ROUTINE ISOLATION OF THE 70 S RNA OF A MURINE ONCORNAVIRUS BY USE OF AN UNESTERIFIED CELLULOSE COLUMN

C.J. LARSEN, M. MARTY, R. EMANOIL-RAVICOVITCH and M. BOIRON

Institut de Recherches sur les Leucémies, Laboratoire d'Hématologie Expérimentale, Hôpital Saint-Louis, 75010 - Paris, France

Received 2 April 1973

1. Introduction

The genome of murine sarcoma virus [MSV-m (MLV)] is a single stranded RNA molecule with a sedimentation constant of approx. 70 S [1, 2]. This molecule is a relatively complex aggregate of 3 or 4 pieces of 3 to 4 x 10^6 daltons each, which contain segments of polyadenylic acid sequences bound by covalent links [3, 4]. Recently it was reported that unesterified cellulose selectively binds poly-(A) containing RNA's in high salt solutions [5]. We have successfully used this method to separate 70 S RNA or its subunit components from the other RNA species which are present inside the viral particle.

2. Materials and methods

2.1. Reagents and products

Synthetic poly-(A) (as the potassium salt) was purchased from Miles Laboratories, Elkhart, Indiana, USA.

RNA from *Torula* yeast (grade VI-Sigma) was phenolized and reprecipitated by 2 vol of ethanol plus 0.1 M NaCl.

Cellulose MN 300 was from Macherey and Nagel (Germany).

High ionic strength Buffer = Buffer H: 0.01 M Tris-HCl, pH 7.6, 0.5 M KCl.

Low ionic strength Buffer = Buffer L: 0.01 M Tris-HCl, pH = 7.6.

All the glassware was autoclaved before use.

2.2. Obtention of viral RNA

MSV-MLV virus was obtained from the supernatant fluids of a chronically infected line ($78A_1$ cell line) [6]. It was labeled and purified as described elsewhere [7]. RNA was isolated by use of SDS-phenol [1]. Potassium polyvinylsulfate (10 μg/ml) was routinely added during the course of isolation. When 70 S RNA was needed, it was separated from the other RNA species by centrifugation through a 5–20% sucrose gradient. [1]

2.3. Cellulose column

0.2 g cellulose were homogenized in 5 ml of Buffer H. A 1 x 0.9 cm column was poured with this slurry and then equilibrated in the same Buffer. RNA samples were dissolved in H Buffer at a concentration not exceeding 10 μg/ml. Buffer H was added through the column until ultraviolet absorbance was decreased to zero. Then, L Buffer was added. One ml fractions were collected.

2.4. Polyacrylamide gels

Composite polyacrylamide-agarose gels were prepared according to procedures of Tiollais et al. [8]. Following electrophoresis, gels were processed for counting as described [8].

3. Results

3.1. Binding of Poly-A to cellulose

Poly-(A) binding capacity of cellulose was tested by adding synthetic poly-(A) dissolved in Buffer H to a column (1.5 × 0.9 cm). No material was recovered in the effluent. Replacement of Buffer H by Buffer L resulted in a quantitative recovery of the poly-(A) (fig. 1A). We verified that the binding effect of cellulose was specific by passing yeast RNA through an identical column. In this case all the ultraviolet absorbing material was found in the washing effluents (fig. 1B). Finally, a mixture of poly-(A) and yeast RNA was loaded on the same column (fig. 1C). Here again, poly-(A) was retained by cellulose at high ionic strength while yeast RNA was not absorbed at all. Ultraviolet

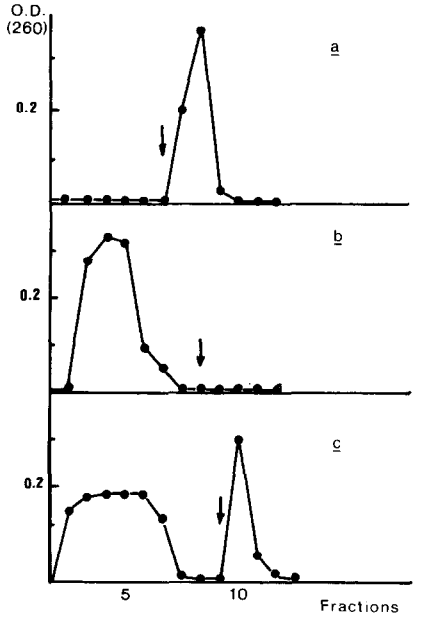

Fig.1. Chromatography on cellulose column of synthetic poly-(A) and *Torula* yeast RNA. a) 6 A_{260} units of poly-(A) were dissolved in 5 ml of H buffer and loaded on a 1.5 × 0.9 cm cellulose column. Elution by L buffer (8 ml) was started at the position indicated by an arrow. b) 1 A_{260} unit of *Torula* yeast RNA was dissolved in 3 ml of H buffer and passed through an identical column, then L buffer was added (arrow). c) A mixture of poly-(A) (0.4 A_{260} unit) and *Torula* yeast RNA (0.9 A_{260} unit) in 6 ml of buffer H was chromatographed in a cellulose column. In the 3 experiments, 1 ml fractions were collected.

