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CRYSTALLIZATION OF THE LARGE RIBOSOMAL SUBUNITS FROM *BACILLUS STEAROTHERMOPHILUS*

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SUMMARY

Well ordered three-dimensional microcrystals (10-100 μm) of *B. stearothermophilus* 50S ribosomal subunits have been obtained *in vitro*. Under suitable conditions crystals appear within several weeks and are stable at 6°C for months. The ribosomal particles within the crystals maintain their biological activity and co-migrate with standard 50S particles in sucrose gradients. The crystals diffract X-rays to ∿3.5 Å, and the optical diffraction pattern of their electron micrographs shows reflection spots between ∿240 Å to ∿40 Å. Hence, the crystals are likely to be suitable for high resolution three-dimensional structure analysis.

INTRODUCTION

Ribosomes are the only cell organelles which occur in all organisms. To understand their role in protein biosynthesis at the molecular level, it is essential to have an exact knowledge of their structure. The shape of the *E. coli* ribosome and its subunits has extensively been studied by electron microscopy (1,2) and by physical-chemical techniques (3).

In spite of the extensive electron microscopical studies there are still differences between the ribosome models developed in various laboratories. This is partly due to changes in the shape of the particles which occur during the preparation of the samples, but is also due to different interpretations of the electron micrographs. More objective interpretations are possible by three-dimensional image reconstruction from electron micrographs of single *E. coli* ribosomal subunits (4) and of two-dimensional sheets of lizard ribosomes formed *in vivo* (5). Unfortunately in the latter case the observed resolution of 90 Å is

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a limiting factor for detailed interpretation. Recently, helical arrays of *E. coli* ribosomal subunits have been obtained *in vitro* (2,6) whose electron micrographs diffract to a resolution of 60 Å.

In this communication we report the first successful three-dimensional crystallization of 50S ribosomal subunits from *B. stearothermophilus*. The stability and X-ray diffraction patterns of the crystals as well as their electron micrographs show that they are appropriate for high resolution three-dimensional structure analysis. The particles in the crystals have biological activity, and they migrate together with standard 50S particles on sucrose gradients. The crystals therefore contain undegraded and functionally active 50S ribosomal subunits.

MATERIALS AND METHODS

Subunits: *Bacillus stearothermophilus* (strain 799) was grown at 60°C to early-log phase in a 50 l fermenter as described (7). 30S and 50S ribosomal subunits were isolated and tested for their biological activity according to ref. 8 and 9. The isolated ribosomal subunits were stored at -80°C at a concentration of at least 200 A_{260} units per ml in TMA-I buffer [10 mM Tris-HCl (pH 7.6 at 25°C), 30 mM NH_4Cl, 10 mM $MgCl_2$, 6 mM ß-mercaptoethanol].

Crystallization: This was carried out using microdialysis, free interface diffusion, vapor diffusion on slides and in capillaries as well as vapor diffusion in hanging drops (10). The crystallization agents were inorganic salts (such as ammonium, magnesium or lithium sulfates, sodium-potassium phosphate, sodium citrate) or low molecular weight alcohols (e.g. ethanol, methanol, *ter*-butanol, 2-methyl-2,4-pentanediol, ethandiol) or organic solvents (such as dioxane, toluene) or polymers (polyethylen glycol and polystyrene sulfonate) or a combination of the above mentioned substances. All together we have used 8 different salts, 11 alcohols, 4 organic solvents and 2 polymers. All experiments were made both in the absence and presence of 5.10^{-3} M spermidine.

The variables for the initial survey were the precipitants, the pH (4,5 - 10,0) and the concentration of the subunits (100 - 500 A_{260} per ml). The concentration of the precipitants was increased if no crystals appeared within the first 3 weeks, and was decreased if a precipitate had formed. All crystallization experiments were carried out at 4 - 7°C.

Electron microscopy and optical diffraction: The crystals were fixed in hanging drops for 2 h at 4°C with 2% glutaraldehyde and treated with 1% OsO_4 for 2 h. The preparations were then dehydrated by rapid passage through an acetone series and embedded in the resin ERL 4206 (11). Thin sections were cut on a Reichert-Ultracut microtome with a diamond knife, mounted on Formvar and carbon-coated grids and stained with 2% uranyl acetate.

Electron micrographs were taken with a Siemens Elmiskop 102 at 30,000 and 60,000x magnification. Diffraction patterns were obtained in an optical diffractometer equipped with a helium-neon laser. They were filtered by means of a Cu foil.

