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Characterization of Ribonucleoproteins and Ribosomes Isolated from Lymphocytic Choriomeningitis Virus

IB R. PEDERSEN* AND ELAINE PRIZANT KØNIGSHØFER

Institute of Medical Microbiology, University of Copenhagen, DK-2100 Copenhagen, Denmark

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Disruption of purified lymphocytic choriomeningitis (LCM) virus with Nonidet P-40 in 0.5 M KCl followed by sucrose gradient centrifugation in 0.3 M KCl led to the isolation of two viral nucleoproteins (RNPs) as well as 40S and 60S ribosomal subunits. The largest viral RNP sedimented heterogenously at 123S to 148S and was associated with 23S and 31S viral RNA. The other viral RNP sedimented at 83S and was associated with 23S viral RNA. The buoyant density in CsCl was determined to be 1.32 g/cm^3 for the viral RNPs. Densities of 1.52 and 1.60 g/cm^3 were determined for the 40S and 60S subunits, similar to those of the BHK-21 cell subunits dissociated by 0.5 M KCl. The viral RNPs were partly sensitive to RNase.

Structural analyses of viruses belonging to the arenavirus group have demonstrated that these viruses are enveloped, with closely spaced projections and unstructured interiors containing a variable number of granules. The granules are unique for the arenaviruses and closely resemble cellular ribosomes (25, 26). Nucleic acid analysis of lymphocytic choriomeningitis (LCM) virus, which is the prototype virus of the arenavirus group, and of Pichinde virus, which is another arenavirus, demonstrated that these viruses (26) contained single-stranded RNA that could be separated into three classes of RNA having sedimentation values of 23S and 31S, 18S and 28S, and 4S to 6S (6, 22), respectively. The small RNAs isolated from LCM virus have been resolved further into 4S, 5S, and 5.5S components (22). The 23S RNA and the 31S RNA are viral specific, amounting to about 50% of the total labeled RNA of the virion, whereas the remaining RNAs are similar to ribosome and ribosome-associated host RNAs and are therefore presumably of host origin (6, 22).

To correlate these viral RNAs with substructures of the arenaviruses, experiments were performed concerning the isolation and characterization of ribosomes and ribonucleoproteins (RNPs) from LCM virus.

MATERIALS AND METHODS

Cultivation and infection of cells. The details for the cultivation of BHK-21 cells in suspension cultures were described previously (20, 22). The suspension cultures were infected with an amount of diluted LCM virus that resulted in 100% infection 18 to 42 h postinfection, as judged by the immunofluorescent assay described below. Viral RNA was labeled for 20 h with 250 μCi of [^{3}H]uridine per 100 ml of culture. In double-labeling experiments, the virus was labeled for 20 h with 25 μCi of [^{14}C]uridine and 500 μCi of ^{3}H-amino acid mixture per 100 ml of culture in medium containing 20% of the normal amount of amino acids. The cells were mycoplasma free when tested for the absence of [^{3}H]uridine-labeled mycoplasma RNA by acrylamide gel electrophoresis (27).

Virus. LCM virus Traub strain as used previously (20, 22) was injected intraperitoneally into 10 special pathogen-free 18- to 20-g C_3H mice. One week after the injection, the virus was harvested from the spleens. It was then sterile filtered and passed in a 10^{-2} to 10^{-3} dilution four times in BHK-21 cell suspension cultures. The virus was harvested from the medium 24 to 48 h postinfection at each passage; at this time, all cells were infected, except for the first passage as judged by the immunofluorescent antibody assay.

Immunofluorescent assay. In the immunofluorescent assay, a pool of hyperimmune mouse antisera having a complement fixation titer of 1:8,000 was used. The ammonium sulfate-precipitated globulins were dialyzed and conjugated with 1 mg of fluorescein isothiocyanate (Baltimore Biological Laboratories, Cockeysville, Md.) per 100 mg of protein in a pH 9.5 phosphate buffer for 1 h, after which the pH was adjusted to 7.2 with a pH 5.3 phosphate buffer. The conjugate was then chromatographed in a Sephadex G-25 (fine) column and in a DEAE-Sephadex A-50 column, which was eluted with 0.3 M NaCl in a pH 7.2 phosphate buffer (28, 29). Fractions having ratios of optical density at 495 nm/280 nm between 0.46 and 0.78 were pooled and stored at −20°C after the addition of 0.1% (wt/vol) bovine serum albumin (BSA). In the immunofluorescent assay of LCM-infected BHK-21 cells, these were fixed with −20°C acetone and stained for 30 min at 37°C with the conjugate diluted 1:10 in Tris buffer (pH 8.4).

