CHARACTERIZATION OF THE SEMLIKI FOREST VIRUS CORE AND ENVELOPE PROTEIN

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The core and envelope proteins of Semliki Forest virus have been purified. Amino acid analysis revealed a high lysine content in the core protein, whereas the envelope protein was found to be more hydrophobic in its character.

Semliki Forest virus (SFV) consists of a nucleoprotein core surrounded by a lipoprotein envelope, which the core particle acquires as it leaves the host cell by budding through the cell membrane (1). Two major structural proteins have been found in SFV (2, 3, 4) and other group A arboviruses (5), one associated with the core and the other with the envelope. Polyacrylamide gel electrophoresis gives a molecular weight of about 35,000 for the SFV core protein and 50,000 for the envelope protein (3).

The present report describes our use of sodium dodecyl sulfate (SDS) to split purified SFV into its major constituents: RNA, protein, and lipid. We have purified the core and envelope proteins by gel filtration and preparative polyacrylamide gel electrophoresis in buffers containing SDS, and determined their amino acid compositions, sedimentation constants, and molecular weights.

MATERIALS AND METHODS

Virus. A prototype strain of SFV grown in monolayer cultures of BHK21 cells was purified as described previously (4). The last purification steps consisted of two successive density gradient centrifugations in potassium tartrate. Virus was labeled with DL-^{3}H-phenylalanine, 5 C/mmole (Amersham), from three to ten hours after infection, with one ug actinomycin D per ml

present throughout the growth cycle. Labeled virus was purified as described before (4). Hemagglutination assays were performed as described previously (4).

Gel filtration. Purified SFV (5-15 mg protein) containing ^{3}H-phenylalanine-labeled virus as marker was incubated with 0.2 M SDS and 0.1% 2-mercaptoethanol in phosphate-buffered saline for 3 hours at 37° C. This mixture was then chromatographed on Sephadex G-200. The column (2.5x90 cm) was fitted for upward flow. The column was equilibrated with 0.1 M tris, pH 7.7 and 0.1% SDS (three times crystallized) and run at room temperature. For calibration five mg samples of purified proteins were used. The void volume was determined with blue dextran and the total volume with 2-mercaptoethanol. The distribution coefficient K_{av} was calculated for each protein as described by Laurent and Killander (6). Radioactivity in effluent fractions was determined in Bray's solution in the Packard Tricarb scintillation spectrometer.

Electrophoresis. Analytical SDS-disc electrophoresis in 10% acrylamide gel was performed as described by Weber and Osborn (7), except that 0.1% mercaptoethanol was included in the buffer (8). The relative mobilities were calculated by their procedure with bromphenol blue as the reference dye. Preparative polyacrylamide gel electrophoresis was performed in the Polyprep apparatus (Buchler) with the gels and the tris-glycine buffer system described previously (9), except that urea was replaced by 0.1% SDS in the upper buffer and in the upper (30 ml) and lower (100 ml) gels. No SDS was added to the elution buffer.

Chemical determinations. Protein determinations were made with bovine serum albumin as the standard using 0.1% SDS in the reaction mixture (10). Phosphorus was measured by the method of Bartlett (11), and SDS by the method of Karush and Sonenberg (12). Qualitative lipid analysis was performed with thin layer chromatography on silica gel (13). Amino acid analysis was performed in the Beckman 120 C amino acid analyzer after hydrolysis in 6 M HCl for 22 hours at 110° C (4). Half cystine was determined as cysteic acid after performic acid oxidation. Tryptophan was determined after $Ba(OH)_2$ hydrolysis

(14). All values were converted from nanomoles of amino acid recovered to moles of amino acid per 1000 moles of all amino acid residues recovered.

Analytical ultracentrifugation. Velocity sedimentation experiments were performed as described previously (15) in a Spinco model E analytical ultracentrifuge equipped with electronic speed control, regulated temperature indicator control units, the split beam photoelectric scanner accessory with a monochromator and the ultraviolet absorption optical system. The sedimentation velocity determinations were carried out in 0.1 M sodium phosphate, pH 7.0 containing 0.1% SDS at a protein concentration of 0.5-1 mg per ml.