Biochemical characterization: Crystals, together with their growth medium, were placed into 0,7 mm X-ray quartz capillaries and were centrifuged at 800 rpm for 3 minutes. The supernatant was removed, and the pellet which contained the crystals was either stored or resuspended with a solution containing the crystallizing agent. The suspension was centrifuged again. Both pellets (after the first and second centrifugation) were dissolved in TMA-I buffer, analyzed on a sucrose gradient and checked for biological activity (8,9).

RESULTS AND DISCUSSION

As mentioned above (see Materials and Methods) several crystallization techniques (10) were employed. The best crystals (size 10-100 μm) were obtained in hanging drops within 3 - 10 weeks. A variety of organic solvents (most of them were low molecular weight alcohols) were found to be suitable agents for the production of crystals. These were obtained over quite a wide range of pH (4.8 to 9.3). Addition of spermidine did not have any detectable effect on crystal growth. One out of seven of the subunit preparations which were used did not produce crystals. The reason for this is not known but is probably due to slight differences in the growth of the bacteria and/or the isolation of the ribosomes and their subunits.

Figs. 1a and b show the shapes of the crystals as seen by light microscopy. The crystals are fairly stable at 6°C, and exposure to X-rays (1500 W) was possible for 30 hours. However, when the crystals were transferred to room temperature, they dissolved within a day. Their shape was maintained even after cross-linking by 0.7% glutaraldehyd within the droplet (Fig. 1c, d).

Electron micrographs of crystals that were grown in 10% ethandiol, pH 7, are shown in Fig. 2a, and their optical diffraction pattern is inserted into the figure. The optically filtered image is depicted in Fig. 2b. The electron micrographs of the sections (thickness ∿500 Å) through the crystals and the optical diffraction both show the regular arrangement of the ribosomal subunits. The detected lowest order diffraction spot is at ∿240 Å which is consistent with the size of the 50S subunit of

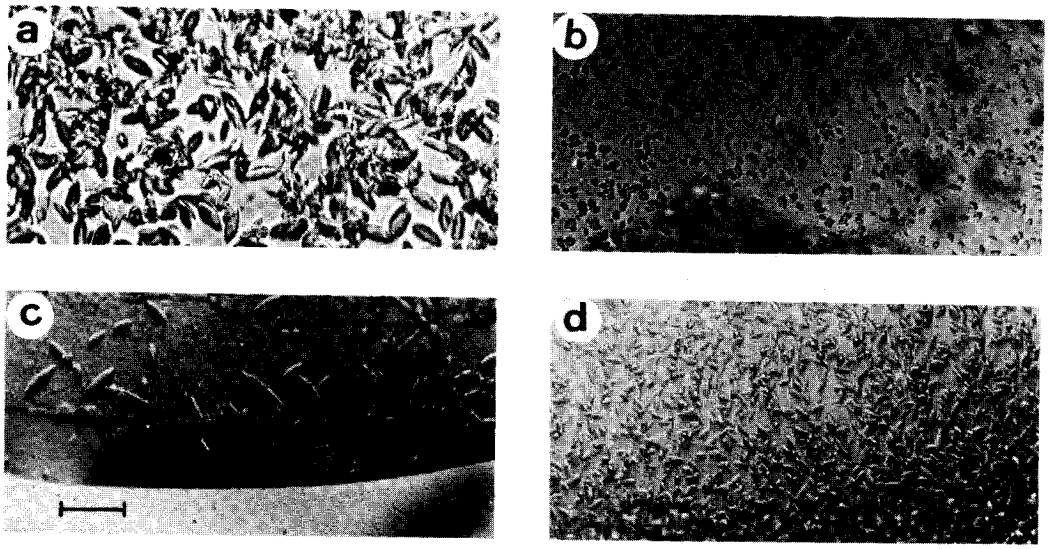

Fig. 1: Shape of crystals as seen by light microscopy. a) Crystals grown in 7% toluene (pH 6.2, adjusted with MES) in the presence of spermidine. b) Crystals obtained with 10% 2-ethyl-1,3-hexandiol (at pH 6.3 in MES buffer). c) Crystals obtained with 10% 2-ethyl-1,3-hexandiol (at pH 6.0 in MES buffer) in the presence of spermidine after crosslinking for 6 hours with 0.7% glutaraldehyde. d) Crystals obtained in 10% 2-ethyl-1,3-hexandiol (at pH 8.8 in glycine-NaOH buffer) in the presence of spermidine after crosslinking for 5 hours with 0.7% glutaraldehyde. All pictures are shown at identical magnifications. Scale bar corresponds to 100 μm.

bacterial ribosomes as studied by other techniques (1-3). Reflections in the resolution range of 40 Å can be identified.

