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A new crystalline form of 30 S ribosomal subunits from *Thermus thermophilus*

M.M. Yusupov, S.V. Tischenko, S.D. Trakhanov*, S.N. Ryazantsev and M.B. Garber

Institute of Protein Research, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region and **Institute of Crystallography, Academy of Sciences of the USSR, Moscow, USSR*

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A new crystalline form of 30 S ribosomal subunits from an extremely thermophilic bacterium *Thermus thermophilus* has been obtained. Positively stained ultrathin sections of the crystals have been analysed by electron microscopy. The crystals show X-ray diffraction to about 12 Å resolution in a synchroton beam at 0°C.

Ribosome; 30 S ribosomal subunit; Three-dimensional crystal; X-ray analysis; (*Thermus thermophilus*)

1. INTRODUCTION

Recent achievements in crystallization of 70 S ribosomes [1,2] and 30 S ribosomal subunits [2,3] are based, at least in part, on the use of an extremely thermophilic bacterium *Thermus thermophilus* as a source of the components of the protein-synthesizing system. The 70 S ribosome crystals are suitable for X-ray analysis at 20–25 Å resolution and native set data collection is in progress now (Trakhanov et al., to be published). Crystals of the 30 S ribosomal subunits which were grown at the Institute of Protein Research in 1985 [2,3] were later also obtained by Glotz et al. [4]. The 30 S crystals had a rod-like form, their length exceeding thickness by 5–10 times [2,3].

This communication describes a new form of 30 S ribosomal subunit crystals from *T. thermophilus* which seem to be more promising for X-ray analysis.

2. MATERIALS AND METHODS

The growth of *T. thermophilus* and purification of 70 S ribosomes and ribosomal subunits were performed according to [5] with slight modifications. The homogeneity of preparations was tested by sedimentation in an analytical ultracentrifuge. The functional activity of the ribosomes was examined as reported in [2]. Microdialysis and the 'hanging drop' method were used for crystallization of ribosomes and ribosomal subunits. Electron microscopy analysis of ultrathin sections of the crystals was carried out as described earlier [2]. SDS-polyacrylamide gel electrophoresis was done according to Laemmli [6].

Correspondence address: M.M. Yusupov, Institute of Protein Research, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

3. RESULTS AND DISCUSSION

Crystallization of the 30 S ribosomal subunits from *T. thermophilus* was described in detail previously [2,3]. Rod-like crystals grow reproductively at 4°C in a solution containing 20 mM Tris-HCl, pH 7.5, 25 mM $MgCl_2$, 75 mM NH_4Cl, 200 mM KCl and 12–20% 2-methyl-2,4-pentanediol. Concentration of the 30 S ribosomal subunits is about 10 mg/ml.

We have found that some preparations of the 30 S ribosomal subunits also produce crystals of another form which are present in crystallization probes among the 'standard' rod-like crystals. These crystals with a hexagonal form are well-shaped and have sizes of 50 × 50 × 20 μm (fig.1). An electron micrograph of an ultrathin section of the crystal is shown in fig.2.

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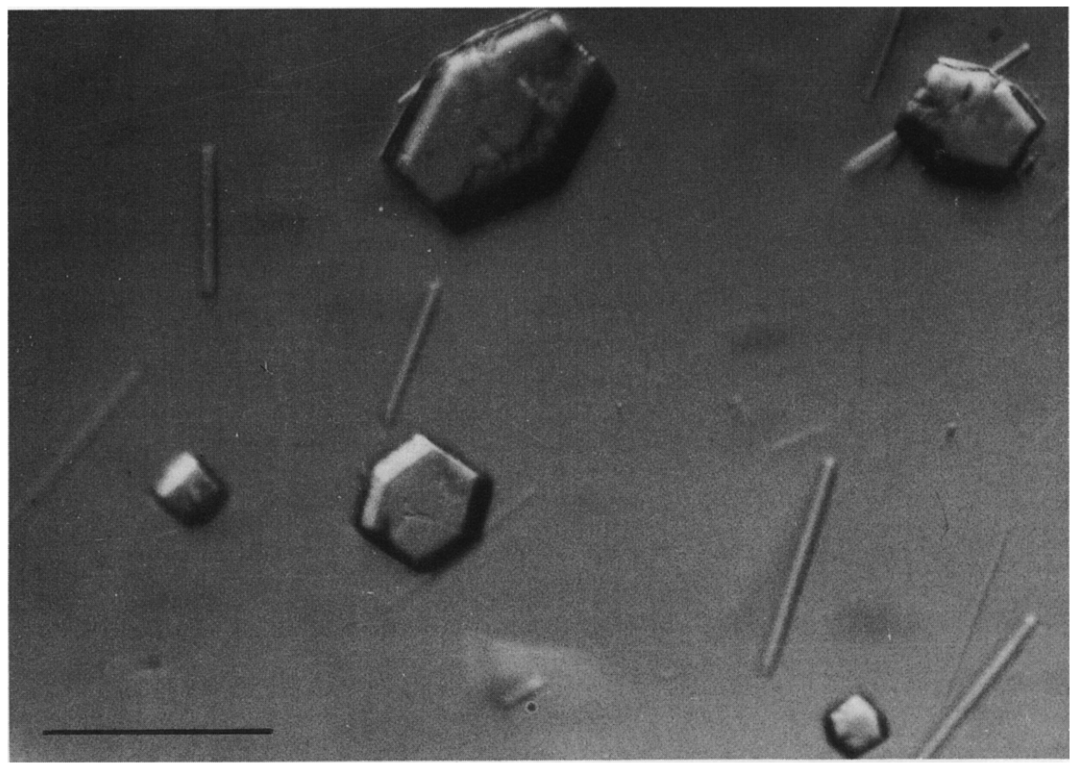

