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THE SEPARATION OF RIBONUCLEIC ACIDS ON SEPHADEX COLUMNS

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SUMMARY

A method was developed for the chromatographic separation of soluble ribonucleic acid (sRNA) and ribosomal ribonucleic acid (rRNA). It involves the use of Sephadex G-75, which was equilibrated with 1.5 *M* NaCl and 0.5 *M* Tris buffer, pH 7.5. After the mixture was embedded, the column was eluted with 0.5 *M* Tris buffer using a 50 ml mixing pot. Excellent separation of sRNA and rRNA was obtained from purified samples as well as crude samples isolated from a human leukemic lymphoblast. Column recoveries were essentially 100%, and the method is fast and very reproducible. Other advantages, such as column capacities, non-denaturation and completeness of separation are discussed.

There are two general procedures usually employed for the separation of soluble ribonucleic acid (sRNA) from ribosomal ribonucleic acid (rRNA). One involves the isolation of the ribosomes from the cytoplasm by ultracentrifugation followed by the precipitation of the respective RNAs, as has been described with yeast[1], *E. coli*[2], and liver[3,4]. The other methods involve the fractionation of whole cell RNA, usually by selective precipitation with NaCl[5], LiCl[6], or streptomycin[7], followed by further purification such as column chromatography[8–10].

During the course of studies involving human leukemic lymphoblasts (CCRF-CEM), a method was developed for the separation of sRNA from rRNA which is applicable to whole cell crude RNA fractions. The procedure is based on "salting out" rRNA on a Sephadex G-75 column, using a solvent of 1.5 *M* NaCl, 0.05 *M* Tris pH 7.5. The sRNA passes through the column in the first few fractions and rRNA emerges in later fractions during a gradient elution with 0.05 *M* Tris pH 7.5. The method is rapid and easily reproducible. No expensive equipment is required, no denaturation occurs and the recoveries are essentially complete. The present report describes this procedure.

MATERIALS AND METHODS

Sephadex G-75-120 was equilibrated (72 h) with 100 ml of a solvent system containing various concentrations of NaCl and 0.05 *M* Tris pH 7.5, and fines were

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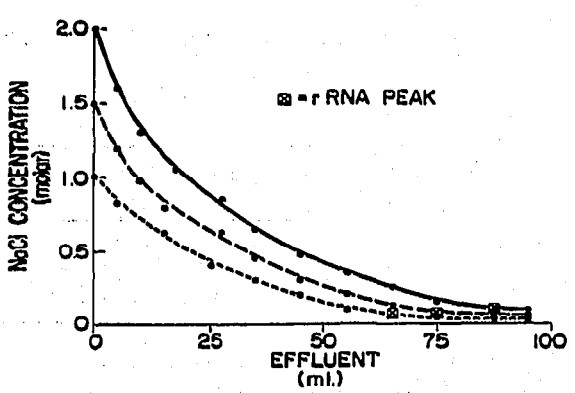
removed three times by aspiration. While the original studies were conducted with 9 mm × 25 cm columns, subsequent investigations utilized 15 mm × 25 cm columns (Lab Glass, Inc., Vineland, N.J.). One and 2.3 g of Sephadex were used in the 9 and 15 mm columns respectively. Before the Sephadex was introduced into the column, 2 ml of a 10% suspension of Whatman cellulose CC-31 (w/v) was placed directly upon the fritted glass disc. The gel was then introduced into the column and flushed with ten volumes of solvent. One milliliter aliquots of the samples were introduced on the column, followed by two 1 ml portions of solvent to embed the material. The column was then filled with NaCl–Tris solution, a 50 ml mixing pot was attached to the column and connected to a reservoir containing 0.05 *M* Tris, pH 7.5 for elution.

