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RNA Subunit Structure of Mason-Pfizer Monkey Virus

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Mason-Pfizer monkey virus 60–70*S* RNA has a molecular weight of 8×10^6 when analyzed on polyacrylamide gels. Dissociation of 60–70*S* RNA of Mason-Pfizer monkey virus and murine leukemia virus by heat or formamide (40%) resulted in conversion to identical subunit structures of 2.8×10^6 daltons; treatment with lower amounts of formamide revealed a partial dissociation to intermediate RNA structures. Complete heat dissociation of Mason-Pfizer monkey virus 60–70*S* RNA released three low-molecular-weight RNA species of 10^5, 3.5×10^4, and 2.5×10^4.

The RNA of oncornaviruses is a highly structured 60–70*S* molecule (6, 7, 9) which can be dissociated to 30 to 40*S* subunits and smaller RNAs utilizing various denaturing conditions (3, 6–9, 13, 21). No subunit structure has thus far been reported for the 60–70*S* RNA of Mason-Pfizer monkey virus (MPMV). This virus, which is neither type B nor type C in morphology (5), was isolated from a mammary carcinoma of a rhesus monkey (5, 15). It represents the first RNA virus associated with a mammary tumor in a non-human primate. MPMV has biochemical and biophysical properties similar to those of the known RNA tumor viruses. These include: (i) a buoyant density of 1.16 g/ml in sucrose (16, 19); (ii) a 60–70*S* RNA (14, 16, 18, 19) which contains polyadenylate sequences of about 200 nucleotides (12, 17); and (iii) an RNA-instructed DNA polymerase (11, 18, 19, 22). We report here a detailed analysis of MPMV RNA and demonstrate its similarity to that of a known RNA tumor virus, i.e., Rauscher murine leukemia virus (MuLV).

Characterization of the RNAs of MPMV and MuLV was derived from electrophoresis in mixed agarose-polyacrylamide gels. Culture media containing ^{3}H-labeled MPMV and [^{32}P]MuLV were mixed, the viruses were co-purified, and the viral RNAs were coextracted. The 60–70*S* RNAs were isolated on glycerol gradients and subjected to electrophoresis in 1.8% polyacrylamide gels containing 0.5% agarose (Fig. 1). By comparing the relative electrophoretic mobilities of vesicular stomatitis virus RNA, and NC-37 ribosomal 28*S* and 18*S* RNAs, the molecular weights of MPMV and MuLV RNAs are estimated to be 8×10^6. This must be taken, however, as an "apparent" molecular weight, as we are dealing with a highly structured molecule.

Studies were undertaken to determine the subunit structure of MPMV and MuLV 60–70*S* RNAs by stepwise dissociation with formamide. 60–70*S* RNA from [^{3}H]uridine-labeled MPMV and from ^{32}P-labeled MuLV were extracted and co-purified. Partial dissociation of both MPMV and MuLV RNA occurred after treatment with 10% formamide (Fig. 2A), whereas complete dissociation was achieved with 40% formamide (Fig. 2B). Disaggregation of the viral RNAs by heating at 80 C for 2.5 min (Fig. 2C) yielded essentially the same result as 40% formamide. The molecular weights of the subunits of MPMV and MuLV were similar and estimated to be 2.8×10^6 (Fig. 2B).

To determine if the time interval between viral release from cells and tissue culture fluid collection affects the size of RNA subunits, [^{3}H]uridine-labeled MPMV was harvested at either 2- or 24-h intervals. The viral RNA isolated from culture fluids from both 2- (Fig. 3A) and 24-h (Fig. 3C) harvests had similar sedimentation values of 60 to 70*S*. After treatment with 40% formamide (Fig. 3B), the 60–70*S* RNA from virus of 2-h collections yielded a major RNA subunit with a sedimentation coefficient of 30*S*. Identical treatment with 40% formamide of 60–70*S* RNA from virus of 24-h collections, however, yielded a heterogeneous array of small RNA molecules (Fig. 3D); this suggests a rapid degradation of RNA within virions after release from cells, a phenomenon which has been found for known oncornaviruses (1, 4a). Treatment of 60–70*S* RNA from virus of 2-h collections with 10 and 20% formamide revealed intermediate RNA structures between

