

The Use of Guanidine-HCl for the Isolation of Both RNA and Protein from RNA Tumour Viruses

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SUMMARY

The RNA components of two C-type RNA viruses, avian myeloblastosis virus and Friend leukaemia virus, have been isolated by treatment of the viruses with 6 M-guanidine-HCl and precipitation with ethanol. The virus proteins were recovered by lyophilization of the guanidine-HCl-ethanol supernatant after thorough dialysis against 0.5 mM-dithiothreitol. This simple method yielded RNA of similar quality to the phenol and sodium dodecyl sulphate (SDS) extraction methods, and the same amount of 60–70S RNA, although a fraction of the smaller (4S) species remained in the protein fraction. The sedimentation patterns of heat-denatured RNA extracted by either method were similar. Electrophoretic analyses of the extracted proteins in polyacrylamide gel gradients containing SDS gave patterns that were very similar to those obtained by direct analysis of SDS disrupted viruses.

The use of guanidine-HCl (GuHCl) for the extraction of RNA tumour virus proteins is a well-established procedure (Fleissner, 1971; Green & Bolognesi, 1974). The mixture of proteins can be fractionated by gel chromatography in this solvent, renatured by dialysis against saline and used for the preparation of antisera as well as for chemical studies. We have found that the RNAs of these viruses can be recovered from the same batch of GuHCl-treated virus.

Double-stranded virus RNAs are soluble in concentrated salt solutions (Baltimore, 1966) as are tRNAs (Barlow *et al.* 1963). Cox (1968) showed that ribosomal RNA can be precipitated from 4 M-GuHCl by ethanol. In this report we demonstrate the application of Cox's method to two RNA tumour viruses, avian myeloblastosis virus (AMV) and Friend leukaemia virus (FLV), on amounts ranging from milligrams to micrograms. We found that while the high mol. wt. 60–70S species were precipitated, some of the low mol. wt. 4S species remained soluble; these can be recovered, however, from the protein fraction by phenol extraction.

The viruses used in these studies were isolated and purified by commonly used procedures, i.e. they were pelleted from the plasma of infected animals and then 'purified' by isopycnic banding on sucrose gradients. Viraemic plasma containing AMV (BA1 strain A) was supplied to Dr Erwin Fleissner and Dr Mukund Modak of this Institute by Dr J. W. Beard, Life Sciences, Inc., St. Petersburg, Fla., U.S.A. The plasma was filtered through four layers of gauze and after centrifuging at low speed (2000 g) was layered over 5 ml of 50% sucrose in TNE buffer (0.1 M-NaCl, 0.001 M- Na_2EDTA , 0.01 M-tris-HCl, pH 7.4) and then centrifuged for 75 min at 27000 rev/min in a Beckman SW 27 rotor. The pelleted virus was recovered from the sucrose cushion, diluted threefold with TNE buffer, and layered on to a 25 to 50% (w/v) linear sucrose gradient in TNE buffer. Each tube contained 25 ml of the gradient solution and 12 ml of virus suspension. The virus was isopycnically banded by centrifugation at 27000 rev/min for 17 h at 5 °C in the SW 27 rotor. The visible band was collected and used immediately or frozen. Friend leukaemia virus was isolated from plasma freshly

