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Note

Hydrophobic chromatography of 28S and 18S ribosomal RNAs on a nitrocellulose column

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It is known that nitrocellulose interacts specifically with single-stranded DNA, poly(A) + mRNA and viral RNAs (for reviews, see refs. 1–3). However, there is little information on the retention of rRNAs on a nitrocellulose column. We have observed that 28S rRNA may be selectively retained on a nitrocellulose column at high ionic strength. Thus, at a sodium chloride concentration of approximately 0.8 *M*, it was possible to achieve a clear separation of 18S polymer from 28S rRNA. 28S rRNA undergoes reversible adsorption, possibly associated with thermal gelation. Under these conditions 18S rRNA elutes in the void volume of the nitrocellulose column.

EXPERIMENTAL

RNA was isolated from liver microsomes of the Novi Sad variant of Wistar rats, as described previously[4], and freed from 4–6S RNAs and other contaminants by precipitation with 1 *M* sodium chloride solution[5]. Poly(A) + mRNA are separated from 28S and 18S rRNAs using poly(U) Sepharose, as described previously[6]. The solution of purified rRNAs in buffer A containing 0.8 M sodium chloride was kept frozen until used [buffer A: 0.1 % sodium dodecyl sulphate (SDS)–0.0025 *M* EDTA, disodium salt, pH 7.5–0.02 *M* Tris-HCl, pH 7.5]. Buffer A was kept at room temperature until used, in order to prevent precipitation of the SDS.

Nitrocel S (Serva, Heidelberg, G.F.R.) was packed in jacketed glass columns; 10–50 ml beds were used with 2–10 mg of RNAs. The columns were carefully equilibrated with 0.8 *M* sodium chloride solution in buffer A before packing. All column operations were carried out at temperatures above 21°C because of the tendency of the SDS to salt out at the relatively high salt concentration employed. The concentration of rRNAs in the range 2–5 mg/ml did not influence the separation. Elution was carried out at 25°C, with a flow-rate of 20–25 ml/h per 100-ml column. Higher temperatures resulted in elution of increasing amounts of 28S rRNA together with 18S rRNA. After elution of 18S rRNA in the void volume of the nitrocellulose column, 28S rRNA was eluted by changing the concentration of sodium chloride in buffer A to 0.1 *M*. Slightly more than one column volume of this buffer was needed for complete elution of the larger rRNA. The nucleic acids were recovered from the eluates by ethanol–ether diethyl concentration and precipitation[4]. The average recovery of the total A_{260} absorbing material applied to the column was 97–99 %. In

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addition, we did not detect UV-absorbing material eluted with 0.1 *M* sodium hydroxide solution after the chromatography of RNAs.

Electrophoresis was performed in 1.25% agar gels prepared with phosphate–citrate buffer (pH 8.0) as described by Tsanev[7]. The electrophoretic bands in the dry agar film were located by direct spectrophotometric analysis at 260 nm.

The nucleotide compositions were determined by the method of Petrović and Brkić[8].

RESULTS AND DISCUSSION

Fig. 1a shows the standard separation of rRNAs using a nitrocellulose column. The electrophoretic mobilities of rRNAs isolated from peaks A and B in Fig. 1 a are shown in Fig. 1b. This analysis suggests that the electrophoretic mobilities of peak A and peak B RNA are similar to those of cytoplasmic 18S and 28S RNA, respectively. Cross-contamination was not observed. The ratio of 28S rRNA to 18S rRNA was in the range 2.15–2.2 (Fig. 1a), which is in agreement with the ratio of their molecular weights and suggests their total separation. The nucleotide composition revealed significant differences between the two isolated rRNAs, especially in overall G–C content (Table I). In this respect the nucleotide composition of rRNA isolated by nitrocellulose chromatography gives results similar to those of Hirsch[9].

