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Note

Hydrophobic interaction of ribosomal ribonucleic acids on a nitrocellulose column

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The non-covalent attachment of polynucleotides to polysaccharide matrices has been extensively used in quantitative biology, starting with the DNA–agar technique[1]. Thus, nitrocellulose can effect an interaction with single stranded DNA, poly(A)+mRNAs and poly(A) (for reviews see refs. 2 and 3). However, there is little information on the retention of rRNA subunits on a nitrocellulose column. We have recently described conditions resulting in selective retention of the larger (28S) rat liver rRNA on a nitrocellulose column[4]. The main purpose of this communication is to compare nitrocellulose with other polysaccharide matrices used previously for selective or less selective retention of individual rRNA species (Sepharoses, agaroses and agar).

EXPERIMENTAL

In view of the need for accurate quantitation at low polynucleotide concentrations, the majority of the retention experiments were done with uniformly labelled rRNAs.

Rat liver rRNAs were isolated essentially as described for microsomal RNAs[5]. Labelling with [6-^{14}C]orotic acid (28 mCi/mmol; Institut Boris Kidrič, Beograd), 0.2–0.4 mCi i.p. per animal, was carried out for at least 48 h. rRNAs were freed from 4–6S RNAs and other contaminants and then stored as described[5,6].

Pea seedling rRNAs were isolated as described by Petrović *et al.*[7] and Huang and Bonner[8]. The seeds were placed in distilled water containing carrier-free $Na_2H^{32}PO_4$ (15 μCi/ml; more than 1000 Ci/g P; Institut Boris Kidrič) and soaked in the same solution throughout the germination period.

The isolation of *Escherichia coli* rRNAs was as described by Petrović *et al.*[7] and the separation of DNA and tRNA from rRNAs was done on Sepharose 4B[9].

Poly(A)+mRNAs were separated from rRNAs using poly U Sepharose as described previously[10].

Sucrose gradient centrifugations for preparative separation of rRNAs were as described by Petrović *et al.*[7].

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The nitrocellulose column (NitroCel S, Serva) was prepared as described by Boezi and Armstrong[11]. Flow-rates necessary for reproducible RNA retention patterns at any given temperature and salt molarity were below 5 ml solvent per hour per ml nitrocellulose bed.

Alkali chlorides used (RCl) were all analytical grade and their actual molarities were routinely checked by refractometry.

RESULTS AND DISCUSSION

The capacity of nitrocellulose columns for retention of unfractionated rat liver rRNAs is shown in Fig. 1. Capacities of 0.14–0.15 mg of rRNA per ml of packed nitrocellulose were obtained in the range of 1.8–2.2 *M* NaCl in buffer A [0.1 %, w/v, sodium dodecyl sulphate (SDS)–0.0025 *M* EDTA–Na, pH 7.5–0.02 *M* Tris–HCl, pH 7.5], at 20°C.

The temperature dependence of rRNA retention was examined in some detail for two high-molecular-weight rRNAs from rat liver. As seen in Fig. 2 and Table I, in the range of 20–40°C, the NaCl molarities necessary for 18S and 28S RNA retention show a slightly saturating increase for both polynucleotides. The retention of rat liver 28S and 18S rRNA on nitrocellulose is dependent on the molarity of NaCl. As shown in Fig. 2, the molarity necessary for rRNA retention suggests that the smaller rRNA is trapped at a higher concentration of NaCl than is the larger rRNA. The larger

TABLE I

TEMPERATURE DEPENDENCE OF SODIUM CHLORIDE CONCENTRATION REQUIRED FOR 50% IMMOBILIZATION OF RAT LIVER rRNAs ($M^{NaCl}_{50\%}$)

Values obtained by graphical extrapolation from the data plotted in Fig. 2A and B.

RNA	$M^{NaCl}_{50\%}$				
	20°C	*Increment (M/°C)*	*30°C*	*Increment (M/°C)*	*40°C*
28S	0.61	0.039	1.0	0.030	1.3
18S	1.05	0.060	1.65	0.055	2.2
NaCl molarity ratio	1.72		1.65		1.69

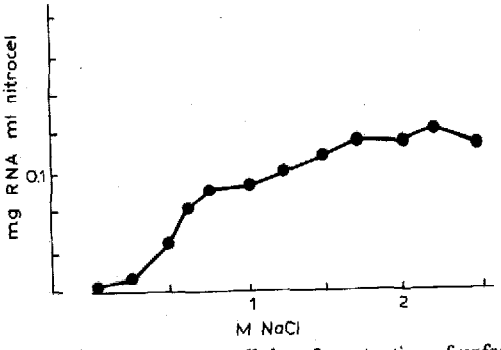

Fig. 1. Capacity of nitrocellulose for retention of unfractionated rat liver rRNAs. A nitrocellulose column (NitroCel S, Serva) of 5 ml was used (20°C). RNA was solubilized in buffer A–NaCl.

