Structure of protein-deficient 50 S ribosomal subunits

Particles without 5 S RNA-protein complex retain the L7/L12 stalk and associate with 30 S subunits

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50 S ribosomal subunit derivatives without the 5 S RNA-protein complex obtained either by splitting with EDTA or by reconstitution from the 23 S RNA and proteins have been studied by electron microscopy. Removal of the 5 S RNA-protein complex is shown to affect neither the overall morphology of the larger ribosomal subunit nor the mode of its association with the small subunit.

Large ribosomal subunit *5 S RNA-protein complex* *L7/L12 stalk* *Electron microscopy*

1. INTRODUCTION

The 5 S RNA forms a specific complex with ribosomal proteins L5, L18 and L25. This complex can be removed from the 50 S subunit as such [1] and can be considered as a more or less autonomous structural domain of the subunit. The complex contributes to the formation of the most prominent morphological feature of the 50 S subunit, i.e. its central protuberance [2–6]. There is evidence that 5 S RNA participates in polypeptide synthesis, but its specific function is unknown [8]. There are several hypotheses concerning this topic. In particular, one proposed function is that the 5 S RNA is indispensable for association of the ribosomal subunits [7]. The purpose of this paper is to elucidate the effect of removal of the 5 S RNA-protein complex on the structure of the 50 S subunit. Here we report the results of such a study using electron microscopy. We have found that removal of the 5 S RNA-protein complex affects neither the overall morphology of the large ribosomal subunit nor the mode of its association with the small subunit. However, some local decompaction of the structure in the region of the central protuberance and, possibly, in the region of the L7/L12 stalk takes place.

2. MATERIALS AND METHODS

Ribosomal 50 S subunits were obtained as in the accompanying paper [9]. The subunits were stored in 10 mM Tris-HCl buffer, pH 7.5, 10 mM $MgCl_2$, 50 mM NH_4Cl, 3 mM mercaptoethanol (buffer A) at −70°C. 50 S derivatives depleted of the 5 S RNA-protein complex were isolated after EDTA treatment by the methods in [1,10] with minor modifications. The subunits were treated with 0.3–0.5 μmol EDTA per μmol nucleotide. The 50 S particles without the 5 S RNA complex (47 S particles) were isolated in a 10–30% linear sucrose gradient in buffer A. 23 S RNA was obtained as in [11] with minor modifications [9]. Total proteins from 50 S (TP 50) subunits were extracted with acetic acid as in [12]. Reconstitution of 47 S particles was done from 23 S RNA and TP 50 according to [13]. The homogeneity of 23 S RNA was checked by agarose-polyacrylamide gel electrophoresis [14]. The protein composition of 47 S particles was determined by two-dimensional electrophoresis [15]. Velocity sedimentation was carried out in a UCA-10 (USSR) ultracentrifuge. Electron microscopy studies were done as in [9].

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3. RESULTS AND DISCUSSION

The RNP particles for the studies were obtained both by depletion of the 5 S RNP complex with EDTA treatment and by reconstitution from purified 23 S RNA and TP 50. The particles had a sedimentation coefficient of 47 S (fig.1) coinciding with a published value [1]. They contained all proteins except L5, L18, L25 (L18 can be seen as traces) (fig.2). Electron micrographs of the 47 S particles are presented in fig.3. It is seen that the majority of the particles in the field contain the L7/L12 stalk (fig.3a). In our study we analysed only such 47 S particles. As expected, the main changes in morphology of the particles took place in the region of the central protuberance. All the 47 S particles, as with the 50 S subunits, have a crown-like region. However, the visible shape of the central protuberance is variable. Fig.3 represents both extreme types of the 47 S particle images in a crown-like view – one type with a very reduced and the other with a nearly normal central protuberance (see fig.3b and c, respectively).

