoplasmic extract, the lysate was clarified by a 30 min centrifugation at 12000 g. The resulting supernatant was precipitated using 3 μl of specific antiserum per 0.5 ml for 2 h at 4 °C, followed by 100 μl of Staph A to render the antigen–antibody complexes insoluble. Immunoprecipitates were collected after a 10 min centrifugation at 10000 g in buffer I (0.2 M-NaCl, 50 mM-Tris–HCl pH 7.4, 0.5% deoxycholate, 0.5% NP40, 0.1% SDS, 1 mM-EDTA). They were filtered twice through a 1 M-NaCl-1 M-sucrose cushion in buffer I and washed twice with buffer I containing 1 M-NaCl. Immunoprecipitates were dissolved, and subjected to electrophoresis according to the method of Laemmli (1970).

**RESULTS**

**EM of SFV-1-infected Cf2Th cells**

EM observation of the uninfected Cf2Th controls demonstrated the absence of any virus-like forms, and particularly of intracytoplasmic type A particles or equivalents. SFV-1-infected Cf2Th cells, however, showed typical SFV particles in their usual location (Rhodes-Feuillette et al., 1979).

**Characterization of viral polypeptides**

In order to determine which polypeptides are specific for SFV-1-infected cells, uninfected and SFV-1-infected cultures were labelled for 10 h with [35S]methionine. Cytoplasmic extracts were treated as described in Methods with rabbit anti-SFV-1 serum. The immune precipitates were analysed by electrophoresis (Laemmli, 1970). Several bands were identified in electrophoreograms of infected cells whereas none were detectable in the controls (Fig. 1).
Fig. 1. Immunoprecipitation of polypeptides from SFV-1-infected and uninfected Cf2Th cells. Extracts from cells labelled with \[^{35}\text{S}]\text{methionine}\) were treated with rabbit anti-SFV-1 serum. Immunoprecipitates were applied to an 8 to 16% gradient polyacrylamide slab gel. Autoradiograms were exposed for 5 days. (a) Mol. wt. markers of 94K, 69K, 45K, 30K and 15K. (b) Several polypeptide bands are identifiable in the sample from infected cells. (c, d) No such bands are seen in samples from uninfected cells, even though the sample in (d) was fourfold more than that of the SFV-1-infected cell extract.

Fig. 2. Isopycnic banding of \[^{35}\text{S}]\text{methionine}-labelled virus purified from Cf2Th cells infected by SFV-1 with a minor peak (A) and a major peak (B).

The efficiency of the extraction methods employed to separate the external polypeptides from the core particles was determined by density gradient centrifugation after \[^{35}\text{S}]\text{methionine}\) labelling, and by EM examination of the peaks obtained.

Isopycnic banding of purified intact virus yielded two radioactive peaks (Fig. 2): a minor peak (A) at 1.22 g/ml and a major peak (B) at 1.16 g/ml. EM of the latter showed enveloped particles with spikes (Fig. 3a). These particles, 90 to 100 nm in diameter, correspond to the complete particles seen budding at the membrane of SFV-1-infected cells. Isopycnic banding of the viral pellet, obtained after extracting the external polypeptides, showed a single peak at 1.22 g/ml. EM of this revealed the presence only of spherical core particles, 50 nm in diameter which were similar to the precursor intracytoplasmic particles found in SFV-infected cells (Fig. 3b). Thus, the extraction procedures applied to separate the external polypeptides from the viral particles, enabled purification of viral cores from pellet P2.
Fig. 3. EM of negatively stained SFV-1 particles, before and after application of the external polypeptide extraction procedures to the viral pellet. Bar markers represent 100 nm. (a) 90 to 100 nm diam. viral particles, with 10 nm spikes, an external envelope and an electron-lucent centre. (b) Viral cores, obtained after extraction of external polypeptides, and measuring approximately 50 nm.
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Fig. 4. PAGE analysis of the steps in purification of the external gp70. (a) Total disrupted virus. (b) Pool G1, corresponding to supernatants S1 and S2. (c) Pool G2 obtained by affinity chromatography of G1 on a Con A column, corresponding to the purified gp70. (d) Mol. wt. markers of 94K, 68K, 45K, 39K, 21K and 13K.