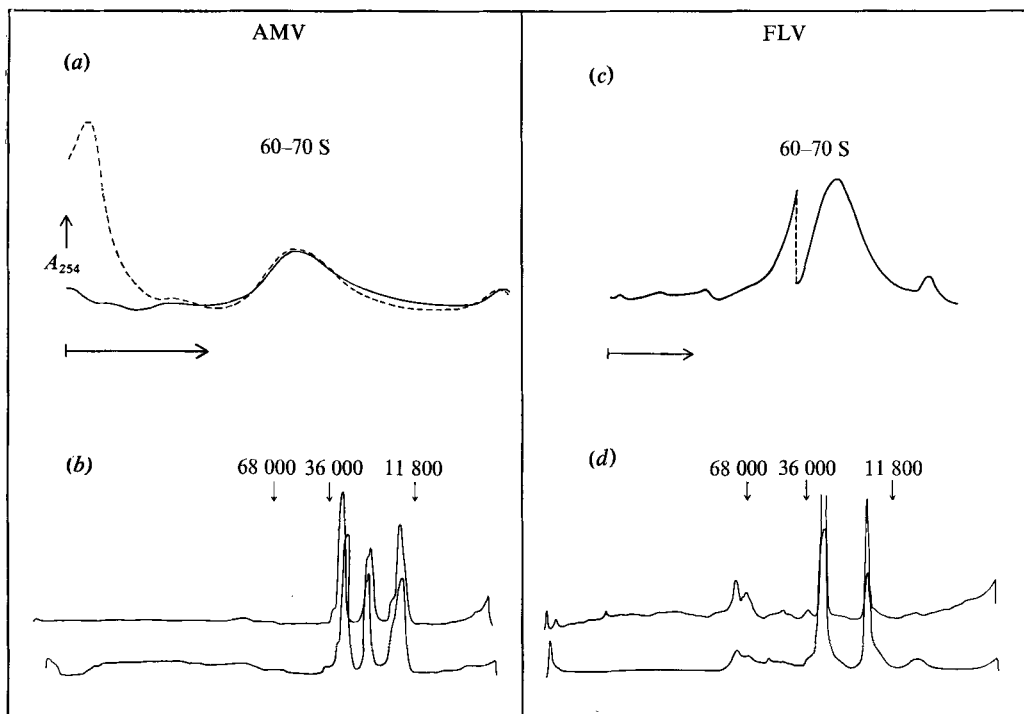


Fig. 1. (a and c). Sedimentation patterns of GuHCl (—) or phenol (---) extracted RNAs of (a) AMV and (c) FLV after centrifugation for 3 h in isokinetic sucrose gradients containing TNE and 0.2% SDS (20 °C) at 40000 rev/min in the Beckman SW 41 rotor. In (c), the discontinuity indicates a change in the monitor range scale. (b and d) Comparison of virus proteins extracted from (b) AMV and (d) FLV by GuHCl with those of virus dissolved in 1% SDS. Electrophoresis in a 5 to 20% gradient of polyacrylamide at alkaline pH in the presence of SDS (Laemmli, 1970). Polaroid photographs of the Coomassie blue stained gels were scanned with a densitometer. Migration is to the positive pole at the right. Upper patterns, virus + SDS; lower patterns, isolated proteins after GuHCl extraction of the virus. The positions of standard proteins, bovine serum albumin (68000), α -glycerophosphate dehydrogenase (36000), and cytochrome c (11800) are indicated by the vertical arrows.

harvested from about 100 leukaemic mice and purified as previously described (Evenson *et al.* 1975a). The virus pellet was immediately resuspended in TNE buffer, layered on to a 15 to 55% (w/v) linear sucrose gradient in the same buffer, and centrifuged at 40000 rev/min for 3 h in a Beckman SW 41 rotor. The banded virus was collected, diluted in TNE buffer and immediately prepared for RNA extraction.

For the extraction of virus RNA, the sucrose solution containing the isopycnicly banded virus was diluted with an equal vol. of cold TNE buffer and brought to about -5°C in an ethanol- CO_2 bath. For convenience, some of the following operations were carried out by weight. To the diluted virus solution, an equal weight of solid GuHCl (ultrapure, Schwarz-Mann, Orangeburg, N.J., U.S.A.) was added with rapid stirring. The mixture was held at about -5°C and stirred for an additional 30 min after the GuHCl crystals dissolved. Dissolution of the GuHCl increases the volume about 1.7-fold. Then a volume of ethanol (-20°C) equal to the original volume of the diluted virus solution was added with constant stirring. Thus diluted virus solution (now about 17% sucrose), solid GuHCl, and ethanol were combined in ratios of 1:1:1 (w:w:v). The suspension was usually stored in Pyrex glass centrifuge tubes at -20°C overnight and then centrifuged for 30 min at 10000 rev/min in

a Sorvall SS34 rotor that had been pre-chilled to -5°C . The RNA precipitate was washed twice with 70% ethanol, 1% sodium acetate and then dissolved in 5 mM-dithiothreitol (DTT) for immediate use, or stored as an ethanol precipitate in small samples at -70°C . When small amounts of FLV (approx. 5×10^{11} particles) were extracted, the RNA precipitate was centrifuged for 45 min at 15000 rev/min in a Sorvall SS34 rotor to ensure good recovery. The use of longer centrifugation times or higher speeds sometimes resulted in the pelleting of small amounts of GuHCl from the nearly saturated solution; this GuHCl could be removed by washing the pellet with ethanol as described above. Alternative procedures that have been used are: dialysis of the virus solution against 6 M-GuHCl, 1 mM- Na_2EDTA , 1 mM-DTT before ethanol precipitation, or direct addition of 6 M-GuHCl to a virus pellet that had been resuspended in a small vol. of TNE buffer.

To recover the virus proteins, the GuHCl-ethanol supernatant was dialysed exhaustively against 6 mM-mercaptoethanol or 0.5 mM-DTT and lyophilized. The proteins were dissolved in a small vol. of 6 M-urea, 1 mM-DTT for analysis by polyacrylamide gel electrophoresis (Laemmli, 1970).

Data are presented in Fig. 1 to illustrate some properties of the RNA and protein fractions obtained by GuHCl extraction of AMV (large scale sample) and FLV (small scale sample). Fig. 1(a) and (c) show the patterns obtained when the distribution of sedimenting species of AMV and FLV-RNAs were examined by sucrose gradient centrifugation. The patterns are similar to those previously published with a main peak sedimenting at 60–70S and some fast-sedimenting material as well as slowly sedimenting components (Gillespie *et al.* 1975).

