SPECIFIC COMPACT SELFPACKING OF THE RIBOSOMAL 23 S RNA

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1. Introduction

A comparative electron microscopical analysis of ribonucleoprotein particles containing the ribosomal 16 S RNA and different sets of proteins provided evidence that the 16 S RNA plays the key role in the formation of the unique three-dimensional structure of the ribosomal 30 S subunit. It has been shown that the 16 S RNA in the complex with only four proteins, S4, S7, S8 and S15, retains all the main features of the 30 S subunit morphology [1]. The 16 S RNA–protein S4 complex has a specific V-shaped structure which is well inscribed into the 30 S subunit contours [2]. Finally under certain ionic conditions the 16 S RNA without any proteins is capable of selfpacking with the formation of a similar compact V-shaped structure which could serve as a ready three-dimensional framework for the arrangement of ribosomal proteins [3,4].

Here, we report the results of an electron microscopical study of the 23 S RNA from the large subunit of *Escherichia coli* ribosomes. It has been shown that the 23 S RNA molecules are capable of acquiring a specific compact conformation which is morphologically close to intact 50 S subunits.

2. Materials and methods

The rRNA was obtained from once-pelleted unwashed *E. coli* MRE 600 70 S ribosomes by splitting off the proteins in 3 M LiCl with 4 M urea [5,6]. The total RNA was dissolved in buffer containing 10 mM Tris–HCl (pH 7.0) 100 mM NaCl, 1 mM EDTA and fractionated by ultracentrifugation in 5–30% sucrose gradient (Spinco SW 27 rotor, 26 000 rev./min, 20 h). The isolated 23 S RNA was re-precipitated 3 times in an ethanol–50 mM ammonium acetate mixture, 2:1 (pH 5.5) and stored at −10°C. The protein contamination in the 23 S RNA preparations, as determined following [7] with some modifications [8], was <1%.

Before the experiment, the RNA was pelleted by centrifugation at 10 000 × *g* for 10 min. The RNA pellet was dissolved either in 50 mM NH_4CH_3COO–10 mM $Mg(CH_3COO)_2$–1 M ethanol, (pH 7.5) (buffer 1) or in the same buffer with 2 mM spermidine. The RNA concentration was adjusted to 0.25 A_{260} units/ml. The RNA solution was heated for 10 min at 40°C. After 1–2 h the solution was clarified by centrifugation for 15 min at 20 000 × *g* and then used for the experiment.

The 23 S RNA was prepared for electron microscopy using the technique of fast freezing to the liquid nitrogen temperature and high resolution shadowing with tungsten–40% rhenium as in [9]. The grids were placed at 26° to the atomic beam. The thickness of the metal layer was 10 Å in the direction of shadowing (i.e., ~5 Å on the specimen surface) as measured with a quartz crystal oscillator. The preparations were examined in a JEM-100C electron microscope equipped with a liquid nitrogen anti-contamination trap. Electron micrographs were taken at 80 kV accelerating voltage, with a 25 μm objective aperture and a magnification of 60 000. The grids were inserted in the electron microscope so that the specimen faced the electron source. The plates were printed with the emulsion away from photographic paper.

3. Results and discussion

Preliminary experiments were performed to determine optimal conditions which provide the maximum compactness of the 23 S RNA in solution. We found that the conformation of the 23 S RNA is greatly affected by polyamines, in the first place by spermidine. Sedimentation behaviour at moderate ionic

strength and neutral pH was analyzed as a function of the various [Mg^{2+}] and [spermidine]. The [Mg^{2+}] and/or [spermidine] were gradually increased. The maximum compact form of the 23 S RNA was found in the buffer 1 (50 mM NH_4CH_3COO, 10 mM $Mg(CH_3COO)_2$, 1 M ethanol, pH 7.5) containing 2 mM spermidine (spermidine trihydrochloride, Sigma). Under these conditions, the 23 S RNA sediments at $s^{\circ}_{20,w}$ = 34.0 ± 0.5 S. The removal of spermidine decreases the $s^{\circ}_{20,w}$ to 31.0 ± 0.5 S and this decrease cannot be compensated by high [Mg^{2+}].

