Journal of Chromatography, 90 (1974) 99–103

CHROM. 7190

FRACTIONATION OF RNA ON A METAL ION EQUILIBRATED CATION EXCHANGER

I. CHROMATOGRAPHIC PROFILES OF RNA ON AN AMBERLITE IR-120 (Al^{3+}) COLUMN

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(First received October 8th, 1973; revised manuscript received November 6th, 1973)

SUMMARY

Amberlite IR-120, a polystyrene sulphonate type of cation exchanger, equilibrated with Al^{3+} ions, has been employed for the fractionation of RNA. This adsorbent affords a quantitative and reproducible recovery of RNA into six fractions.

INTRODUCTION

Although several chromatographic procedures exist for the resolution of RNAs and DNAs[1–6], they do not provide adequate resolution of a complex mixture of functionally different RNAs necessary for the precise study of their metabolic role. In addition, they are not free from operational limitations such as clogging of the column, slow flow-rate leading to irreversible adsorption and consequent loss of biological activity, denaturation, low recovery, etc. In view of the above considerations, alternative methods of fractionation are necessary.

EXPERIMENTAL

Ribonucleic acid

The sodium salt of RNA was isolated from the liver of buffalo (Mammalia, Ruminantia), the chief milking animal of India, essentially by the procedure of Sevag *et al.*[7]. The purity of the isolated RNA was characterized by the usual methods[8–12], and was found to be 80% pure, containing about 10% protein residue and no DNA contamination. Hyperchromic studies showed a 40% increase in the UV absorbance at 260 nm, indicating that the isolated RNA is a native RNA.

Preparation of IR-120 (Al^{3+}) column

A 5-g amount of dry regenerated Amberlite IR-120 (Na^+) resin was allowed to swell in water for about 4–5 h and the slurry obtained was packed in a clean Pyrex glass column (45 × 1 cm). It was then equilibrated with aluminium ions by passing 50 ml of 0.2 *M* aluminium chloride solution through the column at a

flow-rate of 10–15 ml/h. This amount was found to be adequate for equilibration with respect to Al^{3+} ions, as judged from preliminary experiments. Finally, the pH of the column was adjusted to 4.0 by passing a sufficient amount of acetate buffer (pH 4.0, ionic strength, $\mu = 0.05$). The washing with buffer removes all of the loosely retained Al^{3+} ions. The amount of Al^{3+} in the influent, effluent and buffer washings were determined by Vogel's method[13], and it was observed that the resin retained 39.2 mg of Al^{3+} per gram of resin under the conditions used.

Typical chromatographic profiles of RNA

A homogeneous solution of buffalo liver RNA, isolated by the method of Sevag *et al.*[7] and characterized for its purity and nativeness, was prepared in acetate buffer (pH 4.0, $\mu = 0.05$) and was applied to a column containing 5 g of IR-120 (Al^{3+}), previously equilibrated with the above buffer. The RNA solution was allowed to percolate through the column at the rate of 12–15 ml/h, and this slow flow-rate permitted satisfactory equilibrium conditions to be attained. The effluent was collected and the column was washed with four bed volumes of the above buffer in order to remove any loosely retained RNA species.

The adsorbed RNA was then eluted by the continuous passage of 0.05 *M* ammonium acetate solution. The fractions, each of 10 ml, were collected and assayed for their RNA content by the thymol–iron (III) chloride–hydrochloric acid reaction[9].

The percentage of total RNA adsorbed and eluted is given in Table I. Fig. 1 shows typical chromatographic profiles of RNA on the IR-120 (Al^{3+}) column, the total optical density at 590 nm being plotted against the test-tube numbers of the fractions eluted.