Several parallel extractions of FLV by the phenol and GuHCl methods produced RNA in similar yields and of similar quality as judged by the number and sizes of molecules observed by electron microscopy (Evenson *et al.* 1978). For AMV a comparison of the two methods was made by extracting half of a batch of isopycnicly-banded virus by the phenol- CHCl_3 method (Perry *et al.* 1972) and half by the GuHCl method. The sedimentation patterns (Fig. 1a) obtained for phenol- and GuHCl-extracted RNA show that the two methods gave approximately the same amount of 60–70S RNA. However, the amount of slower sedimenting species was significantly diminished in the pattern of the GuHCl-extracted RNA, about half that expected from published values (Bellamy *et al.* 1974). The proportion of slowly-sedimenting species was also low for GuHCl extracted FLV RNA (Fig. 1c). To determine whether the small RNAs could be recovered, the supernatant fraction of GuHCl-ethanol treated AMV was extracted with phenol and the RNA thus obtained was examined by polyacrylamide gel electrophoresis (Varricchio & Ernst, 1975) in parallel with the GuHCl-ethanol insoluble fraction. Both samples contained species that migrated in the region of authentic tRNAs (data not shown).

The subunit integrity of GuHCl-extracted RNA of FLV was compared with RNA extracted by another standard method. One batch of FLV was treated with 1% SDS in TNE. The second was extracted with GuHCl. Both the SDS-released RNA and the GuHCl-extracted RNA were first sedimented in velocity sucrose gradients as in Fig. 1(c). Fractions containing the '60–70S' RNA were pooled and the RNA recovered by precipitation with 2 vol. of ethanol containing 1% sodium acetate. The precipitate was dissolved in TNE – 0.2% SDS, heated for 3 min at 80°C , and after fast cooling to room temperature, layered on to sucrose gradients. Fig. 2 shows that the FLV RNAs isolated by either the GuHCl or SDS method sedimented similarly with peaks at about 35S. Therefore, the GuHCl method does not produce nicks in the 60–70S RNA.

Cox (1968) showed in his detailed study that ribonuclease is inactive in 4 M-GuHCl, but that the RNA precipitate must be washed to ensure its removal. This additional step may be

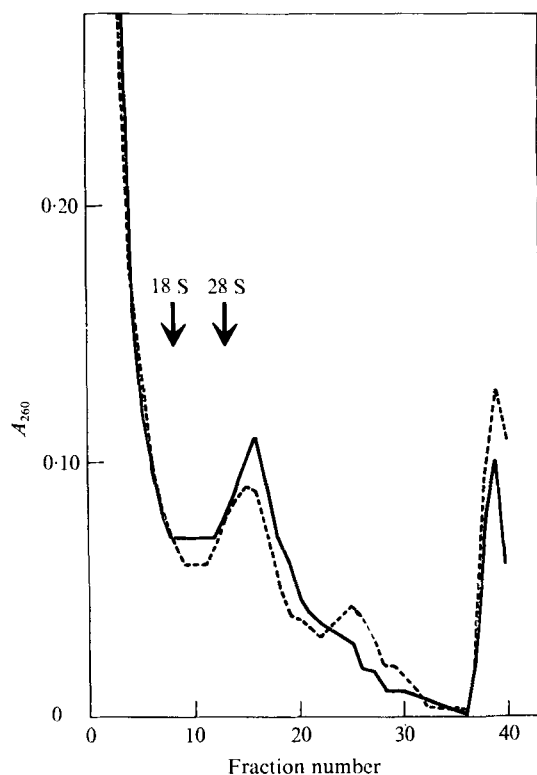


Fig. 2. Velocity sucrose gradient analyses of heat denatured 60–70S FLV RNA extracted by GuHCl (—) or SDS (---). Centrifugation conditions were as described in Fig. 1 (*a* and *b*), except that the time of centrifugation was 5 h. The positions of 18S and 28S ribosomal RNAs were determined by parallel centrifugation of SDS-treated Friend cell ribosomes.

desirable, although FLV-RNA was similar in size as measured by electron microscopy (Evenson, 1977) whether or not the RNA was re-precipitated (data not shown).

Retrovirus RNAs have a high degree of secondary structure as has been shown by electron microscopy (Weber *et al.* 1974; Evenson *et al.* 1975*b*) and in physical studies by Cavalieri (1974). In the experiments of Cavalieri (1974), phenol-extracted AMV-RNA regained its helicity even after heating to 70 °C. In 4 M-GuHCl, base stacking interactions as well as hydrogen bonding are decreased (Cox & Kanagalingam, 1967). Evidence that GuHCl-extracted oncornavirus RNAs are not irreversibly denatured by exposure to that solvent was obtained in a test of their 'transcribability'. With saturating concentrations of AMV reverse transcriptase, the extent of incorporation of labelled deoxyribonucleotides into DNA using GuHCl-extracted 60–70S RNA was comparable to that obtained with 60–70S RNA prepared by the conventional phenol extraction method (data not shown).

The GuHCl-extracted proteins of AMV and FLV have been compared with the proteins of detergent-treated virus (Fig. 1*b* and *d*). The main point to consider is the similarity between the gel electrophoretic patterns obtained for the extracted protein fractions and total virion proteins, demonstrating that there is no preferential loss of proteins in the ethanol precipitation step.

Criteria for a preparative method to be applied to labile macromolecules in short supply should include speed and facility as well as quantitative recovery of pure components. The

ease and convenience of this method of extraction recommend it as an alternative to the more cumbersome phenol method especially when both the RNA and proteins of a retrovirus are to be studied.

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