ELECTRON MICROSCOPY STUDY OF 70 S RIBOSOMES OF *ESCHERICHIA COLI*

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1. Introduction

Drying from the frozen state may lead to better preservation of biological objects in electron microscopy studies. The dimensions of *E. coli* ribosomal 50 S subparticles, freeze-dried in vacuum and contrasted by shadowing, were found to be significantly larger [1, 2] than those of the air-dried 50 S subparticles shadowed [3] or negatively contrasted [4, 5]. It is evident that in drying from the frozen state the ribosomal particles undergo minimal distortion in shape and dimensions.

In this communication, the technique of freeze-drying in vacuum, followed by shadowing, was applied to a study of complete 70 S ribosomes. A limitation of this method is that the high concentration of Mg^{2+} (0.01 M), necessary to maintain the 70 S ribosomes in a non-dissociated state, results in deterioration of quality of the electron-microscopic image. However, replacement of Mg^{2+} by methanol [6] permitted a decrease of Mg^{2+} concentration in the medium down to 0.002 M, without essential dissociation of ribosomes into subparticles.

2. Materials and methods

Ribosomes were isolated by centrifugation of the *E. coli* MRE-600 extract as described by Spirin et al. [7]. The pellets were resuspended in 0.01 M tris-HCl buffer, pH 7.4, containing 0.01 M $MgCl_2$, and the ribosomes were again spun down by centrifugation. These ribosomes were additionally purified by centrifugation through a linear sucrose gradient with the same buffer and then thoroughly dialyzed against 0.01 M ammonium-acetate buffer, pH 7.7, containing 1 M methanol and 0.002 M magnesium acetate. Sedimentation analysis was done on a Spinco Model E analytical ultracentrifuge using absorption ultraviolet optics. According to the sedimentation pattern, the preparation contained about 65% of 70 S ribosomes.

Ribosomes were prepared for electron microscopy studies as follows. A drop of the ribosome suspension was deposited onto the surface of a freshly-cleaved thin mica plate. The excess suspension was sucked off with a filter paper, then quickly placed in a Dewar flask with liquid nitrogen and fixed in a pre-cooled massive metallic sample holder. The holder with the sample was then removed from the Dewar flask and placed in a vacuum chamber. Sublimation of ice from the specimen was accomplished at about $-100\,^{\circ}C$ in a vacuum of 2×10^{-6} torr. The sample was slowly heated to $+50\,^{\circ}C$, cooled to room temperature, shadowed with tungsten at the angle of about 75°, and the carbon layer was deposited from above. The effective tungsten source subtended less than 1°.

The preshadowed carbon replicas were examined and their micrographs taken with a JEM-7A electron microscope at 80 kV accelerating voltage, with a 30 μm objective aperture and a total magnification of 50,000.

3. Results

The general view of the ribosomal preparation is presented in fig. 1. As in the initial suspension, complete ribosomes comprise about 65% of the particles. The dimensions of ribosomes are 260–240 Å × 240–220 Å × 180–160 Å. This is approximately 25% greater than the linear dimensions of air-dried shadowed [3] or negatively contrasted