Fig. 5. Analysis of intracellular SFV-l-specific glycoproteins precipitated by mouse anti-gp70 serum. Cytoplasmic extracts from infected and uninfected Cf2Th cells labelled with $[^3H]$glucosamine were prepared and treated with mouse anti-gp70 serum. Immunoprecipitates were applied to an 8 to 10% gradient polyacrylamide slab gel according to the method of Laemmli. Autoradiograms were exposed for 8 days. (a) Immunoprecipitate of an extract of uninfected cells. (b) Immunoprecipitate of an extract from SFV-l-infected cells showing a major band at 70K. (c) Mol. wt. markers of 94K, 69K, 45K, 30K and 15K.

Characterization of envelope polypeptides

PAGE analysis allowed the major steps leading to the purification of the external polypeptides to be followed (Fig. 4). Supernatant S1, obtained by separation of the external polypeptides as shown by EM (Fig. 3a, b), contains two main polypeptides of 70K and 30K (Fig. 4b). The 70K protein was further purified by chromatography on a Con A-Sepharose column. PAGE analysis of the appropriate fractions obtained by elution with a linear NaCl gradient showed a single 70K band (Fig. 4c). The affinity of this 70K polypeptide for Con A suggested a glycoprotein nature (i.e. gp70). Moreover, immunoprecipitates using extracts of $[^3H]$glucosamine-labelled SFV-l-infected and uninfected Cf2Th cells and a mouse antibody directed towards the purified gp70, showed the presence of a 70K band in infected cells (Fig. 5b), which was absent in uninfected cells (Fig. 5a).
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Fig. 6. Characterization of the gp70 of SFV-1 by immunodiffusion. Purified gp70 was placed in the central well. Rabbit antisera against SFV-1 and SFV-10, and horse antiserum against SFV-2 were respectively placed in wells 1, 2 and 3. The preimmune serum from the rabbit immunized against SFV-1 was placed in well 4. Similar results were obtained with horse anti-SFV-1 serum (results not shown).

When immunodiffusion experiments were carried out by the Ouchterlony method, the results revealed that gp70 interacted exclusively with SFV-1 antiserum (Fig. 6), indicating a type-specificity which is a characteristic of other retroviral envelope glycoproteins.

The p30 external polypeptide showed no affinity for Con A nor was it labelled with \[^3H\]glucosamine. The low yield and poor immunogenic properties of this polypeptide have prevented further characterization.

DISCUSSION

Our results show that the envelope of SFV-1, the prototype primate foamy virus, is composed of two proteins, a major 70K glycoprotein and a minor 30K probably unglycosylated polypeptide.

We were able to obtain a highly purified preparation of gp70, by separating the envelope polypeptides from the cores, employing a procedure derived from that described by Strand & August (1976) for the isolation of external polypeptides of other retroviruses. This protein was shown to bind to Con A and was labelled by \[^3H\]glucosamine, showing it to be glycosylated. It is apparently type-specific, since immunodiffusion experiments, using SFV-1, SFV-2 and SFV-10 antisera, clearly showed strong specific reactivity with SFV-1 antiserum, but no interaction with the others. However, further immunological characterization of the gp70 of SFV-1 appears necessary to confirm the absence of cross-reactions with the gp70 of other serological types since some degree of immunological interaction has been described using seroneutralization and haemagglutination inhibition techniques (Périès & Todaro, 1977).

Another envelope protein, p30, located externally, was not labelled by \[^3H\]glucosamine nor was it retained by the Con A–Sepharose column, suggesting a lack of glycosylation. This p30 could be a contaminant core polypeptide, but the high degree of purification of the envelope components achieved argues against this possibility.

Altogether, the results presented here demonstrate that SFV-1 representing the primate Spumavirinae, differs little from other known retroviruses which contain, beside a major envelope glycoprotein, a secondary unglycosylated envelope polypeptide of lower molecular weight (Stephenson, 1980).
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REFERENCES


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