Fig.1 represents sedimentation patterns of the 23 S RNA in 10 mM Tris–HCl–100 mM NaCl–1 mM EDTA (pH 7.3) (a) and in buffer 1 with 2 mM spermidine (b) where it is in the compact form. The sedimentation pattern demonstrates a high homogeneity of the preparations. Occasionally some aggregates (dimers) have appeared in the buffer with spermidine.

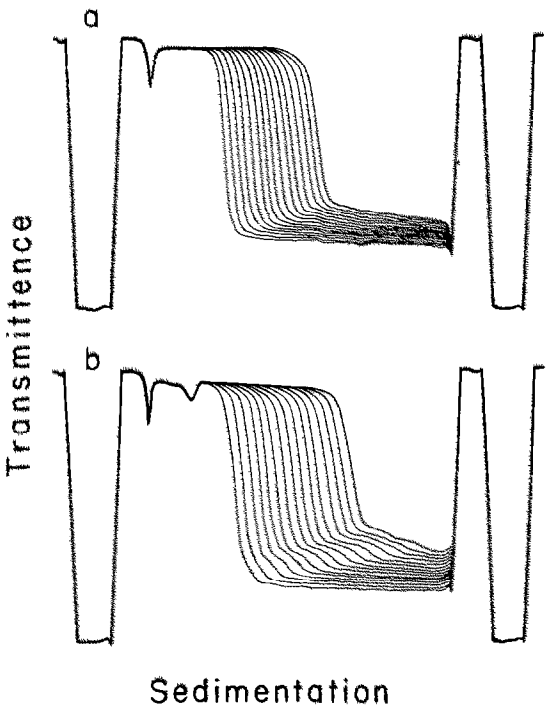

Fig.1. Sedimentation patterns of preparations of the ribosomal 23 S RNA in the buffer containing 10 mM Tris–HCl (pH 7.3), 100 mM NaCl, 1 mM EDTA (a), and in the buffer containing 50 mM NH_4Ac, 10 mM MgAc, 1 M ethanol, 2 mM spermidine (pH 7.5) (b). Centrifugation was done in a UCA-10 analytical scanning ultracentrifuge (USSR) equipped with ultraviolet optics at 40 000 rev./min, 20°C; records were taken at 4 min intervals.

The electron micrographs are presented in fig.2. They show the effect of spermidine on the conformation of the 23 S RNA molecules. The electron microscopy images of the RNA molecules prepared from buffer 1 without spermidine (fig.2a) have random outlines thus indicating no uniformity of the conformations of the RNA molecules under these conditions. Similar images of isolated 23 S RNA were obtained in [10,11].

On the contrary, the electron microscopical images of 23 S RNA molecules prepared from buffer 1 containing 2 mM spermidine have regular shapes and can be classified into several reproducible types (fig.2b). Micrographs of 23 S RNA under the same conditions at a higher magnification are presented in fig.3 together with a schematic depiction of the molecules. Many 23 S RNA images bear a striking similarity to characteristic images of the 50 S subunit in their two main views, such as 'crown' and 'crescent' (or 'kidney') [12–16]. Intact 50 S subunits give asymmetric crown-like images characterized by a central protuberance or nose [12] and two side protuberances of different length and shape [15–18]. Experiments with stripping of 50 S subunits by different agents have shown that the depletion of proteins causes stepwise decrease of the both side protuberances thereby transforming asymmetric crown forms to symmetric ones, while the central protuberance remains [16]. Some images of 23 S RNA molecules (no. 1 in fig.3) are similar to crown-like images of 50 S subunits with reduced side protuberances.

Fig.4 shows the shape and dimensions of the 23 S RNA molecule in the compact form as compared with those of the 50 S subunit in crown view. (50 S subunits were prepared for electron microscopy as in section 2 from the buffer containing 50 mM NH_4CH_3COO, 0.5 mM $Mg(CH_3COO)_2$, 1 M ethanol (pH 7.5)). The length of the RNA particles is 260 ± 20 Å while the length of 50 S subunits is 230 ± 10 Å.

The other type of the 23 S RNA images (no. 2 in fig.3) is very similar to the 'kidney' view of the 50 S subunit. The third type of images looks like 'slit' view of the 50 S subunit, which was described in [12].