Purification of virus. The cells from BHK-21 sus-

pension cultures were spun down by centrifugation (2,500 × g) for 15 min at 4°C. The medium was then cooled in an ice-ethanol mixture, and the following operations were done at 0 to 4°C. The LCM virus was precipitated by the addition of 0.4 M KCl, 0.01 M magnesium acetate, and 10% (wt/vol) polyethylene glycol 6000. The solution was then stirred for 2 h and processed or kept in the cold until the next day. The precipitate was isolated by centrifugation (7,000 × g) for 30 min. It was dissolved in 10 ml of $TK_{100}MD$ buffer (pH 7.4) (see below) and homogenized by 10 strokes in a Dounce glass homogenizer. BSA was added to a final concentration of 0.1% (wt/vol), and the solution was centrifuged (16,300 × g) for 10 min. The supernatant was then diluted to a final volume of 26 ml in $TK_{100}MD$ buffer (pH 7.4) containing 0.1% BSA (wt/vol). The concentrated virus solution was purified by centrifugation on a double cushion consisting of 5 ml of 65% (wt/vol) sucrose and 5 ml of 20% (wt/vol) sucrose in $TK_{100}MD$ buffer. Centrifugation was done in a Spinco SW27 rotor at 26,000 rpm for 90 min. The virus band between the two sucrose layers was collected, diluted to 10 ml with sucrose buffer containing 0.1% (wt/vol) BSA, and layered onto a preformed continuous sucrose gradient made from 12 ml of 65% (wt/vol) sucrose and 13 ml of 20% (wt/vol) sucrose in $TK_{100}MD$ buffer (pH 7.4). Equilibrium centrifugation was performed in an SW27 rotor at 26,000 rpm for 18 h at 4°C. The peak of virus was localized by taking 25 to 50 μl from each fraction and counting the samples. Four to five peak fractions were pooled, and BSA was added to a final concentration of 0.1% (wt/vol). The purified virus pool usually contained between 0.4×10^6 and 1×10^6 cpm from an 800-ml virus culture. The virus from the pools was concentrated in some of the experiments by centrifugation in a Spinco 65 rotor at 55,000 rpm for 60 min. The method used is a modification of that used previously for the purification of LCM virus (20), which involved the polyethylene glycol precipitation procedure as described for the purification of vesicular stomatitis virus (17) and Pichinde virus (6).

Isolation of RNPs from LCM virus. Purified LCM virus in 0.5 to 1.0 ml of cold $TK_{100}MD$ buffer (pH 7.4) was used. The Mg concentration was raised to 5 mM, and the virus was lysed by treatment with the detergent Nonidet P-40 (1%, vol/vol) for 5 min in ice. To split the viral ribosomes into subunits and to release the viral RNPs from other viral material, the KCl concentration was raised to 0.5 M KCl by the addition of 4 M KCl. The solution was then incubated for 5 min at 0°C and for 3 min at 37°C and cooled again in ice. The KCl concentration was lowered to 0.3 M KCl by the addition of $TK_{100}MD$ buffer (pH 7.4). Thereafter, the viral RNPs were separated by centrifugation in a 15 to 30% (wt/vol) continuous sucrose gradient in $TK_{300}MD$ buffer (pH 7.4) containing 0.5% (vol/vol) Nonidet P-40. The centrifugation was performed in an SW27 rotor at 25,000 rpm for 4.5 h at 4°C. The procedure used is a combination of methods described for the isolation of ribosomal subunits from eukaryotic cells (7, 19).

Isolation of ribosomes from cells. The BHK-21 cells were labeled with [^{14}C]uridine (3.2 μCi per 200 ml of culture) for 18 h. The cells were collected by centrifugation (630 × g) at 4°C, suspended in $TK_{100}MD$ buffer (pH 8.4) (23) containing 0.2 M sucrose (7), centrifuged as described above, resuspended in 3 ml of the same buffer, and lysed for 5 min with 0.5% (vol/vol) Nonidet P-40 (4). The nuclei were pelleted by centrifugation (27,000 × g) for 10 min at 4°C. The supernatant containing the ribosomes was diluted to 5 ml in the above-mentioned buffer, layered onto a cushion of 5 ml of 60% (wt/vol) sucrose in $TK_{100}MD$ buffer (pH 8.4), and centrifuged in a Spinco 65 rotor at 55,000 rpm for 1 h at 4°C. The pelleted ribosomes were resuspended in 0.5 ml of $TK_{100}MD$ buffer (pH 7.4) and stored at −70°C. The ribosomes prepared in this way were added to the viral RNPs as centrifugation markers and split into subunits, as described above. The ribosomes were also used as RNA markers during acrylamide gel electrophoresis of viral RNA after the ribosomes had been deproteinized with 1% (wt/vol) sodium dodecyl sulfate. Due to the addition of sodium dodecyl sulfate, these ribosomes had been prepared in buffers containing NH_4Cl instead of KCl.

