THE COMPLEX OF 16 S RNA WITH PROTEINS S4, S7, S8, S15 RETAINS THE MAIN MORPHOLOGICAL FEATURES OF THE 30 S RIBOSOMAL SUBPARTICLE

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Received 29 April 1977

1. Introduction

It has been shown that protein-deficient derivatives obtained by treatment of 30 S ribosomal subparticles with 2.15 M LiCl and containing proteins S4, S6, S7, S8, S15, S17, S18, S19 are compact particles retaining the main morphological features of the intact 30 S ribosomal subparticles [1]. This means that the proteins which are readily split off by monovalent metal salts (S1, S2, S3, S5, S9, S10, S12, S14, S20, S21) do not seem to be required for the principal packing of main elements of the 30 S ribosomal subparticle three-dimensional structure [1].

This paper presents a further electron microscopy study of protein-deficient derivatives of 30 S ribosomal subparticles. The derivatives containing less than a quarter of the full set of the 30 S subparticle proteins (S4, S7, S8, S15 and a small amount of a S16 + S17 mixture) have been obtained. It has been shown that such derivatives are compact particles and retain the main morphological features of the original 30 S ribosomal subparticles.

2. Materials and methods

Ribosomal 30 S subparticles were obtained from *Escherichia coli* MRE-600 by sucrose gradient zonal centrifugation in the presence of 0.5 M NH_4Cl and 1 mM $MgCl_2$ [2]. Protein-deficient derivatives of the 30 S subparticles were obtained by 3.15 M LiCl treatment in the presence of 5 mM $MgCl_2$ [3,4]. A 1.1 vol. 6 M LiCl was added to a suspension of 30 S ribosomal subparticles (5–10 mg/ml) in a buffer consisting of 10 mM Tris–HCl, 100 mM KCl, 10 mM $MgCl_2$, pH 7.15. The mixture was incubated for 22–26 h at 4°C. After incubation the ribonucleoprotein particles were collected by centrifugation at 190 000 × *g* for 6 h and then re-suspended in a buffer consisting of 30 mM Tris–HCl, pH 7.4, 350 mM KCl, 20 mM $MgCl_2$. The particles with a concentration of 400–450 A_{260}/ml were incubated in this buffer for 40 min at 40°C then diluted with the buffer containing 30 mM CH_3COONH_4, 6 mM $(CH_3COO)_2$ Mg, 1 M ethanol, pH 7.5, to a concentration of 0.4 or 0.5 A_{260}/ml. The suspension of particles was clarified from possible aggregates by centrifugation at 20 000 × *g* for 10 min. This preparation was used for electron microscopy studies as described earlier [1,5,6].

The protein composition was checked by two-dimensional polyacrylamide gel electrophoresis [7]. The protein was extracted with 67% acetic acid in the presence of 0.1 M $MgCl_2$ [8].

Reconstitution of 30 S subparticles from the protein-deficient derivatives and the split proteins was done in the buffer containing 30 mM Tris–HCl, pH 7.4, 330 mM KCl, 20 mM $MgCl_2$ and 1 mM dithiothreitol, for 45 min [9]. The molar ratio of split protein to derivative particles was about 4 to 1.

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The biological activity of the reconstituted 30 S subparticles was tested in a cell-free system of polyphenylalanine synthesis. The reaction mixture (0.12 ml) contained 7 μg 30 S subparticles, 14 μg 50 S subparticles, 10 μg poly(U) (K^+-salt), 20–24 μg GTP, 35 μg protein of the total elongation factor fraction (calculated for dry protein) and 80 μg [^{14}C]phenylalanyl-tRNA (650 pmol phenylalanine/mg tRNA; the [^{14}C]phenylalanine was from Amersham, England, 513 mCi/mmol), in a buffer consisting of 10 mM Tris–HCl, pH 7.2, 10 mM $MgCl_2$, 100 mM KCl and 1 mM dithiothreitol [10,11]. 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 [10,11].

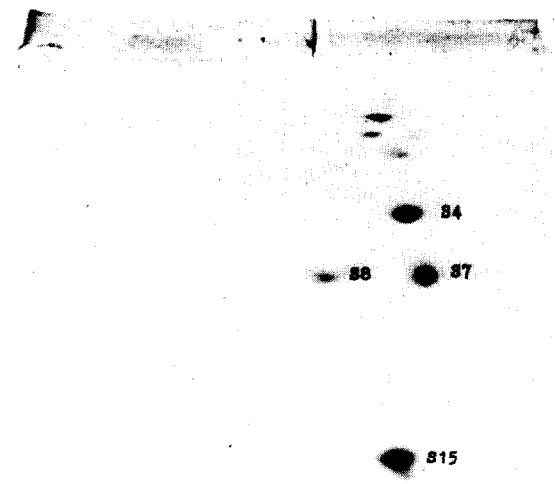

Fig.1. Two-dimensional polyacrylamide gel electrophoresis of the protein-deficient derivatives of the 30 S ribosomal subparticle.