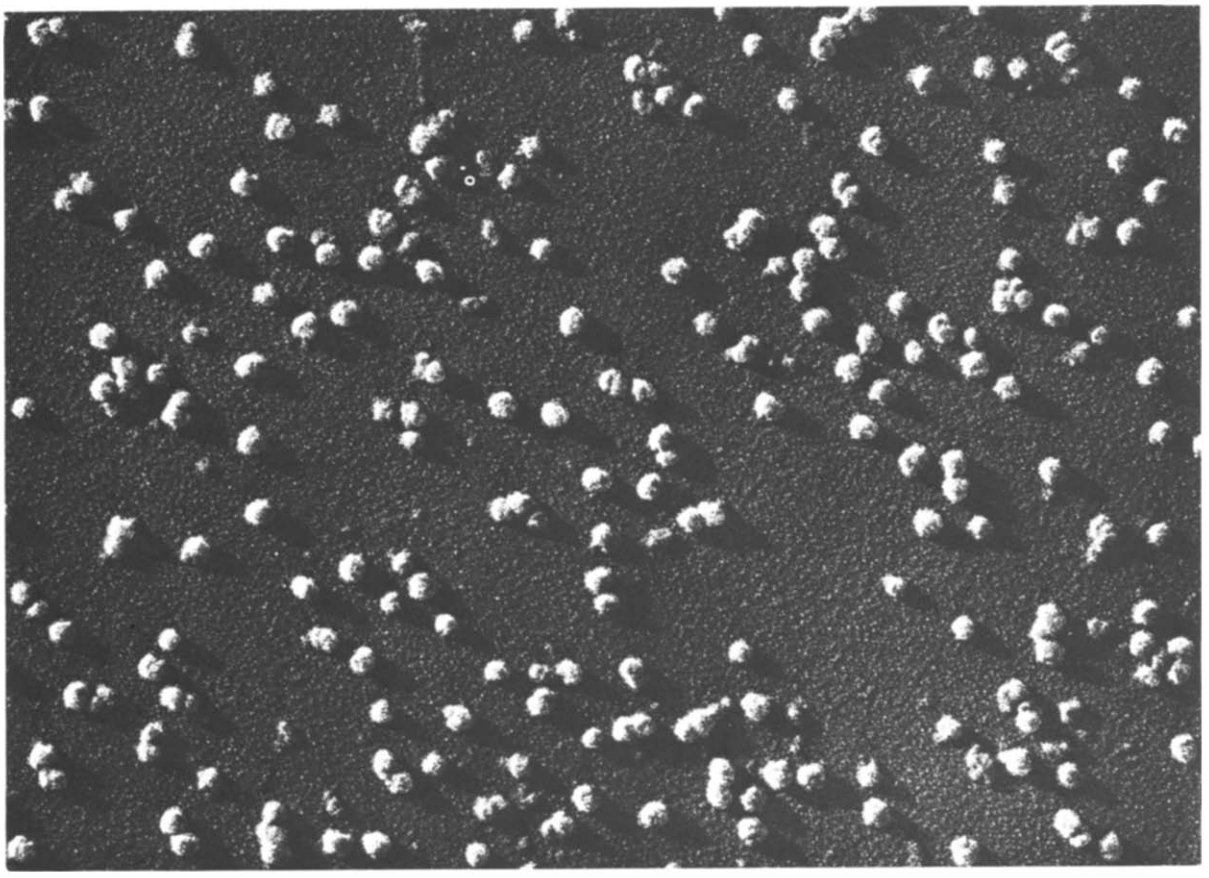

Fig. 1. Freeze-dried *E. coli* 70 S ribosomes. Carbon replica pre-shadowed with tungsten. Ratio of shadow length to object height is 3.5:1 magnification × 135,000.

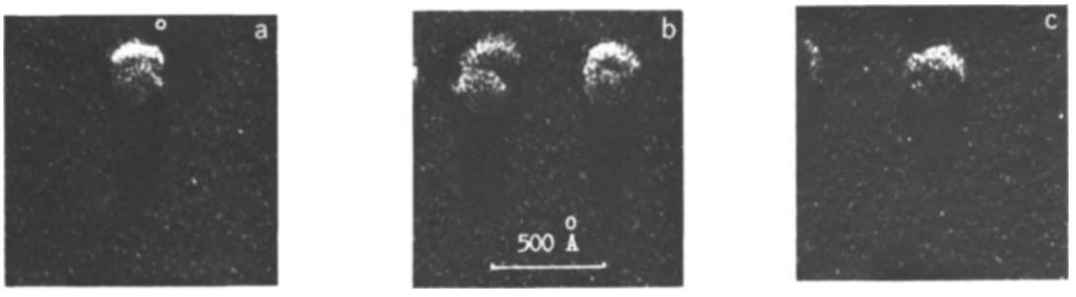

Fig. 2. Separate *E. coli* 70 S ribosomes. (a), (b) view 'from above'; long axis is parallel to direction of shadowing. (c) view 'from below'; long axis is perpendicular to direction of shadowing.

[4, 5] 70 S ribosomes. The length and width of ribosomal particles are measured taking into account a correction for the thickness of the shadowing metal layer increasing the visible dimension along the axis parallel to the shadowing direction. In our experiments the thickness of the tungsten layer was 25 Å. The height of particles was calulated from the shadow length. The particles corresponding to the 70 S ribosomes and their shadows have smooth outlines; no distinct edges are observed. The particles have a complicated shape and only in a rough approximation can be regarded as elliptical bodies with axial ratio of 1:1.35:1.5. Although detailed analyses of the electron-microscopic images of the particles are not presented here and no models are suggested, one essential peculiarity of their morphology should be pointed out: though the ratio of the long and short axes is not large, the particles are almost always oriented by the short axis perpendicular to the mica substrate, predominantly facing it by one of the sides. Thus the view 'from above' is more often observed (fig. 2a and b), which corresponds to the usual idea on the shape of the subparticles within the complete ribosome [3, 4]. According to this concept, the 30 S subparticle is an oblate ellipsoid of revolution, which somewhat deforms during association with the dome-shaped 50 S subparticle, assuming a characteristic convexo-concave form. However , the view 'from below' is sometimes observed, corresponding to the particles lying with their opposite sides on the substrate (fig. 2c). In this case, the 30 S particle is clearly divided by a cleft into two unequal parts and somewhat resembles a telephone receiver in shape. Examination of isolated freeze-dried 30 S subparticles showed that they represent elongated and curved bodies, on the concave side of which there is an asymmetrically located groove or cleft [8]. Thus, the opposite sides of the 70 S ribosome, perpendicular to the short axis, are morphologically different.

The surface of ribosomes has a relief analogous to that observed by Hart [2] and which can be interpreted as the result of packing of the ribonucleoprotein strand or protein globules with a mean diameter of 30 Å. As seen from the microphotographs presented, a shadowing metal layer creates a uniform background with grain dimensions of about 15 Å. In many regions no background details comparable in size to the details of the fine ribosomal structure are present. Therefore, the reality of the observed structure of the ribosome surface is beyond doubt.

4. Conclusions

Freeze-dried *E. coli* 70 S ribosomes have dimensions of 260–240 Å × 240–220 Å × 180–160 Å which corresponds to a volume approximately twice their 'dry' volume. The opposite sides of the 70 S ribosome, perpendicular to their short axis, are morphologically different. The surface of ribosomes is characterized by a parallel striation of its regions with a periodicity of about 30 Å.

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References

[1] R.G.Hart, Biochim. Biophys. Acta 60 (1962) 629.
[2] R.G.Hart, Proc. Natl. Acad. Sci. U.S. 53 (1965) 1415.
[3] C.E.Hall and H.S.Slayter, J. Mol. Biol. 1 (1959) 329.
[4] H.E.Huxley and G.Zubay, J. Mol. Biol. 2 (1960) 10.
[5] V.I.Bruskov and N.A.Kisselev, J. Mol. Biol. 37 (1968) 367.
[6] A.S.Spirin and E.B.Lishnevskaya, FEBS Letters 14 (2) (1971) 114.
[7] A.S.Spirin, N.V.Belitsina, Ö.Gaal and T.M.Pozdnyakova, Molekul. Biol. SSSR 2 (1968) 95.
[8] V.D.Vasiliev (1971) in preparation.