CsCl gradient centrifugation. The viral RNPs and ribosomal subunits from the sucrose gradients were adjusted to 1,200 μl with $TK_{100}M$ buffer (pH 7.4) and fixed at 0 to 4°C by the addition of 300 μl of 25% (vol/vol) glutaraldehyde that had been neutralized to pH 7 with 1.91 M $NaHCO_3$ just prior to use. The fixed RNPs were centrifuged in discontinuous gradients consisting of 4.5 ml of 25% (wt/wt) CsCl and 4.5 ml of 45% (wt/wt) CsCl, whereas the ribosomal subunits were centrifuged in similar 40% to 55% (wt/wt) gradients. The CsCl was made up in $TK_{10}M$ buffer (pH 7.4) containing 0.1% (wt/vol) Brij-35 and 0.25% (vol/vol) glutaraldehyde. The gradients were centrifuged in a fixed-angled Spinco 65 rotor (9) at 55,000 rpm at 4°C for 18 h. The density was measured by weighing in a 100-μl pipette at room temperature. The corresponding protein content was calculated by the method of Perry and Kelley (24), using an RNA density of 1.9 g/cm^3 and a protein density of 1.3 g/cm^3 (12). Similar methods have been used for isopycnic centrifugation of other virus RNPs and ribosomal subunits (1, 16).

Radioactivity determinations. Acid-precipitable radioactivity was collected on Sartorius nitrocellulose membrane filters, as described previously (20, 22), or on Whatman glass-fiber GF/F filters (2.5 cm; pore size, 0.7 μm). After being washed with 5 ml of tricholoroacetic acid, the glass filters were dried at 60°C for 60 min, placed in plastic scintillation vials, and treated with 0.5 ml of NCS solubilizer (Nuclear-Chicago Corp., Des Plaines, Ill.) diluted 1:4 with toluene (2).

Buffers. $TK_{100}MD$ buffer contains: 0.01 M Tris, 0.1 M KCl, 0.003 M magnesium acetate, and 0.001 M dithiothreitol (at pH 7.4 and 8.4). $TK_{300}MD$ buffer contains: 0.01 M Tris, 0.3 M KCl, 0.003 M magnesium acetate, and 0.001 M dithiothreitol (pH 7.4). $TK_{10}M$ buffer contains: 0.01 M Tris, 0.01 M KCl, and 0.003 M magnesium acetate (pH 7.4).

Chemicals. [5-^{3}H]uridine (25,000 to 30,000 mCi/mmol), [2-^{14}C]uridine (>50 mCi/mmol), and tritiated amino acid mixture (1 mCi/ml) were from the Radiochemical Centre, Amersham, England. Polyethylene glycol (Carbowax 6000) was from JJ's Ltd.,

King's Lynn, Norfolk, England. RNase A (bovine pancreas) was from Boehringer, Mannheim, West Germany.

RESULTS

Sedimentation analysis of the viral nucleoproteins. The viral RNPs and ribosomes were labeled with [^{3}H]uridine and analyzed by centrifugation in sucrose gradients. Before centrifugation, the RNPs and ribosomes were released from the purified LCM virus by treatment with the nonionic detergent Nonidet P-40, followed by dissociation of the ribosomes into subunits and release of the viral RNPs from the viral material by treatment with 0.5 M KCl. The centrifugation (Fig. 1) separated the disrupted virus into four components. Two of these components sedimented together with the 60S and 40S BHK-21 ribosomal subunits used as centrifugation markers. The fastest-sedimenting components sedimented rather heterogenously as a broad peak. Repeated sedimentation analysis indicated that two RNPs sedimented in this region; therefore, two areas, named V1 and V2, were analyzed to represent the broad peak. Another viral RNP sedimenting in front of the 60S subunit was named V-3. Using the subunits as sedimentation markers, the V-1, V-2, and V-3 RNPs sedimented at 148S, 123S, and 83S, respectively. These values should be compared with nondisrupted LCM virus, which sediments at 470S (20). Repeated sedimentation analysis demonstrated some variation in the amounts of the different components, although the ratio between the 60S and 40S subunits was found to be close to the expected value of 2.5 (19).

