Volume 220, number 2, 319-322 FEB 05008 August 1987

Crystallization of 70 S ribosomes and 30 S ribosomal subunits from *Thermus thermophilus*

S.D. Trakhanov*, M.M. Yusupov, S.C. Agalarov, M.B. Garber, S.N. Ryazantsev, S.V. Tischenko and V.A. Shirokov

Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region and **Institute of Crystallography, Academy of Sciences of the USSR, Moscow, USSR*

Received June 1987

Well-ordered three-dimensional crystals of 70 S ribosomes and 30 S ribosomal subunits from extremely thermophilic bacteria *Thermus thermophilus* have been obtained. Positively stained thin sections of the crystals have been analyzed by electron microscopy. Redissolved crystalline ribosomes and small ribosomal subunits reveal sedimentation constants of 70 S and 30 S, respectively, and are functionally active in the poly(U)-system.

Ribosome; 30 S Ribosomal subunit; Three-dimensional crystal; Electron microscopy; (*Thermus thermophilus*)

1. INTRODUCTION

The exploration of ribosomes and ribosomal subunits from microorganisms living in very extreme conditions has provided three-dimensional crystals suitable for X-ray analysis. Significant progress has been achieved mainly due to the work of Yonath and co-workers in Wittmann's laboratory [1,2]. High quality crystals of the large ribosomal subunits from the extremely halophilic bacterium *Halobacterium marismortui* diffract to 6 Å resolution [3].

The extremely thermophilic bacterium *Thermus thermophilus* has been selected by us as a source for purification of ribosomes because of an increased stability of bacterial proteins and nucleic acids which are shown to be well crystallizable [4-6]. Recently crystals of the 70 S ribosomes [7] as well as those of the 30 S ribosomal subunits [8] have been obtained at the Institute of Protein Research.

Correspondence address: S.D. Trakhanov, Institute of Crystallography, Academy of Sciences of the USSR, Moscow, USSR

Here we report the conditions for growing a new highly ordered form of the 70 S ribosomes. The growing of crystals from the 30 S subunits, in particular via the seeding procedure, is also described.

2. MATERIALS AND METHODS

Growth of *T. thermophilus*, and purification of the ribosomes and ribosomal subparticles were performed according to [9] with slight modifications. The homogeneity of preparations was tested by sedimentation in an analytical centrifuge. The functional activity of the ribosomes was examined in a cell-free poly(U)-directed polyphenylalanine synthesizing system at 65°C as reported in [8]. The functional activity of the 30 S subunit was measured in a similar way with the exception that a 1.5 molar excess of the 50 S subunits was added.

Microdialysis and the 'hanging drop' method were used for crystallization of the ribosomes.

To identify macromolecules forming the crystals, the latter were pelleted from the mother solution, rinsed with buffer, containing 10% (v/v) 2-methyl-2,4-pentanediol (MPD), and dissolved in a MPD-free buffer. The solution was examined by analytical centrifugation.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

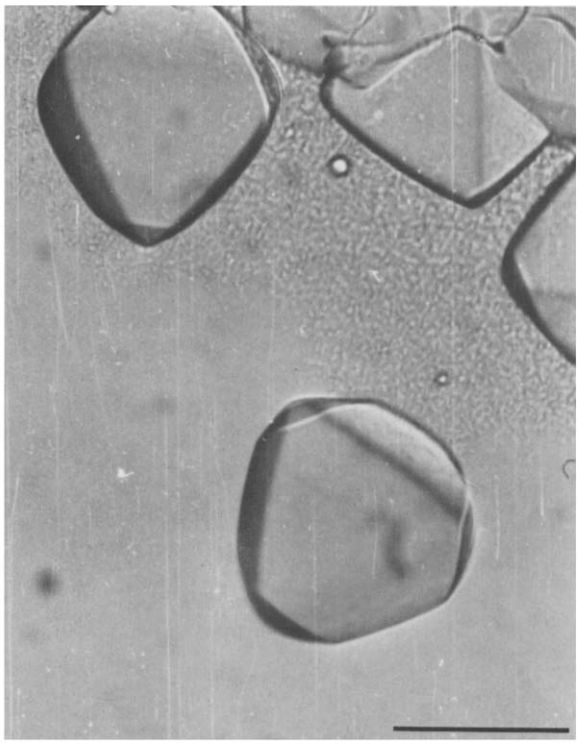


Fig.1. Crystals of 70 S ribosomes from *T. thermophilus* obtained by the 'hanging drop' method. All crystallization trials were done at 4°C. 10 μl drops containing ribosomes (~10 mg/ml), 20 mM Tris-HCl, pH 7.5, 25 mM $MgCl_2$, 75 mM NH_4Cl, 200 mM KCl and 10% MPD were equilibrated for several weeks against 11–20% MPD in the same solution. Bar, 0.1 mm.