A comparative analysis of the 47 S particle and 50 S subunit images shows changes in the character

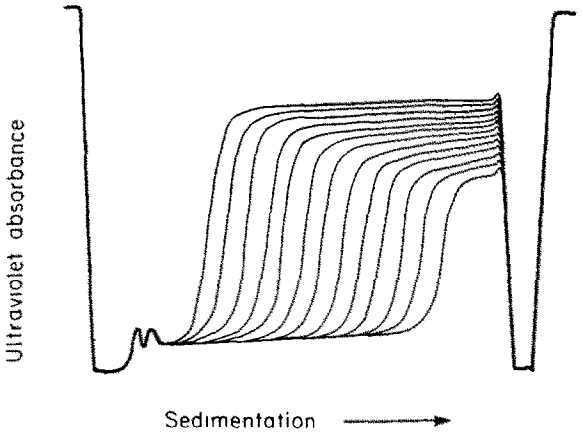


Fig.1. Sedimentation pattern of the 47 S particles reconstructed from 23 S RNA and TP 50. Sedimentation was done in buffer A at 20°C using an analytical ultracentrifuge (UCA-10, USSR). Scanning interval 2 min, 40000 rpm.

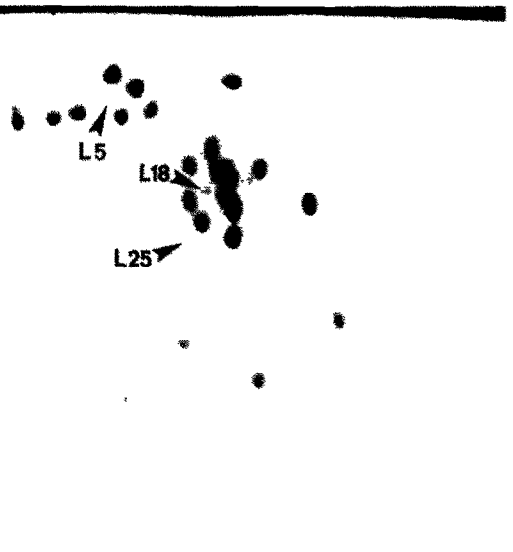
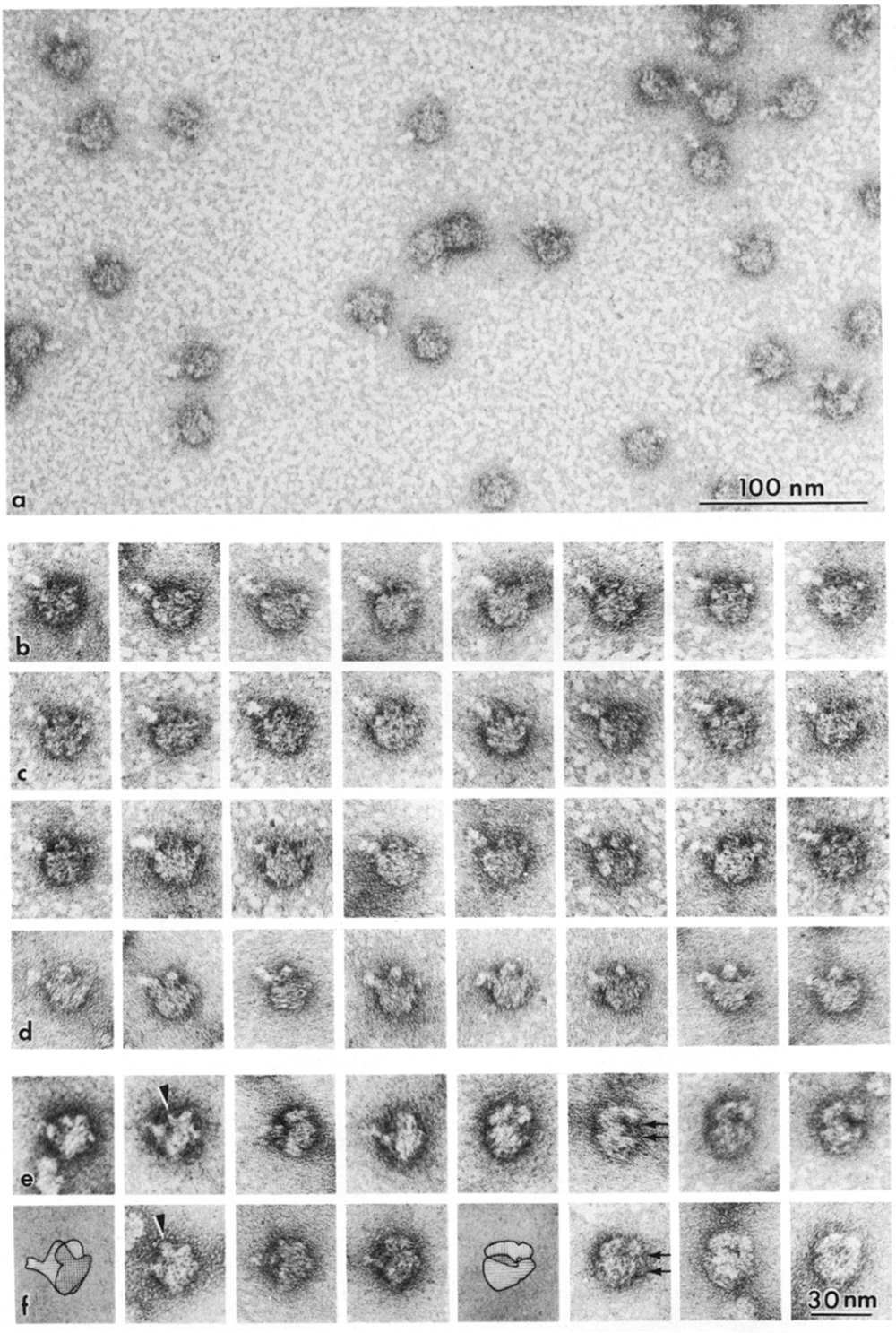


