ISOLATION OF RIBOSOMES FROM COTTON SEEDS

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A rise in the concentration of ions of bivalent metals (Ba^{2+}, Ca^{2+}) promotes the aggregation and precipitation of ribosomes [1, 2]. It is preferable to use magnesium ions for precipitation, since they are already present in the composition of the ribosomes, fulfilling the function of stabilizers of the compact structure. Consequently, magnesium ions [3, 4] and ethanol with an increased concentration of Mg^{2+} [5, 6] are used for the precipitation of ribosomes. In contrast to the classical method of isolating the ribosome fraction by differential centrifugation, precipitation by ethanol in the presence of Mg^{2+} permits the ribosome fraction to be isolated more rapidly and in larger amounts without having recourse to ultracentrifugation.

The aim of our investigations was the rapid isolation in preparative amounts of the cytoplasmatic ribosomes from cotton seeds with the subsequent extraction from them of the 5S-ribosomal RNA for structural investigations.

The isolation of a ribosome fraction from plants by this method has not been described in the literature, and we have therefore studied some parameters for the complete extraction of the ribosome fraction from a homogenate of the seeds. It has been established that raising the concentration of Mg^{2+} to 0.05 M leads to aggregation and to an increase in the amount of material precipitated, and a further rise in the concentration to 0.1 M leads to a decrease in it [3].

We brought the concentration of Mg^{2+} to 0.05 M and varied the amount of added ethanol from 0.2 to 0.4 of the volume of the extract. The completeness of the precipitation of the ribosomes was judged from the presence of 5S rRNA in the supernatant (Fig. 1). Raising the concentration of ethanol to 0.3 volumes enabled the time of centrifugation to be reduced from two hours (at 0.2 volumes of ethanol) to 1 h at 6000 rpm for the complete precipitation of the ribosomes (Fig. 2).

The ribosomes obtained were contaminated with a considerable amount of nonribosomal proteins. Their amount decreased considerably after the ribosomes had been purified by two reprecipitations with ethanol (0.3 volume) in the presence of 0.05 M Mg^{2+}. The results of purification were as follows:

Precipitation	RNA, %	Protein, %	$\frac{E_{260}}{E_{235}}$	$\frac{E_{260}}{E_{280}}$
I	13,7	86,3	0,74	1,3
II	29	71	0,84	1,5
III	36	64	1	1,55

The total ribosomal RNA isolated from the ribosomes by treatment with sodium dodecyl sulfate (SDS)–phenol were purified and the high-molecular-weight rRNA's were eliminated by chromatography on a column containing DEAE-cellulose. On analysis of the total rRNA by electrophoresis in 10% polyacrylamide gel (PAG) it was found that the percentage composition of the RNA's present in it (28S + 18S rRNA, 5S rRNA, and tRNA) changes considerably with a tendency to an increase in the amount of tRNA in the preparation with a rise in the concentration of ethanol from 0.2 to 0.3 volumes:

Amount of ethanol added	High-molecular-weight rRNA's, %	5S rRNA, %	tRNA, %
0,2 V	73,9	5,6	20,5
0,3 V	64,9	4,4	30,7

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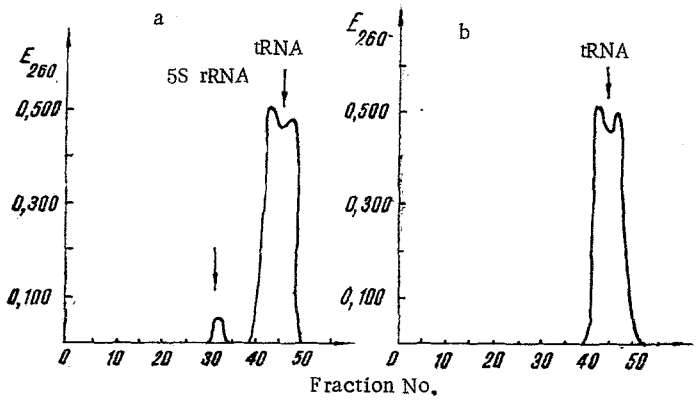