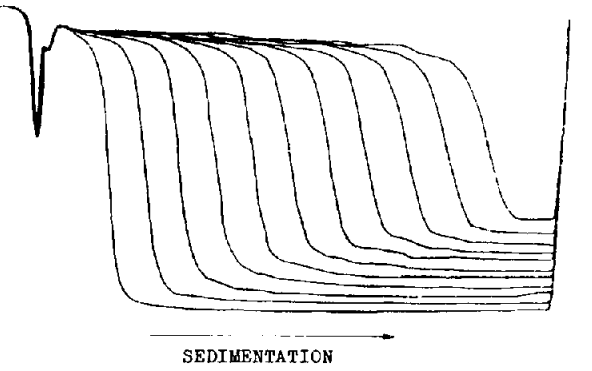
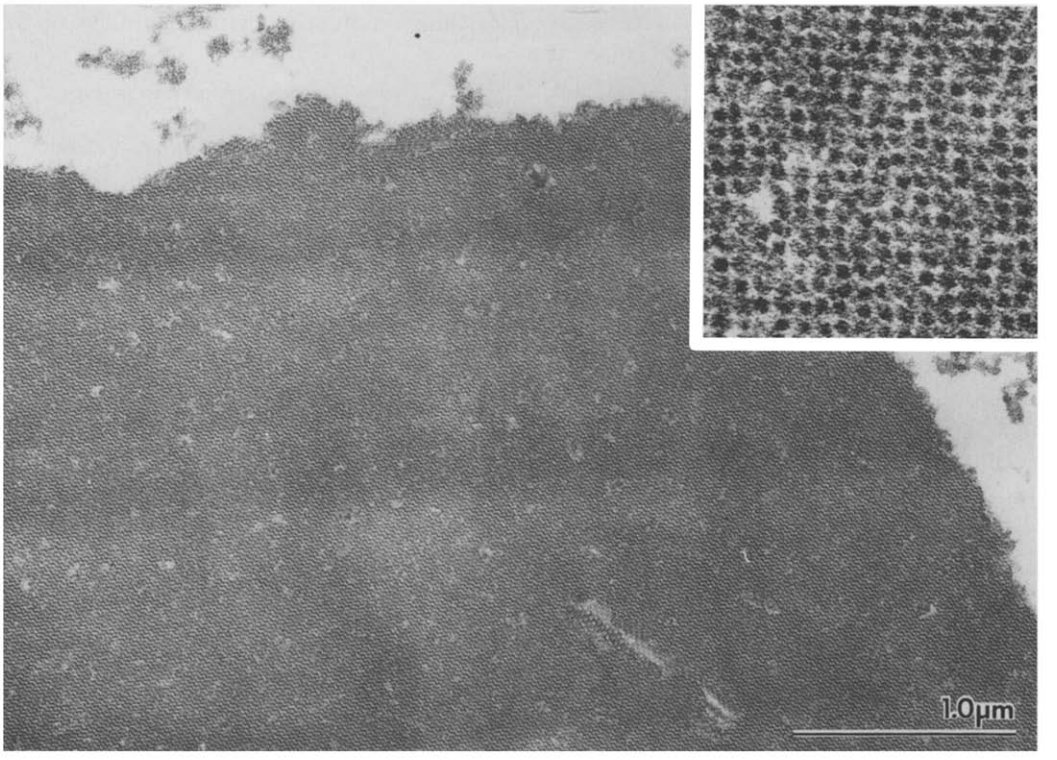


Fig.2. Sedimentation profile of 70 S ribosomes from dissolved crytals.



For electron microscopy investigations the crystals were treated with 0.5% glutaraldehyde by vapor diffusion for 12–20 h at 4°C followed by resuspension in a solution of 0.5% glutaraldehyde in a corresponding buffer for several hours. The pelleted crystals were rinsed twice with water. The fixed crystals were then dehydrated and embedded into the epoxy resin as described elsewhere [7]. Ultrathin sections of the crystals after staining in a saturated solution of uranium acetate according to [10] were visualized using a JEM-100C microscope under a 5000–20 000-fold magnification.