Fig.2. Two-dimensional gel electrophoresis of the reconstituted 47 S particles. Arrowheads indicate the sites occupied by proteins L5, L18 and L25 on the electropherogram of the intact 50 S subunits.

of uranyl acetate staining in the region of the central protuberance. Negative staining of ribonucleoprotein particles with uranyl acetate is always accompanied by some positive staining of RNA, the intensity of which depends on the protein content and RNA compactness. In fact, the micrographs show the predominance of negative staining in the region of the central protuberance of the intact 50 S subunit (see fig.3d), whereas in the same region of the 47 S particles mainly positive staining is observed (fig.3a–c). On the one hand, this means that removal of the 5 S RNA-protein complex results in protein deficiency of the central protuberance and in some local decompaction of the 23 S RNA in this region. On the other, the presence of the positively stained central protuberance in the 47 S particles, as shown in fig.3c, indicates that this protuberance in the 50 S subunit is upheld by 23 S RNA as a core.

It can be suggested that the disappearance of a

Fig.3. Electron micrographs of the 50 S subunit derivatives depleted of 5 S RNA-protein complex (47 S particles) and their couples with the 30 S subunits. Micrographs of the 50 S subunits and 70 S ribosomes are also presented for comparison. (a) Field of the preparation of the 47 S particles. (b,c) Two types of images of the individual 47 S particles (see text). (d) Individual 50 S subunits. The central protuberance is mainly negatively stained. (e) 30 S·47 S couples. The couples are in overlap (first 4 frames) and non-overlap (next 4 frames) views. (f) 70 S ribosomes in overlap and non-



overlap views. Interpretative drawings are given in the first and fifth frames. Arrowheads and arrows in (e) and (f) indicate the region of the central protuberance on the images of the 30 S·47 S couples and 70 S ribosomes in overlap and non-overlap views, respectively.

considerable part of the central protuberance on some images of the 47 S particles (fig.3b) is only apparent and is due to a change in staining of this region. Close inspection of images of the type shown in fig.3b reveals at times a weakly contrasted peripheral region of the central protuberance whose contours correspond to those of the central protuberance on images of the type in fig.3c. An alternative explanation is that after removal of the 5 S RNA-protein complex the central protuberance becomes flexible and is turned up or down on adsorption of the 47 S particle on the carbon film.

Another difference between the 50 S subunits and the 47 S particles is some 'looseness' of the L7/L12 stalk of the latter, although its position does not change.

There is an indication that the 5 S RNA participates in association of the ribosomal subunits [7,8]. We have compared the association of the 50 S and 47 S particles with the 30 S subunits. The curve of the Mg^{2+} dependence of the association for 47 S particles is found to be similar to that for the intact 50 S subunits (fig.4). Thus, the 5 S RNA-protein complex does not seem to be crucial for association of the subunits, at least under in vitro conditions.

