Structure of protein-deficient 50 S ribosomal subunits

Nine core proteins induce the compact conformation of 23 S ribosomal RNA

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The complex of 23 S ribosomal RNA with the nine core proteins L2, L3, L4, L13, L17, L20, L21, L22 and L23 obtained either by the disassembly procedure or by reconstitution has been studied by electron microscopy. This complex is found to be very similar to the intact 50 S subunit both in size and in shape.

Large ribosomal subunit *Core particle* *Electron microscopy*

1. INTRODUCTION

Isolated 23 S ribosomal RNA in buffers with 10 mM magnesium has a sedimentation coefficient of 26 S and is less compact than that in intact ribosomes [1,2]. In the presence of ethanol or spermidine, the 23 S RNA molecule becomes more compact ($s^{\circ}_{w,20} = 34$ S) and acquires an overall shape similar to that of the intact 50 S subunit [1]. 23 S RNA with 18–20 ribosomal proteins (intermediate particles in the process of assembly of 50 S subunits) has a sedimentation coefficient of 41 S [3]. Moreover, it has been shown recently that the complex of 23 S RNA with 9 proteins (L2, L3, L4, L13, L17, L20, L21, L22, L23) has a sedimentation coefficient of 42 S and a radius of gyration close to that of the 23 S RNA within the 50 S subunit [4].

To elucidate the role of ribosomal proteins in the structural organization of 23 S RNA we investigated protein-deficient 50 S particles by electron microscopy.

The results obtained show that the 9 core proteins enumerated above are sufficient for the 23 S ribosomal RNA to acquire a compactness close to that within the ribosomal 50 S subunit. The ribonucleoprotein (RNP) complexes studied are very similar to the intact 50 S subunits in both size and shape.

2. MATERIALS AND METHODS

50 S ribosomal subunits were obtained from ribosomes of *E. coli* MRE-600 as described in [5] with minor modifications [6]. The 50 S particles were stored in 10 mM Tris-HCl buffer, pH 7.5, with 10 mM $MgCl_2$, 50 mM NH_4Cl and 3 mM mercaptoethanol (buffer A) at −70°C. Protein-deficient particles were obtained by treatment of the 50 S subunits with 4 or 5 M LiCl, according to [7,8]. The RNP particles were purified in a 10–30% linear sucrose gradient in buffer A and stored under ethanol. 23 S RNA was isolated as described by Steinhäuser et al. [9] with minor modifications. Ribosomes were overlaid on a 7.5–30% linear sucrose gradient in 10 mM Tris-HCl buffer, pH 7.8, with 0.3 mM $MgCl_2$, 10 mM NaCl and 0.3% SDS. Individual ribosomal proteins were isolated as in [7]. RNP complexes were reconstituted from pure 23 S RNA and the individual proteins L2, L3, L4, L13, L17, L20, L21, L22 and L23, according to Nierhaus et al. [10]

Homogeneity of RNA was checked by electrophoresis [11]. The protein composition of the RNP complexes was determined by gel electrophoresis with SDS [12] and two-dimensional gel electrophoresis [13]. Analytical centrifugation was carried out in a UCA-10 ultracentrifuge (USSR).

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The samples for electron microscopy were dialyzed against 50 mM ammonium acetate, pH 7.5, with 10 mM magnesium acetate and negatively stained with 1% uranyl acetate, according to Valentine et al. [14]. Samples were examined in a JEM-100C microscope at an operating voltage of 80 kV and a magnification of 60000. The micrographs represent particles as viewed from the specimen side.

