STRUCTURE OF THE RIBOSOMAL 16 S RNA–PROTEIN S4 COMPLEX AS REVEALED BY ELECTRON MICROSCOPY

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1. Introduction

It has been shown that protein-depleted ribonucleoprotein derivatives of ribosomal 30 S subparticles containing either, in one case, proteins S4, S6, S7, S8, S15, S16, S17, S18, S19, or, in another case, just proteins S4, S7, S8, S15, are compact particles retaining the main morphological features inherent to original 30 S subparticles [1,2]. This means that the four proteins – S4, S7, S8, S15 – seem to be sufficient for the formation of the main elements of the unique spatial arrangement of the 30 S ribosomal subparticle [2,3].

This paper presents a result of electron microscopy study of the ribosomal 16 S RNA–protein S4 complex. Such a complex is compact, and its structure is similar to that of the original 30 S ribosomal subparticle.

2. Materials and methods

The ribosomal 16 S RNA was isolated from 30 S subparticles by separating the protein in 3 M LiCl with 4 M urea [4,5]. The protein contamination in the ribosomal 16 S RNA preparations was less than 1.5%. Protein S4 was isolated from the total 30 S ribosomal protein by fractionating on a column with phosphocellulose [5]. Purity of the protein S4 preparation was tested by electrophoresis in polyacrylamide gel according to Moore et al. [6]. Figure 1 shows protein S4

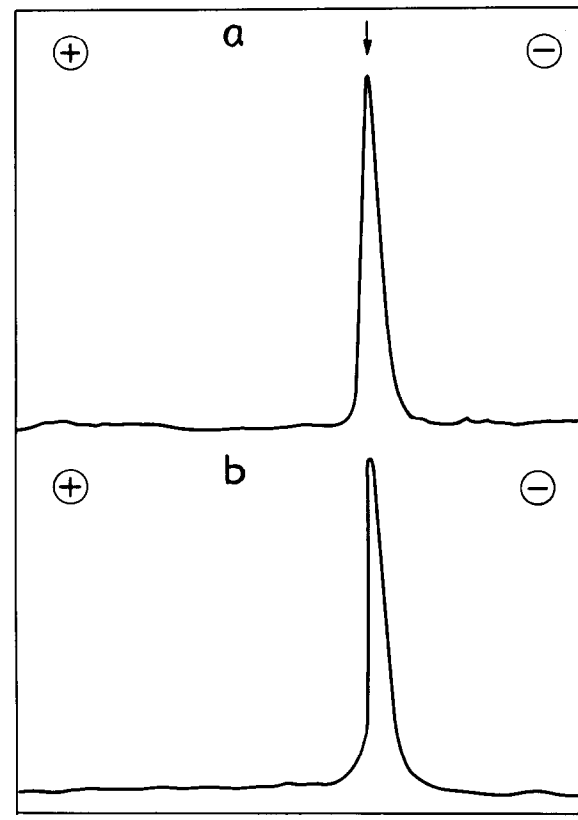

Fig.1. Densitogram of the gel after electrophoresis of the protein S4 preparation stained with amido black. Gels were scanned at 640 nm in the spectrophotometer 'Acta C3' (Beckman, USA). (a) Initial protein preparation. (b) Protein S4 extracted from the complex with 16 S ribosomal RNA.

densitograms. It is seen that the purity of the protein S4 is no less than 95%.

