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THREE-DIMENSIONAL CRYSTALS OF RIBOSOMES AND THEIR SUBUNITS FROM EU- AND ARCHAEBACTERIA

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SUMMARY

Ordered three-dimensional crystals of 70S ribosomes as well as of 30S and 50S ribosomal subunits from various bacteria (E. coli, Bacillus stearothermophilus, Thermus thermophilus and Halobacterium marismortui) have been grown by vapour diffusion in hanging drops using mono- and polyalcohols. A new compact crystal form of 50S subunits has been obtained, and it is suitable for crystallographic studies at medium resolution. In addition, from one crystal form large crystals could be grown in X-ray capillaries. In all cases the crystals were obtained from functionally active ribosomal particles, and the particles from dissolved crystals retained their integrity and biological activity.

INTRODUCTION

In order to investigate the molecular structure of ribosomes, three-dimensional crystals of ribosomal particles from several bacterial species were grown (1-7). Several of them were suitable for X-ray analysis (5-7). Furthermore, two-dimensional crystalline sheets were obtained from 50S and 70S ribosomal particles (8-11). Three-dimensional image reconstruction of these sheets led to the derivation of models of the whole ribosome (10) and its large subunit (11).

In this paper we describe the growth of three-dimensional crystals of 30S and 50S ribosomal subunits as well as of 70S ribosomes from eu- and archaebacteria.

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MATERIALS AND METHODS

Ribosomes and their subunits were isolated essentially as previously described (1,3). The integrity of the ribosomal particles was checked by sucrose gradient centrifugation, and their biological activity was determined in the poly(U)-system (1,12).

Crystallization was carried out by vapour diffusion in hanging drops or in X-ray capillaries (3,13). Crystallographic studies were performed using synchrotron radiation at station X31 of DESY in Hamburg. The internal order of the crystals was examined by electron microscopy of positively stained sections of crystals embedded in Epon resin (1).

RESULTS

At present, the three-dimensional crystals described in this paper are (with the exception of those of 50S ribosomal subunits from B. stearothermophilus) too small for efficient crystallographic studies. However, information concerning their packing arrangement and the unit cell parameters can be obtained from diffraction patterns of electron micrographs of thin sections through embedded crystals.

30S ribosomal subunits

The first indications for our ability to crystallize the small ribosomal subunits were somewhat disordered microcrystals of 30S subunits from E. coli ribosomes which were obtained at 4°C using 30% ethyl butanol as precipitant (Fig. 1). The same conditions were used for the crystallization of 30S subunits from Thermus thermophilus. In this case, it was possible to grow crystals in hanging drops and consequently to reduce the number of nucleation centers and to extend the time needed for diffusion of the alcohols into the crystallization solution (13). This resulted in the growth of crystals with unit cell dimensions of 110 Å x 400 Å and $\beta = 95°$ (Fig. 2).

The 30S subunits from H. marismortui (Fig. 3) were crystallized from polyethylene glycol in the presence of salts which mimic the natural environment of the halophilic ribosomes. This procedure has already been developed for the crystallization of the 50S subunits from the same bacteria (7). Although the

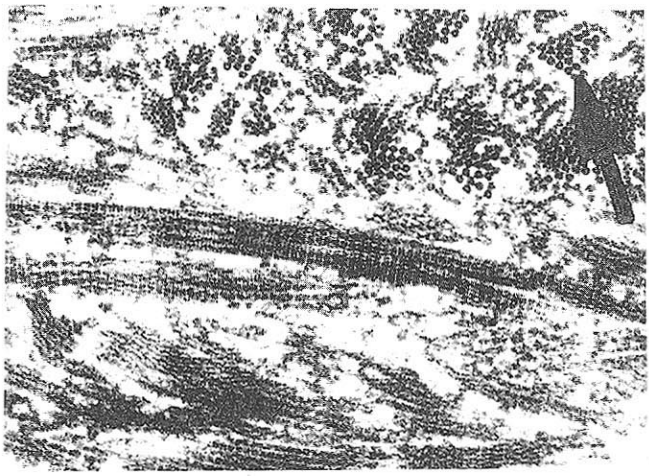

Fig. 1: Electron micrograph of a positively stained section through several microcrystals of 30S ribosomal subunits from E. coli. Some crystals were sectioned parallel to their long axis and others perpendicular to it (marked by an arrow). The microcrystals were obtained at 4°C in hanging drops using 30% ethyl butane, pH 8.2.

