A SIMPLE METHOD FOR THE SEPARATION OF SOLUBLE RIBONUCLEIC ACID FROM RIBOSOMAL RIBONUCLEIC ACIDS USING Zn^{II} AS A PRECIPITANT

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For the preparation of soluble RNA (sRNA) from micro-organisms on a large scale, procedures based on the extraction of cells with phenol (Monier et al., 1960; Holley, 1963; Altunina et al., 1964; Brubaker and McCorquodale, 1963) or with isoamyl alcohol (Rammler et al., 1965) have been used. These methods are not suitable for the preparation of ribosomal RNA (rRNA). The extraction of animal tissues or micro-organisms with phenol (Kirby, 1956; Scherrer & Darnell, 1962; Zubay, 1962) or with hot detergent solutions (Crestfield et al., 1955; Ofengand et al., 1961) yields a mixture of rRNA and sRNA which are usually separated by precipitating rRNA with NaCl. Though the NaCl-soluble RNA has enhanced aminoacid-acceptor activity (Smith, 1960; Wicks et al., 1965), the separation is not complete. The use of $(NH_4)_2SO_4$ (Littauer & Eisenberg, 1959) and of 2M LiCl (Barlow et al., 1963) for the separation of rRNA and sRNA have been reported; though the fractions were characterized by analytical ultracentrifugation or sucrose density gradient centrifugation, the aminoacid-acceptor activity of the sRNA fraction has not been given. It is clearly desirable to characterize the fractions both by physicochemical methods and by measurement of biological activity since RNA fractions without aminoacid-acceptor activity have been reported to sediment in the 4S region on a sucrose density gradient (Brown, 1963; Greenman et al., 1964).

We have observed that Zn^{II} at a concentration of 0.1-0.2M specifically precipitates rRNAs from preparations which contain both the rRNAs and sRNA. The "zinc-soluble" RNA, when freed of Zn^{II}, sediments in the 4S region on sucrose gradient and has aminoacid-acceptor activity. The "zinc-insoluble" RNA contains the two rRNAs and is free from aminoacid-acceptor activity. The method is applicable to total RNA derived from rat liver or Escherichia coli and may provide a convenient procedure for the simultaneous preparation of both rRNA and sRNA on a large scale.

Materials and Methods

E. coli, grown on nutrient medium, was harvested during the log phase of growth. The cells were suspended in 0.01M acetate buffer (pH 5.1) containing bentonite (5 mg/ml), sodium dodecylsulphate (5 mg/ml), polyvinyl sulphate (5 mg/ml) and EDTA (0.001M), and extracted with an equal volume of phenol at 50° for 4 min. The aqueous and phenol layers were separated by centrifugation and the latter re-extracted with the buffer. RNA was precipitated from the combined aqueous layers with 2.5 vol of cold ethanol and dialysed.

The livers of rats fasted for 48 hr were homogenized in the above buffer medium and RNA extracted as above.

To obtain the precipitation curves, experiments were done in 0.1M acetate buffer at pH 4.5 in the case of E. coli RNA and at pH 5.5 in the case of rat liver RNA. Two ml aliquots of RNA solution (1 mg/ml) were mixed with 2 ml aliquots of zinc acetate solution (in the appropriate buffer) of varying Zn^{II} concentration, and left in the cold for 4 hr. The mixtures were then centrifuged to remove the precipitated RNA and from the O.D. readings of the supernatants at 260 mμ, the proportion of RNA precipitated at various Zn^{II} concentrations was calculated. An O.D. of

1.0 at 260 mμ in a 1 cm cell was taken as equivalent to 40 μg of RNA/ml.

When the zinc-soluble and zinc-insoluble RNA fractions were needed for characterization, 20 ml of a solution of E. coli RNA (pH 4.5) was mixed with 20 ml of 0.4M Zn^{II} solution. After standing for 4 hr, the precipitate and supernatant were separated by centrifugation; the precipitate was dissolved in a minimum volume of 0.1M EDTA adjusted to pH 7. This solution and the supernatant were then dialysed against water to remove Zn^{II}; the zinc-soluble RNA, after dialysis, was passed through a column of DOWEX 50W-X8 (potassium form). The RNA was precipitated with 2.5 vol of cold ethanol. With rat liver RNA the same procedure was followed excepting that 0.2M Zn^{II} solution and pH 5.5 were used.

Results and Discussion

Fig. 1 shows that in the case of both E. coli and rat liver total RNA preparations, increasing amounts of RNA were precipitated as the Zn^{II} concentration was raised from 0.01M upwards. At a Zn^{II} concentration of 0.1M, about 85% of RNA was precipitated; there was no further increase in precipitation with an increase in Zn^{II} concentration upto 0.5M. Since the proportion of "zinc-soluble" RNA corresponded approximately to sRNA content of the RNA preparations used, we were led to characterize both the RNA fractions, zinc-soluble and zinc-insoluble, by sucrose gradient centrifugation, and to measure their aminoacid-acceptor activity.

