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Note

Fractionation of RNA on a metal ion-equilibrated cation exchanger

III. Chromatographic profiles of RNA: methodology and protein association

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Most methods of isolation do not result in the complete recovery of RNA contained in the starting material. Fractionation may therefore take place during the course of isolation, and RNA samples isolated from the same starting material by different isolation procedures are likely to differ in their composition and physicochemical properties as well as associated impurities[1].

Also, although different isolation procedures are similar in principle as far as the extraction of the ribonucleoprotein is concerned, they involve the use of various deproteinizing agents for the removal of protein from RNA. These agents include mild agents such as concentrated sodium chloride solution, drastic deproteinizing agents such as phenol, sophisticated agents such as sodium dodecyl sulphate (SDS) and enzymatic digestion. These deproteinizing agents are likely to be different in their mechanisms of deproteinization and can give RNAs that have different macromolecular conformations or base composition and also RNAs that have different amounts of residual protein associated with them.

It was, therefore, thought worth while to study if the methodology, *i.e.*, the method of isolation and deproteinization in particular, has any effect on the chromatographic profiles of RNA on an IR-120 (Al^{3+}) column.

METHOD OF ISOLATION

RNA samples were isolated from fresh buffalo liver after deproteinization according to the following methods:

(1) Sevag's *et al.* chloroform–amyl alcohol method[2];
(2) Kay and Dounce's SDS method[3];
(3) Kirby's phenol method[4].

RNA samples thus isolated were native and fairly pure (70–75%). They were, however, found to be associated with varying amounts (5–10%) of residual protein.

The RNA samples isolated by the above methods were chromatographed on an IR-120 (Al^{3+}) column as described in detail earlier[5]. The fractions were assayed for their RNA contents by the thymol–iron(III) chloride–hydrochloric acid reaction[6]. The percentage retention of RNA and the percentage elution of total

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF RNA ISOLATED BY DIFFERENT METHODS ON AN IR-120 (Al^{3+}) COLUMN

Method of isolation	*Retention (%)*	*Elution (%)*	*Profiles*
Sevag *et al.*[2]	100	100	6 fractions (F_1–F_6)
Kay and Dounce[3]	100	95	6 fractions (F_1–F_6)
Kirby[4]	100	97	6 fractions (F_1–F_6)