Fig.3e and f presents images of the 30 S·47 S couples and the 70 S ribosomes, respectively. The particles are in overlap (the first 4 frames of each row) and non-overlap projections [16,17]. The images of the 30 S·47 S couples in the overlap projection differ somewhat from those of the 70 S ribosomes only in the size of the central protuberance. The contours of the 30 S·47 S couple images in the non-overlap projection almost coincide with those of the 70 S ribosomes as well, the only difference being some shortening of the central protuberance in the couples.

Thus, removal of the 5 S RNA-protein complex affects neither the overall morphology of the large ribosomal subunit nor the mutual arrangement of the subunits in the ribosome. Such a removal results only in some local decompaction of the structure in the region of the central protuberance and, possibly, in the region of the L7/L12 stalk. This is in good agreement with the findings on the attachment of the 5 S RNA-protein complex at the last step of 50 S assembly [8].

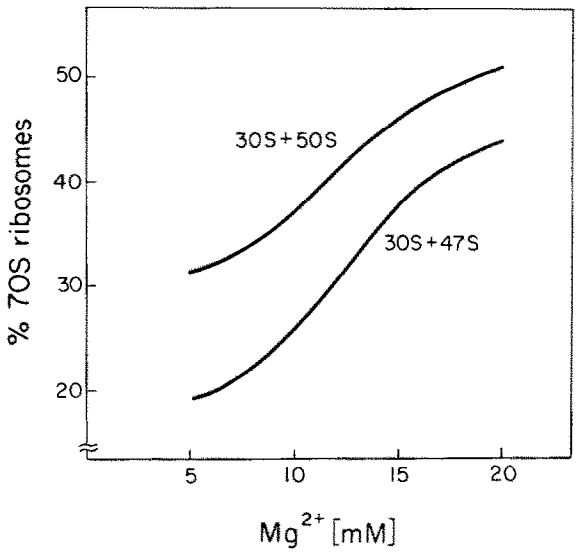


Fig.4. Mg^{2+} dependence of association of the subunits. Buffer A.

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REFERENCES

[1] Sarkar, N. and Comb, D.G. (1969) J. Mol. Biol. 39, 31–44.
[2] Shatsky, I.N., Evstafieva, A.G., Bystrova, T.F., Bogdanov, A.A. and Vesiliev, V.D. (1980) FEBS Lett. 121, 97–100.
[3] Evstafieva, A.G., Shatsky, I.N., Bogdanov, A.A. and Vasiliev, V.D. (1985) FEBS Lett. 185, 57–62.
[4] Stöffler-Meilicke, M., Noah, M. and Stöffler, G. (1983) Proc. Natl. Acad. Sci. USA 80, 6780–6784.
[5] Noah, M. Stöffler-Meilicke, M. and Stöffler, G. (1983) Proc. 10th Int. Congr. Electron Microsc. 3, 101–102.
[6] Stöffler, G., Lotti, M., Noah, M. and Stöffler-Meilicke, M. (1984) Proc. 8th Eur. Congr. Electron Microsc. 2, 1551–1552.
[7] Azad, A.A. (1979) Nucleic Acids Res. 7, 1913–1929.
[8] Dohme, F. and Nierhaus, K.H. (1976) Proc. Natl. Acad. Sci. USA 73, 2221–2225.
[9] Gongadze, G.M., Selivanova, O.M., Gudkov, A.T. and Vasiliev, V.D. (1986) FEBS Lett. 197, 74–78.
[10] Blobel, G. (1971) Proc. Natl. Acad. Sci. USA 68, 1881–1885.

[11] Steinhäuser, K.G., Wooley, P., Dijk, J. and Epe, B. (1983) Eur. J. Biochem. 137, 337–345.
[12] Hardy, S.J.S., Kurland, C.G., Voynow, P. and Mora, G. (1969) Biochemistry 8, 2897–2905.
[13] Nierhaus, K.H. and Dohme, F. (1974) Proc. Natl. Acad. Sci. USA 74, 4713–4717.
[14] Peacock, Q.C. and Dingman, C.W. (1968) Biochemistry 7, 668–673.
[15] Madjar, J.J., Michel, S., Cozzone, A.J. and Reboud, J.-P. (1979) Anal. Biochem. 92, 174–182.
[16] Lake, J.A. (1976) J. Mol. Biol. 105, 131–159.
[17] Vasiliev, V.D., Selivanova, O.M., Baranov, V.I. and Spirin, A.S. (1983) FEBS Lett. 155, 167–172.