Fig. 2 indicates the following: (1) the total RNA preparations used contained the two rRNAs and sRNA; (2) in the case of rat liver RNA, the zinc-insoluble RNA fraction was virtually free of sRNA and the zinc-soluble RNA fraction free of rRNA, although the latter fraction seemed to be contaminated with a small quantity of a component with sedimentation characteristics intermediate between those of rRNAs and sRNA; and (3) the zinc-insoluble RNA fraction from E. coli contained the two rRNAs

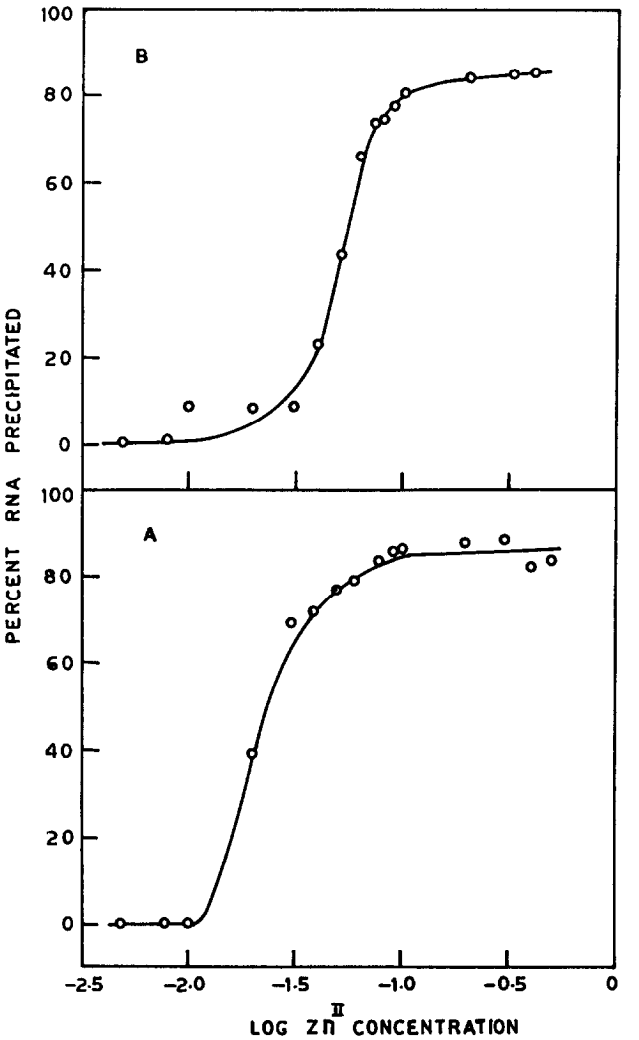

Fig. 1 Precipitation curves of RNA with Zn^{II}. A, rat liver RNA; B, E. coli RNA

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and a trace amount of a RNA sedimenting in the sRNA region, while the zinc-soluble RNA fraction was free from rRNAs and contained only sRNA.

The measurement of aminoacid-acceptor ability showed that this activity was associated with the zinc-soluble RNA fraction in the case of both E. coli and rat liver RNA; the zinc-insoluble RNA did not accept