The content of a crystallization droplet was used for "powder" moderate angle X-ray diffraction studies. The sample diffracts to a minimum value of ∿3,4 Å. Its pattern is sharp and partially oriented. It contains the main features that have been previously reported (12,13) for gels of ribosomes and extracted RNA. Details will be published elsewhere.

In order to substantiate that the crystalline material represent 50S ribosomal subunits, the isolated crystals were dissolved in TMA-I buffer and analyzed by sucrose gradient centrifugation (Fig. 3). Since the dissolved crystalline material comigrated with standard radioactive 50S ribosomal subunits it was

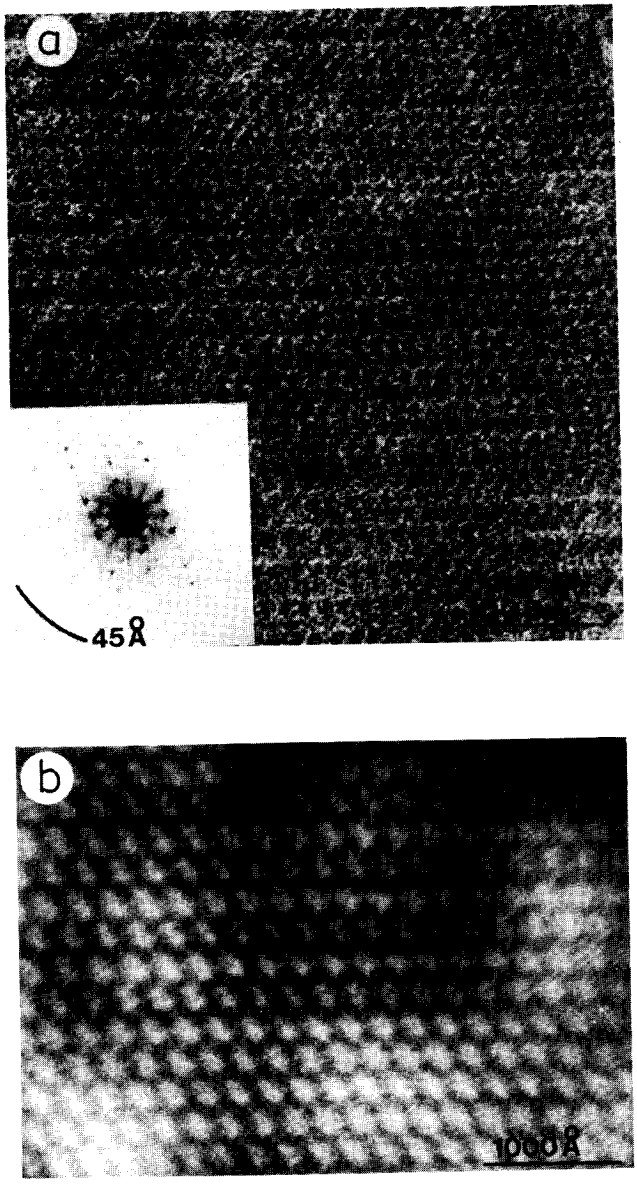

Fig. 2: a) Electron micrographs of a thin section of crystals grown in 10% ethandiol, pH 7.0 (MES). Insert: optical diffraction of the electron micrograph. The lowest order spot is at d ∿240 Å. The diffraction pattern has a maximum resolution of ∿40 Å.
b) Optically filtered image of Fig. 2a.