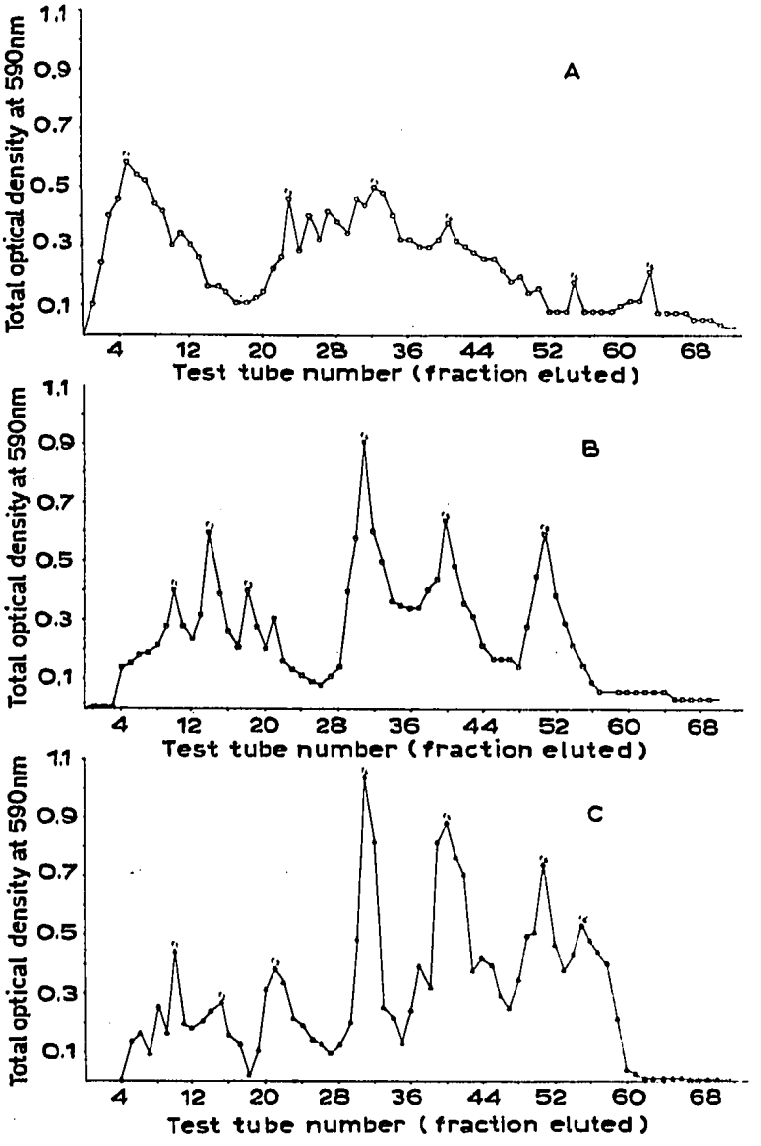

Fig. 1. Chromatographic elution profiles of buffalo liver RNA deproteinized by different methods, on an Amberlite IR-120 (Al^{3+}) column. Method: A, Sevag *et al.*[2]; B, Kirby[4]; C, Kay and Dounce[3].

adsorbed RNA are given in Table I. The chromatographic elution profiles are given in Fig. 1.

Effect of protein association

RNA samples containing different percentages of associated protein were deliberately isolated from buffalo liver by the method of Sevag *et al.*[2]. The amount of residual protein depends upon the number of deproteinizing treatments the ribonucleoprotein (RNP) solution is given with chloroform–amyl alcohol. Different percentages of associated protein can thus be obtained by controlling the number of deproteinizing treatments.

The amount of protein associated with different batches of RNA preparations thus varied largely owing to the number of deproteinisation treatments given. The treatments given ranged from a single deproteinization to extensive deproteinization with chloroform–amyl alcohol and also involving the use of pepsin digestion.

The RNA samples thus obtained were native, fairly pure and contained various amounts of protein (1.0–20%). Protein was estimated by Lowry's reaction[7]. RNA samples thus characterized for their nativeness and purity were dissolved in acetate buffer (pH 4.0; 0.05 *M*) and chromatographed on an IR-120 (Al^{3+}) column as described previously[5]. The fractions were assayed for their RNA contents by the thymol–iron(III) chloride–hydrochloric acid reaction[6].

The percentage retention of RNA and the percentage elution of the total adsorbed RNA associated with different amounts of protein are given in Table II. Fig. 2 gives the elution profiles of different RNA samples.

RESULTS AND DISCUSSION

Methodology

It is evident from Table I that RNAs isolated by all of the above mentioned methods are completely retained and eluted quantitatively into well-defined fractions.

RNA isolated by the method of Sevag *et al.*[2] furnishes typical chromatographic profiles yielding six reproducible fractions. The chromatographic elution pattern of RNA isolated by Kay and Dounce's method[3] involves significant changes in the elution pattern and percentage distribution. From Fig. 1, it is clear that the elution profile is shifted to the right and increasing amounts of RNA are eluted late in the chromatogram, indicating that RNA isolated by Kay and Dounce's method binds more firmly to the IR-120 (Al^{3+}) column and the dissociation of this binding requires a larger amount of 0.05 *M* ammonium acetate solution as an eluting agent.

The chromatographic elution pattern of RNA isolated by Kirby's method[4] also involves two changes, in the elution pattern and in the percentage distribution (Fig. 1). As with RNA isolated by Kay and Dounce's method[3], the profiles are also shifted towards the right. In case of Sevag *et al.*'s RNA, a large proportion of RNA is eluted earlier, while the later fractions are spread over large volumes and are displaced in comparison with the fractions obtained with the other two preparations.