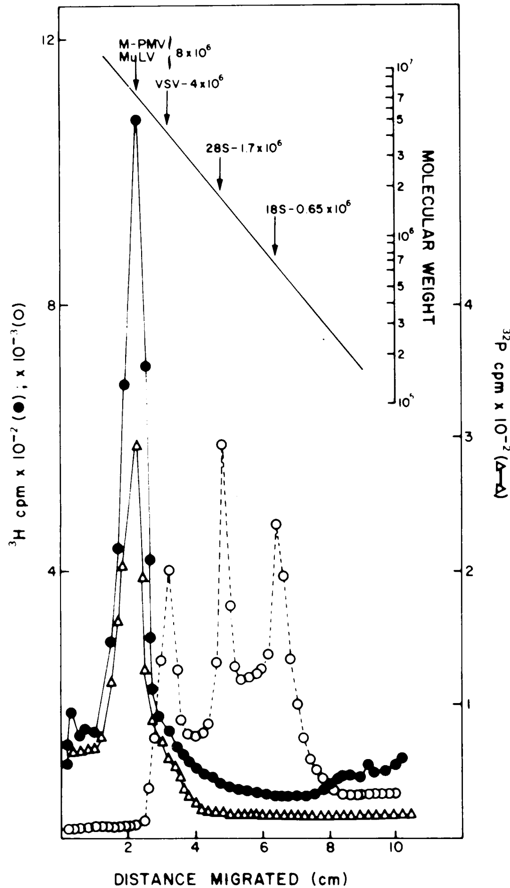

FIG. 1. *Polyacrylamide gel electrophoresis of MPMV and MuLV high-molecular-weight RNA. [^{3}H]MPMV was grown in CMMT cells, and [^{32}P]-MuLV was grown in NIH-Swiss 3T3 cells. RNAs were prepared by the addition of [^{3}H]uridine or ^{32}P to the tissue culture medium in roller bottle cultures. [^{3}H]uridine was added to a final concentration of 100 μCi/ml. The tissue culture medium was replaced after 2 h with fresh medium and harvested thereafter at 2-h intervals for 8 h. Pooled tissue culture fluid was clarified by centrifugation at 6,500 × g for 10 min and stored at −80 C until use. For ^{32}P labeling, media lacking phosphates was used and supplemented with 10% dialyzed fetal calf serum. The cells were washed twice with the phosphate-free medium and incubated with phosphate-free medium containing ^{32}P, carrier-free (100 μCi/ml), as described above. Virus was precipitated from the tissue culture fluids by stirring with polyethylene glycol 6000 (7.5% wt/vol) in 0.5 M NaCl for 2 h at 4 C. The virus precipitate was collected by centrifugation (3,500 × g for 30 min), suspended in 0.01 M Tris-hydrochloride, pH 8.3, 0.15 M NaCl, and 0.001 M EDTA (TNE) and centrifuged through 20% glycerol (vol/vol) in TNE. Viral RNA was extracted as previously described (17). Viral RNA was resuspended in 0.01 M Tris-hydrochloride, pH 8.3, 0.15 M NaCl, 0.001 EDTA, and 0.2% sodium-N-lauroyl sarcosinate (TNES), layered onto a linear 10 to 30% glycerol gradient in TNE and centrifuged for 3.5 h at 40,000 rpm in an SW41 rotor at 4 C. The 60–70S RNA regions were pooled and ethanol was precipitated. The RNA was resuspended in electrophoresis buffer and analyzed by polyacrylamide gel electrophoresis, as described in the legend to Fig. 2.*

