DISSOCIATION AND DENSITY CHARACTERISTICS OF RIBOSOMES OF PLANT CELLS

M.A. AJTKHOZHIN, A.B. BEKLEMISHEV and L.M. NAZAROVA
Institute of Botany, Academy of Sciences of the Kazakh SSR, Alma Ata, Kazakh SSR, USSR

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1. Introduction

Up to the present numerous data have been published on conditions of dissociation of bacterial and animal cell ribosomes. Such reports about plant cell ribosomes are rare [1–3]. At present there are no reliable data on the buoyant density characteristics of plant ribosomes and their subunits. The importance of investigating this field is to a significant degree determined by the necessity of differentiating various types of ribonucleoprotein particles in cell extracts on the basis of their sedimentation and density characteristics.

In this paper conditions of dissociation of plant cell ribosomes into subunits and density characteristics of the ribosomes and their subunits in cesium chloride density gradients are reported. Of the conditions tested, the optimal for the dissociation of plant cell ribosomes was found to be 0.06 M phosphate buffer pH 7.6 with 0.0005 M $MgCl_2$ and 0.001 M KCl; in this buffer the plant ribosomes studied dissociated into 2 subunits, the 60 S and 40 S, and reassociated upon increase of the Mg^{2+} concentration. The buoyant density values of monoribosomes (80 S), of the large subunit (60 S), and of the small subunit (40 S) in CsCl were found to be 1.54–1.55 g/cm^3, 1.56–1.57 g/cm^3 and 1.52–1.53 g/cm^3, respectively.

2. Materials and methods

5 to 6 day old pea (*Pisum sativum*) seedlings grown in an aqueous culture were used for the investigation. Tissue grinding and preparation of extracts were by standard techniques [4]. Ribosomes from the extract were obtained by twice repeated ultracentrifugation in 0.02 M triethanolamine-HCl, 0.005 M $MgCl_2$, 0.05 M KCl, 0.005 M mercaptoethanol, pH 7.4 buffer. The compositions of other buffer solutions used are denoted in the legends to figures. The ribosomal subunits were isolated by centrifugation in a linear 15–30% sucrose gradient in the SW 25.2 and SW 50 Spinco L rotors. After separation of subunits, the fractions were taken from the tubes with a special collector connected to a micropump. The collected fractions were diluted and their UV-absorption at 260 nm was measured on the SF-4 spectrophotometer (USSR). The sedimentation coefficients of ribosomes and their subunits were determined by the method of Martin and Ames [5]. Buoyant density analysis of the particles was done by centrifugation in a preformed CsCl gradient after preliminary fixing with formaldehyde [6, 7].

3. Results and discussion

Treatment of ribosomes with EDTA, 2.2 μmoles per mg of ribonucleoprotein, resulted in a complete dissociation of the ribosomes into 2 subunits. The sedimentation coefficients of the subunits were found to be of 50 S and 27 S. Increase of the EDTA concentration to 7.5 μmoles per mg of ribonucleotprotein also led to dissociation into 2 subunits, but their sedimentation coeffients were much lower, 32 S and 19 S. Apparently in these conditions the dissociation of the ribosomes into subunits was accompanied by their partial unfolding (loosening).

A heterogeneous sedimentation pattern with a few peaks distributed over a wide band of sedimentation

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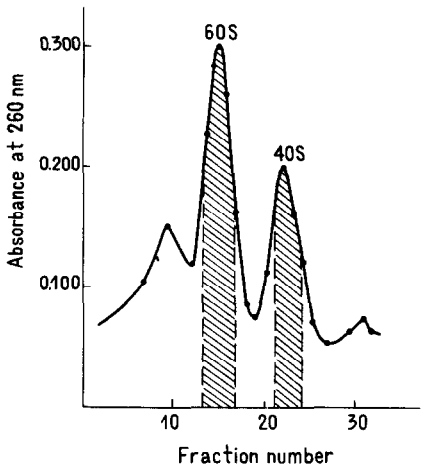

Fig. 1. Sedimentation distribution in a sucrose gradient (15 – 30%) of ribosomes dissociated for 3.5 hr in a 0.06 M phosphate buffer with 0.0005 M $MgCl_2$ – 0.001 M KCl. The suspension of dissociated ribosomes was fixed with 4% formaldehyde and layered on the sucrose gradient prepared in a buffer of the following composition: 0.002 M triethanolamine-HCl, 0.005 M $MgCl_2$, 0.005 M KCl, 4% formaldehyde, pH 7.6. Centrifuged in the SW 25.2 rotor for 18 hr at 21,000 rpm.

coefficients was observed upon introduction of the ribosomes into 0.001 M sodium pyrophosphate without magnesium ions. The addition of magnesium ions up to 0.005 M improved the sedimentation patterns but nevertheless did not lead to a distinct separation into 2 subunits. Lower concentrations of pyrophosphate (below 0.001 M) did not lead to dissociation of the ribosomes at all.