As far as the nature of the elution is concerned, it seems to be governed by the stability of RNA–Al^{3+} complexes on the IR-120 (Al^{3+}) column. Weaker complexes, being more easily dissociated, appear earlier, while the more stable complexes appear later in the chromatogram.

TABLE II

CHROMATOGRAPHIC PROFILES OF BUFFALO LIVER RNA ASSOCIATED WITH VARYING PERCENTAGES OF PROTEIN ON AN IR-120 (Al^{3+}) COLUMN

Protein associated with RNA (with respect to RNA) (%)	*Retention (%)*	*Elution (%)*	*Profiles*
20.0	100	100	6 fractions (F_1–F_6)
10.0	100	100	6 fractions (F_1–F_6)
1.0*	100	99	6 fractions (F_1–F_6)

* Extensive digestion with pepsin at pH 4.0 at 37° for 24 h.

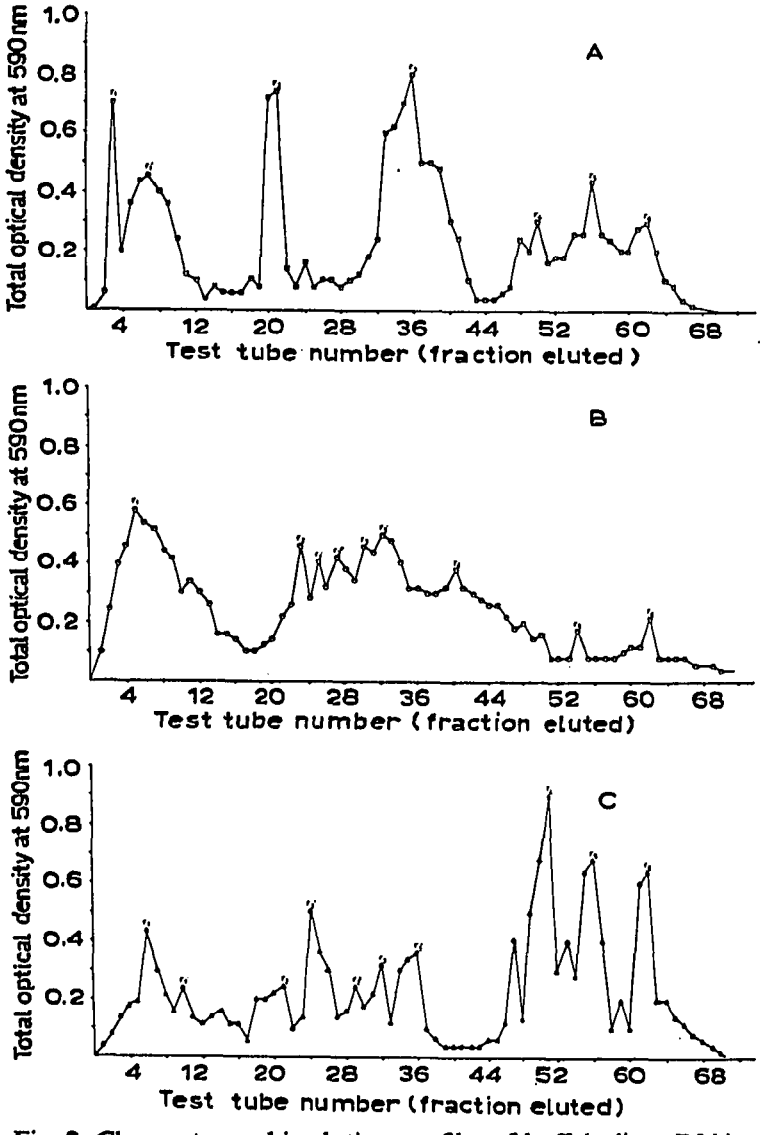

Fig. 2. Chromatographic elution profiles of buffalo liver RNA with different percentages of protein association, on an IR-120 (Al^{3+}) column. Protein association: A, 1%; B, 10%; C, 20%.