The complex of ribosomal 16 S RNA with protein S4 was obtained by their incubation in a buffer containing 0.03 M Tris–HCl, 0.02 M $Mg(CH_3COO)_2$, 0.33 M KCl, 0.006 M β-mercaptoethanol, $pH_{20°}$ 7.8 for 30 min at 40°C [7] at a molar ratio of protein S4 to 16 S RNA of 3–5:1. After incubation the mixture was cooled to 0°C and kept for 1 h. The precipitate of excess protein was removed by centrifugation at 10 000 × *g* for 1 h. The supernatant was layered over 2.7 ml 12% sucrose in a buffer containing 0.03 M Tris–HCl, 0.02 M $Mg(CH_3COO)_2$, 0.33 M KCl, 0.006 M β-mercaptoethanol, $pH_{20°}$ 7.8 and was centrifuged for 7 h at 50 000 rev/ min in a SW 50.1 rotor ('Beckman', USA). The pellet obtained of the ribosomal 16 S RNA–protein S4 complex was suspended in a buffer containing 0.03 M CH_3COONH_4, 0.006 M $Mg(CH_3COO)_2$, 1 M ethanol, $pH_{20°}$ 7.5 to a concentration of 0.4–0.5 A_{260}/ml. Aggregates were removed from the solution by centrifugation at 20 000 × *g* for 10 min. The preparation was heated for 10 min at 40°C and then was used for electron microscopy study as described [1,8,9].

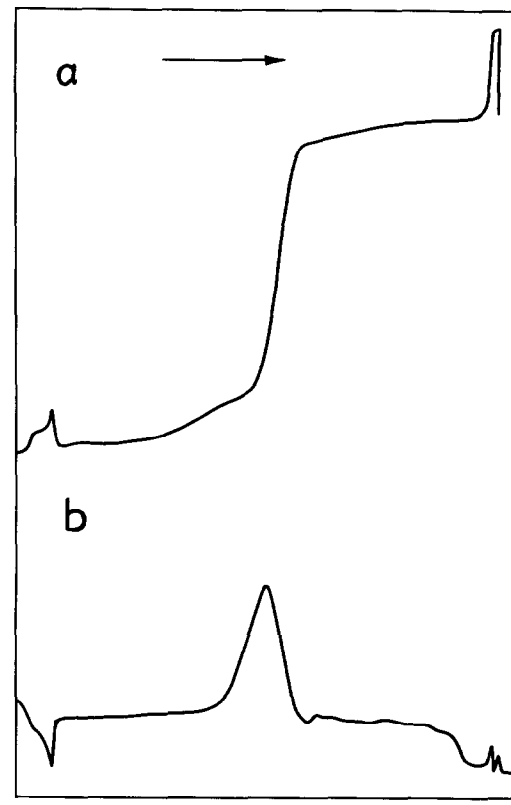

Fig.2. Sedimentation analysis of the preparation of 16 S RNA–protein S4 complex in the buffer containing 0.03 M CH_3COONH_4, 0.006 M $(CH_3COO)_2Mg$, 1 M ethanol, pH 7.5. Centrifugation was done at 44 000 rev/min, 20°C, in an analytical ultracentrifuge Spinco model E (USA) with ultraviolet optics. (a) Integral pattern. (b) Differential pattern.

3. Results

The ribosomal 16 S RNA–protein S4 complex has a sedimentation coefficient $s^{\circ}_{20,w}$ 22.5 ± 0.5 in a buffer containing 0.03 M CH_3COONH_4, 0.006 M $Mg(CH_3COO)_2$, 1 M ethanol, pH 7.5. Sedimentation analysis (fig.2) testifies of the high homogeneity of the complex.

Figure 3 shows the general view of the particles of the 16 S RNA–protein S4 complex. It is seen that the preparation mainly consists of compact elongated particles and does not contain large aggregates or noticeable amounts of unfolded and/or degraded particles. The histogram of particle distribution by length (fig.4) has a maximum at 240 Å. The distribution of the particles studied by length is less uniform than the usual distribution of the 30 S ribosomal subparticles [1].