3. RESULTS AND DISCUSSION

Three-dimensional crystals of the 70 S ribosomes were first obtained by Wittmann and coworkers in 1982 [11]. These crystals had a needle-like form 200 μm in length. 70 S ribosomes from *T. thermophilus* were crystallized in 1986 at the Institute of Protein Research [7]. The crystals have a rod-like form with a maximal length of 200 μm. We found conditions for growing a new form of 70 S ribosome crystals which seems to be more suitable for X-ray analysis. Their micrographs are shown in fig.1. The crystals are well formed bipyramids reaching 200 μm in size. The conditions of crystallization are indicated in the legend to fig.1. Fig.2 represents an analytical sedimentation pattern of the dissolved crystals. The sedimentation coefficient was 70 S. The solution of the crystals exhibits a functional activity comparable to that of the starting material. Fig.3 shows a micrograph of ultrathin sections of 70 S ribosome crystals.

The seeding procedure, applied to a solution of the small subunits, has yielded crystals of increased sizes up to 300 μm (fig.4a). The solvent composition was the same as in [8]. Fig.4b,c represents micrographs of the 30 S subunit crystals growing from solutions at different MPD concentrations. It is evident that the morphology of the crystals depends markedly on the MPD concentration (fig.4b,c) without a difference in internal order as visualized by electron microscopy (not shown). Electron microscopy patterns of crystal thin sections are shown in fig.5. The dissolved crystals are functionally active in the cell-free system of polyphenylalanine synthesis.

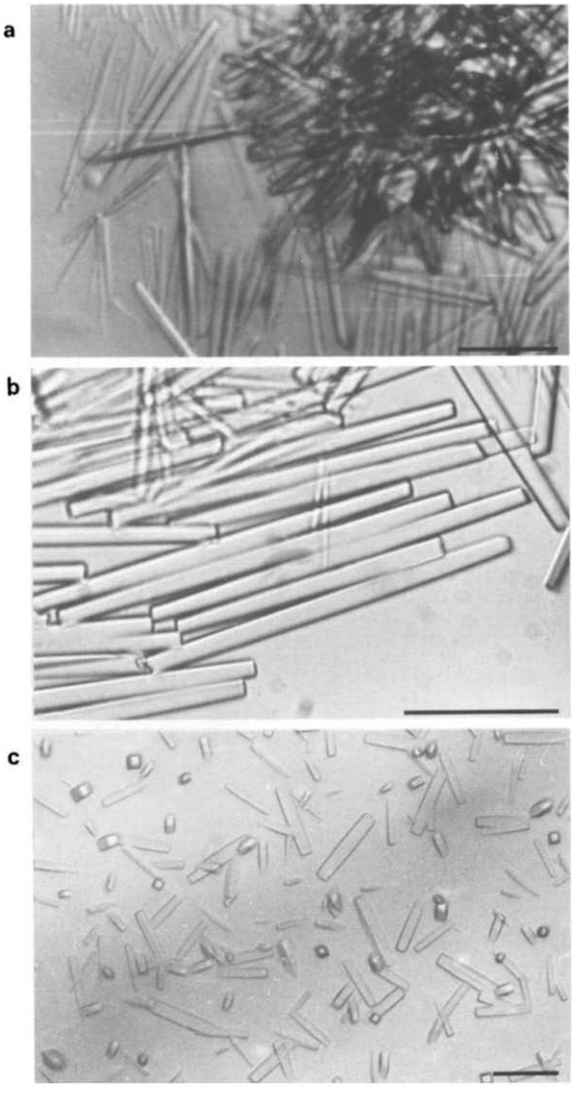


Fig.4. Crystals of 30 S ribosomal subunits from *T. thermophilus*, grown as reported in [8]. (a) Result of the seeding procedure; (b) crystals obtained by the 'hanging drop' method at a 15% MPD concentration; (c) the same as b, but the MPD concentration was increased to 20%. Bar, 0.1 mm.

At present studies are being done to find optimal

Fig.3. Electron micrographs of thin sections of embedded crystals of 70 S ribosomes; grown under the same conditions as in fig.1. The insert is a higher magnification of the crystal.