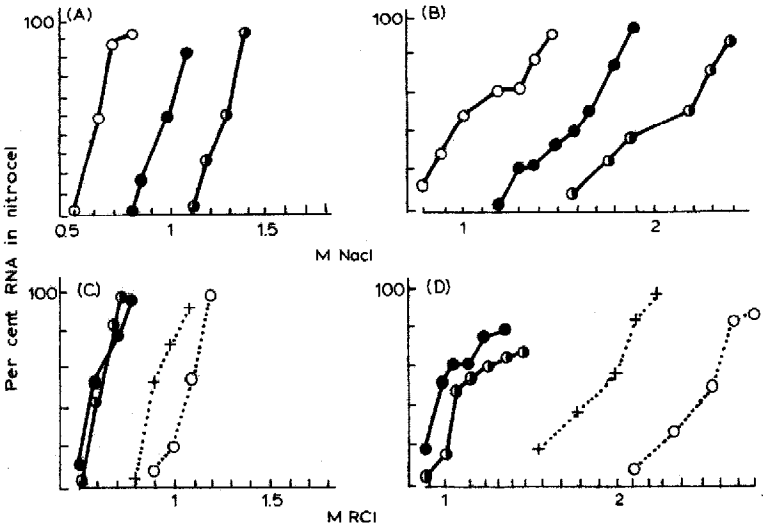

Fig. 2. A, B, Retention of rat liver rRNAs on a nitrocellulose column as a function of sodium chloride concentration and temperature. 10-ml nitrocellulose column with 0.1–0.15 mg RNA labelled with [6-^{14}C]orotic acid (0.2 mCi *in vivo*, for 48 h). A, Retention of 28S RNA (○—○, 20°C; ●—●, 30°C; ◑—◑, 40°C); B, retention of 18S RNA (details as in A). C, D, Retention of rat liver rRNAs on nitrocellulose columns as a function of the concentration of various alkali chlorides (RCl). Column as in A, B. C, for 28S RNA, ●—●, LiCl; ◑—◑, NaCl; + ··· +, KCl; ○ ··· ○, CsCl; D, for 18S RNA, details as in C.

RNA was retained at concentrations between 0.5 and 0.7 *M* NaCl in buffer A (20°C) with a 50% retention at 0.61 *M* NaCl ($M_{50\%}^{NaCl}$). The smaller rRNA was retained at concentrations between 0.9 and 1.7 *M* NaCl (20°C) with $M_{50\%}^{NaCl} = 1.05$. Thus, at concentrations between 0.7 and 0.9 *M* NaCl (20°C) it is possible to achieve a clear-cut selective retention of 28S RNA.

Table I suggests that the temperature dependence of the retention of 18S RNA is greater than that of the retention of 28S RNA (increment, M °C 0.030–0.039 for 28S NA and 0.060–0.55 for 18S RNA).

To determine the dependence of the retention process on the nature of the cation species, the retention of both 28S and 18S rRNAs from rat liver were studied in solutions of LiCl, NaCl, KCl and CsCl (Fig. 2C and D). It was found that the retention of both rRNAs displays cation selectivity in the order $Li^+ > Na^+ > K^+ > Cs^+$. Thus, the effects of neutral salts on the adsorption process do not follow the order of their chaotropic effects, *i.e.*, the Hofmeister series. The $M_{50\%}^{RCl}$ ratios at 20°C (Fig. 2) showed some correspondence with ratios of the crystal ionic radii[7,12].

Both molecular weight and base composition may be important parameters for RNA immobilization on nitrocellulose columns. Fig. 2 (A and B) shows that two rat liver rRNAs which differ greatly in molecular weight and base composition[13,14] show large differences in retention molarities. On the other hand, the two invertebrate rRNAs with less pronounced differences in both these parameters[15,16] showed more similar retention molarity ranges (Fig. 3A and B). These relationships were not linear, however. For example, 25S rRNA having a considerably higher molecular weight than 18S rRNA, had the same $M_{50\%}^{NaCl} = 1.05$ and the same saturating retention (Figs. 2B and 3B).