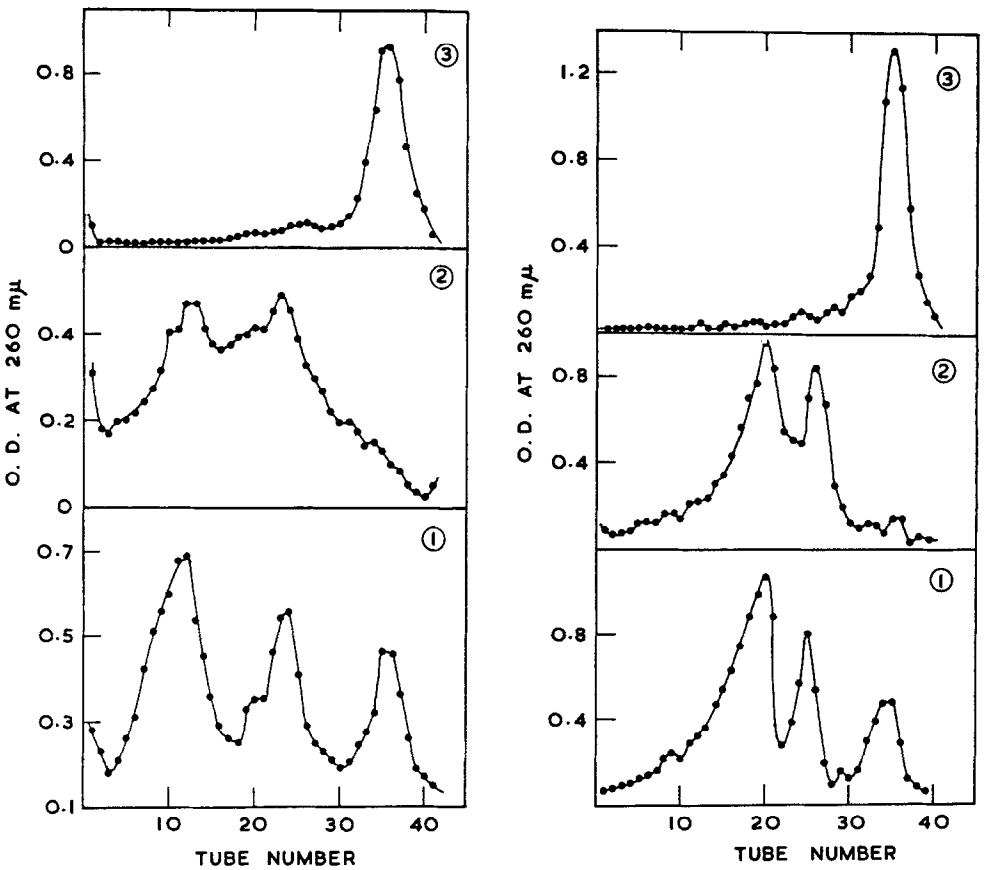

Fig. 2 Sucrose density gradient centrifugation patterns. A linear gradient of 5–20% sucrose in 0.01M acetate buffer (pH 5.0) containing 0.1M NaCl was used. After centrifugation for 16 hr at 24,000 rpm at 4° in SW Rotor 25.1 in a Spinco Model L ultracentrifuge, tubes were punctured, approximately 1 ml samples collected, and O.D. read at 260 mμ after dilution to 2 ml.

A, Rat liver RNA: (1) total RNA; (2) zinc-insoluble RNA; (3) zinc-soluble RNA.

B, *E. coli* RNA: (1) total RNA; (2) zinc-insoluble RNA; (3) zinc-soluble RNA.

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any aminoacids (Table 1). Further, this ability of zinc-soluble RNA fraction was of the same order as that of homologous sRNA ('standard sRNA') obtained by chromatography of total RNA on Sephadex G-100. This

shows that treatment of RNA with Zn^{II} does not result in a loss of aminoacid-acceptor activity.

Table 1

Assay for aminoacid-acceptor ability

A mixture (total vol 0.5 ml) containing RNA (50-200 µg), 50 µmoles of tris, 5 µmoles KCl, 5 µmoles $MgCl_2$, 5 µmoles ATP, 2.5 µmoles phosphoenol pyruvate, 40 µg pyruvate kinase and 3 µc (^{14}C)Chlorella protein hydrolysate was equilibrated at 36° for 6 min, and 0.1 ml of "pH-5 enzyme" fraction (6.0 O./ml at 280 mµ) was added. The mixture was incubated for 25 min and the reaction stopped by the addition of an equal volume of 10% TCA; 1 mg of BSA was used as a carrier for the precipitation of RNA. The precipitate was washed thrice with 5% TCA containing a mixture of unlabelled amino acids; TCA was removed by washing once with ethanol saturated with sodium acetate. The precipitate was dissolved in 2 ml of 2N NH_4OH and an aliquot plated on an aluminium planchet. Radioactivity was measured in a Tracerlab Counter with 5% efficiency for ^{14}C. The "pH-5 enzyme" was obtained from the 105,000 x g supernatant of lysed E. coli, and purified by adsorption on DEAE-cellulose (equilibrated with phosphate buffer, 0.02M, pH 7.5) and elution with the same buffer containing 0.3M KCl.

The activity measurements were made in the region where activity increases linearly with RNA concentration.

	Activity in counts/min/mg RNA
E. coli RNA	
Zinc-soluble RNA	12,975
Standard sRNA	14,350
Zinc-insoluble RNA	nil
Rat liver RNA	
Zinc-soluble RNA	5920
Standard sRNA	7200
Zinc-insoluble RNA	nil

In our experience two factors are of importance for good separation of rRNA and sRNA using Zn^{II}: (1) pH of precipitation and (2) absence of degraded material in RNA preparations. We have found that the best operating conditions are pH 5.5 (acetate buffer) for rat liver RNA and pH 4.5 (acetate buffer) for *E. coli* RNA, and that the pH has to be carefully controlled. The presence of degraded material in the RNA preparations introduces uncertainty in precipitation and should therefore be removed by prior dialysis. This investigation does not indicate the extent of contamination of zinc-soluble RNA (sRNA) and zinc-insoluble RNA (rRNAs) fractions with minor RNA components such as 5S RNA and messenger RNA.

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