3. Results

The protein-deficient derivatives obtained by treatment of 30 S ribosomal subparticles with 3.15 M LiCl had a sedimentation coefficient $s^0_{20,w} = 23.5 \pm 0.5$ S in the buffer containing 30 mM CH_3COONH_4, 6 mM $(CH_3COO)_2Mg$, 1 M ethanol, pH 7.5. According to two-dimensional polyacrylamide gel electrophoresis (fig.1), the particles studied contained proteins S4, S7, S8, S15 and a mixture of S16 + S17 in a reduced amount. The ability of the derivatives to assemble into biologically active 30 S subparticles was studied under reconstitution conditions at 20°C and 40°C. It is seen in table 1 that during incubation at 40°C the particles attach the split proteins and form biologically active 30 S subparticles. Incubation at 20°C for 45 min does not result in the formation of biologically active 30 S subparticles.

Electron microscopy studies of the protein-deficient derivatives containing mainly four proteins (S4, S7, S8, S15) demonstrate the high uniformity in their dimensions and shape. Figure 2a shows the micrograph of a field of about 170 particles which

Table 1
Activity of ribosomes containing reconstituted 30 S subparticles in the cell-free system of polyphenylalanine synthesis

Ribosomal components	Reconstitution temp. (°C)	Polyphenylalanine precipitated by 5% trichloroacetic acid, radioactivity of [^{14}C]Phe(pmol)	% Activity
50 S + original 30 S	–	21.4	100
50 S + reconstituted 30 S	40	17.7	83
50 S + reconstituted 30 S	20	1.1	5
50 S + derivatives of 30 S	40	1.1	5
50 S	–	1.1	5