TABLE I

TYPICAL CHROMATOGRAPHIC PROFILES OF BUFFALO LIVER RNA ON AN IR-120 (Al^{3+}) COLUMN

Percentage retention	*Percentage elution by 0.05 M ammonium acetate*						*Total elution* (F_1–F_6)	*Total recovery* (%)
	F_1	F_2	F_3	F_4	F_5	F_6		
100	32.18	19.33	15.04	19.67	1.97	4.63	92.82	100

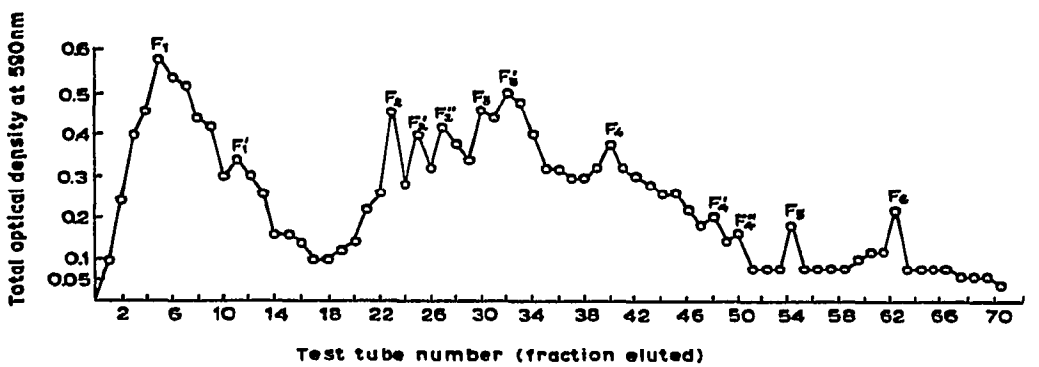

Fig. 1. Typical chromatographic elution profiles of buffalo liver RNA (method of Sevag *et al.*[7]) on an IR-120 (Al^{3+}) column.

Re-chromatography

Re-chromatography of a given fraction or a peak corresponding to a given fraction is an important aspect of any chromatographic technique. Reproducible chromatographic behaviour can be regarded as a sound criterion of the homogeneity of the material eluted in a specific fraction.

As fraction one (F_1) was the largest and most distinct fraction, it was chosen for re-chromatographic studies. It was extensively dialyzed against physiological saline at 4° in order to remove ammonium acetate completely, then re-chromatographed on a fresh IR-120 (Al^{3+}) column, as described above. The fraction was completely adsorbed and was eluted in a single peak and at the same position on the chromatogram (Fig. 2).

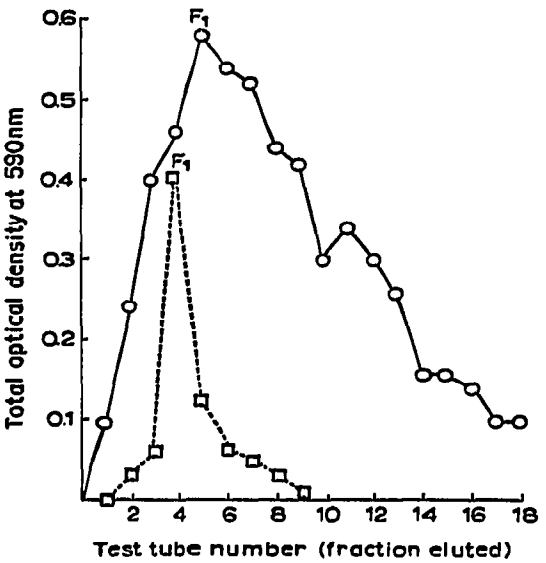

Fig. 2. Re-chromatography of fraction F_1 on an IR-120 (Al^{3+}) column: o—o, chromatography; □– – –□, re-chromatography.

Base composition

Different RNAs may differ in their base composition and/or the sequence of bases in the chain. In order to ascertain whether the IR-120 (Al^{3+}) column fractionates RNAs according to their composition, the base composition of three of the major isolated fractions was therefore studied.

RNA fractions were hydrolyzed by incubating them with 0.3 *M* potassium hydroxide solution at 37° for 16 h [14]. The resulting nucleotides were further subjected to hydrolysis with perchloric acid in order to liberate the bases.

From this digest, adenine was determined by the method of Woodhouse[15] and guanine by the Folin phenol reaction according to Hitchings[16]. After the determination of purine bases, they were quantitatively removed by precipitation as silver salts[17] and the pyrimidines, *viz.*, cytosine and uracil, were determined according to the method of Soodak *et al.*[18]. From this mixture, cytosine was then removed

by adsorption on Decalso cation exchanger[17] and uracil was determined as mentioned above[18]. The results are given in Table II.