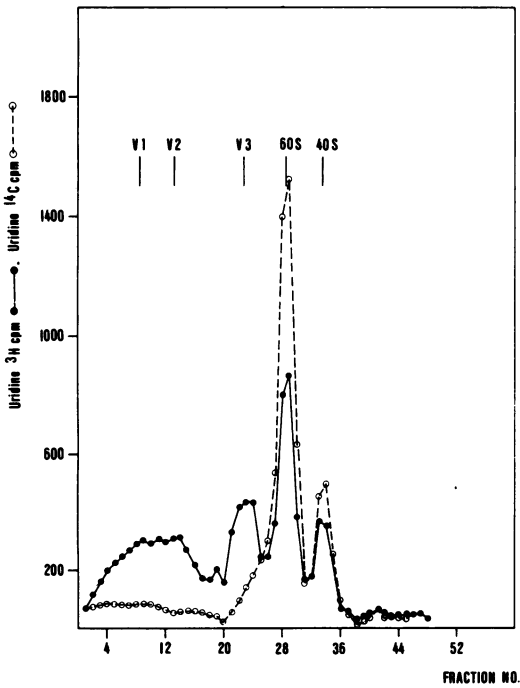

FIG. 1. *Rate-zonal centrifugation of LCM virus RNPs and BHK-21 cell ribosomal subunits used as markers. Centrifugation was performed in a 15 to 30% continuous sucrose gradient containing 0.3 M KCl, in an SW27 rotor for 18 h. Symbols: ●, [^{3}H]uridine-labeled LCM virus RNPs (counts per minute); ○ [^{14}C]uridine-labeled BHK-21 cell 60S and 40S subunits (counts per minute).*

Different methods were used to establish whether the sedimentation profile could be changed. Thus, the sedimentation of V-3 was examined after the dissociation of the virus was performed in the presence of 0.01 M puromycin, since V-3 could be due to or associated with monosomes, which are known to sediment in this region. Although puromycin in the presence of KCl at 37°C causes complete conversion of 80S monosomes into subunits (3), no change in region of V-3 was observed after this treatment. Furthermore, the resolution of the different components was not improved if EDTA was added to the gradient at a concentration of 1 mM. Finally, if detergent was omitted from the gradient, the V-3 RNP sometimes sedimented at 113S and the V-1–V-2 RNPs sedimented at 170S.

Further characterization of the nucleoproteins. In the following experiments, analyses of the protein and RNA of the RNPs were performed to determine whether the viral RNPs and ribosomal subunits could be considered as distinct molecules, or whether one of the RNPs was derived from one of the others. In the first of these experiments, both the RNA and the proteins of the LCM virus were labeled. The RNA was labeled with [^{14}C]uridine and the proteins were labeled with a ^{3}H-amino acid mixture. Purified virus was then analyzed on a sucrose gradient (Fig. 2). The labeling ratio between protein and RNA is similar in the V-1–V-2 and V-3 nucleoproteins and is about six times higher than that in the viral subunits. About 65% of the protein label stays on top of the gradient in the last five fractions, which probably represent the envelope proteins.

Buoyant density determination. In the next experiment, the buoyant density of the RNPs in CsCl gradients was determined, since this density reflects the RNA-to-protein ratio (24). The viral RNPs were separated on a sucrose gradient (Fig. 1). Three to four fractions of each peak were pooled and fixed with glutaraldehyde. The buoyant densities were then determined by CsCl gradient centrifugation (Fig. 3). It should be noted that reported density values

vary slightly due to the different method of preparation, since subunits prepared in the presence of a high concentration of salts have a higher buoyant density (12, 16).

Our results demonstrate that the V-1, V-2, and V-3 RNPs have similar buoyant densities of 1.32 g/cm^3, corresponding to a protein content of about 95%. Thus RNP V-3, which sediments at 83S, has a considerably higher protein content than that expected for monosomes. The density of 1.32 g/cm^3 of the viral RNPs should also be compared with that of the LCM virion, which was determined to be 1.22 g/cm^3 in CsCl (10). The density of the 40S viral subunit was determined to be 1.52 g/cm^3, corresponding to a protein content of 54%, and the density of the 60S subunit was 1.60 g/cm^3, corresponding to a protein content of 41%. Similar densities were obtained for the 60S and 40S subunits prepared from uninfected BHK-21 cells.

Analysis of RNA associated with the viral components. The RNA content of the [^{3}H]uridine-labeled LCM RNPs and ribosomal subunits was examined by acrylamide gel electrophoresis, after these had been separated on a sucrose gradient. The electrophoresis (Fig. 4) demonstrated that the V-1 and V-2 viral RNPs contained similar amounts of both 31S and 23S viral RNAs, as had been demonstrated previously in RNA isolated directly from LCM virus (21, 22; R. M. Welsh, P. W. Lampert, P. A. Burner, and M. B. A. Oldstone, Virology, in press). The V-3 RNP contained th 23S viral RNA only and no RNA. The 60S viral RNP contained 28S RNA, and the 40S RNP contained the 18S RNA. There was very little cross-contamination of the RNA from the different RNPs and ribosomal subunits.

