THE 30 S RIBOSOMAL SUBPARTICLE RETAINS ITS MAIN MORPHOLOGICAL FEATURES AFTER REMOVAL OF HALF THE PROTEINS

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1. Introduction

Ribonucleoprotein particles resembling natural ribosomal precursors were obtained in experiments on assembly–disassembly of ribosomal particles in vitro [1–4]. Later the self-assembly of the 30 S ribosomal subparticle was investigated in more detail [5–7]. Intermediate particles deficient in protein (RI-particles) formed in the process of self-assembly at 0°C were shown to be capable of attaching additional proteins only after their transformation into activated 30 S subparticle precursors (RI*-particles) [5–7]. The RI → RI* transition strongly depended on temperature and led to formation of more compact particles [5–7]. It is not excluded that in the process of the RI → RI* transition a unique compact packing of the protein molecules and 16 S RNA regions is formed which does not change essentially after the subsequent attachment of the remaining proteins [8].

This paper presents the results of an electron microscopy study of the derivatives of *Escherichia coli* MRE-600 30 S ribosomal subparticles containing proteins S4, S6, S7, S8, S15, S16, S17, S18 and S19, i.e., proteins which are requisite for the formation of RI*-particles [7]. It is shown that such derivatives are compact particles and retain the main morphological features intrinsic to the original 30 S ribosomal subparticles. Hence, the loss of half of the structural proteins does not lead to a considerable change of the 30 S subparticle morphology.

2. Materials and methods

Ribosomal 30 S subparticles were obtained from *E. coli* MRE-600 by sucrose-gradient zonal centrifugation in the presence of 0.5 M NH_4Cl and 1 mM $MgCl_2$ [9]. Derivatives of the 30 S subparticles were obtained by 2.15 M LiCl treatment in the presence of 5 mM $MgCl_2$ [8,10]. Details of the procedure and characteristics of the preparations will be given elsewhere [11].

Proteins were extracted from 30 S subparticles and derivative particles by 67% acetic acid in the presence of 0.1 M $MgCl_2$ [12]. The protein composition was established by two-dimensional polyacrylamide gel electrophoresis [13].

Reconstitution of 30 S subparticles from the protein-deficient derivatives and the protein was done in 30 mM Tris–HCl buffer, pH 7.4, containing 330 mM KCl, 20 mM $MgCl_2$ and 1 mM dithiothreitol, for 45 min [6]. The protein-to-particles molar ratio was approximately 4:1.

The biological activity of the reconstituted 30 S subparticles was tested in a cell-free system for polyphenylalanine synthesis. The reaction mixture (0.12 ml) contained 7 μg 30 S subparticles and 14 μg 50 S subparticles, 10 μg poly(U) (K^+-salt), 20–24 μg GTP, 35 μg protein of the total elongation factor fraction, 80 μg [^{14}C]phenylalanyl-tRNA (810 pmol phenylalanine/mg tRNA, the initial [^{14}C]phenylalanine was from Amersham, England, 513 mCi/mmol), in a buffer consisting of 10 mM Tris–HCl, pH 7.2, 13 mM $MgCl_2$, 100 mM KCl and 1 mM dithiothreitol [14,15]. Incubation was done at 25°C for 60 min. The radioactivity of hot 5% trichloroacetic acid-insoluble [^{14}C]polyphenylalanine was estimated as described earlier [14,15].

For the electron microscopy study of the derivative particles and the original 30 S subparticles we used

the method of freeze-drying in vacuum [16]. The particles were suspended in a solution containing 30 mM $(NH_4)_2CO_3$, pH 7.2, 6 mM $MgCl_2$ and 1 M ethanol at a concentration of 0.4 A_{260}/ml. As substrates we used carbon films 20–30 Å thick which were obtained as described earlier [17,18]. Shadowing was done with tantalum-tungsten from a distance of 15 cm using an electron gun. The ratio of the shadow-length to the object-height was about 2:1. The thickness of the metal-layer was measured by a quartz oscillator KIT-1 (USSR). The preparations were studied in a JEM-100C microscope at 80 kV without an objective aperture at 30 000 and 60 000 magnifications.