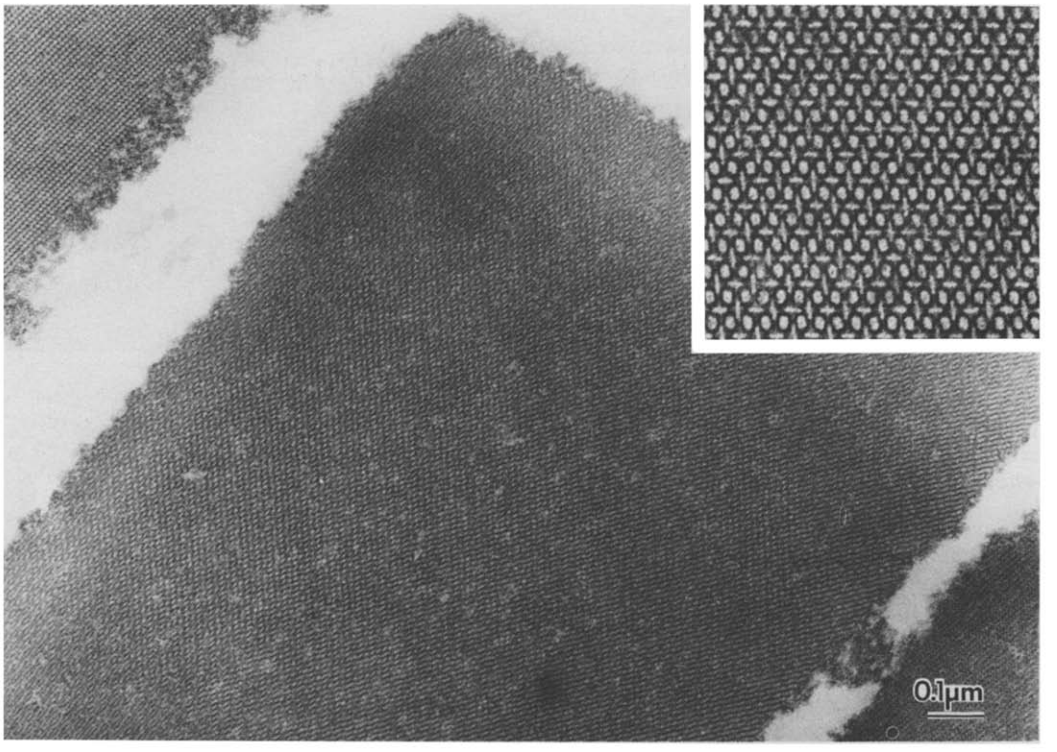


Fig.5. Electron micrographs of thin sections of 30 S subunit crystals. Inset: higher magnification of the crystal.

conditions for growing large crystals of the 70 S and 30 S particles, suitable for X-ray examination.

ACKNOWLEDGEMENTS

We are very grateful to Professors A.S. Spirin and B.K. Vainstein for stimulating discussions and support of our study.

REFERENCES

[1] Yonath, A., Saper, M.A., Makowski, I., Müssig, J., Pietke, J., Bartunik, H.D., Bartels, K.S. and Wittmann, H.G. (1986) J. Mol. Biol. 187, 633–636.
[2] Yonath, A., Saper, M.A. and Frolow, F. (1986) J. Mol. Biol. 192, 161–162.
[3] Makowski, I., Frolow, F., Saper, M.A., Shoham, M., Wittmann, H.G. and Yonath, A. (1987) J. Mol. Biol. 193, 819–822.
[4] Morikawa, K., Kawakami, M. and Takemura, S. (1982) FEBS Lett. 145, 194–196.
[5] Reshetnikova, L.S. and Garber, M.B. (1983) FEBS Lett. 154, 149–150.
[6] Sedelnikova, S.E. (1987) Biopolimery i Kletka (USSR) 3, 163–166.
[7] Karpova, E.A., Serdyuk, I.N., Tarkhovsky, Yu.S., Orlova, E.V. and Borovyagin, V.L. (1986) Dokl. Akad. Nauk SSSR 289, 1263–1266.
[8] Yusupov, M.M., Trakhanov, S.D., Barynin, V.V., Borovyagin, V.L., Garber, M.B., Sedelnikova, S.E., Selivanova, O.M., Tischenko, S.V., Shirokov, V.A. and Edintsov, I.N. (1987) Dokl. Akad. Nauk SSSR 292, 1271–1274.
[9] Gogia, Z.V., Yusupov, M.M. and Spirina, T.N. (1986) Mol. Biol. (USSR) 29, 519–526.
[10] Reynolds, E.S. (1963) J. Cell. Biol. 17, 208–212.
[11] Wittmann, H.G., Müssig, J., Pietke, J. Gewitz, H.S., Rheinberger, H.J. and Yonath, A. (1982) FEBS Lett. 146, 217–220.