Sensitivity of LCM RNPs to RNase. To compare and further characterize the ribosomes and RNPs isolated from LCM virus, their sensitivity to treatment with RNase was tested. The [^{3}H]uridine-labeled LCM RNPs were separated on a sucrose gradient (Fig. 1) and used in the RNase sensitivity experiment. This was performed by incubation with pancreatic RNase (1 μg/ml at 25°C) for different length of time (Fig. 5). The incubation was done both with and without the addition of EDTA (10 mM final concentration). In the absence of EDTA, the V-1, V-2, and V-3 RNPs, as well as the 60S ribosomal subunits, were degraded by about 40% within 30 min of incubation. The 40S subunit was degraded by about 50%. In the presence of EDTA, the rate of degradation of the V-1, V-2, and V-3 RNPs was not changed significantly as compared with the 40S and 60S ribosomal subunits, which were degraded rapidly. Similar experiments were performed without EDTA,

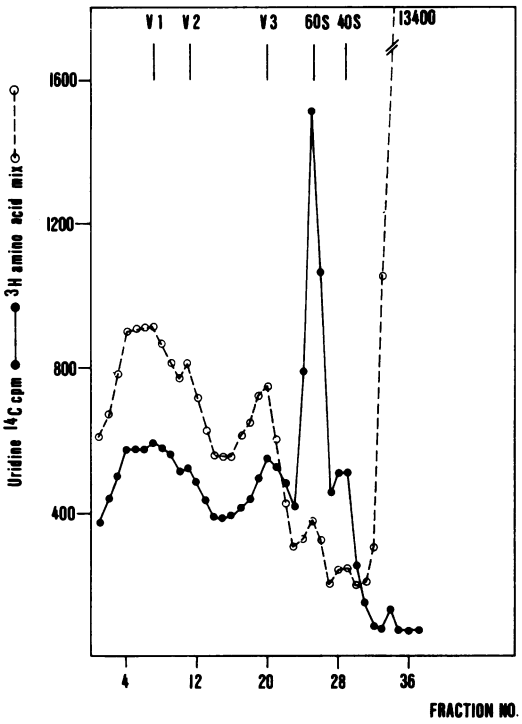

FIG. 2. *Rate-zonal centrifugation of RNA- and protein-labeled LCM virus RNPs similar to the conditions given in the legend of Fig. 1. Symbols: ○, ^{3}H-amino acid mixture-labeled viral proteins (counts per minute); ●, [^{14}C]uridine-labeled viral RNA (counts per minute).*

using a 10-fold higher concentration of RNase at 25 and 37°C (curves not shown). At 25°C with 10 μg of RNase per ml, the degradation of the five RNPs was increased about 10% after 30 min of incubation, but even after 90 min of incubation the degradation was not complete, as there was still 20 to 25% which were resistant. At 37°C, the V-1, V-2, and V-3 RNPs were degraded about 85% after 10 min of incubation and 93% after 30 min, whereas the ribosomal subunits were degraded about 97% in 30 min. In conclusion, these RNase sensitivity experiments showed no significant differences among the V-1, V-2, and V-3 LCM RNPs. In the presence of EDTA, however, a clear difference was seen between the V-1, V-2 and V-3 RNPs as a group and the viral ribosomal subunits.

DISCUSSION

Dissociation of purified LCM virus by treatment with Nonidet P-40 in 0.5 M KCl and sedimentation analysis in sucrose gradient containing 0.3 M KCl led to the isolation of ribosomal subunits from the virus, which were indistinguishable from that of the BHK-21 host cells