Fig. 1

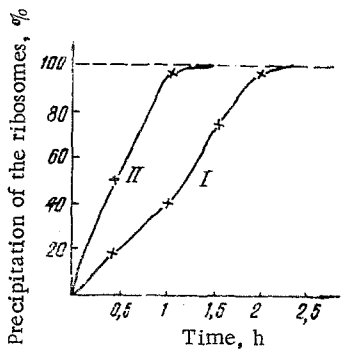

Fig. 2

Fig. 1. Electrophoresis of the total RNA from the supernatant after the column containing DEAE-cellulose; 10% gel, 5 mA per gel; a) 0.05 Mg^{2+} and 0.2 volumes of ethanol; centrifugation at 6000 rpm for 2 h; b) 0.05 M Mg^{2+} and 0.3 volumes of ethanol; centrifugation at 6000 rpm for 1 h.

Fig. 2. Dependence of the precipitation of the ribosome fraction on the time of centrifuging at 6000 rpm. Precipitation of the ribosomes: I) at 0.05 M Mg^{2+} and 0.2 volume of ethanol; II) at 0.5 M Mg^{2+} and 0.3 volume of ethanol.

Consequently, in order to decrease the amount of tRNA associated with the ribosomes, and also the amount of nonribosomal proteins, in the isolation process we performed a preliminary treatment of the extract with KCl at a final concentration of 0.5 M before the Mg^{2+}—ethanol precipitation. At this concentration of KCl the ribosomes dissociate into subunits and are freed from labilely bound tRNA.

The amount of tRNA in the total RNA after this treatment falls sharply, but the treatment with 0.5 M KCl leads to an increase in the amount of ribosomes in the supernatant, i.e., to their incomplete precipitation under the same conditions (0.05 M Mg^{2+} and 0.3 volumes of ethanol). The precipitation of the ribosomes was judged from the amount of 5S rRNA in the ribosomal fraction and in the supernatant. Below we give the percentage of rRNA included in the composition of the total RNA isolated by the use of 0.3 volumes of ethanol (I), and of 0.5 M KCl and 0.3 volumes of ethanol (II), and in the total RNA from the supernatant using 0.5 M KCl and 0.3 volumes of ethanol (III):

Total RNA	High-molecular weight rRNA	5S rRNA	tRNA
I	64,9	4,4	30,7
II	83,3	6,6	10,1
III	4,4	5,9	89,7

An increase in the amount of ethanol (to 0.4 volumes) in the extract led to the complete precipitation of the ribosomes from a solution with a relatively smaller tRNA content. The percentage composition of the components of the total rRNA (I) and the total RNA from the supernatant (II) isolated by the use of 0.5 M KCl and 0.4 volumes of ethanol were as follows:

Total RNA	High-molecular weight rRNA	5S rRNA	tRNA
I	77,5	9	13,5
II	0	0	100

It can be seen from the facts given below that the degree of purity of the ribosomes (I) obtained by precipitation with 0.05 M Mg^{2+} and 0.4 volumes of ethanol, after treatment with KCl, is already comparable with that of the reprecipitated ribosomes and is even superior in some indices (see above):

Ribosome	RNA, %	Protein, %	$\frac{E_{260}}{E_{235}}$	$\frac{E_{260}}{E_{280}}$
I	20,6	79,4	1	1,6

EXPERIMENTAL

Isolation of the Ribosomes from Cotton Seeds. The defatted flour of cotton seeds from variety 108-F (40 g) was homogenized with cooling (0°C) in 600 ml of buffer A: 0.05 M Tris-HCl, pH 7.5, containing 0.01 M $MgSO_4$, 0.05 M KCl, with the addition of sucrose to a concentration of 0.25 M. A ratio of 1:15 (wt/volume) was determined experimentally. To inhibit RNase activity we used heparin [7] (sodium salt) in a concentration of 0.25 mg/ml. The homogenate was centrifuged at 6000 rpm in a TsLR-1 centrifuge for 1 h. Dry KCl was added to the supernatant to a final concentration of 0.5 M. After the salt had dissolved, a 1 M solution of $MgCl_2$ to a final centration of 0.05 M and 0.4 of the volume of the extract of ethanol (96%` cooled to −20°C were added with vigorous stirring. After centrifuging at 6000 rpm for an hour, the resulting precipitate of the ribosome fraction (C) was rinsed out of the test-tube with buffer A, and, after being suspended in the same buffer, was used for the isolation of the total rRNA.

