Large-Scale Purification of Ribonucleic Acid Tumor Viruses by Use of Continuous-Flow Density Gradient Centrifugation

D. P. GRANDGENETT, K. BRACKMANN, AND M. GREEN

Institute for Molecular Virology, Saint Louis University School of Medicine, St. Louis, Missouri 63110

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This report describes a 200- to 300-fold increase in the quantity of ribonucleic acid tumor virus particles previously isolated at one time by zonal centrifugation.

The development of the model K zonal centrifuges (1, 6) has permitted the application of continuous-flow density gradient centrifugation to the purification of ribonucleic acid (RNA) tumor viruses from larger volumes of cell culture fluids than is possible with other zonal centrifuges (5) or with conventional methods of RNA tumor virus purification (4). This report describes a procedure for the use of the zonal centrifuge for the purification and concentration of different RNA tumor viruses directly from cell culture fluids and from culture media in which the virus was precipitated by polyethylene glycol prior to zonal centrifugation.

Avian myeloblastosis virus (AMV), which was previously precipitated by polyethylene glycol from tissue culture fluid, was purified in an RK 6 rotor with a K-3 core (Fig. 1A) by using a continuous-flow density gradient centrifuge. The quantity of AMV (9 × 10^14 virus particles) utilized represented a 200- to 300-fold increase in the quantity of RNA tumor virus particles previously isolated at one time by zonal centrifugation (6). The peak of absorbance at 260 nm is not coincident with the RNA-directed deoxyribonucleic acid (DNA) polymerase activity located in the virion. The same absorption profile was obtained at 280 nm. The majority of this material which absorbs at 260 nm and peaks at fraction 12 (Fig. 1A) is containing material which is most likely derived from materials precipitated by polyethylene glycol from the cell culture medium utilized in the growth of the virus. The larger size virus particles can be readily separated from this contaminating material by velocity sedimentation (Fig. 2A). These two-step gradients resulted in a 86% decrease in protein concentration with only a 7% loss of RNA-directed DNA polymerase activity in the purified cushioned virus. This purified virus had 14 polypeptides when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These same polypeptides were observed with AMV which was purified in this laboratory by sucrose gradient centrifugation (4). In addition, the polypeptide pattern obtained was almost identical to that previously published for AMV (2).

Toplin and Sottong (6) previously reported the presence of a small fraction (5 to 10%) of low density (1.11 to 1.13 sucrose g/cm^3) banding RNA tumor virus not sedimenting with the principal virus band (1.14 to 1.17 g/cm^3). Similar results were obtained with the AMV virus (Fig. 1A). Analysis of this pooled virus (13–16) by equilibrium sedimentation again resulted in the virus banding at this lower density. This virus also had both DNA polymerase activities which are associated with the 1.14 to 1.17 g/cm^3 density purified AMV (3). The AMV virus banding at higher densities (1.17 to 1.23 g/cm^3) is probably aggregated and/or associated with debris and could not be recovered.

