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SOME ASPECTS OF THE FRACTIONATION OF DNA ON AN IR-120 Al^{3+} COLUMN

V. THE EFFECT OF RNA CONTAMINATION ON THE CHROMATOGRAPHIC PROFILES OF DNA

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SUMMARY

The effect of RNA contamination on the chromatographic behaviour of DNA using an IR-120 Al^{3+} column has been studied. For this purpose, DNA and RNA were simultaneously isolated from fresh buffalo liver by the method of SEVAG *et al.*[1], purified and characterised for their purity as well as nativity. The adsorbability of RNA on IR-120 Al^{3+} column was studied prior to studying its effect upon the DNA profiles. It was noted that RNA is retained more tenaciously by the IR-120 Al^{3+} column than DNA. DNA contaminated with RNA was chromatographed and it was found that RNA contamination has no significant influence on the DNA profiles using IR-120 Al^{3+} column.

INTRODUCTION

According to the modern concept of protein biosynthesis, all the genetic information is transferred from DNA to RNA and the latter acts as a functional expression of DNA for the protein synthesising system. In view of their close association and functional relationship in multiple biological systems, it was thought worthwhile to study the effect of RNA contamination on the chromatographic profiles of DNA on an IR-120 Al^{3+} column. The term "RNA contamination" here means the deliberate addition of RNA to DNA *i.e.* RNA supplied exogenously, in contrast to a "DNA–RNA hybrid" which shows distinct characteristics, *viz.* nondigestibility by pancreatic ribonuclease etc.

EXPERIMENTAL

The IR-120 Al^{3+} column

10 g of dry regenerated Amberlite IR-120 (Na^+ form), polystyrene sulphonate, were equilibrated with a 0.2 *M* aluminium chloride solution to give the IR-120 Al^{3+} column[2,3]. Glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) was percolated through the column till the pH of the influent and effluent were the same. This column was then

used for studying the adsorbability of RNA and also for studying the effect of RNA contamination on the chromatographic profiles of DNA.

Ribonucleic acid

Yeast RNA (B.D.H. Great Britain). This preparation was about 80 % pure as judged from the phosphorous analysis data and the presence of orcinol positive material. It contained 1–2 % protein residue.

Buffalo liver RNA. This preparation was isolated from fresh buffalo liver by the method of SEVAG *et al.*[1]. It was characterised for its purity as well as nativity, and found to be a highly polymerised preparation, about 85 % pure as seen from the phosphorous analysis data and the presence of orcinol positive material. It contained about 5 % protein residue.

Although the method of RNA isolation followed may not be an ideal one, it was used to avoid any complications[4], *viz.* the effect of methodology.

Deoxyribonucleic acid

The sodium salt of DNA, used in these experiments, was isolated simultaneously from buffalo liver by the method of SEVAG *et al.*[1]. It was a white fibrous and fairly pure preparation[2]. Its purity and nativity were examined by the usual methods[2].

Procedure

Chromatography of RNA alone. The RNA samples were dissolved in glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) to obtain homogeneous solutions. At this pH, RNA is not very stable for long periods and is likely to be slowly degraded to its constituent mono- and oligonucleotides. However, as the objective was to ascertain whether RNA is retained on an IR-120 Al^{3+} column under the conditions which are optimal for DNA retention[5], the adsorption was studied at pH 8.6.

RNA solutions were separately loaded on to IR-120 Al^{3+} columns, and were allowed to percolate at the rate of 10–15 ml/h. The effluents were collected and the column washed with at least three bed volumes of the above buffer to remove any loosely retained RNA. Elution of the adsorbed RNA was attempted by following a discontinuous gradient elution procedure with the usual sequence of eluting agents[2] employed for DNA. The fractions, 25 ml each, were analysed for their RNA content by BIAL's orcinol reaction[6,7].

The results are given in Table I. Fig. 1 gives the percent retention of RNA, the

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF RNA ON AN IR-120 Al^{3+} COLUMN

Source of RNA	*Retention* (%)	*Elution* (%)	*Elution profiles*
Yeast	60	95	Major fraction in 0.5 *M* saline. Minor fraction in 1.0 % EDTA
Buffalo liver	100	100	Elutable only by 0.1 *M* sodium hydroxide