The ribosomes (B) from the extract were also isolated by the addition of a 1 M solution of $MgCl_2$ to a final concentration of 0.05 M and 0.3 volumes of ethanol without the use of KCl.

Purification and Characterization of the Ribosomes. The ribosomes (B) were suspended in 300 ml of buffer A and the suspension was centrifuged at 6000 rpm for 1 h. To the supernatant were added 1 M $MgCl_2$ solution to a final concentration of 0.05 M Mg^{2+} and 0.3 volumes of ethanol cooled to −20°C, and the mixture was centrifuged at 6000 rpm for 1 h. The precipitate of ribosomes was suspended in 150 ml of buffer A and the suspension was centrifuged at 6000 rpm for 1 h. To the supernatant were added a 1 M $MgCl_2$ solution to a final concentration of 0.05 M and 0.3 volumes of ethanol (96%) cooled to −20°C and the suspension was centrifuged at 6000 rpm for 1 h. The precipitate of the ribosome fraction was suspended in 75 ml of buffer A and the suspension was centrifuged at 6000 rpm for 1 h. The resulting solution of the ribosome fraction was used for determining its RNA and protein contents.

In order to follow the degree of purification, after each stage of reprecipitation the RNA and protein contents and also such characteristics of the purity of the preparation as the E_{260}/E_{235} and E_{260}/E_{280} ratios were found.

The total rRNA in the ribosomes was determined by A. S. Spirin's method [8], and the protein by Lowry's method [9]. The UV spectrum of the solution of ribosomes was taken on an SF-4 spectrophotometer.

Isolation and Purification of the Total rRNA from the Ribosomes. The ribosomes (C) were suspended in 300 ml of buffer A containing 1% of SDS, and then 150 ml of phenol saturated with buffer A was added and the mixture was shaken for 30 min. After centrifuging at 6000 rpm for 45 min, the aqueous phase was separated off, and the phenol treatment was repeated (0.5 of the volume of the aqueous phase). Then 2.5 volumes of ethanol (96%) cooled to −20°C was added to the cooled aqueous phase and the mixture was left in the refrigerator for 12 h. The precipitate of total rRNA that deposited was collected by centrifuging, dissolved in 50 ml of 0.2 M NaCl in buffer B (0.25 M CH_3COONa, pH 5.6 [10]) and deposited on a column (3 cm × 3.14 cm^2) of DEAE-cellulose previously equilibrated with 0.05 M NaCl in buffer B. The column was washed with 50 ml of 0.2 M NaCl in buffer B and elution was carried out with 1 M NaCl in buffer B. The RNA was precipitated from the solution with 2.5 volumes of ethanol cooled to −20°C. The precipitate was collected by centrifuging and washed with ethanol and with ether and was dried in vacuum.

Isolation and Purification of the Total RNA from the Supernatant. The supernatant after separation from the precipitate of ribosomes was diluted twofold since in the phenol treatment there was poor layer separation after centrifuging because of the presence of sucrose (0.25 M). Sodium dodecyl sulfate to a final concentration of 1% and an equal volume of water-saturated phenol were added, and the mixture was shaken for 30 min and was then centrifuged at 6000 rpm for 1 h. The aqueous phase was separated off and was treated with chloroform (0.5 of the volume of the aqueous phase), and the mixture was shaken for 30 min. Centrifuging was carried out at 6000 rpm for 1 h, and the aqueous phase was again treated with water-saturated phenol (0.5 of the volume of the aqueous phase). After centrifuging at 6000 rpm for an hour, the aqueous phase was separated off, 2.5 volumes of ethanol cooled to −20°C was added, and the mixture was allowed to stand in the refrigerator for the formation of a precipitate of RNA (2-2.5 h). The total RNA was collected by centrifuging and was dissolved in

50 ml of 0.2 M NaCl in buffer B. The solution was chromatographed on a column of DEAE-cellulose just as in the case of the rRNA. The resulting RNA solution was cooled, 2.5 volumes of ethanol cooled to −20°C was added, and the mixture was left in the refrigerator for 12 h. The precipitate of total RNA that deposited was collected by centrifuging, washed with ethanol and with ether, and dried in vacuum.