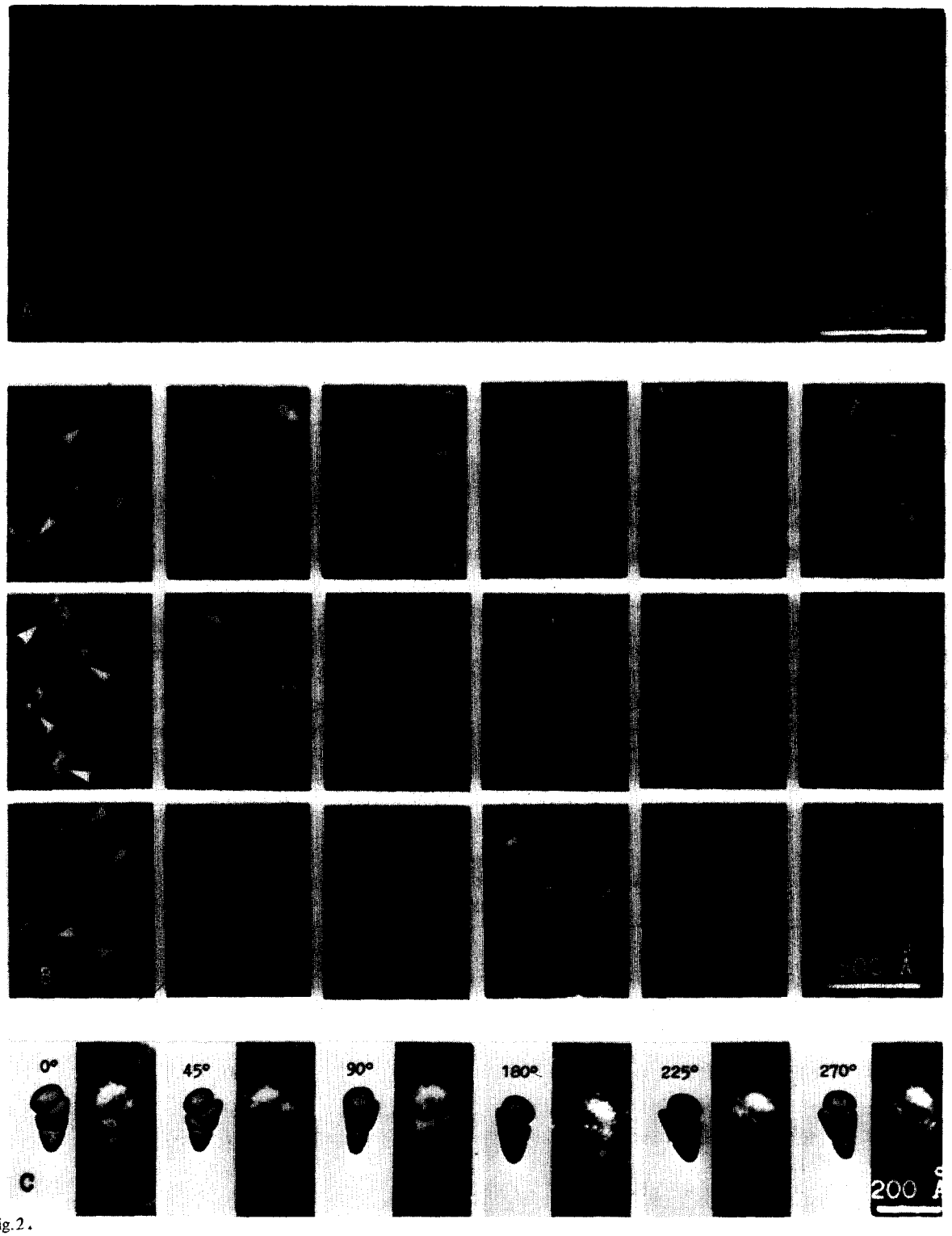

Fig.2.

has only a few dimers and large aggregates. The length of the particles is 210–230 Å, i.e., about 5% less than the length of the 30 S subparticles (220–240 Å) [5,6].

The 30 S subparticle can be roughly characterized as an elongated asymmetric structure, with an axial ratio of about 2:1, and 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 [1]. The particles studied are similar to the 30 S subparticles in these main morphological features. The electron micrographs of the individual particles are given in fig.2b. They have characteristic images similar to those which correspond to different projections of the three-dimensional structure of the 30 S subparticle (fig.2c) [5,6]. In contrast to the 30 S subparticles the derivative particles appear more flattened and have less distinct outlines. It can be thought that the protein-deficient particles have a very similar but less rigid structure.

Thus, the derivatives of 30 S subparticles containing only four proteins, such as S4, S7, S8, S15, in contrast to the derivatives containing nine proteins, such as S4, S6, S7, S8, S15, S16, S17, S18, S19 [1] i.e., proteins which are requisite for the formation of RI*-particles [12], do not assemble into biologically active 30 S subparticles in reconstitution conditions at 20°C. Nonetheless, the particles with the four proteins, just as the particles with nine proteins, which are somewhat protein depleted structural analogs of RI*-particles [1], retain the main morphological features of the 30 S subparticles. This indicates that the difference in the ability of protein-deficient particles to assemble into biologically active 30 S subparticles does not affect the general morphological features reflecting the main elements of the unique three-dimensional structure of 30 S ribosomal subparticles.

4. Conclusion

Electron microscopy studies of the morphology of the protein-deficient derivatives of the 30 S ribosomal subparticles containing proteins S4, S7, S8, S15 show their great similarity with intact 30 S subparticles in their dimensions, shape and subdivision into two unequal parts. The following conclusions can be made: (1) Proteins S4, S7, S8, S15 in the complex with 16 S ribosomal RNA are sufficient for packing of the main elements of the unique three-dimensional structure of 30 S subparticles; (2) The other proteins, such as S1, S2, S3, S5, S6, S9, S10, S11, S12, S13, S14, S16, S17, S18, S19, S20, S21, do not play any important role in fundamental structural rearrangements during RI → RI* transition.

Acknowledgements

We express our gratitude to Professor A. S. Spirin for encouragement and valuable discussion. We also thank Professor A. A. Bogdanov for help and Dr A. S. Girshovich for critical remarks.

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Fig.2. Electron micrographs of the protein-deficient derivatives freeze-dried in vacuum. Shadowing with tantalum–tungsten. Shadow length to object height ratio was about 2:1. Metal layer thickness was 15 Å. (a) Electron micrograph of a field of particles. (b) A gallery of electron micrographs of particles. Arrows in the left vertical row of micrographs indicate the subdivision of the particles into 'head' and 'body'. (c) A model and characteristic images of the 30 S subparticles which correspond to the different projections of their three-dimensional structure [5,6].

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