Hence, these micrographs demonstrate similarity in the overall shape and dimensions between the 23 S RNA molecule in the compact conformation and the 50 S subunit. Their common structural feature is the central protuberance. Our data on the 16 S RNA [3] and the 23 S RNA show that the both high M_r ribosomal RNAs, without ribosomal proteins, are capable

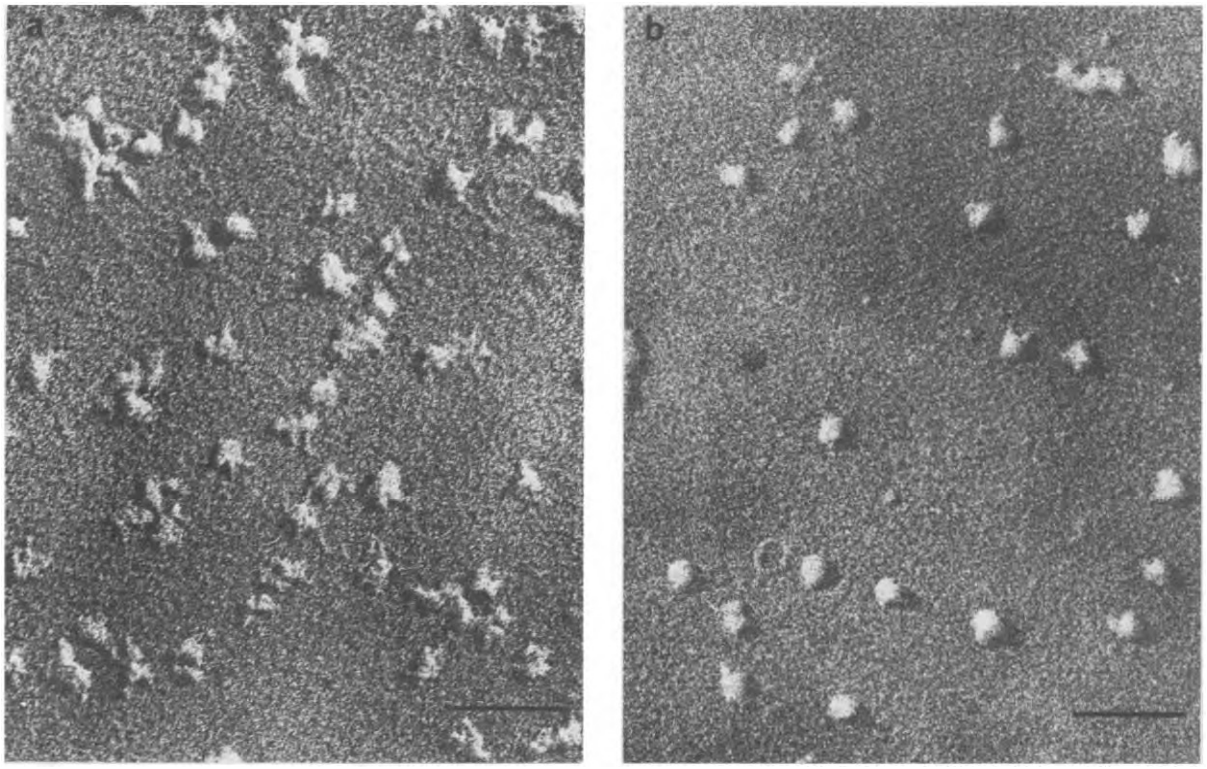

Fig.2. Electron micrographs of 23 S RNA preparations freeze-dried in vacuum. Shadowing with tungsten–40% rhenium. Ratio of the shadow length to the object height is ~2:1. The metal cap is 10 Å thick. (a) 23 S RNA in buffer 1 ($s^{\circ}_{20,w}$ = 31.0 ± 0.5 S); (b) 23 S RNA in buffer 1 containing 2 mM spermidine ($s^{\circ}_{20,w}$ = 34.0 ± 0.5 S); bar = 1000 Å.