3. Results

The protein-deficient derivatives obtained by treatment of 30 S ribosomal subparticles with 2.15 M LiCl had a sedimentation coefficient $s^0_{20,w}$ = 25.5S ± 0.5 in 10 mM Tris–HCl buffer, pH 7.2, containing 30 mM KCl and 5 mM $MgCl_2$. A semiquantitative characteristic of the protein composition of the derivative particles according to electrophoretic analysis data [11] is given in table 1. It is seen that they contain mainly proteins S4, S7, S8, S15, S16, S17, and a reduced amount of proteins S6, S18 and S19. For comparison the same table gives the set of proteins required for the formation of RI*-particles [7].

The ability of the ribosomal derivatives to attach the missing proteins and to be assembled into biologically active 30 S subparticles was studied under reconstitution conditions at different temperatures. It appeared (table 2) that during 45 min of incubation at 20°C, the derivative particles already attach the missing proteins and form biologically active 30 S subparticles.

Table 1
Comparison of the set of proteins in the protein-deficient derivative particles with that which is necessary for formation of RI*-particles

Proteins	Presence in the derivative particles[a]	Requirement for formation of RI*-particles[b]
S1	–	–
S2	–	–
S3	–	–
S4	++	++
S5	–	±
S6	+	–
S7	++	++
S8	++	++
S9	–	±
S10	–	–
S11	±	±
S12	–	±
S13	±	–
S14	–	–
S15	++	+
S16	++	++
S17	++	+
S18	+	±
S19	+	++
S20	–	–
S21	–	–

[a](++) Present in an amount comparable with that in the original 30 S subparticle, (+) present in a reduced amount, (±) traces, (–) not found

[b](++) Absolutely, (+) moderately, (±) weakly, (–) not necessary [7]

Table 2
Activity of ribosomes containing reconstituted 30 S subparticles in the cell-free polyphenylalanine synthesis system

Ribosomal components	Reconstitution temperature (°C)	Radioactivity of [^{14}C]Phe, nmol[a]	(%) Activity
50 S + original 30 S	–	54.2	100
50 S + reconstituted 30 S	40	49.6	91
50 S + reconstituted 30 S	20	43.5	80
50 S + derivatives of 30 S	40	3.3	6
50 S	–	2.7	5

[a]Polyphenylalanine precipitated by 5% trichloroacetic acid

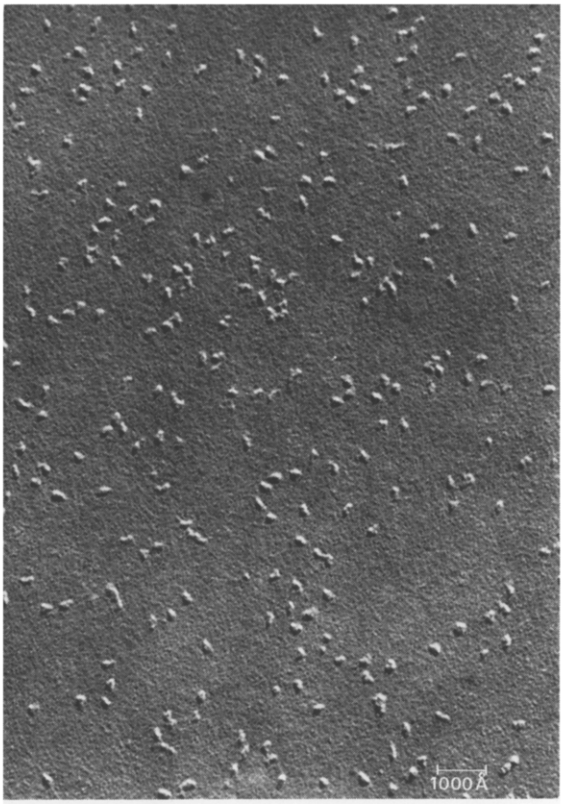

Fig.1. General view of the preparation of the protein-deficient derivative particles freeze-dried in vacuum. Shadowing was done with tantalum-tungsten.