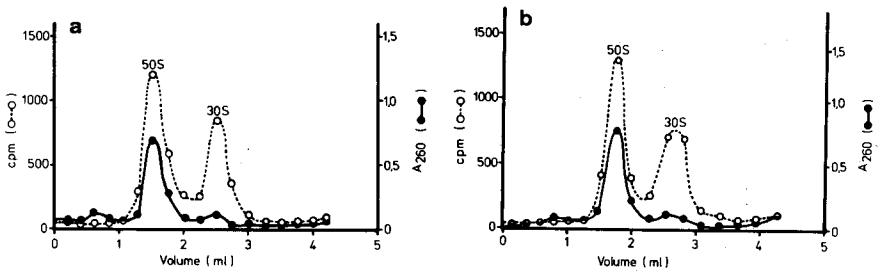

Fig. 3: Sucrose gradient analysis of (a) twice centrifuged crystals and (b) supernatant fraction (samples 5 and 6, respectively of Table I). 0.5 A_{260} unit samples, in 50 µl, were placed on top of a linear (4,2 ml) 10 - 30% sucrose gradient (SW60 Ti rotor) in TMA-II buffer (same as TMA-I buffer except for $MgCl_2$ concentration, which was 3 x 10^{-4} M). Each gradient contained reference *E. coli* [^{3}H] 50S and 30S ribosomal subunits (∿2000 cpm and ∿0.015 A_{260} units of each) obtained from Dr. R. Brimacombe. Centrifugation was carried out for 125 min at 4°C and 50 000 rpm. Four-drop fractions were collected after puncturing the tube, and A_{260} and ^{3}H radioactivity were determined by standard procedures.

Table I: Test for biological activity in a poly(U) system

Sample	Complete poly(U) system minus 50S subunits	cpm	Activity in poly(U)
1. 50S (standard)	+	7050	100%
2. Blank (minus 50S)	+	98	0%
3. 50S from crystals* after first centrifugation	+	4670	66%
4. 50S from the supernatant of sample 3	+	2885	41%
5. 50S from crystals* after second centrifugation	+	4230	60%
6. 50S from the supernatant of sample 5	+	2070	30%

*Crystals were grown in 8.5% 2-methyl-2,4-pentanediol at pH 7,0 - 9,3 (sample 3) or at pH 6,0 - 8,1 (sample 5).

concluded that the crystals contain *B. stearothermophilus* 50S ribosomal subunits, and that the crystallization conditions do not cause gross morphological changes in the ribosome structure. However, in one experiment (samples 3 and 4 in Table I) the dissolved crystalline material migrated with sedimentation coefficient of 47S.

Further, the crystallized 50S ribosomal subunits were tested for their biological activity. The results are summarized in Table I and demonstrate that the crystallized subunits retained at least 60% of their biological activity in a poly(U) directed polyphenylalanine synthesizing system. We therefore conclude that the crystallized material represents 50S ribosomal subunits with a high preservation of their structure as reflected by their biological activity.

In spite of the progress reported in this paper we are aware of the limitations imposed by the size and quality of the crystals. Considerable effort is still needed to optimize and refine the conditions for their growth in order to make them more suitable for three-dimensional structure analysis, e.g. by low and high angle X-ray crystallography, electron microscopy, neutron diffraction, image reconstruction and light scattering.

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REFERENCES

1. Stöffler, G., Bald, R., Kastner, B., Lührmann, R., Stöffler-Meilicke, M., Tischendorf, G. and Tesche, B. (1980) In "Ribosomes" (G. Chambliss et al., eds), pp. 171-205, Univ. Park Press, Baltimore.
2. Lake, J.A. (1980) In "Ribosomes" (G. Chambliss et al., eds), pp. 207-236, Univ. Park Press, Baltimore.
3. van Holde, K.E. and Hill, W.E. (1974) In "Ribosomes" (M. Nomura, A. Tissières and P. Lengyel, eds), pp. 53-92, Cold Spring Harbor Laboratory Press, Long Island, N.Y.
4. Knauer, V. and Hoppe, W. (1980) In "Electron Microscopy" (P. Brederoo and W. de Priester, eds), pp. 702-703, Seventh Europ. Congr. on Electron Microscopy, Leiden.
5. Unwin, P.N.T. (1977) Nature *269*, 118-122.

6. Clark, M.W., Hammon, M., Langer, J.A. and Lake, J.A. (1979) J. Mol. Biol. *135*, 507-512.
7. Erdmann, V.A., Fahnestock, S., Higo, K. and Nomura, M. (1971) Proc. Nat. Acad. Sci. USA *68*, 2932-2936.
8. Cronenberger, J.H. and Erdmann, V.A. (1975) J. Mol. Biol. *95*, 125-137
9. Wrede, P. and Erdmann, V.A. (1973) FEBS Lett. *33*, 315-319.
10. McPherson, A. (1976) In "Methods of Biochem. Analysis" (Glick, ed.), pp. 249-345, John Wiley and Sons, New York.
11. Spurr, A.R. (1969) J. Ultrastruct. Res. *26*, 31-43.
12. Zubay, G. and Wilkens, M.H.F. (1960) J. Mol. Biol. *2*, 105-112.
13. Klug, A., Holmes, K.C. and Finch, J.T. (1961) J. Mol. Biol. *3*, 87-100.