3. RESULTS AND DISCUSSION

The RNP particles obtained by 4 M LiCl treatment of 50 S subunits (4 M LiCl core particles) contain 9 proteins: L2, L3, L4, L13, L17, L20, L21, L22 and L23 (fig.1a). Similar particles but with a reduced amount of proteins L2 and L20 and with additional protein L29 were reported in [15]. This difference may be connected with different magnesium concentrations used under conditions of protein depletion (100 mM here and 10 mM in [15]). The sedimentation coefficient of the core particles is 42 S in buffer A (fig.2a). The RNP complex reconstituted from 23 S RNA and the 9 proteins has the same sedimentation coefficient (42 S) and the same set of proteins as the LiCl core particles (fig.1b).

The sedimentation coefficient of 23 S RNA in buffer A is 26–27 S. 23 S RNA with the additional mass of the 9 proteins should have a sedimentation coefficient of about 31 S if the proteins are added in such a way as to cause no change in the shape or hydration of the particles (the expected sedimentation coefficient of the 23 S RNA with 18 proteins is approx. 34 S [2]). Therefore, the $s^{\circ}_{w,20}$ of 42 S found for the complex of 23 S RNA with 9 core proteins suggests a significant compactness of the 23 S RNA in this complex. In contrast to isolated 23 S RNA, ethanol and spermidine virtually do not affect the sedimentation coefficient of the

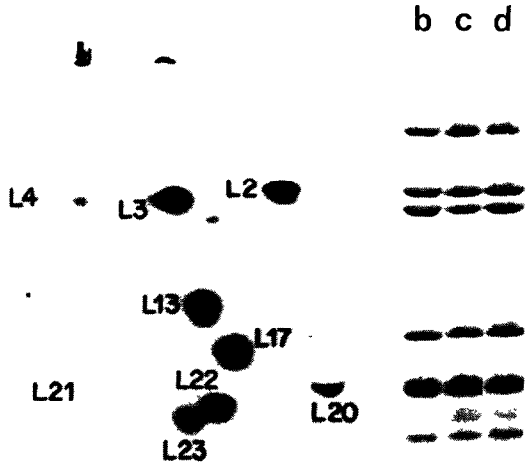

Fig.1. Protein content of the 42 S core particles: (a) Two-dimensional gel electrophoresis [8] of 42 S 4 M LiCl core particles. (b) Electrophoresis in polyacrylamide gel with SDS [10] of an equimolar mixture of the 9 proteins. (c) The same for proteins from 4 M LiCl core particles. (d) The same for proteins from the reconstituted 42 S particles (see section 2).

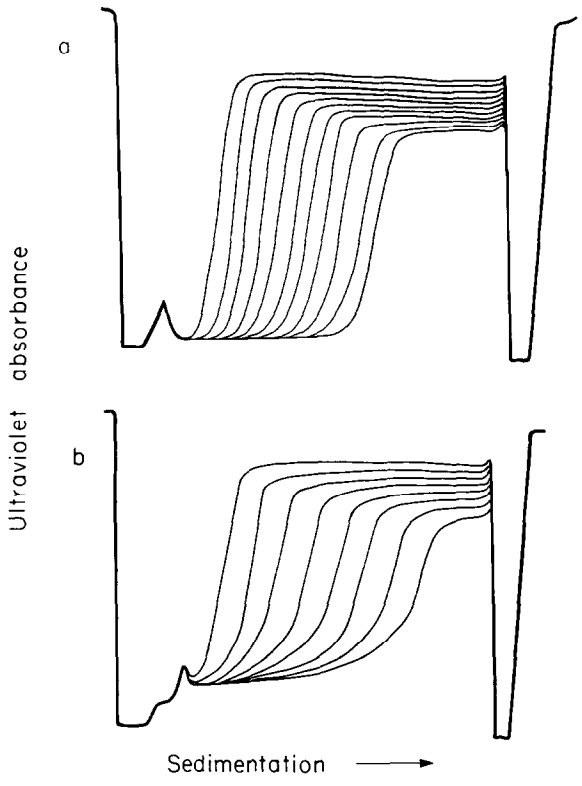

Fig.2. Sedimentation patterns of the RNP preparations. Sedimentation was done in buffer A at 20°C using an analytical ultracentrifuge (UCA-10, USSR) equipped with ultraviolet optics. (a) 4 M LiCl core particles (42 S). Scanning interval 4 min, 30000 rpm. (b) Reconstituted 23 S RNA-9 protein complex. Scanning interval 4 min, 40000 rpm.