RESULTS AND DISCUSSION

Purified Semliki Forest virus which had been treated with SDS and mercaptoethanol could be separated into four fractions by filtration on Sephadex

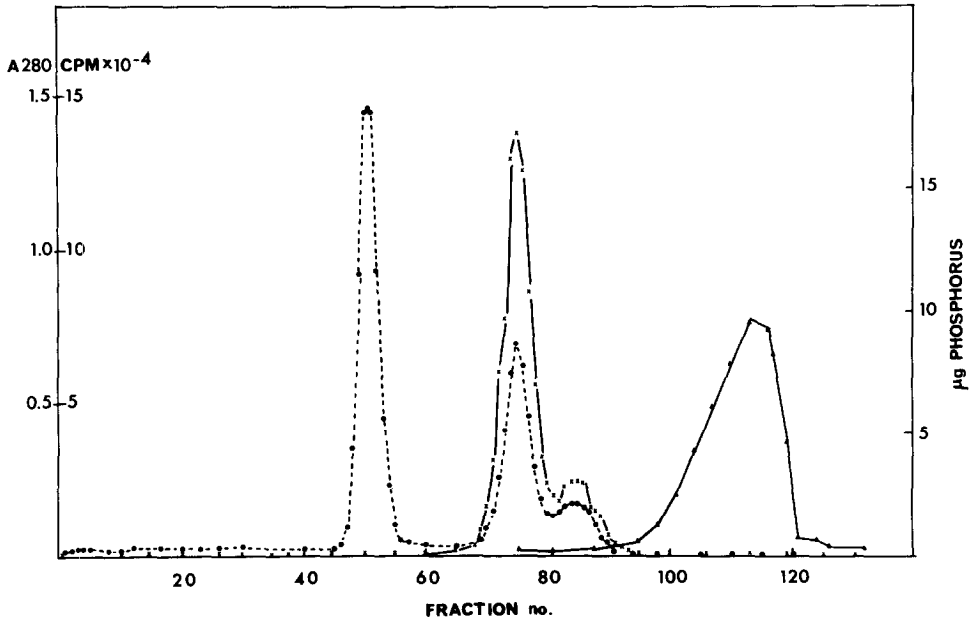

Figure 1. Sephadex G-200 filtration of purified SFV (15 mg protein) after treatment with SDS and mercaptoethanol. Fraction volume 2.5 ml. Absorbance at 280 nm ●----------●, radioactivity per fraction x ——— x, and phosphorus per fraction (determined only from fractions 74-130) ▲———▲

G-200. The elution diagram of one typical separation is shown in Fig. 1. The first fraction which eluted in the void volume contained the viral RNA free from protein and lipid. The absorption spectrum of this fraction had a minimum at 230 nm, and the 280:260 nm ratio was 0.45. Sucrose density gradient analysis (16) showed that most of the material sedimented at about 42 S, which is the sedimentation constant of the SFV RNA. The second and the third fractions contained the envelope and core proteins respectively. This was established by SDS-disc electrophoresis which can be used to identify these proteins (4). After exhaustive dialysis the protein fractions were tested for hemagglutinating activity, but none could be found. Screening by qualitative thin layer chromatography showed that the viral lipids were in a fourth fraction eluting after the proteins. The amount of phospholipid in this fraction was quantitated by phosphorus analysis as shown in Fig. 1. Similar separation of the protein and lipid components of E.coli membranes by SDS-gel filtration has recently been reported by Jones and Kennedy (17).

The envelope and core protein fractions were contaminated by each other after the gel filtration. These fractions were further purified by preparative polyacrylamide gel electrophoresis in SDS. After this purification step the envelope and core proteins appeared to be homogeneous as judged by analytical SDS-disc electrophoresis (Fig. 2) and analytical ultracentrifugation. The purified envelope and core proteins sedimented as single components with s_{20} values of 2.9 and 2.3 S respectively. Thus the virus contains only two proteins of different size. It should be pointed out, however, that the purification steps we used separated proteins according to their size, and would not detect other types of heterogeneity.

The amino acid composition of the purified envelope and core proteins are shown in Table 1. The core protein had a high lysine content. This is consistent with its role as the structural protein forming the virus core particle in association with the RNA. The ratio of mole percentages of the hydrophilic and the hydrophobic amino acids was calculated as described by Hatch and Bruce

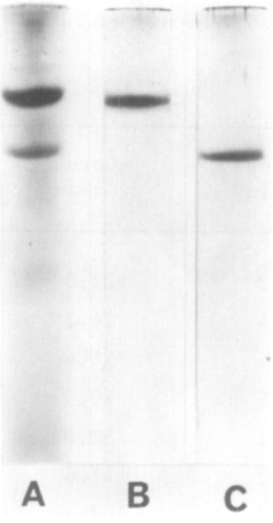

Figure 2. SDS-disc electrophoresis of A. SFV, B. purified envelope protein, and C. purified core protein. Anode at the bottom.

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(18), and was found to be 1.98 and 2.55 for the envelope and core proteins, respectively. Apparently the envelope protein is adapted for protein-lipid interactions, and this is reflected in its relatively high content of apolar amino acids compared to the lysine-rich core protein.

