CHROMATOGRAPHIC SEPARATION OF RAT LIVER RIBONUCLEIC ACIDS AS CONSTITUENTS OF CYTOPLASMIC RIBONUCLEOPROTEIN COMPLEXES

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1. Introduction

We report here the results of the fractionation of RNA samples from rat liver cytoplasmic ribonucleoprotein particles absorbed on a celite column. The dissociation of these adsorbed RNP particles and subsequent elution of the released RNA's were effected by the salt and temperature gradient procedure as described previously for the fractionation of RNA on the MAK column [1,2].

The binding of RNP to the celite occurs exclusively through the protein component since purified RNA could not be adsorbed by the column. It is reasonable to surmise that such a technique which permits the fractionation of native RNP particles opens a way for the separation of RNA samples according to the tightness of RNA–protein bonds in the complexes.

2. Materials and methods

Cytoplasmic RNP particles were isolated by the magnesium precipitation procedure applied to the rat liver postmitochondrial supernatant [3]. The preparation consisted of polyribosomes, ribosomes and ribosome subparticles along with informosomes [4–7].

The RNP solution in 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.6, was passed through a column of celite 545 at room temp. followed by washing out of the unadsorbed material. The salt–urea dissociation of the RNP was then carried out using a linear gradient from 5 mM $MgCl_2$ – 20 mM Tris-HCl, pH 7.6 to 2.0 M LiCl – 4.0 M urea – 5 mM $MgCl_2$ – 20 mM Tris-HCl, pH 7.6 at 37°. When the salt–urea gradient elution was completed the salt–urea concentration of the eluent was kept constant and the temperature was raised from 37° to 98° (1.3°/min). The elution profile was registered with a Uvicord LKB (0.5 cm light path, A_{254}). The results of the spectrophotometric assay [8] of the ultraviolet absorbing material eluted showed that it represented nucleic acids containing practically no protein contaminants.

Abbriviations. RNP, ribonucleoprotein; rRNA, ribosomal RNA; MAK, methylated albumin-kieselguhr.

3. Results and discussion

Cytoplasmic RNP particles when subjected to celite column chromatography appear to display different characteristics in terms of RNA–protein interactions. The RNA components I, II and III could be eluted in the salt–urea gradient whereas heating was needed to obtain component IV (fig. 1).

The question arises as to the nature of the RNA fractions separated by this technique. To answer this question the nucleotide composition (table 1) and the ultracentrifugation velocities of the corresponding RNA samples in a sucrose gradient (fig. 2) were determined. Unfortunately, the amount of component I was too small (and its separation from component II in some cases was rather poor) to be analyzed by sucrose-density-gradient centrifugation.

The most plausible interpretation of the results presented concerning the nature of components II and III would be that they were 18 S and 28 S rRNA,

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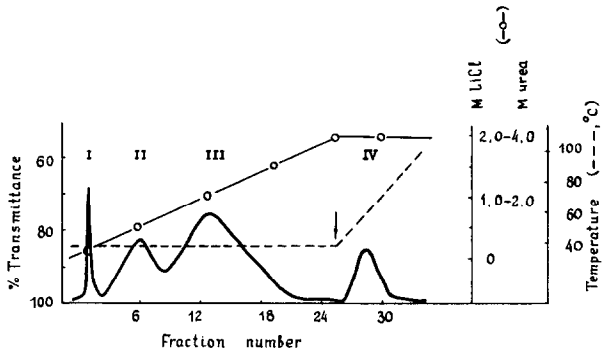

Fig. 1. Fractionation of cytoplasmic RNP particles on the celite column. Solution of RNP particles (about 35 optical units at A_{260}) in 15 ml 5 mM $MgCl_2$ – 20 mM Tris-HCl, pH 7.6, was passed through the celite column at room temp. The nonadsorbed material was washed out of the column with the same buffer. A salt–urea gradient from 5 mM $MgCl_2$ – 20 mM Tris-HCl, pH 7.6, to 2.0 M LiCl–4.0 M urea–5 mM $MgCl_2$ – 20 mM Tris-HCl, pH 7.6, at 37° was used. When the salt–urea gradient elution was completed the salt–urea concentration was kept constant and the temperature was increased from 37° to 98°. The arrow indicates the start of the temperature gradient. (——): % transmittance at 254 nm; (o——o——o): salt–urea concentration; (– – –): temperature gradient.

Table 1
Nucleotide composition of rat liver RNA fractions separated by celite chromatography.

Fraction	Amount of nucleotide (mol. %)				(G+C)/(A+U) ratio
	CMP	AMP	GMP	UMP	
Total RNA from postmitochondrial supernatant	25.6	15.0	37.0	22.4	1.68
Components I	26.1	21.3	29.0	23.6	1.23
II	25.4	22.1	31.2	21.3	1.31
III	29.0	16.7	34.8	19.5	1.76
IV	27.2	17.5	33.0	22.3	1.50

Nucleotide composition was determined by the method of Katz and Comb [11] after hydrolysis of RNA fractions with 0.3 N KOH at 37° for 18 hr.