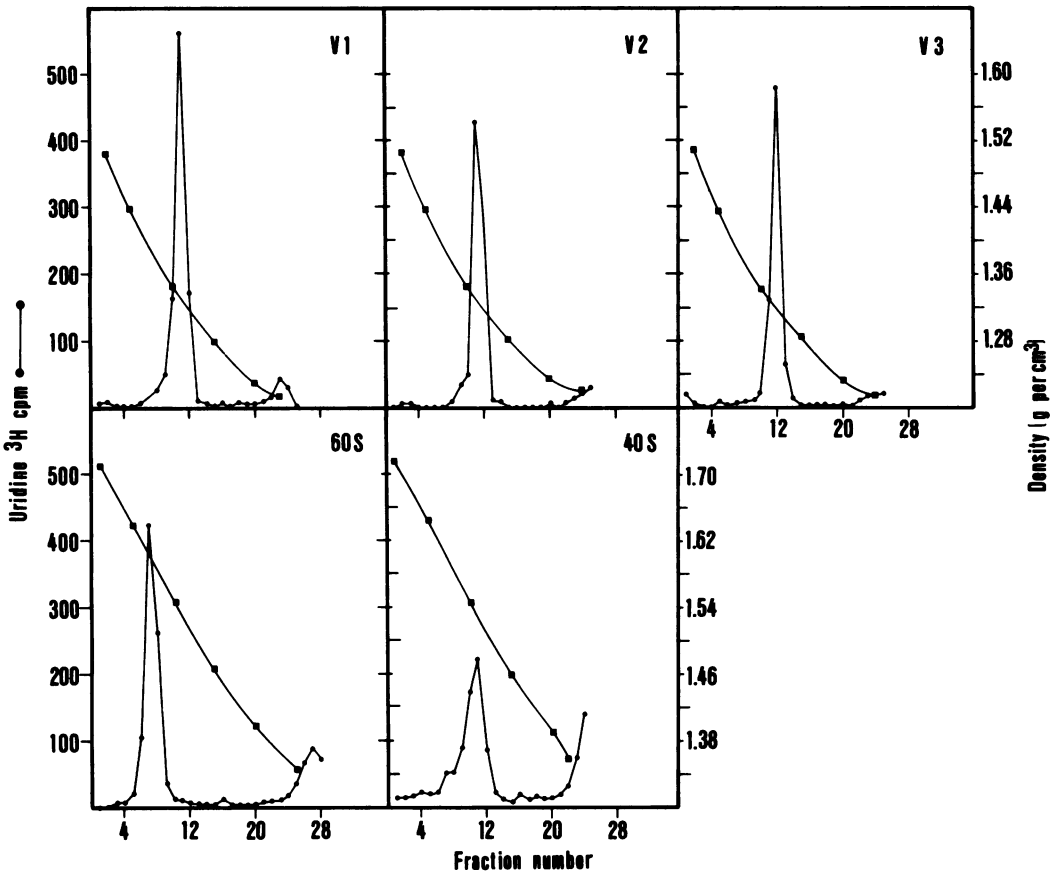

FIG. 3. *Equilibrium centrifugation in CsCl gradients of the V-1, V-2, V-3, 60S, and 40S LCM virus RNPs pooled from the rate-zonal sucrose gradient, as shown in Fig. 1. The CsCl centrifugation was performed in a no. 65 rotor at 55,000 rpm for 18 h. Symbols: ●, [^{3}H]uridine-labeled RNPs (counts per minute); ■, solution density (grams per cubic centimeter). Note the difference in the density scale for the viral RNPs and the viral subunits.*

with respect to sedimentation in sucrose gradients, RNA and protein content, and sensitivity to RNase. The determined densities of 1.60 and 1.52 g/cm^3 in CsCl for the 60*S* and 40*S* subunits, respectively, correspond to reported values for eukaryotic ribosomes dissociated in 0.5 M salt (12). This demonstrates that the ribosomes must exist in a rather undegraded form in the LCM virion, but it does not exclude the existence of minor differences between ribosomes isolated from the virion or the host cells, since a eukaryotic ribosome consists of 70 different proteins, some of which could easily be released during the preparation (30).

Sedimentation analysis of RNPs isolated from LCM virus revealed one distinct RNP (V-3) sedimenting at 83*S*, which contained the 23*S* viral-specific RNA only. Another RNP containing the 31*S* RNA was expected which sediments faster than V-3 at about 112*S*. However, a broad-sedimenting RNP containing both 31*S* and 23*S* RNA was found. Two regions from this band, named V-1 and V-2, were analyzed. Both contained the same amount of protein, and they contained the 31*S* RNA and the 23*S* RNA with the same amount of uridine labeling. Both showed similar sensitivity to RNase. Assuming an equimolar labeling of the 23*S* and 31*S* RNA, which have a molecular weight of 1.1×10^6 and 2.1×10^6, respectively (21), the fast-sedimenting RNP contained two molecules of 23*S* RNA for each 31*S* RNA. This indicates that the fast-sedimenting RNP represents one RNP containing the 31*S* RNA that is aggregated to V-3, resulting in a broad sedimentation profile.

The LCM RNPs had densities of 1.32 g/cm^3 in CsCl, corresponding to 95% protein. This is similar to the RNP composition of other large enveloped RNA viruses represented by the paramyxoviruses and rhabdoviruses (5), but it is