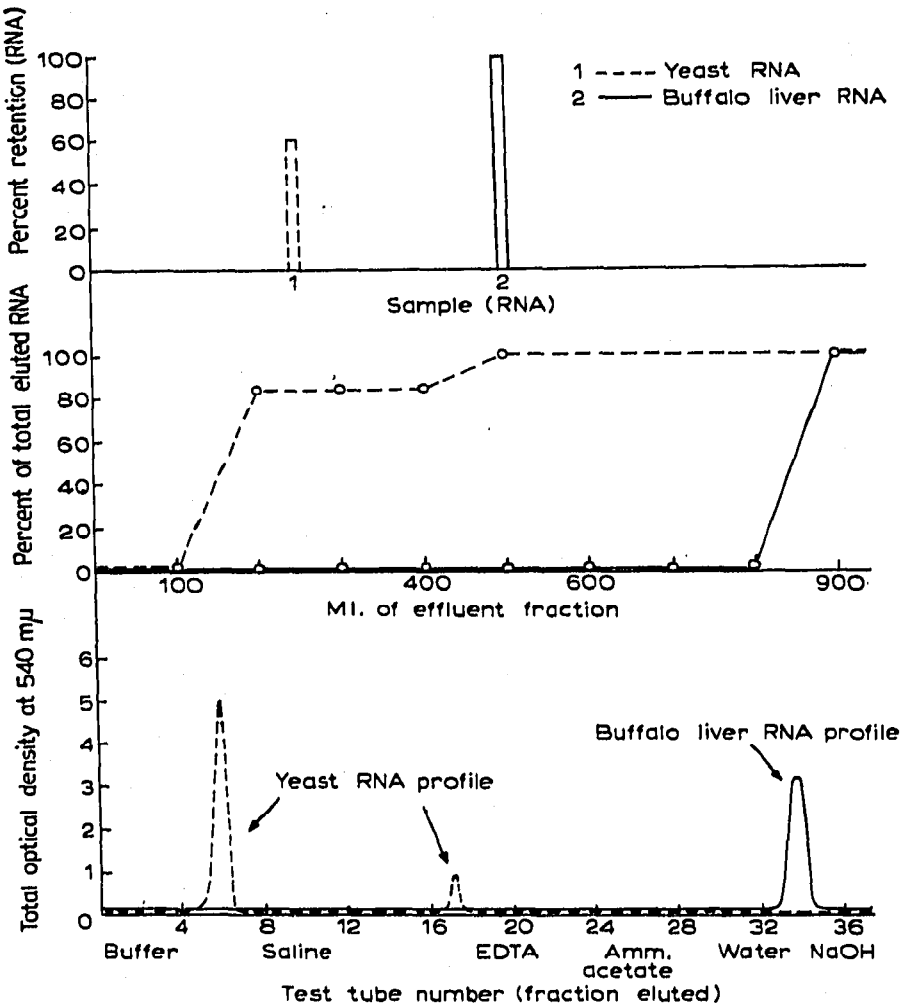

Fig. 1. Chromatographic behaviour of RNA on an IR-120 Al^{3+} column.

TABLE II

EFFECT OF RNA CONTAMINATION ON THE CHROMATOGRAPHIC PROFILES OF DNA ON AN IR-120 Al^{3+} COLUMN

System loaded	*Retention* (%)	*Elution* (%)	*Elution profiles*
DNA alone	100	100	Seven typical fractions (F_1–F_7)
DNA + 5% RNA	100	100	Seven typical fractions

percent of total eluted RNA in the different fractions obtained, as well as the elution profiles with the usual sequence of eluting agents wherein total optical density at 540 mμ, *i.e.* orcinol positive material, is plotted against the test tube number or the fractions eluted.

Chromatography of DNA in presence of RNA. Homogeneous solutions of DNA and RNA were obtained in glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*); RNA

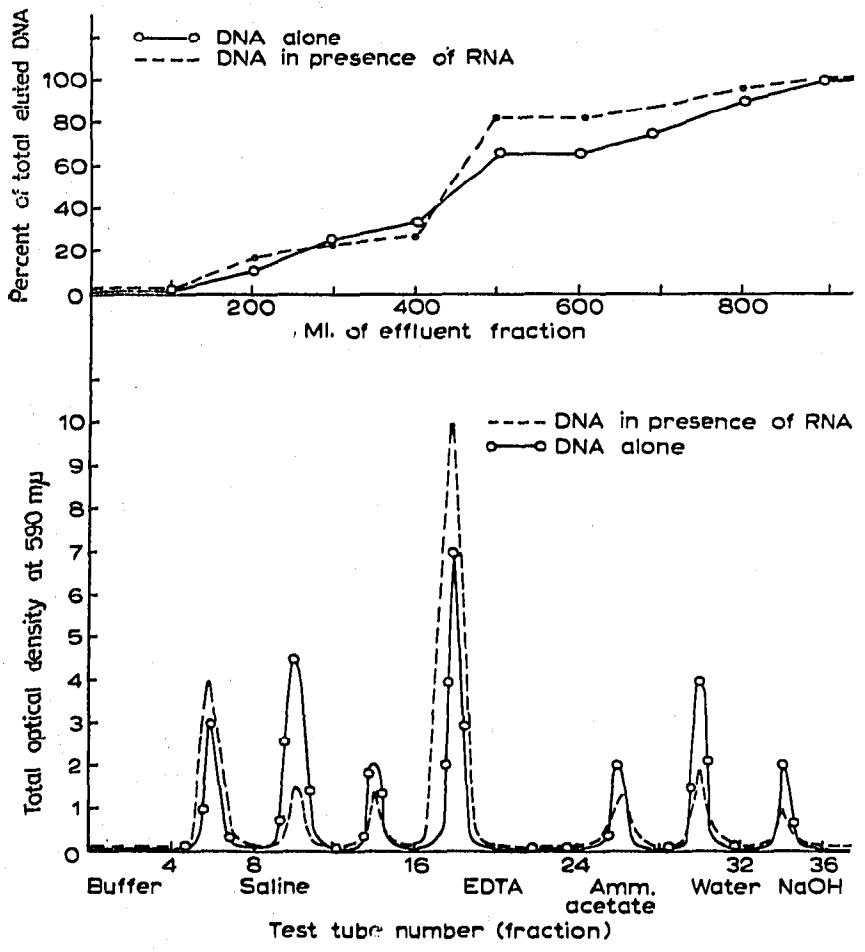

Fig. 2. Effect of RNA contamination on chromatographic profiles of DNA on an IR-120 Al^{3+} column.