Different concentrations of phosphate buffer, from 0.01 M to 0.08 M, without Mg^{2+}, were found to dissociate the ribosomes, but sedimentation patterns again revealed a complicated picture with a number of peaks.

It was possible to achieve a distinct separation of subunits with normal sedimentation coefficients of 40 S and 60 S and a normal ratio between the large and the small subunits by using a 0.06 M phosphate buffer, pH 7.6, with 0.0005 M $MgCl_2$ and 0.001 M KCl after preliminary incubation of the ribosomes in this buffer for 3.5 hr at 4° (fig. 1). A complete reassociation of the subunits and a restoration of the initial sedimentation coefficients was observed after prolonged dialysis of the dissociated ribosomes against a buffer with an increased concentration of magnesium

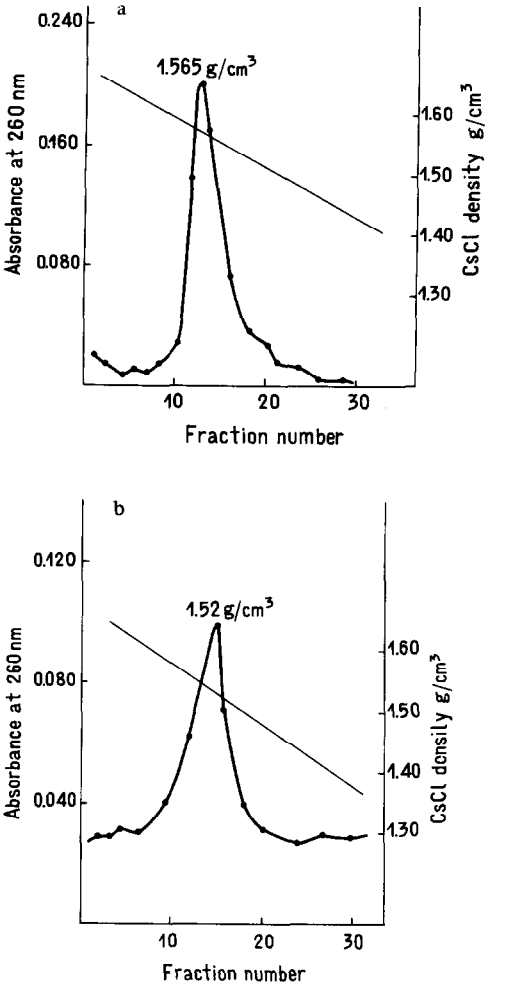

Fig. 2. Density distribution of ribosomal subunits in cesium chloride gradients:
a) the large subunit (60 S);
b) the small subunit (40 S).
Centrifuged in the SW 50 rotor for 16 hr at 38,000 rpm.

ions (0.005 M) thus demonstrating that no irreversible changes occur during such a dissociation.

To determine the buoyant density characteristics of the ribosomes and their subunits in cesium chloride density gradients, the suspension of dissociated ribosomes was fixed with 4% neutral formaldehyde, layered on a linear sucrose gradient and centrifuged in the SW 25.2 rotor. After centrifugation the fractions corresponding to the subunit zones were collected (marked regions in fig. 1) and either spun down in the SW 50 rotor for 6 hr or dialyzed against a buffer to

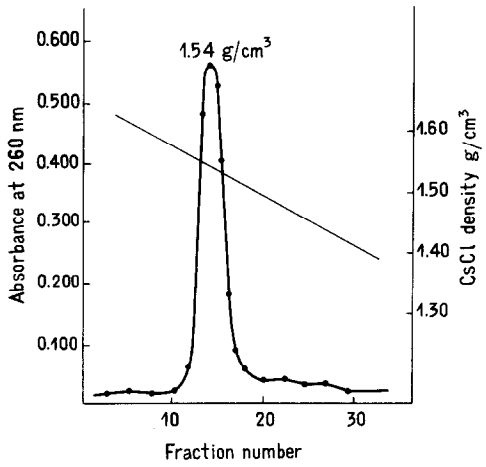

Fig.3. Density distribution of non-dissociated monoribosomes in a cesium chloride gradient.
Centrifuged in the SW 50 rotor for 16 hr at 38,000 rpm.

remove sucrose. After this up to 4% formaldehyde was added to the preparations obtained and they were centrifuged in a cesium chloride density gradient.

Fig.2 (a and b) shows typical patterns of subunit density distribution in CsCl. From a number of repeated experiments the buoyant density of the large subunit (60 S) was determined as 1.565 g/cm^3 ± ± 0.005 g/cm^3 and that of the small subunit (40 S) as 1.525 g/cm^3 ± 0.005 g/cm^3.

The density distribution of non-dissociated monoribosomes is given in fig.3. The density of plant cell monoribosomes was found to be 1.545 g/cm^3 ± ± 0.005 g/cm^3. The buoyant density of reassociated ribosomes proved to be identical to that of the non-dissociated monoribosomes. The RNA: protein ratio, estimated from the results of buoyant density analysis in cesium chloride gradients [8], is about 50% RNA and 50% protein for these plant cell monoribosomes.

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