Fig.1. Hexagonal crystals of the 30 S ribosomal subunits from *T. thermophilus*. Bar, 0.1 mm.

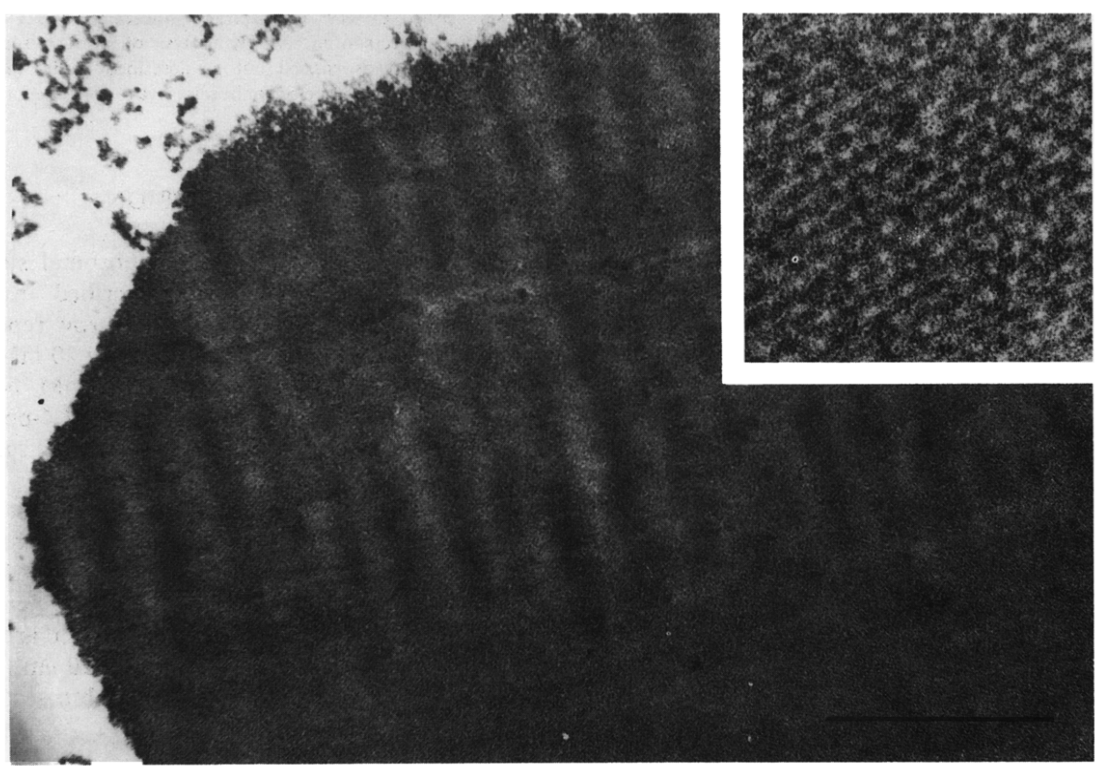

Fig.2. An electron micrograph of an ultrathin section from embedded hexagonal crystals of the 30 S ribosomal subunits. Bar, 1 μm. The inset is a micrograph of the same section at a higher magnification.

To identify the macromolecules in the hexagonal crystals, the latter were picked out from the mother solution, rinsed with the buffer containing 15% 2-methyl-2,4-pentanediol and dissolved in the buffer for SDS-electrophoresis. Electrophoresis has shown that the distribution and intensities of the protein bands are the same as those in the starting 30 S preparation.

Seeding of the initially obtained hexagonal 30 S crystals into the fresh solution of 30 S ribosomal subunits has yielded crystals of an increased size, up to $100 \times 100 \times 30$ μm. Preliminary examination of the crystals has been carried out in the high-intense X-ray beam at the LURE synchrotron station (Orsey, France) at Professor D. Moras' laboratory (unpublished). The crystals are rather stable under irradiation at 0°C and diffract X-rays to about 12 Å resolution.

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