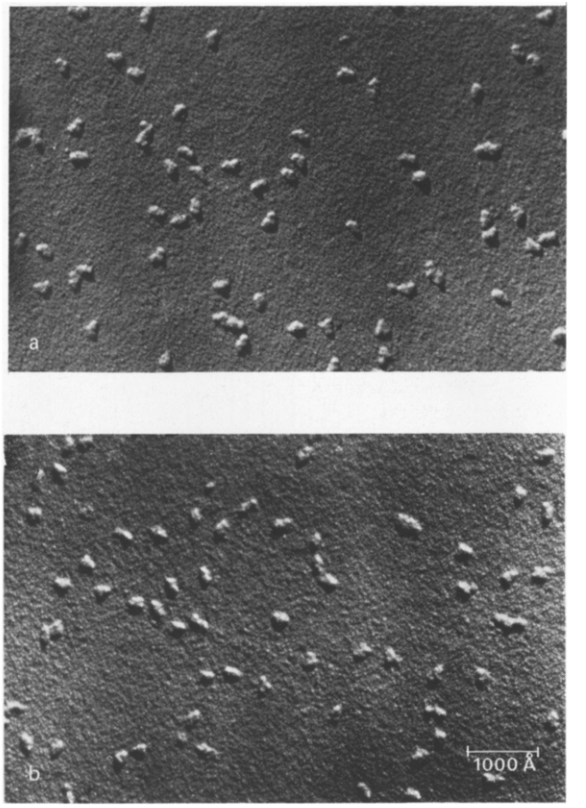

Fig.2. Original 30 S ribosomal subparticles (a) and protein-deficient derivative particles (b) freeze-dried in vacuum.

Thus, the derivatives of 30 S subparticles obtained contain proteins which are necessary for the formation of RI*-particles [7]. The derivatives are capable of forming biologically active 30 S subparticles even at 20°C. This allows us to consider the derivatives obtained as somewhat protein-depleted structural analogs of RI*-particles.

Figure 1 shows the general view of the protein-deficient particles at a small magnification. It can be seen that the preparation mainly consists of particles uniform in dimensions and shape and does not contain large aggregates or noticeable amounts of unfolded and/or degraded particles.

Figure 2 represents electron micrographs of the original 30 S subparticles (a) and the protein-deficient derivatives (b) at a large magnification.

The small *E. coli* ribosomal subparticles were investigated by electron microscopy by several groups using different contrasting methods [17–21]. In the corresponding reports, models of the small (30 S) subparticle differing in fine details of structure were proposed. From the totality of data reported by different authors at least two firmly established main morphological features of the 30 S subparticle can be emphasized. Firstly, the 30 S subparticle represents an elongated asymmetric structure with an axial ratio of about 1:2. Secondly, the 30 S subparticle is subdivided, perpendicularly to its long axis, into two unequal parts: a rounded 'head' and an approximately twice longer 'body' narrowing towards the opposite end.

A comparison of electron micrographs of the protein-deficient derivatives and the 30 S subparticles (fig.2) reveals their great similarity in these two main

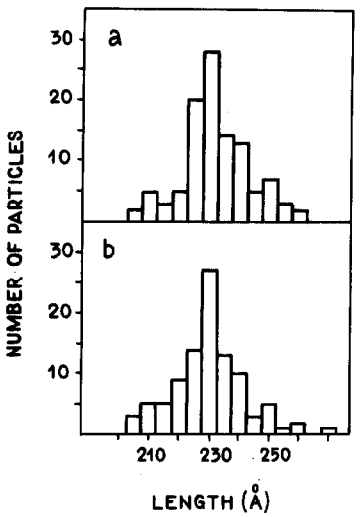

Fig.3. Distribution of ribosomal particles by dimensions: (a) original 30 S subparticles. (b) Protein-deficient derivative particles. Dimensions corrected for metal-cap.

features. Most of the electron micrographs of the derivative particles show their subdivision into the 'head' and the 'body'. The length of the derivative particles as well as the original 30 S subparticles is 230 ± 10 Å. Histograms of length distribution of both types of particles are shown in fig.3 to be practically identical. In contrast to the 30 S subparticles the derivative particles have somewhat less distinct contours, and their width is 5–10% smaller.

4. Conclusion

The comparative electron microscopic study of the morphology of the 30 S subparticles and their protein-deficient derivatives demonstrates their great morphological similarity. The derivative particles are similar to the 30 S subparticles in shape and dimensions and, like them, are divided into two unequal parts. A loss of at least a half of the 30 S subparticle proteins does not lead to a considerable change of its morphology. Hence, the proteins easily detached by monovalent salts, such as S1, S2, S3, S5, S9, S10, S12, S14, S20 and S21, do not seem to take part in the formation of the main morphological features of the 30 S ribosomal subparticles.

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