The batch-production of CCRF-CEM cells (20–25 g wet weight per 15 l suspension culture) has been described elsewhere[11]. Cells were harvested from such suspension cultures in a continuous flow Sorvall centrifuge, and whole cell crude RNA was extracted after removal of DNA according to the method described by KAY[12]. For control studies, sRNA and rRNA were isolated from yeast according to the methods described by HOLLEY *et al.*[13] and CRESTFIELD *et al.*[14], respectively. RNA was determined by ALBAUM AND UMBREIT'S modification of the orcinol method[15], and sodium determinations (calculated as NaCl) were made in a Perkin Elmer flame photometer.

RESULTS

Yeast rRNA was embedded on the several columns which had been equilibrated with different concentrations of NaCl. The columns were then eluted (by gradient) with Tris buffer, and some results are illustrated in Fig. 1. The rRNA peaks emerged at the 65, 75, and 85 ml fractions when the initial solvent system contained 1.0, 1.5, and 2.0 *M* NaCl, respectively, and the concentrations of NaCl in the eluates containing rRNA was found to be of the order of 0.01 *M*. When sRNA was embedded on the



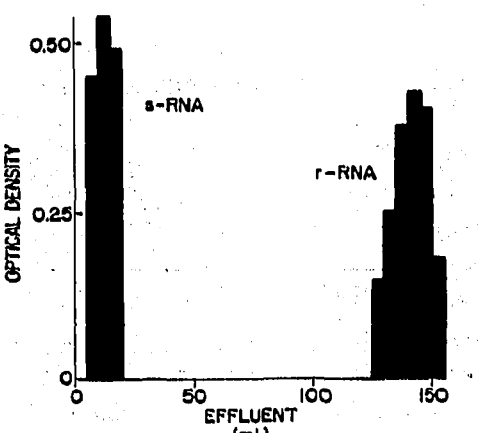


Fig. 1. The gradient elution of rRNA when embedded with solutions containing different concentrations of NaCl in Tris buffer. The mixing pot was 50 ml of embedding solution, and a solution of 0.05 *M* Tris buffer, pH 7.5 was the eluting solvent. For experimental details, see text.

Fig. 2. A typical elution pattern for the separation of sRNA from rRNA. The column was charged with a mixture of yeast sRNA (2.0 mg) and rRNA (2.0 mg). The column used was 15 mm × 25 cm containing 2.3 g of Sephadex G-75-120 equilibrated with 1.5 *M* NaCl, 0.05 *M* Tris, pH 7.5, and eluted with 0.05 *M* Tris buffer. A 50 ml mixing pot was used for the gradient elution.

column, the peaks were consistently eluted in the first 15 ml irrespective of the concentration of NaCl used. As a result, 1.5 *M* NaCl and 0.05 *M* Tris, pH 7.5 was selected as the embedding solution.

When mixtures of yeast sRNA and rRNA were placed on a 15 mm column, the peaks emerged in slightly different fractions (as compared to the 9 mm column). A typical elution pattern is shown in Fig. 2. sRNA was found between the 5th and the 20th ml while rRNA emerged in the 125th to 155th ml fractions. The capacity of the column was then tested and satisfactory separations were obtained when as much as 20 mg each of sRNA and rRNA were used. U.V. analyses were made in a Beckman DU spectrophotometer and sedimentation coefficients were determined on the starting material and the eluted samples. Both the initial and final material had the same absorbance and the same S rates.

Some recovery experiments were done to determine the column efficiency. Table I shows the results, using purified yeast sRNA and rRNA. With the exception of the 0.16 mg sample of rRNA, the recoveries from chromatography are in the order of 95–100 %, irrespective of whether the samples were embedded individually or

TABLE I

RECOVERY OF ISOLATED YEAST sRNA AND rRNA FROM SEPHADEX G-75 COLUMNS

Amount added (mg)		*Amount recovered (mg)*		*% recovered*	
sRNA	*rRNA*[a]	*sRNA*	*rRNA*	*sRNA*	*rRNA*
0	0.16	0	0.14	—	88
0	0.40	0	0.39	—	97
2.0	0	1.99	0	99	—
5.0	0	4.98	0	99	—
5.0	0.40	4.80	0.39	96	97

[a] Small amounts of rRNA were used due to the limited amount of material available.