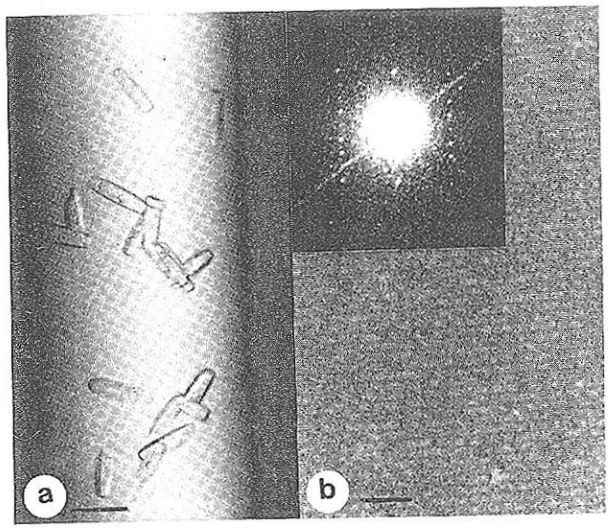

Fig. 2: (a) Crystals of 30S ribosomal subunits from Thermus thermophilus grown at 4°C in X-ray capillaries from a mixture of 20% ethyl butanol and 10% ethanol at pH 8.2. Bar: 0.1 mm. (b) Electron micrograph of a positively stained thin section of crystals shown in (a). Bar: 1000 Å. A diffraction pattern of the section is inserted.

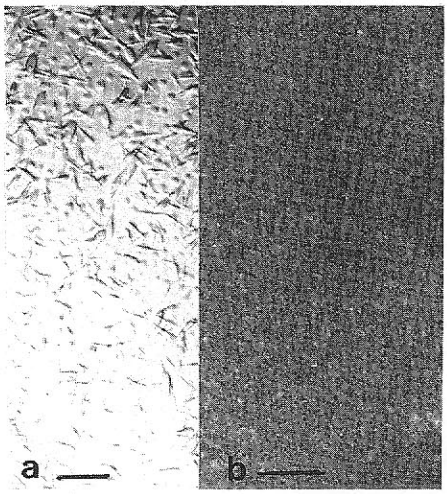

Fig. 3: (a) Crystals of 30S ribosomal subunits from Halobacterium marismortui grown at 19°C in hanging drops from 5% polyethylene glycol in the presence of 2 M KCl, 0.5 M NH_4Cl and 0.1 M $MgCl_2$, pH 5.7. Bar: 0.1 mm. (b) Electron micrographs of a positively stained thin section of crystals shown in (a). Bar: 1000 Å.

crystals of the 30S subunits are much smaller than those of the 50S subunits, they are well shaped and densely packed. Thus they are potentially suitable for crystallographic studies.

50S ribosomal subunits

Large and diffracting crystals of 50S subunits from B. stearothermophilus have previously been obtained (6). They were grown directly in X-ray capillaries using mixtures of methanol and ethylene glycol. Although it was possible to obtain valuable crystallographic information from these crystals (6) there were substantial difficulties in handling them. Therefore, we searched for other conditions for the growth of crystals from this source. This procedure was based on our ability to obtain two-dimensional sheets of ribosomal particles from B. stearothermophilus using mixtures of alcohols and salts and on our observation that the lower the Mg^{++} concentration in the reaction mixture is, the thicker the crystals are. Along these lines, crystals could be grown in hanging drops using very low concentrations of poly-

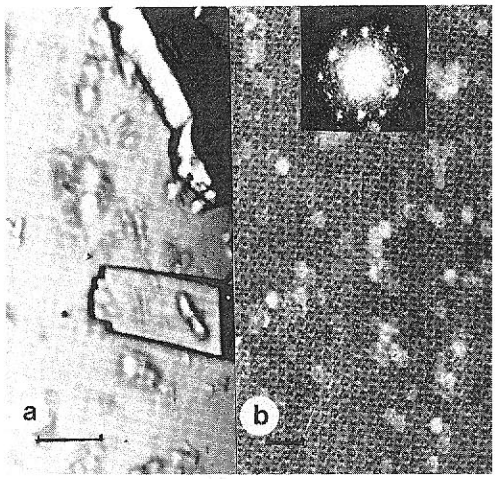

Fig. 4: (a) Crystals of 50S ribosomal subunits from Bacillus stearothermophilus (form I) grown in hanging drops at 4°C. Bar: 0.2 mm. Crystallization mixture: 2.5% polyethylene glycol, 0.5 M KCl, 0.18 M $(NH_4)_2SO_4$, 0.03 M $MgCl_2$, pH 6.6. (b) An electron micrograph of a positively stained thin section through a crystal as shown in (a). Bar: 1000 Å. A diffraction pattern of the section is inserted.