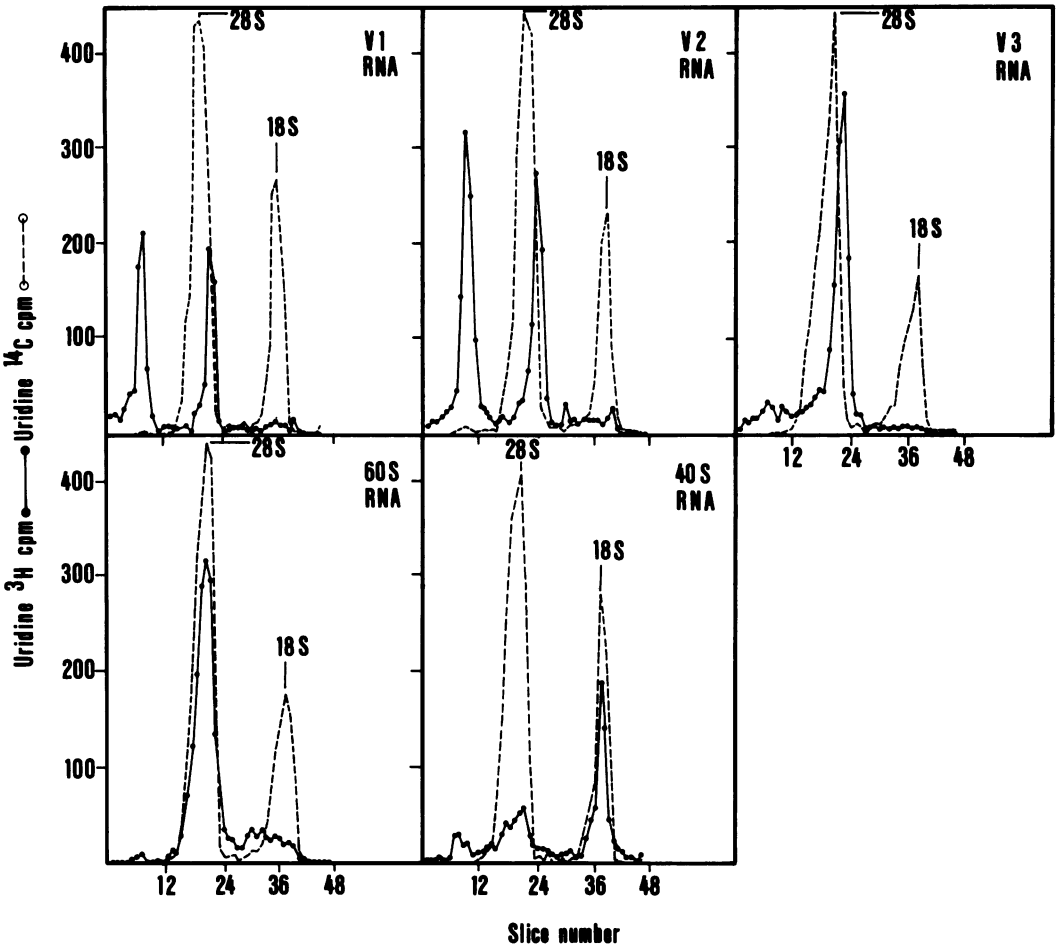

FIG. 4. *Acrylamide gel electrophoresis of LCM virus RNAs from the V-1, V-2, V-3, 60S, and 40S RNPs that were deproteinized with sodium dodecyl sulfate. The RNPs were separated initially in a sucrose gradient (Fig. 1), and the peak fractions were pooled. To the pools was added unlabeled carried RNA that had been extracted with phenol from BHK-21 cells (20, 21), and the RNPs were then precipitated with 2 volumes of 96% ethanol, collected by centrifugation, and dissolved in the electrophoresis buffer containing 1% (wt/vol) sodium dodecyl sulfate. The electrophoresis was performed together with a BHK-21 cell rRNA marker in tubes (0.6 by 6 cm) containing 2.7% acrylamide for 4 h at a current of 6 mA per gel, as described previously (21, 22). Symbols: ●, [^{3}H]uridine-labeled viral RNA (counts per minute); – –, [^{14}C]uridine-labeled BHK-21 cell rRNA (counts per minute).*

somewhat different from the RNPs of influenza viruses, which have densities of 1.34 g/cm^3, corresponding to 88% protein (15). When Pichinde virus, another arenavirus, was examined by rate-zonal centrifugation in low salt, i.e., 0.01 M NaCl, 40S, 60S, and 80S ribosome structures were detected together with fast-sedimenting polysome-like structures (8). Viral 31S and 22S RNA was detected only in the fast-sedimenting structure and in combination with rRNA (8). The ribosomes from unfractionated virus had a density of 1.61 g/cm^3, and the RNP material had a density of 1.37 g/cm^3 in CsCl. Since the Pichinde virus was purified in EDTA-containing buffers and the virus was not dissociated to the same extent as that reported for LCM virus, the results are not fully comparable.