The methods most commonly used for the separation of rRNAs are density gradient centrifugation[10] and agar or acrylamide gel electrophoresis[7,11]. Although both provide good separations, they have limitations and are not easily used for the

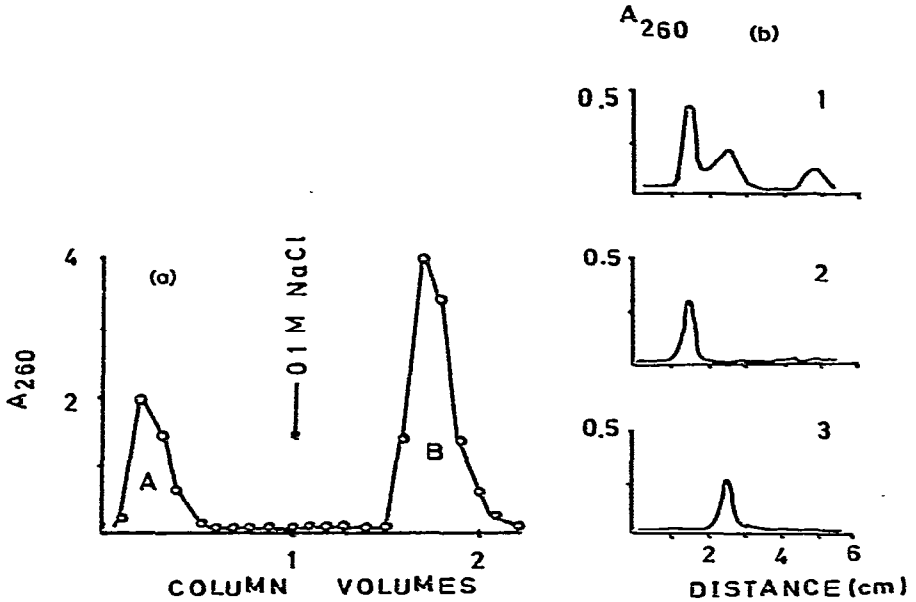

Fig. 1. (a) Fractionation of 3.1 mg of rat liver 28S and 18S mixtures on a 50-ml bed of Nitrocel S. Elution with 0.8 *M* NaCl in SDS–Tris–EDTA buffer (see text) and then with 0.1 *M* NaCl in the same buffer. (b) Electrophoretic mobility of diethyl ether–ethanol precipitation of 28S and 18S rRNAs, separated by nitrocellulose chromatography run as in (a). Electrophoretic patterns of rRNAs isolated from peak A (3) and peak B (2) from Fig. 1 left, compared with cytoplasmic rat liver rRNAs (1).

TABLE I

NUCLEOTIDE COMPOSITION OF rRNAs ISOLATED BY NITROCELLULOSE CHROMATOGRAPHY

The data are algebraic means of four determinations. Numbers in parentheses are standard deviations.

RNA	*Amount (moles per 100 moles of nucleotide)*				*G + C (mole-%)*
	Cytidylic acid	*Adenylic acid*	*Uridylic acid*	*Guanylic acid*	
18S	27.5 (1.1)	24.8 (1.5)	21.5 (1.3)	27.1 (1.8)	56
28S	32.1 (1.8)	18.9 (1.4)	17.1 (1.7)	31.2 (1.2)	64

separation of large amounts of material. Other methods have been reported that have not achieved the same popularity, including column chromatography on methylated albumin on Kieselguhr[12], reversed-phase chromatography[13] and selective immobilization of rRNA on agarose[14] or agar[4] gel chromatography at high ionic strength. The nitrocellulose chromatography of rRNAs has same advantages in comparison with agarose or agar chromatography: (1) nitrocellulose columns give higher and constant flow-rates; (2) the flow-rate does not decrease with increasing concentration of RNA as it does in gel chromatography; (3) the nitrocellulose column does not contain UV-absorbing material, whereas in gel chromatography traces of UV-absorbing material owing to resin destruction and resin pigments; (4) 18S rRNA is eluted in the void volume of the nitrocellulose column, so that 28S rRNA may be eluted immediately; this is not so in gel chromatography, where a molecular sieving phenomenon exists (18S rRNA has a K_d value in the range 0.55–0.65). This is why the nitrocellulose column is faster.

Experiments at elevated temperatures revealed that the adsorption of the larger rRNA progressively decreased as the temperature increased. At 40°C nearly all rRNAs eluted within the void volume. This observation seems to support the idea that the established separation results from preferential thermal-dependent gelation of the larger rRNA under conditions of high ionic strength.

In conclusion, this paper has presented a potentially useful method for the rapid separation the two rRNA species found in eucaryotic ribosomes.

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