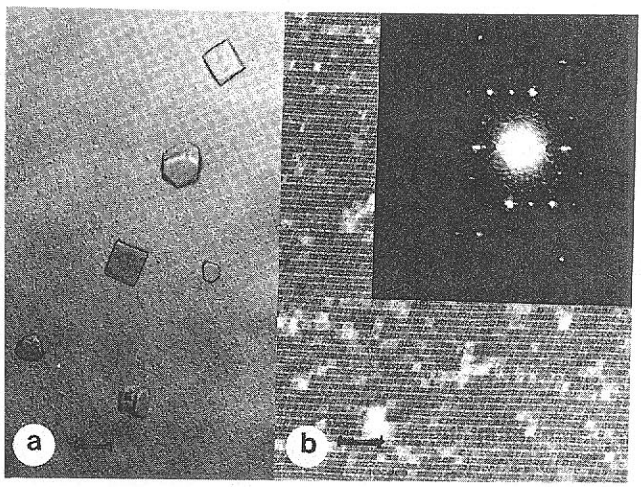

Fig. 5: (a) Crystals of 50S ribosomal subunits from Bacillus stearothermophilus (form II) grown in hanging drops at 4°C. Bar: 0.1 mm. Crystallization mixture: 2.5% polyethylene glycol, 0.18 M $(NH_4)_2SO_4$, 0.02 M $MgCl_2$, pH 6.4. (b) An electron micrograph of a positively stained thin section through a crystal as shown in (a). Bar: 1000 Å. A diffraction pattern of the section is inserted.

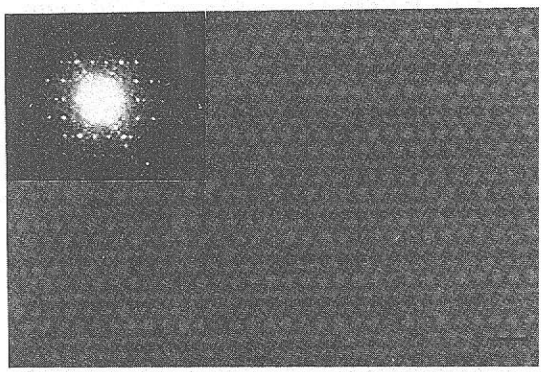

Fig. 6: A positively stained thin section of a crystal of 70S particles from Thermus thermophilus, grown in hanging drops at 4°C from 15% methyl pentane diol, pH 7.5. Bar: 1000 Å. A diffraction pattern of the section is inserted.

ethylene glycol as well as of the other ions in slightly higher concentrations than are necessary for maintaining the integrity of the particles. As seen in Figs. 4 and 5, two forms of well ordered crystals of a bulky shape and a fair size could be obtained. Crystals of form I (Fig. 4), can be handled and are suitable for crystallographic studies with synchrotron radiation. X-ray diffraction patterns extend to about 16 Å resolution and show periodic spacing of about 260 ± 10 Å, 300 ± 15 Å and 600 ± 20 Å (14). The crystals shown in Fig. 5 were too small for crystallographic studies. However, they were examined by electron microscopy. Interestingly, there is a similarity in the unit cell dimensions of the two forms (form II has 270 x 360 Å), in spite of the differences in growth conditions (form I grows in the presence of 0.5 M KCl, whereas in the crystallization solution of form II there is no KCl).

70S ribosomes

70S ribosomes from E. coli have yielded well ordered microcrystals with pseudohexagonal packing and unit cell dimensions of 340 Å x 340 Å x 590 Å and $\beta = 120°$ (2). Fig. 6 shows positively stained thin sections through crystals of 70S ribosomes from Thermus thermophilus, which are packed in unit cells of 420 x 470

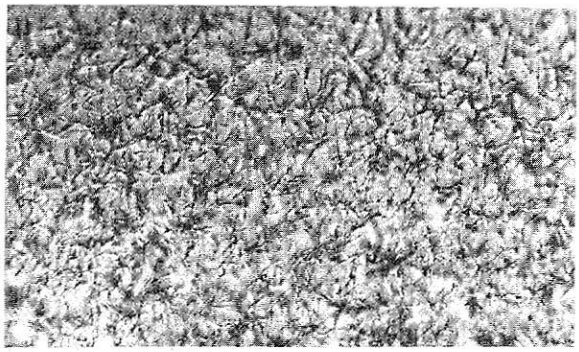

Fig. 7: Crystals of 70S from B. stearothermophilus grown at 4°C, pH 6.6, from 2.5% PEG in the presence of 0.5 M KCl, 0.18 M $(NH_4)_2SO_4$ and 30 mM $MgCl_2$.

x 300 Å. In Fig. 7 we show microcrystals of 70S ribosomes from B. stearothermophilus which seem to be potential candidates for X-ray crystallographic studies.

CONCLUSION

As described in this paper it is possible to crystallize all (30S, 50S and 70S) ribosomal particles under appropriate conditions using pure, homogeneous and biologically active samples of ribosomes and their subunits. The crystals were obtained under a large variety of crystallization conditions, and the crystalline ribosomal particles retain their biological activity.

Acknowledgements

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