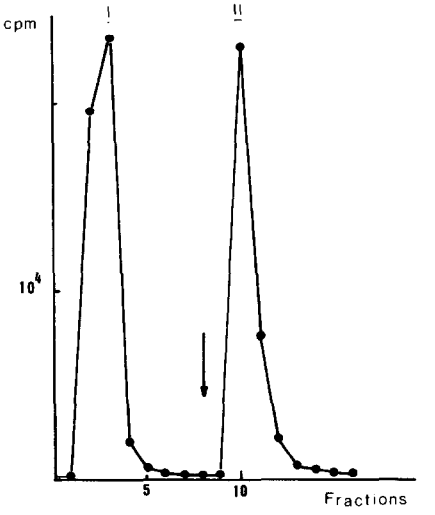

Fig.2. Chromatography on cellulose column of MSV-m(MLV) RNA. Total RNA's were extracted from MSV-m(MLV) preparations labelled for 24 hr with ^{32}P. They were dissolved in 0.5 ml of H buffer and loaded on a 1 × 0.9 cm gravity-packed column. Recovery of the adsorbed material was done by 8 ml of L buffer. Materials of peaks I and II were separately pooled and precipitated for polyacrylamide gel electrophoresis.

spectrum of the fraction eluted by Buffer L was that of pure poly-(A). All these results are in total agreement with those of Kitos et al. [5].

3.2. Fractionation of viral RNA

^{32}P-labeled MSV-MLV RNA was dissolved in 0.5 ml of H Buffer and passed through a column (0.9 × 15 cm) containing 0.2 g of gravity packed cellulose. 9 ml of Buffer H were then added, followed by 5 ml of Buffer L. One ml fractions were collected and radioactivity in each fraction was determined (fig.2). From the results of the preceding experiment, it could be assumed that material of peak II was poly-(A) containing RNA, e.g. 70 S RNA. On the other hand, peak I should contain other viral RNA species, e.g. low molecular weight RNA. To ascertain this assumption, material of peak I and II was separated, pooled and analyzed in 1.7%–0.5% polyacrylamide–agarose gels. The gel profiles are presented in fig.3. The material of the peak I which has no affinity for cellulose is composed of low molecular weight RNA, especially 4 S and 8 S species (fig.3A). Traces of 18 S and 28 S ribosomal RNA are also visible. On the other hand, all the materi-