Electron microscopic images of individual particles of the complex are represented in fig.5. The particles studied are elongated asymmetric structures with an axes ratio of about 2:1 (fig.5a,b). It is possible to observe in some of these particles the subdivision into two unequal parts: the smaller 'head' and the larger 'body', which is characteristic of 30 S ribosomal subparticles. Electron microscopic images of such particles are represented in fig.5b. These images are similar to those of the 30 S subparticle in its lateral view. Figure 5c shows a model of the 30 S subparticle [8,9]. According to the model, lateral views of the 30 S subparticle correspond to the angles of its rotation around the long axis of 0°, 45°, 180°, 225°, relative to the supporting surface. The amount of the particles such as those represented in fig.5b is about 20% of the total amount of particles. It should be noted that this

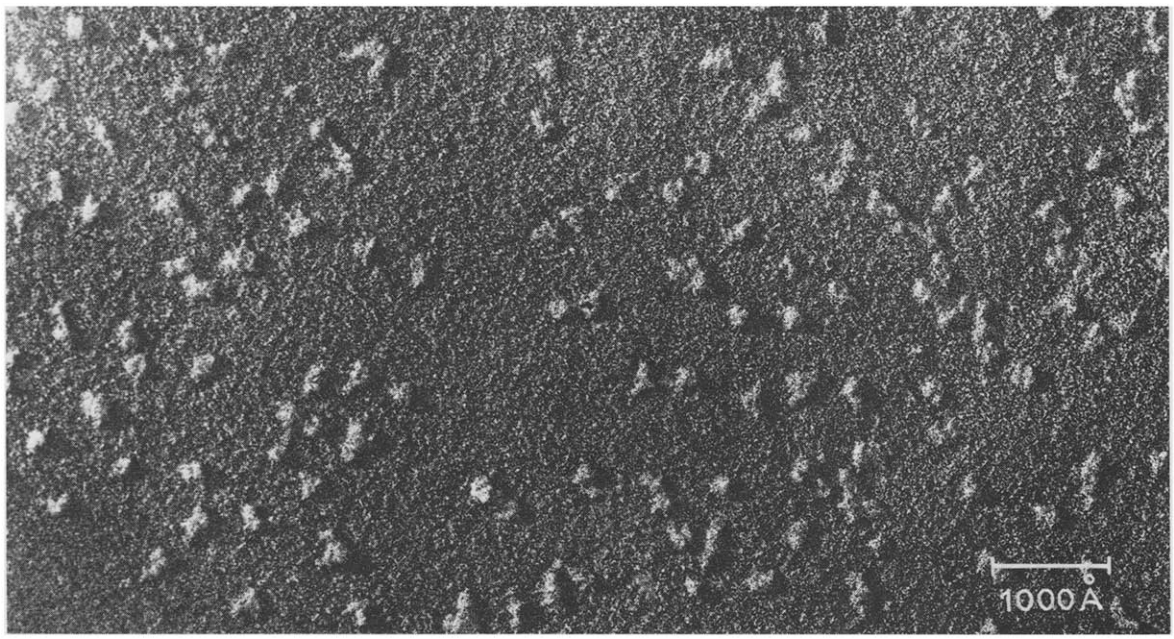

Fig.3. General view of the preparation of ribosomal 16 S RNA–protein S4 complex freeze-dried in vacuum. Shadowing with tantalum–tungsten. Shadow length to object height ratio was about 2:1. The metal layer thickness was 15 Å. Microscope JEM-100C.

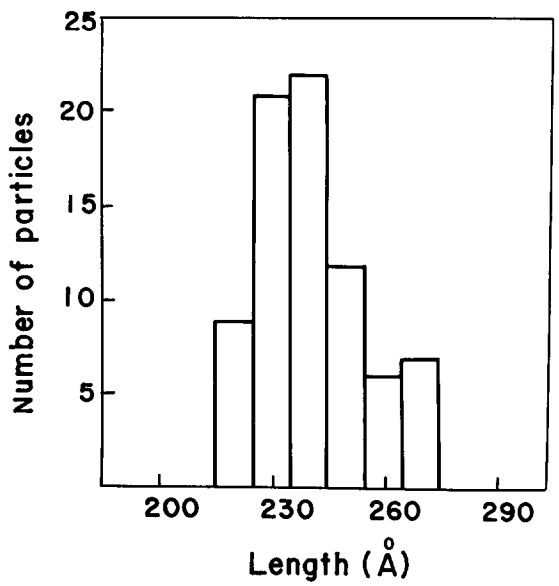

Fig.4. Distribution of the particles of ribosomal 16 S RNA–protein S4 complex by length.

type of particle has a smaller head than the original 30 S subparticles.

