The Separation of High and Low Molecular Weight RNA by Precipitation with N-Cetyl-N,N,N-trimethyl-ammoniumbromide

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Separation of Different RNAs, Various Objects

RNA with a sedimentation constant of 28S and 18S can easily be separated from 4S and 5S RNA.

The method depends on the different solubulities of nucleic acids in solutions of the cationic detergent N-cetyl-N,N,N-trimethyl-ammoniumbromide at various ionic strengths. The separation can be achieved with high efficiency in one step.

Introduction

The study of kinetics of protein synthesis often requires pulse labeling of RNA with high radioactivities. In this context, the rapidly labeled mRNA is often of special interest. Its separation from the high molecular weight ribosomal RNA as well as from the rapidly labeling 4S and 5S RNA raises some problems. These problems become serious when mRNA has only short or no poly-A sequences at all, so that the preparation of mRNA by affinity chromatography does not work properly. The preparative separation of such classes of RNA may be accomplished by sucrose density centrifugation or electrophoresis on agarose-acrylamide composite gels. This methods may be replaced by less tedious and less expensive methods. Recently a procedure basing on differential solubilities of RNA species at varying salt concentrations has been published [1]. We here describe a single step method for the efficient separation of high- and low molecular weight RNA by fractionated precipitation with the cationic detergent N-cetyl-N,N,N-trimethyl-ammoniumbromide (CTAB), which is a variation of the separation procedure for RNA and DNA [2, 3].

Materials and Methods

The moss *Polytrichum commune* was pulse labeled with [^{3}H]-Adenosine (38 Ci/mmol) and homogenized in liquid nitrogen as described previously [4].

RNA was prepared from gametophytes of the moss by the method of Aviv and Leder [5]. The total nucleic acid fraction was freed of DNA by treatment with deoxyribonuclease I (Boehringer, Mannheim) and subsequent deproteinisation with phenol [6]. RNA was precipitated by addition of sodium acetate to 2% and 2 volumes of ethanol standing over night at −20 °C. Poly-A containing RNA was adsorbed to columns of Poly-U-Sepharose 4B (Pharmacia, Uppsala) according to Puckett et al. [7] and eluted with salt free buffer at 45 °C. To minimise the amount of RNA bound unspecifically to Poly-U-Sepharose, the procedure was repeated.

RNA was analyzed on 2.5% or 3.4% polyacrylamide-agarose slab gels in the buffer system of Cupello and Hydén [8]. The size of the gel slab was 100 mm × 50 mm × 1 mm. Electrophoresis was achieved in an electrophoresis apparatus from Blum and Reichert (Mainz). The gels were run at 70 V and 35 mAmp for 90 min. Gels were stained in stains all [9] and read in a Zeiss densitometer.

For the determination of radioactivity, gels were sliced into pieces of 1.2 mm, solubilized and counted according to Adesnik and Darnell [10].

For the fractionated precipitation of RNA, total RNA was solubilized in NaCl 1 M, CTAB 1% (Merck, Darmstadt) at pH 8.0. Ionic strength was lowered by addition of appropriate volumes of salt free CTAB 1% (pH 8.0) under stirring. Conductivity of this solution was controlled by a conductometer from Michaelis, Mainz. Insolubilized RNA was sedimented by centrifugation at 40 000 × g for 20 min. All solutions and glassware had been sterilized before use.

Results and Discussion

0.5 – 1% of the total RNA of *Polytrichum commune* are insoluble in 1 M NaCl-1% CTAB. From this RNA nearly 4% are retained by Poly-U-Sepharose 4B. Electrophoretic analysis of this RNA shows some 28S and 18S ribosomal RNA. Most of the RNA does not penetrate the 2.5% acrylamide gel, probably since it has been aggregated artificially into a very high molecular weight product.

The RNA soluble in 1 M NaCl-1% CTAB has been fractionated by lowering the salt concentration in steps of 0.025 M NaCl. The amount of RNA precipitated at the different salt concentrations was checked by measuring the radioactivity of the precipitates in the liquid scintillation counter. As Fig. 1 shows, a high amount of total RNA precipitates at a salt concentration of 0.5 M, no detectable precipitation can be found between 0.4 and 0.35 M NaCl, while a second maximum of precipitation can be found between 0.3 and 0.2 M NaCl.