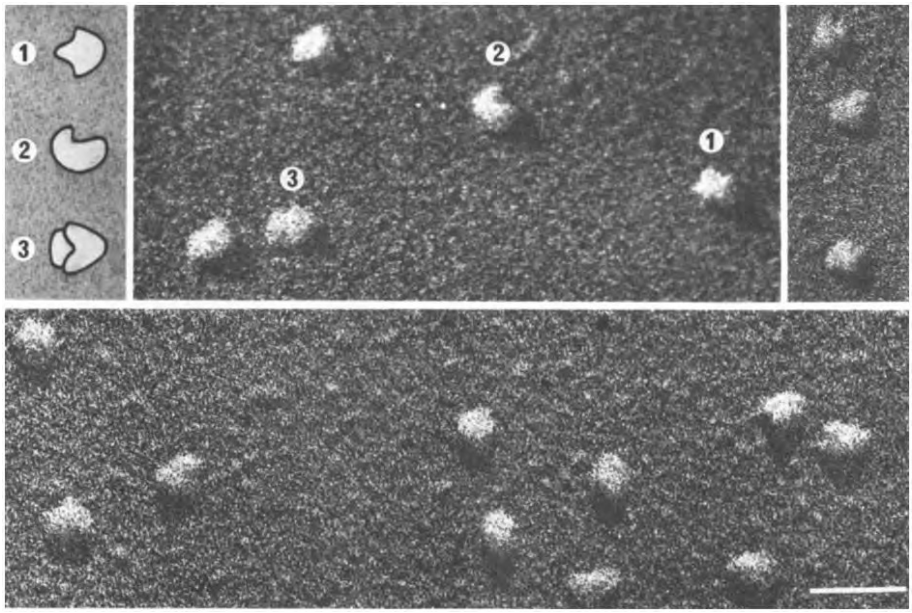

Fig.3. Main types of electron micrographical images of 23 S RNA molecules in a compact form and their schematic interpretation; bar = 500 Å.

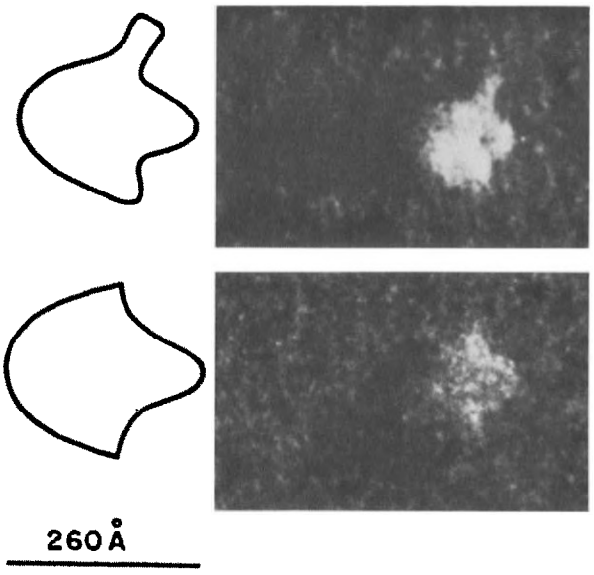

Fig.4. Comparison of the dimensions and shape of the 50 S subunit (left) and the compact 23 S RNA molecule (right) in crown view.

of forming a specific compact structures, which reflect, in the main, the three-dimensional organization of the ribosomal subunits.

Most recently, an electron microscopy analysis of rRNAs prepared under reconstitution conditions (from the buffer containing 20 mM Tris–HCl (pH 7.6), 4 mM Mg^{2+}, 400 mM NH_4Cl) has been done [11]. The freeze-drying and the shadow-cast technique also was used in this study. Irregular structures with some domains, such as presented in fig.2a, were found in the (23 S + 5 S) RNA preparation. The 16 S RNA molecules were described as empty baloons with a smooth surface and a diameter of 180 Å. Two comments should be done in connection with these results.

(1) Washing with water of the rRNAs absorbed on the carbon film was used. This seems to be a very drastic treatment for such a polyelectrolyte as RNA and can result in a great distortion of its structure

(2) Some amount of the elongated and V-shaped particles similar to those described by us [3] can be detected in fact on the micrograph of the 16 S RNA preparation (see fig.1 in [11]).

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