About 60% of particles of 16 S RNA–protein S4 complex have a less clear subdivision into the head and body. Most of these particles are V-shaped; one shoulder is thicker than the other and often has at its end a bulge resembling the 'head' (fig.5a). These structures can be considered as slightly unfolded structures of the type represented in fig.5b. It can be presumed that in solution the 16 S RNA–protein S4 complex has a structure similar to that of the 30 S subparticle, but less rigid.

4. Conclusion

The electron microscopy study of the ribosomal 16 S RNA–protein S4 complex shows its structural similarity to the 30 S subparticle. It can be concluded that the 16 S RNA in the complex with protein S4 is able to form the main elements of the unique three-dimensional structure of the 30 S ribosomal subparticle.

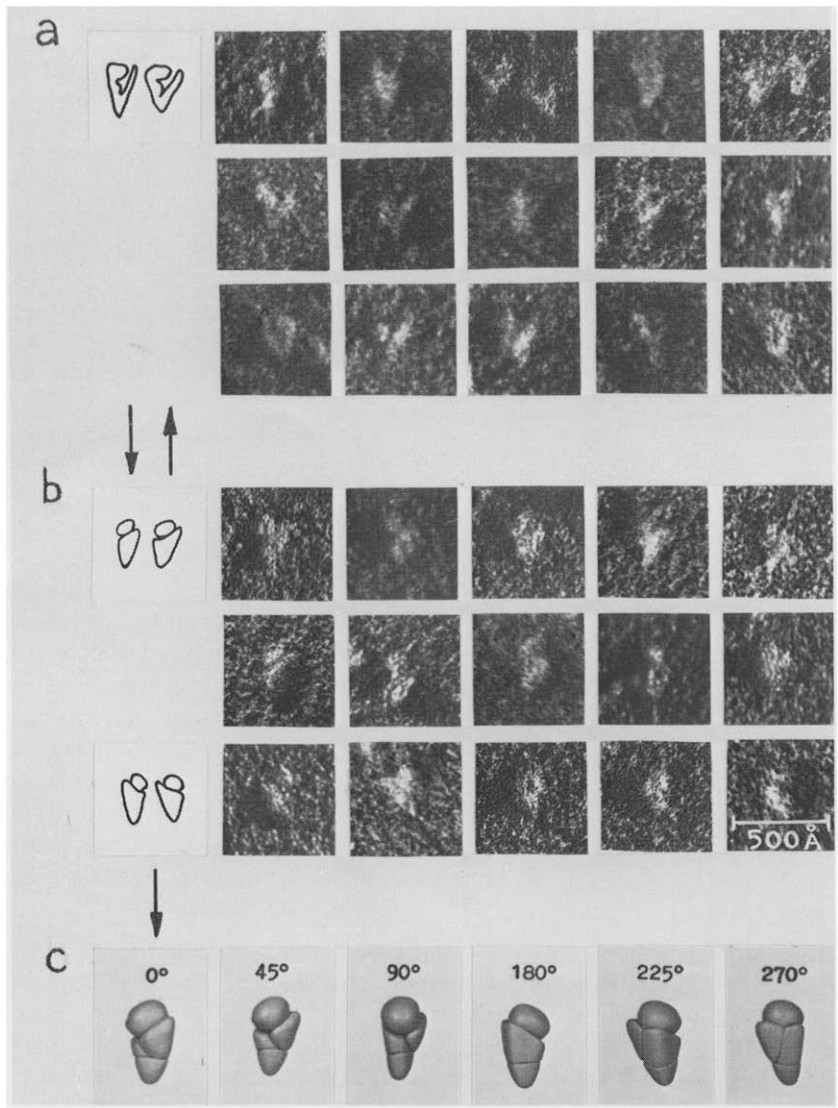

Fig.5. Electron microscopy images of individual 16 S RNA–protein S4 complex particles and their schematic representation. (a,b,c) see the text.

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