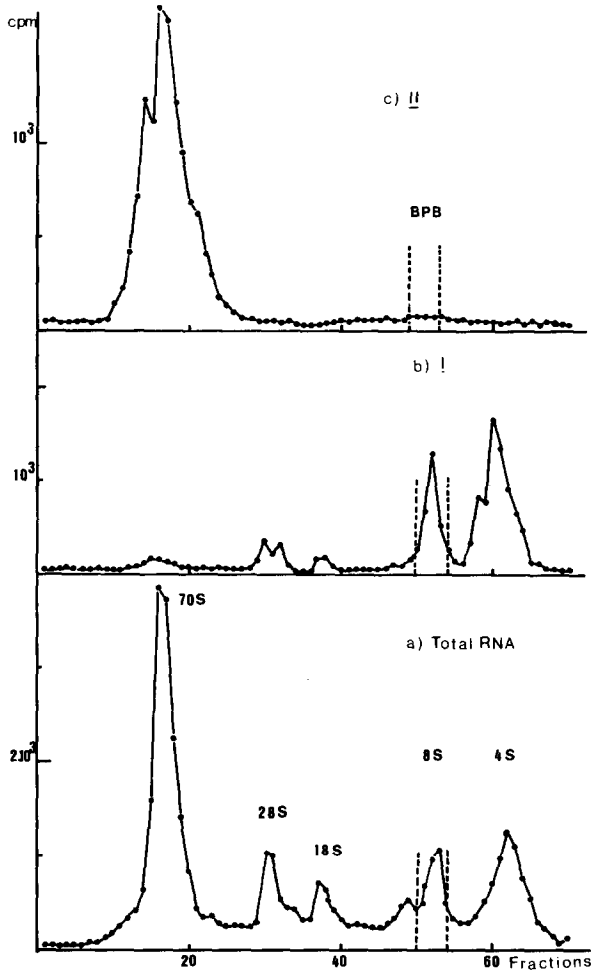

Fig.3. Polyacrylamide gel electrophoresis of cellulose fractionated MSV (MLV) RNA. RNA was analyzed in 1.7%–0.5% polyacrylamide–agarose gels. Migration was performed at 10 V/cm for 3 hr. 1.5 mm slices were cut out and hydrolyzed for 1 hr at 60° with 0.5 ml of NH_4OH. 5 ml of Bray's solution were added [12] and couting was done in a Packard scintillation counter.
a) Control RNA.
b) Viral RNA of the peak I of the cellulose column.
c) Viral RNA of the peak II of the cellulose column.

al adsorbed by cellulose migrates in the gel at the position of 70 S RNA. By comparison with the pattern of total viral RNA presented on fig.3C, there is no extra-peak of degraded RNA (although the peak of 70 S RNA passed through cellulose is somewhat broader than that of the control sample). We concluded that the chromatographic step did not alter the viral RNA.

In another set of experiments, 70 S RNA was previously purified by centrifugation in a 5–20% sucrose gradient before being chromatographed on a cellulose column [1]. More than 90% of the RNA was retained by cellulose and could be eluted by L buffer.

4. Discussion

In this report, we have established that cellulose is capable of binding 70 S RNA of murine sarcoma virus, MSV-m (MLV), but has no affinity for the other RNA components present in the virions. This property probably relies upon the existence of polyadenylic acid segments in every subunit which composes the 70 S complex since it has been shown in this paper and by others [5] that poly-(A) molecules are adsorbed by cellulose. The exact mechanism of this binding is not known but it is presumably a consequence of the particular secondary structure of adenylic homopolymers.

Recent results suggest that 70 S RNA is the ultimate product found in mature virions. In nascent viral particles, there is a majority of 30–40 S RNA molecule which are converted into the 70 S complex during the extracellular maturation of the virions [10–11]. It would be of obvious interest to look at the interactions of these precursors with cellulose. Such studies are currently under investigation.

To date, sucrose gradient centrifugation appears as the most convenient procedure for separating the 70 S RNA from the low molecular weight species. However, isolation of false 70 S molecules resulting from aggregation of smaller non viral RNA components cannot be excluded by this procedure. To conclude, the cellulose column offers a rapid and reliable method which could replace the sucrose gradient, especially when isolation on a large scale is required.

Acknowledgements

This work was performed pursuant to Contract NE 72 3263 with the National Cancer Institute, National Institute of Health. It was also supported in part by a grant from the Commissariat à l'Energie Atomique for the radioactive products.

References

[1] F. Galibert, C. Bernard, P. Chenaille and M. Boiron, C.R. Hebd. Séances Ac. Sci., Paris, 261 (1965) 1771.
[2] W. Robinson, A. Pitkanen and H. Rubin, Proc. Natl. Acad. Sci. U.S. 54 (1965) 137.
[3] P.H. Duesberg, Proc. Natl. Acad. Sci. U.S. 60 (1968) 1511.
[4] M.C. Lai and P.H. Duesberg, Nature 235 (1972) 383.
[5] P.A. Kitos, G. Saxon and H. Amos, Biochem. Biophys. Res. Commun. 47 (1972) 1426.
[6] C. Bernard, J. Lasneret and M. Boiron, C.R. Hebd. Séances Acad. Sci. Paris 264 (1967) 2170.
[7] C.J. Larsen, R.E. Ravicovitch, A. Samso, J. Robin, A. Tavitian and M. Boiron, (1973) in press.
[8] P. Tiollais, F. Galibert and M.A. Auger, Biochimie 54 (1972) 331.
[9] C.J. Larsen, R.E. Ravicovitch, J. Robin, M. Mauchauffe, M. Bazilier and M. Boiron, C.R. Hebd. Séances Acad. Sci. Paris, Série D, 274 (1972) 1396.
[10] K.S. Cheung, R.E. Smith, M.P. Stone and W.K. Jocklik, Virology 50 (1972) 851.
[11] E. Canaani, K.V.D. Helm and P. Duesberg, Proc. Natl. Acad. Sci. U.S. 70 (1973) 401.
[12] G.A. Bray, Anal. Biochem. 1 (1960) 279.