TABLE II

BASE COMPOSITION OF RNA FRACTIONS ON AN IR-120 (Al^{3+}) COLUMN

Concentrations of bases are expressed as moles/100 moles of phosphorus.

Type of RNA	*Base contents*						
	A	*G*	*C*	*U*	$\frac{A}{U}$	$\frac{G}{C}$	$\frac{A+U}{G+C}$
Unfractionated	12.00	40.26	39.57	9.45	1.42	1.02	0.26
Fraction 1 (F_1)	11.80	40.40	39.72	8.48	1.39	1.02	0.25
Fraction 2 (F_2)	11.80	40.00	39.00	8.40	1.41	1.03	0.26
Fraction 3 (F_3)	19.50	35.30	37.89	7.35	2.65	0.93	0.37

RESULTS AND DISCUSSION

The failure of the IR-120 (Na^+) column to adsorb RNA[19] and the large increase in retention on the IR-120 (Al^{3+}) column shows that interaction with Al^{3+} ions on the IR-120 (Al^{3+}) column is the basis of adsorption. Dissociation of the Al^{3+}–RNA complex and its subsequent removal could be the basis for resolution–elution.

There are likely to be reversal types of linkages that bind RNA molecules to the IR-120 (Al^{3+}) column. In order that specific RNA molecules might progress down the column, these multiple linkages must be dissociated or disrupted simultaneously. Reagents such as EDTA, citrate and fluoride elute RNA from the IR-120 (Al^{3+}) column by their intrinsic ability to form a complex with Al^{3+} ions and thus displace RNA. With such reagents, most of the RNA is eluted in a single fraction, making resolution impossible. In such instances, the elution characteristics are less dependent on the concentration of the eluting agent than on its chemical nature. A changing gradient of these eluting agents will not, therefore, be of much help in such instances, when a basic technique has had to be established in order to study broadly the heterogeneity of RNA. In view of these considerations, a simple system consisting of only one eluting agent was employed, which resulted in complete elution.

The ability of ammonium acetate solution of low concentration (0.05 *M*) to effect elution, in spite of its not being a chelating agent, suggests that the binding between RNA and Al^{3+} ions is weak. The fact that 0.05 *M* ammonium acetate solution is able to effect complete elution together with fractionation indicates that the mechanism which governs this column chromatographic behaviour is probably a distribution between two phases.

Quantitative elution of a fraction in a single peak after re-chromatography not only shows that the eluted fraction represents one group of RNA, but also suggests that the fractionation procedure is reproducible.

Base composition studies indicated that the unfractionated buffalo liver RNA and the fractions (F_1–F_3) obtained after passage through the IR-120 (Al^{3+}) column have similar base compositions, and that they are guanine–cytosine rich.

The results suggest that the fractionation of RNA on an IR-120 (Al^{3+}) column is likely to be independent of base composition, and similar observations were reported by Mahler *et al.*[20] and Miura and Suzuki[21].

CONCLUSIONS

As the present studies were aimed mainly at understanding the heterogeneity of RNA and studying broadly its chromatographic behaviour, sub-fractionation or finer resolution was not attempted. It can be stated that the present fractionation procedure does not appear to be of a conventional ion-exchange chromatographic type because, in addition to the resin, Al^{3+} ions are also involved in the adsorption.

The present investigation has shown that the use of the IR-120 (Al^{3+}) column is a promising technique for the fractionation of RNA. Further, the IR-120 (Al^{3+}) resin is reasonably stable at elevated temperatures, over a wide range of pH and at high salt concentrations, and the technique offers a simple, reproducible and inexpensive system for the fractionation of nucleic acids. In addition, certain factors such as the amount of adsorbent, concentration of the adsorbate, marginal variation in flow-rate and ageing of RNA, do not have a significant effect on the chromatographic behaviour of RNA on the IR-120 (Al^{3+}) column.

ACKNOWLEDGEMENTS

The authors are grateful to Professor H. J. Arnikar, Senior Professor and Head of the Department of Chemistry, University of Poona, for providing laboratory facilities and for his interest in the work. One of the authors (V.S.) is thankful to the University Grants Commission and the University of Poona for financial assistance.

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