RNP complex ($s^{\circ}_{w,20}$ = 43 S in the buffer with 1 M ethanol).

The radius of gyration (R_g) of the 42 S RNP complex is 73 Å [4], this value being only 7 Å higher than the R_g of 23 S RNA within the 50 S subunit. Isolated RNA in a compact form (34 S)

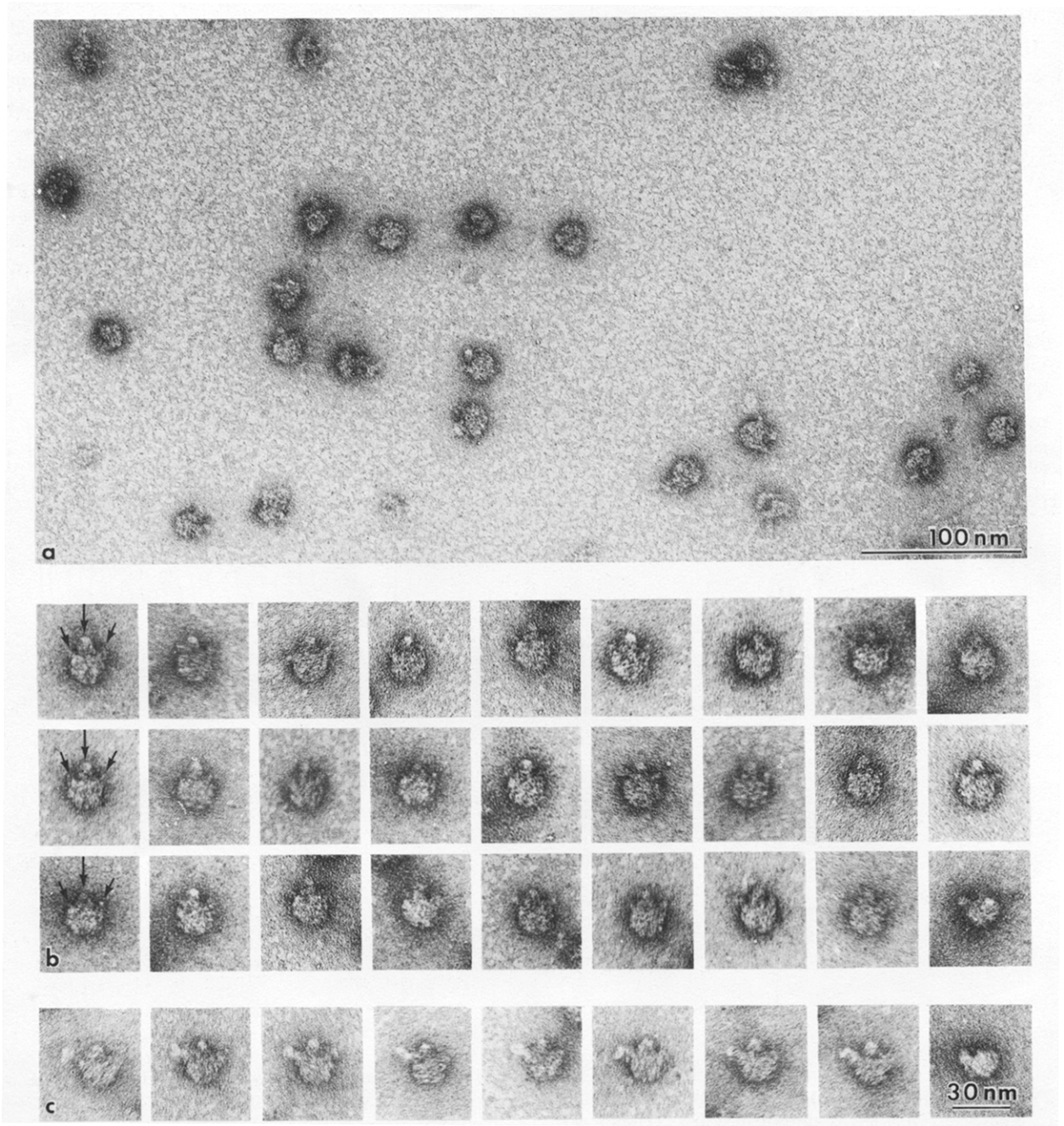

Fig.3. Electron micrographs of the reconstituted 42 S particles. Micrographs of the intact 50 S subunit are also presented for comparison. (a) Field of the preparation. (b) Gallery of the individual 42 S particles in a crown view. Arrows indicate the crown-like region of the particles in the left row. The last frame represents the 42 S particle in a lateral view. (c) Intact 50 S ribosomal subunits.

has an R_g of 100 Å [4]. Hence, the 9 proteins induce a significant compactness of the 23 S RNA.

Attempts to obtain compact RNP complexes with a set of less than 9 proteins have failed. For instance, 5 M LiCl core particles containing 4 or 5 proteins (L3, L4, L13, L17, L21) were not homogeneous and their maximal sedimentation coefficient was 32–33 S.

Electron microscopy studies demonstrate that the 23 S RNA in the complex with the 9 core proteins has a specific compact conformation. The reconstituted 42 S complex proved to be very stable over a wide range of ionic conditions. This permitted study of the complex by negative staining without preliminary fixation of the samples. The morphology of 50 S subunits is well documented in the literature. Detailed descriptions of their two orthogonal projections have been given and very similar three-dimensional models of the 50 S subunits have been proposed [16–18]. The main 50 S projection is an asymmetric crown-like one with a central protuberance and two side protuberances of different length and shape. Another projection is kidney-shaped.