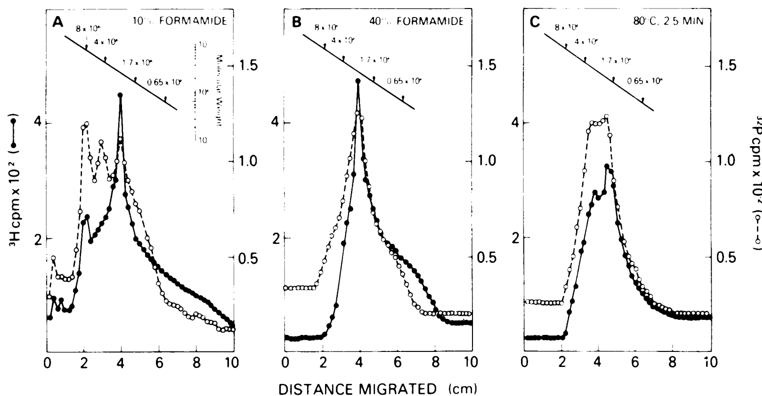

FIG. 2. *Stepwise dissociation of MPMV and MuLV high-molecular-weight RNA by formamide and heat. [^{3}H]MPMV RNA (●) and [^{32}P]MuLV RNA (○), isolated as described in Fig. 1, were denatured with (A) 10% formamide (deionized) for 10 min at 37 C, (B) 40% formamide for 10 min at 37 C, or by (C) heating at 80 C for 2.5 min. The resultant dissociation products were analyzed by mixed agarose polyacrylamide gel electrophoresis. PAGE (polyacrylamide gel electrophoresis) of viral 60–70S RNA was performed on 10-cm long, 1.8% polyacrylamide gels (2), containing 0.5% agarose. Electrophoresis was performed at 5 mA per gel until the tracking dye (phenol red) reached the bottom of the gel. The gels were then sliced into 1-mm fractions and two consecutive fractions were placed in each vial. The RNA was eluted overnight in 10 ml of toluene-based scintillation fluid containing 3% protosol (New England Nuclear Co.). Samples were counted in a Beckman scintillation counter. Electrophoresis on 10% gels was performed as above with no added agarose.*

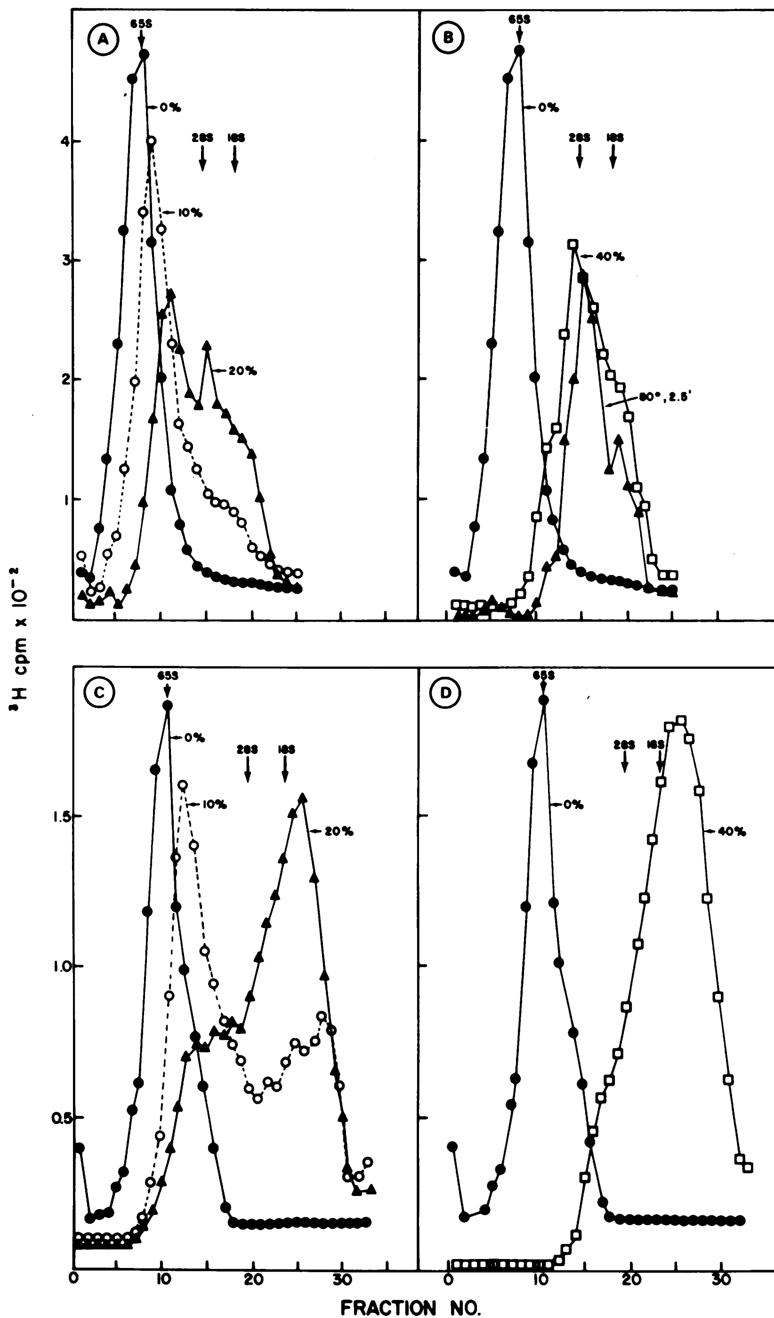

FIG. 3. *Stepwise dissociation of MPMV high-molecular-weight RNA from virions of 2- and 24-h cell culture fluid harvests. [^{3}H]uridine-labeled MPMV RNA was isolated from 2- (A and B) and 24-h (C and D) cell culture fluid harvests of MPMV. The RNAs were denatured by treatment with increasing concentrations of formamide: 0% (●), 10% (○), 20% (▲), 40% (□), or by heating to 80 C for 2.5 h (▲), and analyzed on 10 to 30% glycerol gradients in 0.01 M Tris-hydrochloride, pH 8.3, 0.15 M NaCl, 0.001 EDTA, and 0.2% sodium-N-lauroyl sarcosinate buffer, as described in Fig. 1. Ribosomal 28S and 18S RNAs from NC37 cells were used as markers.*