The sensitivity of the LCM RNPs to the action of pancreatic RNase showed that the treatment with 1 or 10 μg of RNase per ml for 30 min at 25°C degraded the viral RNPs similarly (to about 40 to 60% only). Similar treatment of Newcastle disease virus and influenza virus showed that the RNP of the former was resistant and influenza RNP was sensitive to RNase

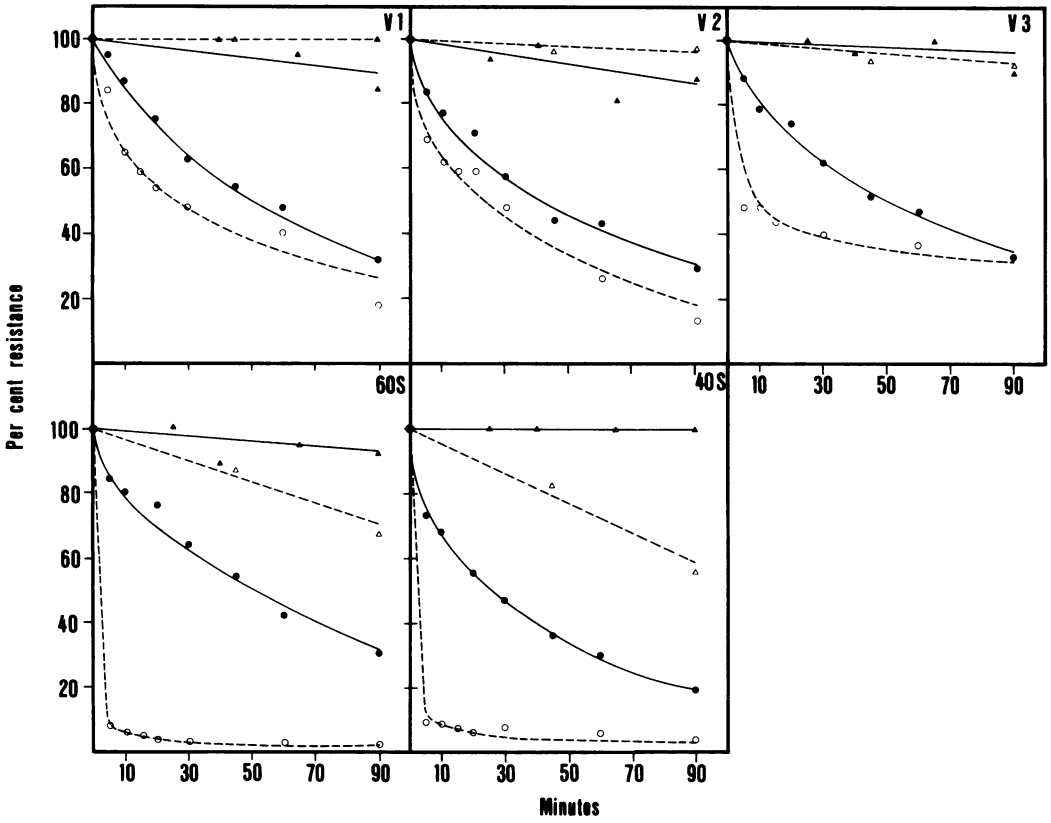

FIG. 5. *Sensitivity of the V-1, V-2, V-3, 60S, and 40S LCM virus RNPs to 1 μg of RNase per ml at 25°C. The RNPs were separated in a sucrose gradient (Fig. 1), and the peak fractions were pooled. The RNPs were diluted in 0.01 M Tris buffer (pH 7.4) to a final concentration of 0.1 M KCl and preincubated for 5 min, and 0.4-ml portions of the RNase-treated and 0.2 ml of the nontreated controls were removed. Thereafter, the trichloroacetic acid-precipitable counts were determined. The experiment was done with and without, respectively, 10 mM EDTA. The RNP pools contained initially between 4,000 and 12,5000 cpm. Symbols: ●, [³H]uridine-labeled LCM RNP which was RNase treated without EDTA; ○, [³H]uridine-labeled LCM RNP which was RNase treated with EDTA; ▲, [³H]uridine-labeled LCM RNP control without RNase and EDTA; △, [³H]uridine-labeled LCM RNP control without RNase and with EDTA.*

(13, 14). At 37°C, LCM virus RNPs were about 93% degraded after 30 min, but measles virus RNP was still resistant at this temperature (11). These observations suggest that the structure of the LCM RNPs is different from that of the myxo- and paramyxoviruses, although they have a protein content similar to that of the paramyxoviruses.

In conclusion, the LCM virus contained two RNPs, one of which was clearly separated from the other viral components. The RNPs have great similarities to RNPs from other large enveloped RNA viruses. Furthermore, it is now well documented that the granular structures which characterize the arenaviruses indeed are ribosomes and not simple contaminants attached to the virus surface (8, 22, 25). The ribosomes incorporated in the virus can be synthesized in the cell both before and after the virus infection (6, 22). It is still uncertain whether the ribosomes have any function in the arenavirus multiplication, as they could very well be incorporated in the virus accidentally during the budding process. It is possible that they exist only on the large granular type of LCM virus and not in the smaller nongranular type observed by Mannweiler and Lehmann-Grube (18). The large ribosome-containing particles may therefore be defective, and the ribosomes incorporated in the arenaviruses may have no function at all in the virus multiplication.

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