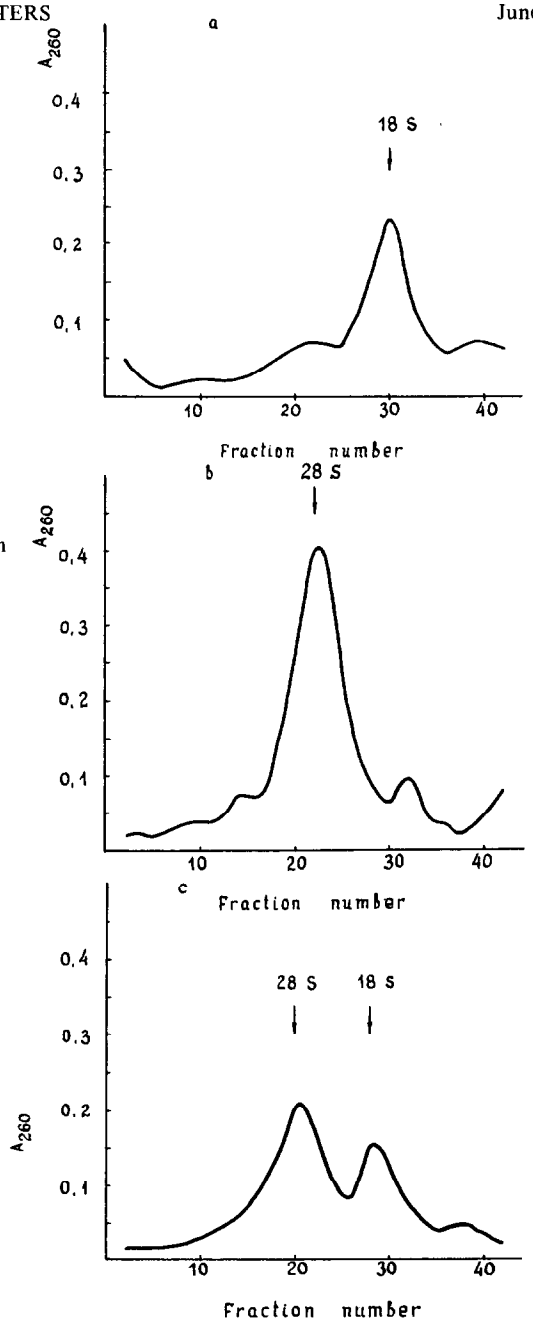

Fig. 2. Sucrose-density-gradient centrifugation of a) component II, b) component III and c) component IV of RNA samples separated by celite chromatography. Centrifugation of RNA samples was effected in a linear (5–20%) sucrose density gradient containing 100 mM NaCl, 10 mM sodium acetate, 1 mM EDTA, pH 5.1, on a Spinco model L2 centrifuge, with a SW 50 rotor at 37,000 rpm for 200 min at 4°. The S values were calculated by the method of Martin and Ames [12]. Tubes were pierced and 3-drop fractions were collected for A_{260} measurements.

respectively, They have both nucleotide compositions and sedimentation coefficients similar to those of rRNA components. In addition, the quantitative ratio of these components (III/II) is about 2.5 which is characteristic of the rRNA species.

Thus the small ribosomal subparticle seems to be less resistant to the dissociating action of the salt–urea eluent than the large one in spite of the fact that the former contains a higher proportion of protein [9,10]. In other words, there is no strict correlation between the relative proportion of protein within a RNP complex and its resistance to these dissociating factors.

The nature of minor components (I and IV) is less clear. As to component I, its nucleotide composition differs from that of rRNA being closer to the AU-type. Component IV can be identified with 18 S and 28 S rRNA judging from both the sedimentation velocity and nucleotide composition (fig. 2, table 1). This fact suggests that there is a small fraction within the pool of cytoplasmic ribosomes with rather unusual properties, namely a high resistance to the dissociating action of LiCl and urea. However, we cannot rule out at present an alternative proposition that component IV may be contaminated with the salt–urea rRNA fraction.

Experiments were carried out to test the possibility of redistribution of RNA in the course of the elution procedure, i.e. whether certain RNA molecules liberated from their association with the original proteins would complex again at random with alien proteins. Unlabelled cytoplasmic particles were adsorbed on the celite column and subjected to dissociation by using the standard elution precedure described above. Once the elution was started the labelled exogenous nuclear DNA-like RNA was introduced into the eluent. Measurements of the radioactivity showed that the labelled RNA was not adsorbed and passed straight through the column thus indicating that no redistribution occurred (fig. 3).

Thus it has been established that cytoplasmic RNP particles are different in respect of their susceptibility to the salt–urea dissociating action and their RNA could be fractionated according to this principle.

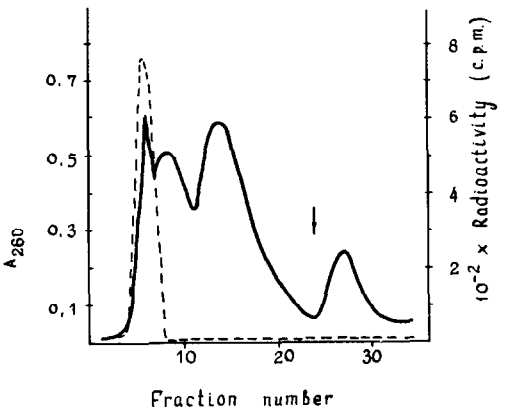

Fig. 3. Pattern of the distribution of exogenous labelled RNA added to the RNP particles adsorbed on the celite column. Unlabelled cytoplasmic RNP particles were adsorbed on the celite column under the conditions given in the legend to fig. 1. Rat liver nuclear RNA labelled after an actinomycin D-induced block of rRNA synthesis was isolated by the method of Georgiev and Mantjeva [13]. Labelled RNA (15 μg) was dissolved in 0.2 M LiCl–0.4 M urea–20 mM Tris-HCl, pH 7.6–5 mM $MgCl_2$ and passed through the celite column on which RNP particles were previously adsorbed. Then the salt–urea (from 0.2 M LiCl–0.4 M urea to 2.0 M LiCl–4.0 M urea) and the temperature gradients were effected as shown for fig. 1. (——) A_{260}; (– – –): radioactivity (cpm).

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