We have previously found that the ratio of envelope:core proteins is about 4:1 in the virus particle assuming uniform radioactive labeling with ^{14}C protein hydrolysate (4). When this ratio was used to calculate the amino acid composition of the whole virus the values obtained were in good agreement with the previously determined total amino acid composition of the virus (Table 1).

The molecular weight of the envelope and core proteins were estimated using both SDS-gel filtration and SDS-disc electrophoresis. The results obtained are shown in Fig. 3. The values for the core protein were in good agreement: 35,000 by gel filtration and 33,000 by electrophoresis. There was some discrepancy in the values obtained for the envelope protein: 60,000 by

Table 1. AMINO ACID COMPOSITION (MOLES/1000 MOLES) OF ENVELOPE AND CORE PROTEINS AND OF SEMLIKI FOREST VIRUS

Amino acid	Envelope protein[a]	Core protein[a]	SFV (calculated)[b]	SFV (determined)[c]
Lys	58.2	127.0	72.0	70.0
His	38.4	26.2	35.9	36.4
Arg	39.4	51.6	41.8	43.2
Asp	87.2	80.5	85.9	83.5
Thr	80.1	58.8	75.8	74.1
Ser	66.2	41.4	61.3	56.6
Glu	87.3	107.5	91.5	91.6
Pro	71.3	82.9	73.6	76.8
Gly	70.9	91.7	75.0	68.6
Ala	83.4	87.9	84.3	85.4
1/2-cys	30.8	16.4	27.9	30.7
Val	78.9	64.6	76.0	79.7
Met	18.7	26.4	20.3	21.7
Ile	42.7	39.5	42.1	42.8
Leu	57.8	38.4	53.9	53.7
Tyr	42.0	25.0	38.6	40.6
Phe	33.1	24.1	31.3	31.9
Trp	13.6	10.1	12.9	12.7

a Mean of 6 determinations from three different preparations.
b Calculated assuming 80% envelope protein and 20% core protein in the virion (4).
c Previously determined from phenol extracts of purified SFV (4).

gel filtration and 50,000 by electrophoresis. These methods depend upon comparison with standard proteins of known molecular weight. This kind of comparison may be inaccurate if the protein being studied binds more SDS than the standard proteins as might be expected for lipophilic proteins like the envelope protein.

To find out how much SDS the virus proteins bind we carried out equilibrium dialyses in 0.1 % SDS and 0.1 M sodium phosphate, pH 7.5. The core pro-

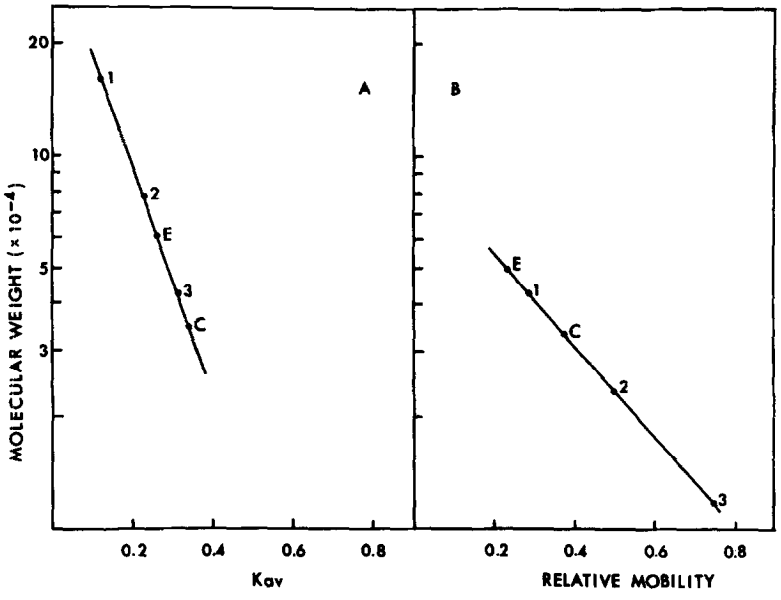

Figure 3. Apparent molecular size determination of the envelope (E) and core protein (C). A. SDS-gel filtration with the following marker proteins 1. IgG, 2. transferrin, and 3. ovalbumin. B. SDS-disc electrophoresis with the following marker proteins 1. ovalbumin, 2. trypsin, and 3. cytochrome C.

tein was found to bind 0.8 mg SDS per mg protein whereas the envelope protein bound as much as 2.2 mg of SDS per mg protein. Most proteins bind about 0.7-1.4 mg SDS per mg protein in similar conditions (19). Other methods are therefore needed to determine the exact molecular weight of the envelope protein of SFV.

This and previous investigations (5) show that the protein composition of the group A arbovirus envelopes is remarkably simple compared to the complex compositions of cell membranes (20).

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