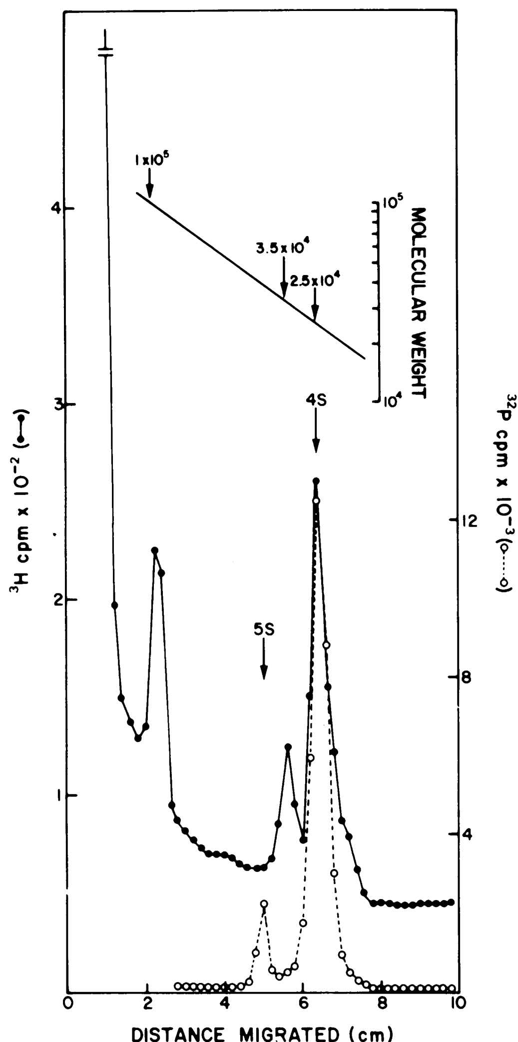

FIG. 4. *Polyacrylamide gel electrophoresis of low-weight RNAs associated with MPMV 60–70S RNA. [^{3}H]uridine-labeled 60–70S MPMV RNA was denatured by heating to 80 C for 2.5 min and the dissociated low-molecular-weight RNAs* (●) *were analyzed on 10% polyacrylamide gels as described in Materials and Methods. [^{32}P]4S and 5S RNA markers were obtained from NC37 cytoplasmic RNA* (O).

65*S* and 30*S* (Fig. 3A). Similar treatment of 60–70*S* RNA from virus of 24-h collections, however, yielded more heterogeneous peaks with much lower sedimentation values (Fig. 3C). Heating of the 60–70*S* RNA from virus of 2-h collections at 80 C for 2.5 min yielded RNA subunit structures similar to those obtained with 40% formamide (Fig. 3B).

To characterize any low-molecular-weight RNAs associated with MPMV 60–70*S* RNA, purified [^{3}H]MPMV 60–70*S* RNA from 2-h viral harvests was heated at 80 C for 2.5 min and electrophoresed on a 10% polyacrylamide gel (Fig. 4). When compared to [^{32}P]cellular 4*S* and 5*S* RNA markers, three small RNAs with molecular weights of 2.5×10^4, 3.5×10^4, and 10^5 were evident. These molecular weights were calculated (20) to correspond to approximate sedimentation coefficients of 4*S*, 4.5*S*, and 7*S*, respectively. They are similar to those found in Rous sarcoma virus (4, 8, 10). The 4*S*, 4.5*S*, and 7*S* RNAs were present in counts per minute ratios of 8:3:4 and therefore represent approximately 4, 1.5, and 2%, respectively, of the total counts per minute of the original 60–70*S* RNA molecule.

The stepwise dissociation of MPMV 60–70*S* RNA by limited formamide treatment (Fig. 2 and 3) suggests that the RNA subunits of MPMV may be linked by differentially stable hydrogen-bonded regions. Similar results have been obtained by Travnicek and Riman (21) with the 60–70*S* RNA of avian myeloblastosis virus. The studies reported here demonstrate the similarities of MPMV RNA in size, subunit structure, and associated RNAs to those of known avian and mammalian RNA tumor viruses.

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