solution being added to the DNA solution so that the effective concentration of RNA in the resulting mixture was 5 % by weight. The mixture was then loaded on to an IR-120 Al^{3+} column. The adsorbed system was eluted with the usual sequence of eluting agents[2]. The fractions, 25 ml each, were assayed for both DNA and RNA by BURTON's diphenylamine reaction[8] and by BIAL's orcinol reaction[6,7], respectively.

The percent retention, percent elution and nature of the elution profiles are given in Table II. Fig. 2 shows a graph of the percent of total eluted DNA against different fractions obtained with different eluting agents. It also gives the elution profiles obtained, wherein the total optical density at 590 mμ, *i.e.* diphenylamine positive material, is plotted against the test tube number or the fractions eluted. (The adsorption and elution of RNA is not shown.)

RESULTS AND DISCUSSION

Behaviour of RNA on the IR-120 Al^{3+} column

It can be seen from Table I and Fig. 1 that yeast RNA is 60 % retainable, while buffalo liver RNA is 100 % retainable. The percent elution is, however, 95 and 100 % respectively. Yeast RNA is eluted as a major fraction in 0.5 *M* saline and a minor

fraction in 1 % EDTA, whereas buffalo liver RNA is eluted by 0.1 *M* sodium hydroxide only.

Experimental observations showed that (i) buffalo liver RNA was difficult to dissolve, while yeast RNA was easily soluble; (ii) buffalo liver RNA was precipitated by acid, while the 40 % nonretainable fraction of yeast RNA was not readily precipitated by similar treatment; (iii) maximum precautions were necessary during the isolation of the buffalo liver RNA in order to obtain a highly polymerised preparation; the use of acid, alkali or heat was scrupulously avoided. The isolation procedure was carried out in cold solutions, at a near neutral pH with the addition of citrate to control and minimise the ribonuclease action. On the other hand, yeast RNA, a commercial preparation, may have undergone depolymerisation or degradation during its isolation resulting in scission at certain *loci*, giving a comparatively degraded product.

It seems from the above that the buffalo liver RNA is a highly polymerised preparation, while the yeast RNA is a comparatively degraded product, which may be reflected in the adsorption behaviour of these entities. It has already been reported[9] in the case of DNA that the IR-120 Al^{3+} column requires a finite polymerised state for adsorption and has least affinity for nucleotides. Similar requirements may also be a prerequisite for RNA adsorption. Recent studies by SHANKAR AND JOSHI[10] possibly point in the same direction.

Effect of RNA contamination on the profiles of DNA on an IR-120 Al^{3+} column

From Table II, it appears that there is no difference in the percent retention and chromatographic profiles of DNA in the absence and presence of RNA. Fig. 2 shows small differences in the percent distribution, which may not be of much significance.

As the total amount of DNA and RNA loaded is much less than the capacity of the column, the column may not be saturated. Competition, therefore, need not occur between DNA and RNA for adsorption sites, hence DNA profiles may not in any way be influenced in the presence of RNA. It may, however, be remembered that the effect of RNA contamination and not of the DNA–RNA hybrid is being studied.

Only one fraction, elutable with 0.1 *M* sodium hydroxide, indicated the presence of RNA as judged by BIAL'S orcinol reaction (not shown in Table II or Fig. 2). This observation indicated that complex formation between IR-120 Al^{3+} and RNA is stronger and more stable than that with DNA, which can be eluted by a simple salt gradient or even by distilled water. Elution of RNA only in 0.1 *M* sodium hydroxide solution, substantiates the fact that RNA has no role in the fractionation of DNA. A similar observation has also been made by KIT[11]. He has shown that RNA contamination (about 5 % as judged by the difference between UV positive and diphenylamine positive material) has no effect on the chromatographic behaviour of DNA on an Ecteola-cellulose column. It was further noticed that RNA was spread over many fractions. It has also been reported[12,13] that RNA not elutable by salt solutions could be eluted by 0.1 *M* ammonia in 2.0 *M* saline. The present observations are somewhat similar except that RNA is only eluted in one fraction by 0.1 *M* sodium hydroxide.

MAIN *et al.*[14] have, however, found that RNA is eluted earlier from a calcium phosphate column in the presence of DNA, suggesting the displacement of RNA from the column by DNA. Displacement of RNA by DNA has also been noted by MANDELL AND HERSHEY[15] on a methylated serum albumin kieselguhr column. Existing data does not support the displacement phenomenon in the present studies.

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