The purification of the Moloney strain of murine sarcoma-leukemia virus (MSV-MLV) (M) directly from cell culture fluids by zonal centrifugation is described in Fig. 1B. The absorption profile at 260 nm obtained with MSV-MLV(M) is similar to profiles obtained with other RNA tumor viruses purified directly from cell culture media (6). The utilization of the reverse transcriptase assay to identify MSV-MLV(M) as well as AMV gives a more clearly defined location of RNA tumor viruses than previously established by density and ultraviolet absorption (6). Purification of the pooled MSV-MLV(M) (Fig. 1B; 8–13) by velocity sedimentation resulted in purified virus which was then analyzed by equilibrium centrifugation (Fig. 2B). The polypeptide patterns obtained
(4) was used throughout this study. Previous as well as later steps in the utilization of the model RK zonal centrifuge (Electro-Nucleonics, Inc., Fairfield, N.J.) were previously described (6). Two-step gradients consisting of 700 ml of 20% sucrose (wt/vol) in NTE buffer [0.1 M NaCl, 0.01 M tri(hydroxyethyl)aminomethane (Tris)-hydrochloride (pH 7.8) and 0.001 M ethylenediaminetetraacetic acid] and 500 ml of 60% sucrose (wt/vol) in NTE buffer were pumped into an RK-6 titanium rotor with a K-3 Noryl core (1.6-liter capacity). A total of 35 g (wet weight) virus (9.0 × 10¹⁴ virus particles) in 2.8 liters of cell culture medium was diluted to 20 liters with 5% sucrose in NTE buffer and purified at a flow rate of 5 liters/h (panel A). Upon completion of the centrifuge run, 50-ml fractions were collected from the bottom of the rotor and assayed for RNA-directed DNA polymerase activity by using poly (A)-oligo(dT)₁₂₋₁₈ (3), and the absorbance at 260 nm and the sucrose concentrations (g/ml) were determined (panel A). Prior to assaying for DNA polymerase activity, a sample from each fraction was diluted 1:10 with 0.5% Nonidet P-40 in 0.05 M Tris-hydrochloride, pH 8.0 (lysis buffer), and 10-ml samples of virus lysate were used. Separate fractions were diluted (1:100) with NTE buffer prior to reading absorbance at 260 nm. MSV-MLV(M) was grown as previously described (4). Twenty liters of cell culture fluid was processed without dilution through the zonal centrifuge (panel B) as described above. Fractions (50 ml) were collected and processed as before except that the virus fractions were diluted (1:1) with lysis buffer prior to DNA polymerase assay and they were not diluted prior to reading absorbance at 260 nm.

Fig. 1. Gradient profile from continuous-flow recovery of AMV and MSV-MLV(M) cell culture supernatant in RK-6 rotor. Tissue culture-grown AMV (generously supplied by J. W. Beard, Duke Univ.) which was precipitated and concentrated (×25) by polyethylene glycol similar to published procedures.

Fig. 2. Buoyant density analysis of AMV and MSV-MLV(M) zonal-purified virus. AMV fractions (6-12, Fig. 1A) were combined and diluted with NTE buffer to 700 ml and cushioned onto 1.5 ml of 60% sucrose through 5 ml of 20% sucrose in an SW27 rotor at 26,000 rpm for 1 h at 4 C. This step-gradient-purified virus was diluted to 250 ml with NTE buffer and recushioned onto a 1-ml 60% sucrose cushion. The double-cushioned virus was diluted to 90 ml with NTE to a final protein concentration of 11.5 mg/ml. A sample was layered upon a 20 to 60% sucrose gradient and centrifuged in an SW27 rotor at 27,000 rpm for 6 h at 4 C (panel A). Fractions (1.2 ml) were collected from the bottom and analyzed as previously described in Fig. 1 except that the virus was diluted (1:3) with NTE prior to the 260 nm absorbance reading and was diluted (1:1) with lysis buffer prior to DNA polymerase assay. MSV-MLV(M) fractions (8-13, Fig. 1B) were combined and diluted with NTE to 600 ml and cushioned once through 20% sucrose onto a 60% sucrose cushion as described above. The cushioned virus was diluted to 20 ml with NTE at a final protein concentration of 1.5 mg/ml. The virus was layered on a 20 to 60% sucrose gradient and centrifuged in an SW41 rotor at 36,000 rpm for 2 h at 4 C. Fractions (0.8 ml) were collected and absorbance was read at 260 nm and assayed for DNA polymerase as described above (panel B).
with this cushioned virus were the same as those of MSV-MLV(M) purified by sucrose density centrifugation (4). Purification of feline sarcoma-leukemia virus (Gardner) gave similar results as observed with MSV-MLV(M) (data not shown).

The purification of RNA tumor viruses on the scale reported here makes possible the structural characterization of enzymes found in RNA tumor viruses.

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