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PREPARATION AND BIOCHEMICAL CHARACTERIZATION OF LASSA VIRUS SUBVIRION FRACTIONS

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Summary. — ^{14}C-labelled Lassa virus was purified by isopycnic centrifugation and split to subvirion fractions. The purified virus was treated with nonionic detergents Nonidet P-40 (NP-40) and with octylglycoside. After ultracentrifugation in urographin density gradient, two subvirion fractions with buoyant density of 1.24—1.26 and 1.08—1.10 g/cm^3 were obtained. The first fraction corresponded to the nucleocapsid of Lassa virus: it contained a protein with molecular mass of 60 kDa, the L and S segments of the genomic RNA. The second one contained a protein with molecular mass of 48 kDa and represented, apparently, envelope fraction of virus particles.

Key words: *Lassa virus*; *subvirion fractions*; *protein composition*

Introduction

Lassa virus is the aetiologic agent of human haemorrhagic fever bearing the same name. It is the most pathogenic member of family *Arenaviridae* (Johnson, 1985). Because of its highly contagious nature and its ability of transmission by contact and probably by aerosol from man to man, Lassa fever represents a potential danger not only for West African countries but also for non-endemic territories (Drozdov and Sergiev, 1984). In opinion of WHO experts, real need exists for preparation of an effective anti-Lassa virus vaccine.

In connection with this problem, of great interest is the study of antigenic, immunogenic and protective properties of subvirion components of Lassa virus. The first stage in solving this problem is the preparation of radioactively labelled subvirion fractions of Lassa virus and their biochemical analysis.

Materials and Methods

Lassa virus was kindly provided by Dr. C. Van der Groen from the Institute of Tropical Medicine, Antwerp, Belgium. Propagation, concentration and purification of the virus were done as described previously (Lukashevich *et al.*, 1985; Lukashevich and Lemeshko, 1985). Purified virus was resuspended in TNE buffer (0.01 mol/l Tris-HCl, pH 7.4, 0.1 mol/l NaCl, 0.001 mol/l EDTA) and treated with Nonidet P-40 (NP-40, BDH) or octyl-β-D-glucopyranoside (Serva) in the presence of 1 mol/l KCl. Final concentration of detergents was 3 % and 2 %, respectively. After addition of detergents and KCl, the virus suspension was incubated 20 min

at 22 °C, then it was loaded on a linear 20—50 % urographin gradient and centrifuged for 18 hr in Beckmann SW 27.1 rotor at 24 000 rev/min and 4 °C. The fractions of the gradient were collected using a peristaltic pump. From each fraction 5—10 μl aliquots were taken to determine activity of their acid-insoluble sediment trichloracetic acid-precipitable radioactivity. To determine the buoyant density values, the fractions of in parallel prepared control gradient loaded with TNE buffer were used.

Virus proteins present in the subvirion fractions were analysed by radioimmunoprecipitation. The samples were allowed to react for 18 hr with convalescent serum of a patient recovered from Lassa fever (No. 095312 CDC, Atlanta, U.S.A.). The immune precipitates were adsorbed to *Staphylococcus aureus* (strain Cowan) cells. Then the virus proteins were eluted into lysis buffer (60 mmol/l Tris- HCl,pH 6.8, 5 % SDS, 10 % mercaptoethanol, 10 % glycerine, 0.005 % bromphenol blue) and analysed by SDS-PAGE according to Laemmli, (1970). Vertical gel electrophoresis system 2001 (LKB) was used at 50 V, 22 °C for 20 hr in 50 mmol/l Tris-HCl buffer, pH 8.3 containing 0.83 mol/l glycine and 0.1 % SDS. The dried gels were exposed to ^{3}H-Ultrofilm (Amersham).

RNA for electron microscopic examinations was prepared according to method of Davis *et al.* (1971) under highly denaturing conditions using formamide and urea hyperphase in concentrations of 80 % and 4 mol/l, respectively. The RNA film was laid on a copper grid with Formvar support and coated with platinum-paladium at an angle of 8 °.

Lassa virus RNA molecules and eucaryotic RNA prepared in the same manner were examined in electron microscope JEM-100B. The length of molecules was measured and counted with the help of Hewlett Packard system composed of calculator 982 5A, computer 9864A and a graphical registration device 9862A.