TABLE II

RECOVERY OF YEAST sRNA AND rRNA AND HUMAN LEUKEMIC LYMPHOBLAST RNA FROM SEPHADEX G-75 COLUMNS

Milligrams added			*Total milligrams found*		*Yeast RNA*			
					Milligrams recovered[a] (calc.)		*% recovered*	
Crude CCRF-CEM RNA fraction[b]	*Yeast RNA*[c]							
	sRNA	*rRNA*	*sRNA*	*rRNA*	*sRNA*	*rRNA*	*sRNA*	*rRNA*
7.2	—	—	0.94	2.25	—	—	—	—
7.2	28.0	—	29.16	—	28.22	—	101	—
14.4	—	10.0	1.71	14.20	—	9.70	—	97
7.2	30.0	7.0	30.03	9.20	29.09	6.95	97	99

[a] Milligrams of yeast RNA recovered is calculated by subtracting the RNA found in the crude CCRF-CEM fraction from the total milligrams found. This calculated value is then compared to the amount of purified yeast RNA added to determine % recovery.

[b] The crude fraction was isolated by the method of KAY after removal of DNA[12].

[c] Commercial source: RNA (soluble) Type III and rRNA type XI (Sigma, St. Louis, Mo.).

as a mixture. When purified yeast sRNA was added to CCRF-CEM whole cell crude RNA, the recoveries of yeast RNA were approximately 100 % (see Table II). It was of interest to note that the whole cell crude RNA fraction contained approximately 43 % RNA. This, however, should not be considered as a reflection on the method of isolation, since KAY's procedure was not designed to isolate RNA in purified form[12]. Nevertheless, the column recoveries support the validity of the presently reported separation method.

DISCUSSION

There have been a number of chromatographic methods reported for the separation of RNAs. For example, DEAE cellulose[16,17], and ECTEOLA cellulose[18,19], have been used to develop satisfactory separation procedures. However, some degradation of rRNA occurs when these materials were used. Methylated serum albumin on Kieselguhr has been similarly used[20], but this system has a very low column capacity. Recently, BARBER[21] reported the use of unmodified cellulose and NaCl–ethanol solvent system for the separation of these nucleic acids. sRNA was recovered in the 2nd to the 13th (5 ml) fraction. At the end of the 14th fraction, the eluting solvent was changed to distilled water and rRNA was collected in the 15th to the 20th fraction. When ^{14}C-isoleucine-labeled sRNA was used on the unmodified cellulose column, the resulting elution pattern suggested a slight overlapping of the sRNA and rRNA fractions. With respect to column capacity, BARBER's method is superior to columns containing methylated albumin on kieselguhr in that 15 mg of rRNA could be adsorbed and recovered from a column containing 4 g of Whatman's cellulose powder CF-11. In the present studies, 2.3 g of Sephadex G-75 could readily separate 20 mg each of sRNA and rRNA. U.V. absorption and S-rates of the purified yeast RNAs before and after chromatography were found to be the same. This, in conjunction with the recovery experiments, indicated that no degradation of RNA occurred while it was on the column.

Some mention should be made of the relative amounts of sRNA and rRNA isolated from the CCRF-CEM cells. While KAY's method[12] of isolation was not intended to be quantitative, rRNA represented more than 70 % of the total RNA isolated from the cell. This observation is of particular interest since MCCARTHY *et al.*[22] reported that cytochemical analyses of the CCRF-CEM cells indicated a mean RNA:DNA ratio of 0.7:10, which is somewhat unusual for mammalian cells. These observations will be considered in more detail elsewhere.

The method of separating sRNA from rRNA described herein is easy to run, and is highly reproducible. It is readily applicable to the use of fraction collectors and does not require any attention during elution. The recoveries are approximately 100 %, no denaturation occurs, the column capacities are high, and it is readily applicable to whole cell RNA isolated from mammalian cells.

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