Electrophoresis on polyacrylamide-agarose slab-gels shows, that all RNA precipitated at salt concentrations above 0.4 M represents RNA with a sedimentation constant of more than 5S, while the 4S and 5S RNA precipitates at salt concentrations lower than 0.35 M. At 0.1 M NaCl about 98% of the RNA

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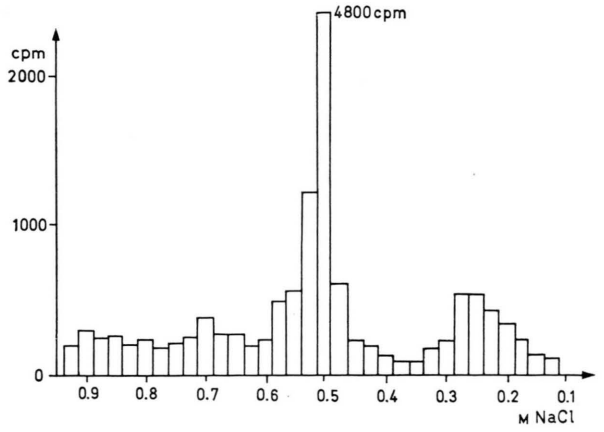

Fig. 1. Precipitation of [^{3}H]Adenosine pulse-labeled total RNA as CTA-salt at different NaCl-concentrations.

is precipitated from the solution, while only 2% of total radioactivity can be found in the supernatant. Half of it (i. e. 1%) can be precipitated with ethanol. It is 4S RNA mostly.

Fig. 2 shows the pherograms of RNA precipitated at 0.5 M NaCl and at 0.25 M NaCl. In the gel with 4S and 5S RNA no radioactivity can be found above 5S, while the other gel shows a high specific radioactivity in the region of 9 – 12S at the migration distance of 15 – 25 mm, were mRNA is to be expected. The CTA-salt of pure Polytrichum 8 – 12S mRNA which had been prepared by affinity chromatography, precipitated at salt concentrations above 0.4 M NaCl and thus cannot be separated by this method from ribosomal RNA (Fig. 3). It can however be demonstrated that mRNA does not precipitate at the same ionic strength as 4S and 5S RNA. Low molecular weight RNA, prepared with CTAB does not contain any RNA which is retained by Poly-U-Sepharose 4B. mRNA itself cannot be separated into different size-classes by the fractionated precipitation with CTAB. All fractions obtained show similar patterns by gel electrophoresis.

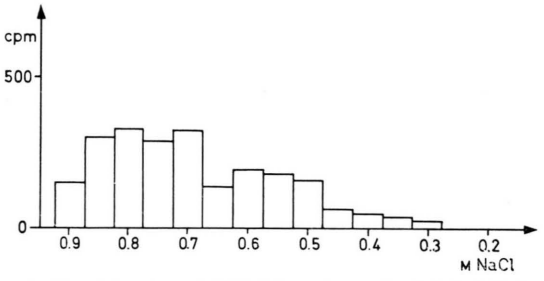

Fig. 3. Precipitation of [^{3}H]Adenosine pulse-labeled Poly-A containing mRNA as CTA-salt at different salt concentrations.

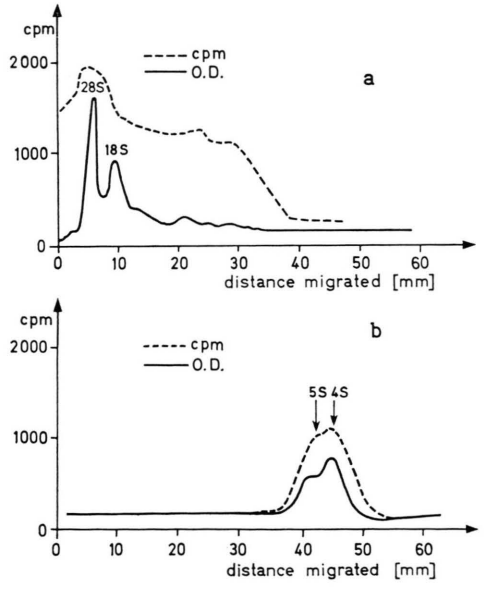

Fig. 2. Separation by electrophoresis on 3.4% polyacrylamide-0.7% agarose slab gels of high and low molecular weight RNA obtained by fractionated precipitation with CTAB. Fig. 2 a shows RNA precipitated at 0.5 M NaCl, Fig. 2 b shows RNA which precipitated at 0.3 M NaCl.

The method described, shows a convenient way to separate in a single step high molecular weight RNA from 4S and 5S RNA. It has been applied with good success to the RNA of the moss *Polytrichum commune*, as well as to RNA of mouse L-cells, rat liver and rat testes. For routine experiments, total RNA is dissolved in 0.9 M NaCl, 1% CTAB. This solution is then diluted 2-fold with 1% CTAB. This precipitates all high molecular weight RNA, leaving the 4S and 5S RNA in solution. When only ribosomal RNA and not mRNA is of interest, the method can be used for the separation of DNA and ribosomal RNA too. As described previously [2, 3, 11], DNA becomes insoluble at a concentration of 0.7 M NaCl and can so be efficiently separated from RNA. In order to avoid degradation of RNA, all glassware and solutions used have to be free of any ribonuclease activities, since under the conditions used, we could not find any ribonuclease inhibition by the cationic detergent solution.

In view of the fact that this applies to all steps at any stages it is especially advantageous that the separation described here, only comprises one single step.

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