Results

Characterization of purified virus

^{14}C-labelled Lassa virus, purified in discontinuous urographin gradient and isopycnic concentration sucrose gradient was located at the density of 1.17—

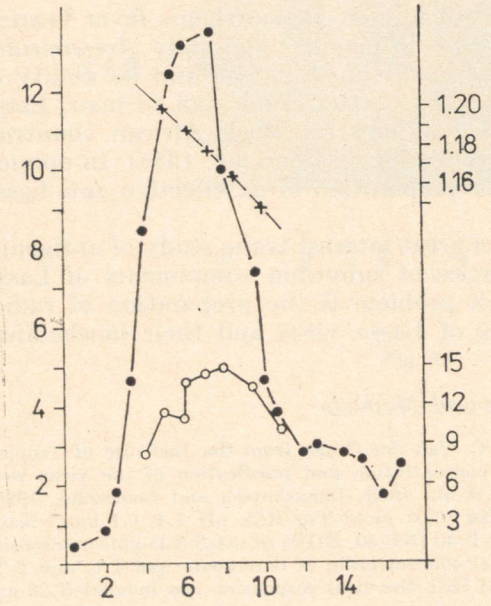

Fig. 1.
Purification of Lassa virus in 20—50 % sucrose density gradient (SW 27.1, 24 000 rev/min, 18 hr, 4 °C)
o radioactivity
● infectivity
× buoyant density of fractions
Fractions 8 and 9 were used as preparations of purified virus.
Abscissa: No of fractions; ordinates: in the left — radioactivity (cpm/min 10^{-2}), in the right — buoyant density (g/cm^{3}) upper part — infectious radioactivity (%).

1.18 g/cm³ (Fig. 1). Maximum infectious activity by plaque titration was detected in fractions showing the highest radioactivity. These were collected, the virus was centrifuged (rotor SW 60, 35 000 rev/min, for 1 hr, at 4 °C), resuspended in TNE buffer and used for further investigations. The buoyant density of the virus purified in this way were 1.15 g/cm³ in urographin and 1.19 g/cm³ in caesium chloride, respectively.

Separation of Lassa virus subvirion components in urographin gradient

To obtain the subvirion components, Lassa virus was treated with the nonionic detergent NP-40 and/or octylglycoside in the presence of KCl. Disintegrated virus particles were centrifuged in urographin concentration gradient. Results are shown in Fig. 2-I. The viral material was divided into two components. The "heavy" subvirion component (first fraction) was situated near the bottom of the gradient and had a buoyant density of 1.24—1.26 g/cm³. It represented 10 % of the whole radioactive material. The "lightest" material (second fraction) was situated in the upper zone of

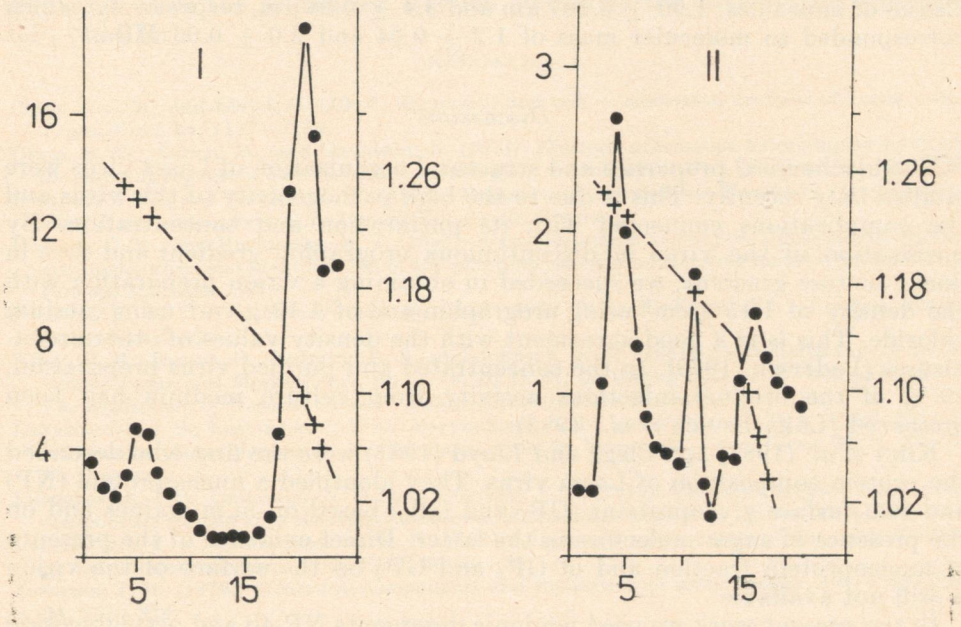

Fig. 2.