The differences in the chromatographic behaviour of RNA preparations isolated by different methods may be due to (a) different deproteinizing agents have different abilities to extract different RNAs, (b) different deproteinizing agents, depending upon their mechanism of deproteinization, bestow different conformations to the RNA upon deproteinization, yielding protein-free RNA entities (*i.e.*, having different three-dimensional structures), and (c) depending upon their ability, different deproteinizing agents leave different amounts of residual protein and deproteinizing agent with RNA, which in turn may play a role in the fractionation procedure.

Goldthwait and Kerr[8] observed that different methods of isolation of RNA from the same source gave different profiles under identical experimental conditions. They also showed that RNA isolated by SDS (Duponol) as a deproteinizing agent furnishes different elution patterns on ECTEOLA-cellulose, compared with that isolated using phenol as a deproteinizing agent. Mirza and Cannon[9] also showed that different methods of preparation of RNA yield different elution patterns on DEAE-cellulose and on polyacrylamide gel electrophoresis. Klee and Staehelin[10], however, found that after extensive purification, RNAs isolated by the phenol method and the SDS method are physically and enzymatically indistinguishable. Their observation that RNA isolated by the concentrated sodium chloride method shows different physicochemical properties is interpreted on the basis that it may have undergone considerable degradation during isolation. Such a possibility of differential degradation using different methods may exist. The present observations on the IR-120 (Al^{3+}) column are similar to the observations of Goldthwait and Kerr[8].

King[11] noted that sRNA prepared by Moldave's method[12] and Brunngraber's method[13] differed not only in their RNA content but also in their disc electrophoretic behaviour. Leisinger and Vogel[14] showed that *Escherichia coli* tRNAs had different distributions in different peaks, presumably as a result of the isolation procedure used or depending upon the stability of individual species of arginyl tRNA.

Protein association

It is clear from Table II that RNA preparations isolated from buffalo liver by the method of Sevag *et al.*[2] containing various amounts of protein (1.0–20%) were found to be completely retained on the IR-120 (Al^{3+}) column. Complete retention of RNA, in spite of the large variations in protein association in different preparations, indicates that the retentivity of RNA on an IR-120 (Al^{3+}) column is independent of the amount of protein associated. Complete elution is achieved in each case but there is a considerable difference in the elution patterns, depending upon the percentage of protein associated.

The profiles of RNA with 20% protein show a tendency towards late elution, indicating firm binding on the IR-120 (Al^{3+}) column. It was shown by Butler *et al.*[15] that even a small degree of protein association markedly alters the physicochemical and chromatographic properties of DNA; it may also be applicable to RNA. High protein association with consequent late appearance in the chromatogram indicates that protein might be altering the molecular weight of RNA by its close association, thus giving it an apparently large size and therefore firmer binding to the column, resulting in late elution. Fractionation on the IR-120 (Al^{3+}) column is to some extent dependent on molecular weight in addition to a finite three-dimensional structure, as has been shown by earlier workers during the frac-

tionation of DNA[16] and proteins[17]. Chromatographic profiles may be altered due to protein association, if the protein functioned to cross-link RNA molecules to form aggregates. This is particularly true if the fractionation is markedly dependent on molecular weight. It has been observed[18] that proteins also bind to IR-120 (Al^{3+}) over the pH range employed and this in turn can strengthen the binding of RNA.

RNA with 10% protein also shows a different elution pattern to that for RNA with 1.0% protein contamination. There is an apparent shift towards the left in the chromatographic elution pattern with a decreasing amount of protein, with respect to earlier emerging fractions, indicating that protein plays a significant role in the fractionation of RNA by altering the elution profiles, although it does not affect retention.

Singh and Keller[19] observed in their studies with wheat embryo RNA on a methylated serum albumin on a Kieselguhr column that slight protein association has no effect on the chromatographic elution profiles. Creaser and Spencer[20] observed that the separation of nucleoproteins on ECTEOLA-cellulose is influenced by the protein content of the nucleoprotein in addition to the base ratios. The observations on the IR-120 (Al^{3+}) column, with respect to protein content, are similar to those of Creaser and Spencer[20].

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