It can be seen in fig.3 that electron microscopy images of the 42 S RNP particles have a well-formed crown-like region with a central protuberance and two reduced (in comparison with the 50 S subunit), but well-identified side protuberances. Thus, the particles have virtually the same shape as the 23 S RNA in the compact conformation [1] but are 15–20% smaller. The length of the 42 S particles is 230 ± 20 Å and coincides with that of the 50 S subunits. Many of the particles have a longitudinal groove on the side opposite to the central protuberance which is observed in the 50 S subunits [18]. The lateral projection of the 42 S particle is also equivalent to that of the 50 S subunit. Hence, the 42 S particles have morphological features in common with intact 50 S subunits and are nearly as compact as the latter.

These results contradict the observation of Boublik et al. [19] on the unfolded conformation of 4 M LiCl core particles; the reason for this discrepancy remains unclear.

Thus, the complex of the 23 S RNA with the 9 core proteins (L2, L3, L4, L13, L17, L20, L21, L22, L23) obtained by either the disassembly procedure or reconstitution has a sedimentation coefficient of 42–43 S. This complex is rather stable over a wide range of ionic conditions and is resistant to the negative staining procedure for electron microscopy. The complex is very similar to the intact 50 S subunit in both size and shape. A comparison of the overall shapes and dimensions as well as the sedimentation coefficients and radii of gyration for the 23 S RNA-9 protein complex with those for the isolated 23 S RNA in a compact state shows that: (i) the overall specific folding of the 23 S RNA seems to be governed by its own intramolecular interactions; (ii) the 9 core proteins seem to induce an additional folding up of the 23 S RNA which acquires a compactness similar to that within the ribosomal 50 S subunit (see also [1,4]). The same conclusions as to the structural role of the ribosomal RNA and proteins have been drawn earlier from studies of the complex of 16 S RNA with a minimal set of core proteins [20–22].

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