Electrophoretic Analysis of the Total rRNA and the Total RNA from the Supernatant. Buffer C [11] for electrolysis was prepared — 0.04 M Tris, 0.02 M CH_3COONa, and 0.002 M disodium salt of EDTA, pH 7.2 (CH_3COOH was added at room temperature). The total rRNA and the total RNA from the supernatant were dissolved in buffer C containing 20% of sucrose to a final concentration of 100-120 O.U./ml. The tubes were prepared with 10% PAG (0.3% of N,N'-methylenebisacrylamide; 0.033 ml of TEMED and 0.33 ml of 10% ammonium persulfate solution).

In portions of 50 µl (5-6 O.U.) the total rRNA and the total RNA from the supernatant were layered onto the gels, and electrophoresis was carried out at 5 mA per tube with the dye Bromophenol Blue. As soon as the dye had migrated to the bottom of the tubes, electrophoresis was stopped, the gel was removed from the tubes and was frozen with dry ice or liquid nitrogen. The gel was cut into pieces 1.5 mm thick. Shapes as shown below (60 shapes)

the length of which corresponded to the length of a stainless steel razor blade and the height of which was 2-3 mm less were cut from Plexiglas [~Leucite; Perspex] 1.5 mm thick. They were collected into a pile, razor blades were inserted between the pairs, they were glued with a solution of Plexiglas in dichloroethane, and packed into boxes. The gel was placed on the surface of this device and was gently pressed down with a Plexiglas plate until the edges of the razor blades appeared. Then the pieces of gel were taken out with a wire hood and distributed into test tubes (forming 55 fractions). To each test tube was added 3 ml of 0.3 N KOH and they were incubated at room temperature for 12 h. As control we used pieces of gel from the tube containing the dye. The optical densities were recorded on an SF-4 instrument at 260 nm and graphs were plotted of the distribution profiles of the total rRNA and total RNA over the fractions (28S + 18S rRNA, 5S rRNA, and tRNA).

SUMMARY

1. It has been established that the method of precipitation with ethanol in the presence of an increased concentration of Mg^{2+} can be used for the rapid isolation of the ribosome fraction from plant material.

2. The optimum conditions for the complete precipitation of the ribosome fraction from a homogenate of cotton seeds have been selected.

3. By treating an extract of the seeds with 0.5 M KCl it was possible to reduce the amount of tRNA in a preparation of the total rRNA.

LITERATURE CITED

1. M. G. Hamilton and M. L. Peterman, J. Biol. Chem., 234, 1141 (1959).
2. B. A. Larkins and E. Davies, Plant Physiol., 52, 655 (1973).
3. Ts. V. Grancharova and T. A. Getova, Phytochemistry, 15, 1431 (1976).
4. H. Diesperger, C. R. Müller, and H. Sandermann, FEBS Lett., 43, 155 (1974).
5. M. Koka and T. Makamoto, Biochem. Biophys. Acta, 262, 381 (1972).
6. M. S. Kaulenas, Anal. Biochem., 41, 126 (1971).
7. N. G. Avadhani and D. E. Buetow, Biochem. Biophys. Res. Commun., 50, 443 (1973).
8. A. S. Spirin, Biokhimiya, 23, 656 (1958).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
10. P. J. Payne and T. A. Dyer, Biochem. Biophys. Acta, 228, 167 (1971).
11. H. Maurer, Disk-Elektrophorese, Walter de Gruyter, Berlin (1968).