Isopycnic analysis of Lassa virus subvirion fractions. Ultracentrifugation in urographin linear density gradient (20-50 %), (SW 27.1, 24 000 rev/min, 18 hr, 4 °C) and fractionation

I — ^{3}H-glucosamine labelled and NP-40 treated virus

II — ^{14}C-hydrolysate labelled and octylglycoside treated virus

Abscissa: numbers of fractions; ordinate: left — radioactivity (cpm/min 10^{-2}), right — buoyant density (g/cm³).

the gradient. Its buoyant density was 1.08—1.10 g/cm³ and represented 43 % of the whole gradient radioactivity.

When the β-octylglycoside treated labelled virus was subjected to ultracentrifugation in urographin concentration gradient, again two main fractions were obtained with the same values of buoyant density (Fig. 2-II). The first fraction contained 33 % and the second one 18 % of the whole gradient radioactivity. The rest of the material (8 %) had a buoyant density corresponding to that of non-disrupted virus.

Biochemical characterization of Lassa virus subvirion components.

The protein composition of the both fractions was determined by immune precipitation and analysed by PAGE. The results are shown in Fig. 3. Purified Lassa virus contained 3 proteins with the electrophoretic mobility corresponding to molecular mass 60, 48 and 34 kDa. Convalescent serum of the patient with Lassa fever precipitated from the first fraction a protein of m. m. of 60 kDa and from the second one a protein with m.m. of 48 kDa.

From the first fraction RNA was isolated by phenol extraction and examined by electron microscopy (Fig. 4). Its length measurements revealed two classes of molecules: 1.90 ± 0.007 μm and 3.4 ± 0.08 μm, respectively, which corresponded to molecular mass of 1.2 ± 0.04 and 2.0 ± 0.05 MDa.

Discussion

The biochemical properties and structural organization of Lassa virus were studied only recently. This is due to the high pathogenicity of this virus and the complications connected with its purification and concentration. By purification of the virus in discontinuous urographin gradient and then in linear sucrose gradient, we succeeded in obtaining a virion preparation with the density of 1.15 g/cm³ using urographin and of 1.19 g/cm³ using caesium chloride. This is in a good agreement with the density values of other arenaviruses (Pedersen, 1979). In the concentrated and purified virus preparation, 10 % of the original infectious activity from culture medium had been preserved (Lukashevich *et al.*, 1985).

Kiley *et al.* (1981) and Clegg and Lloyd (1983) were the first who described the protein composition of Lassa virus. They identified a nucleoprotein (NP) and two surface glycoproteins (GP_1 and GP_2) based on m.m. values and on the presence of sugar molecules in the latter. Direct evidence of the presence of nucleoprotein fraction and of GP_1 and GP_2 on the surface of the virion is still not available.

In the present work we used nonionic detergents NP-40 and octylglycoside with subsequent ultracentrifugation in urographin density gradient. We succeeded to separate the nucleocapsid fraction of Lassa virus with the density of 1.24—1.26 g/cm³. Analogous values were obtained for nucleocapsids of lymphocytic choriomeningitis virus and Machupo virus (Howard and Buchmeier, 1983; Lukashevich and Lemeshko, 1985). In the same fraction we found the L and S segments of the Lassa virus RNA. It is worth to note,

to our knowledge this is the first electron-microscopic examination of Lassa virus RNA. From the nucleocapsid fraction we precipitated a protein with m.m. of 60 kDa (Fig. 3, lane 2) which seems to be the main protein component in this fraction.

From the solubilized fraction of Lassa virus a convalescent human serum precipitated a protein with m.m. of 48 kDa (Fig. 3, lane 3). The second glycosylated protein with m.m. of 34 kDa (GP_2) was not detected in immune precipitates, although the convalescent serum precipitated this protein from the lysate of structural proteins (Fig. 3, lane 1). Very likely, the conditions of virus fractionation led to its partial degradation. It is also possible that in our preparations the GP_2 presents only a small proportion difficult to detect by the methods used.

Summing up, we describe the data on Lassa virus fractionation with nonionic detergents NP-40 and octylglycoside in the presence of 1 mol/l KCl. With the subsequent ultracentrifugation in urographin density gradient we differentiated two subvirion fractions. The "heavier" fraction with the density of 1.24—1.26 g/cm³ contained the virion RNAs and the NP protein (60 kDa) while the solubilized fraction contained the envelope glycoprotein GP_1 (48 kDa).

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Explanation to Figures (Plates XIII—XIV):

Fig. 3. Electrophoretic analysis of protein immunoprecipitated from subvirion of Lassa virus.
1 — structural proteins of Lassa virus
2 — proteins immunoprecipitated from the first fraction
3 — proteins immunoprecipitated from the second fraction

Fig. 4. Electron microphotographs of RNA isolated from the first subvirion fraction of Lassa virus.
I — L segment, magn. × 49 100
II — S segment, magn. × 47 750