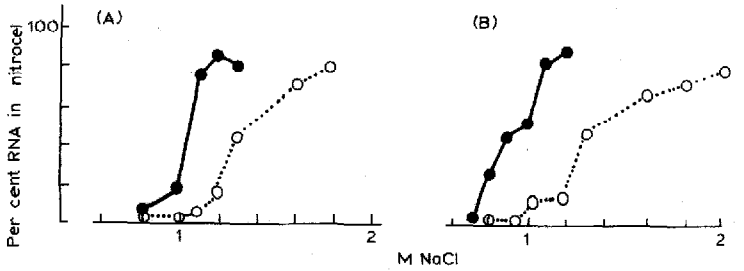

Fig. 3. Retention of bacterial (A) and plant rRNA (B) on NitroCel as a function of sodium chloride concentration at 20°C in buffer A. A, ●—●, 23S RNA *E. coli*; ○---○, 16S RNA *E. coli*; B, ●—●, 25S RNA pea seedlings; ○---○, 17S RNA pea seedlings. 5-ml nitrocellulose column charged with 0.1 mg of ^{14}C-labelled *E. coli* rRNAs or [U-^{32}P]rRNAs of pea seedlings (see Experimental).

Gel-like aggregation of rRNA subunits at high ionic strength or in polar solvents has been used for many years to effect their separation from DNA and tRNAs or to attempt their fractionation (for reviews see refs. 17, 18). Later, the open particle structure of polygalactoside resins was employed as a support for gelation (Sepharoses[19], agaroses[7] and agar[5]). These helical polysaccharide suports were used for hydrophobic selective or non-selective chromatography of various rRNAs. Immobilization on polygalactoside resins has also been employed to isolate and separate specific[20,21] or non-specific mRNAs[10,22] and as a general method for the separation of three major classes of nucleic acids in living systems (DNA, tRNAs and rRNAs[9]).

The retention process on NO_2-polyglucose (nitrocellulose) is in many respects similar to that on polygalactosides (Sepharoses[19], agaroses[7] and agar[5,23]). For example, in all cases the capacity for rRNA retention was limited, arguing strongly against non-specific aggregation or random precipitation as the mechanism of immobilization. The capacities for unfractionated rat liver rRNAs were (mg rRNA per ml adsorbent): 0.4–0.42 for agaroses[7]; 0.34–0.35 for agar[5]; 0.14–0.15 for nitrocellulose (Fig. 1). Thus it seems that increasing the content of charged groups in a polysaccharide diminishes its capacity for rRNA retention. (It is known that agaroses contain less sulphate and carboxyl groups than agar[24,25]; nitrocellulose is not a very appropriate designation[26] since in addition to possessing NO_2 groups the cellulose is

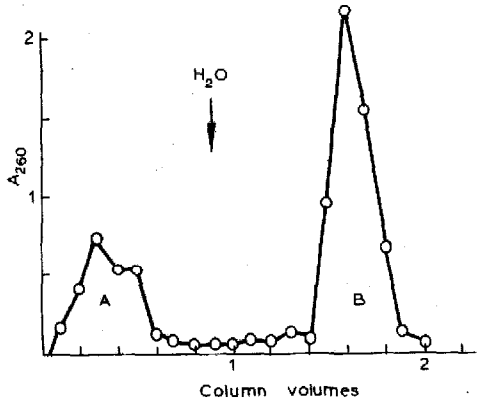

Fig. 4. Standard separation of 1.5 mg of unfractionated rat liver cytoplasmic rRNA subunits. For details see ref. 4.

also acetylated[27].) It is difficult to explain his phenomenon.

It is known that the electrostatic interaction decreases upon increasing the ionic strength[25,28]. Thus, at a concentration of 1.0 *M* (or higher), NaCl tends to quench charge effects[29]. We emphasize that hydrophobic binding is actually stabilized by certain salts[30,31]. A plausible explanation may lie in the open, well hydrated, particle structure of polygalactoside resins, whereas nitrocellulose is a fibrous, less hydrated support. The ratio $M_{50\%}^{NaCl}$ (20°C) for 18S and 28S rRNA is 1.72 (Table I), indicating that the separation capacity of nitrocellulose for high-molecular-weight rRNAs (rat liver) is slightly lower than that calculated for agaroses (2.56[7]) and agar (2.8[23]). However, in the range of 0.7–0.9 *M* NaCl a clear separation of rat liver rRNA is possible (Fig. 4). We note that the identification, purity and other characteristics of 18S and 28S RNA were as described previously[4]. It seems that a better separation of bacterial and plant rRNAs can be achieved when using nitrocellulose than agaroses. Thus, the partial purification of rRNAs on nitrocellulose is probably possible (Fig. 3), whereas on agaroses the retention profiles fully overlapped[7]. These differences support the conclusion that the retention process appears to be dependent on both the molecular size and structure of rRNAs as well as on the chromatographic support used for rRNA immobilization.

It is possible that nitrocellulose is a more complex chromatographic support for the retention of rRNAs than Sepharoses, agaroses and agar. Beside hydrophobic effects, electrostatic forces may interact especially at sodium chloride concentrations less than 1.0 *M*[29]. Some types of charge-transfer interaction might also be involved[32–34] (aromatic–aromatic interaction between the rRNAs and the lignin and polylignin components of cellulose and nitrocellulose).

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