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Characterization and Preliminary Crystallographic Studies on Large Ribosomal Subunits from *Thermus thermophilus*

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Diffracting crystals, suitable for X-ray crystallographic analysis, have been obtained from large (50 S) ribosomal subunits from *Thermus thermophilus*. These crystals, with $P4_12_12$ symmetry and a unit cell of 495 Å × 495 Å × 196 Å, reach typically a size of 0·15 mm × 0·25 mm × 0·35 mm. Using synchrotron radiation at cryo-temperature, these crystals diffract X-rays to better than 9 Å resolution, and do not show any measurable decay after a few days of irradiation. They complete a series of crystals, grown by us, from ribosomal particles of the same source, including a 30 S subunits, 70 S ribosomes and complexes of the latter with: (1) an oligomer of 35 uridine residues and (2) the same oligonucleotide together with approximately two Phe-tRNA^{Phe} molecules. Crystallographic analysis of the various members of this series should provide information for investigating the conformational changes that take place upon the association of ribosomes from their subunits as well as upon binding of non-ribosomal components that participate in protein biosynthesis.

As a result of a systematic exploration of conditions and development of innovative experimental techniques we were able to establish procedures for *in vitro* crystallization of intact ribosomal particles from bacterial sources (for a review, see Yonath & Wittmann, 1988; Yonath *et al.*, 1988*a*). Most of the crystal types grown so far in our laboratory proved suitable for crystallographic analysis at resolution limits ranging from almost molecular, 4·5 Å (1 Å = 0·1 nm), to rather low, 25 Å (Yonath & Wittmann, 1989; Berkovitch-Yellin *et al.*, 1990*a*; Hansen *et al.*, 1990).

In this communication we describe the crystals of intact large (50 S) ribosomal subunits from *Thermus thermophilus*. Like the other crystals of ribosomal particles grown in our laboratory, these crystals were obtained from active particles, and the crystalline material retained its integrity and biological activity for long periods.

The crystals of the 50 S subunits from *T. thermophilus* grew spontaneously or by seeding at 19°C in hanging drops and reached an average size of 0·15 mm × 0·25 mm × 0·35 mm (Fig. 1). They are packed in unit cells of 495 Å × 495 Å × 196 Å with a tetragonal ($P4_12_12$) symmetry. Despite their rather small size and weak diffraction power, we observed distinct reflections at 9 Å resolution (Fig. 2).

Preliminary crystallographic studies were carried out by the rotation method on film, using a well-collimated synchrotron X-ray beam at cryo-temperature (−180°C). For the shock cooling we employed a procedure similar to that developed recently by us (Hope *et al.*, 1989; Yonath *et al.*, 1988*b*; Berkovitch-Yellin *et al.*, 1990*b*). Before cooling the crystals were immersed in a solution containing all the components present in their mother liquor together with ethylene/glycol (15%/18%), which served as a cryogene. After soaking for about an hour, a single crystal, together with its soaking solution, was placed between the two glass films of a double-layer glass spatulum (the "sandwich" spatulum; Yonath *et al.*, 1988*b*), which was soldered to a brass pin. The spatulum holding the crystal was instantaneously frozen in liquid propane, at its liquefying temperature (about a 100 K), and transferred at that temperature to the X-ray rotation camera. Throughout the entire period of data collection the crystals were surrounded by a stream of nitrogen gas at about 90 K. As observed for other crystals of ribosomal particles, the crystals of the large ribosomal subunits from *T. thermophilus* hardly decay when irradiated for days at cryo-temperature. Thus, a complete set of diffraction data could be collected from a single crystal. Crystallographic data were

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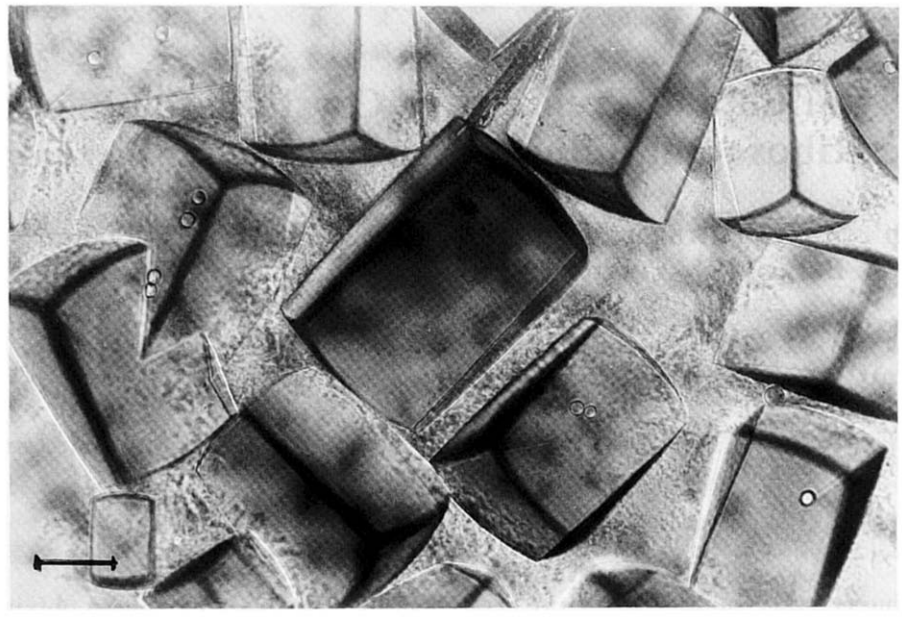

Figure 1. Crystals of 50 S ribosomal subunits from *T. thermophilus* grown within 6 to 12 days by vapor diffusion in hanging drops in Linbro dishes at 19 °C from 6 to 8 μl of solutions containing 5 mg 50 S subunits/ml, 1·1 M-$(NH_4)_2SO_4$, 0·01 M-magnesium chloride, 1 % polyethylene glycol (6000) and 0·1 M-MES buffer at pH 5·6, which were equilibrated with 1 ml of the reservoir of 1·6 M-$(NH_4)_2SO_4$ and all the other components of the drop. Bar represents 0·1 mm.

collected as a series of rotations of 1·5°. We have measured a total of 7365 reflections, of which 2528 were found to be unique. The $R^{(I)}_{scal}$, a measure of the internal consistency in the observed intensity of symmetry-related reflections, was calculated to be 10·34 % (completeness of 85 %) for a resolution of 20·4 Å and 10·77 % (completeness of 70 %) for 17·8 Å.

In fact, these crystals survive the shock-cooling treatment better than those of the large subunits from *Halobacterium marismortui* and *Bacillus stearothermophilus*. They diffract to the same resolution at 4 °C and at cryo-temperature and hardly develop cracks when mounted on spatulas.

The shape of the large ribosomal subunits from another thermophilic bacterium, *B. stearothermophilus*, has been reconstructed by us from electron micrographs of tilt series of two-dimen-

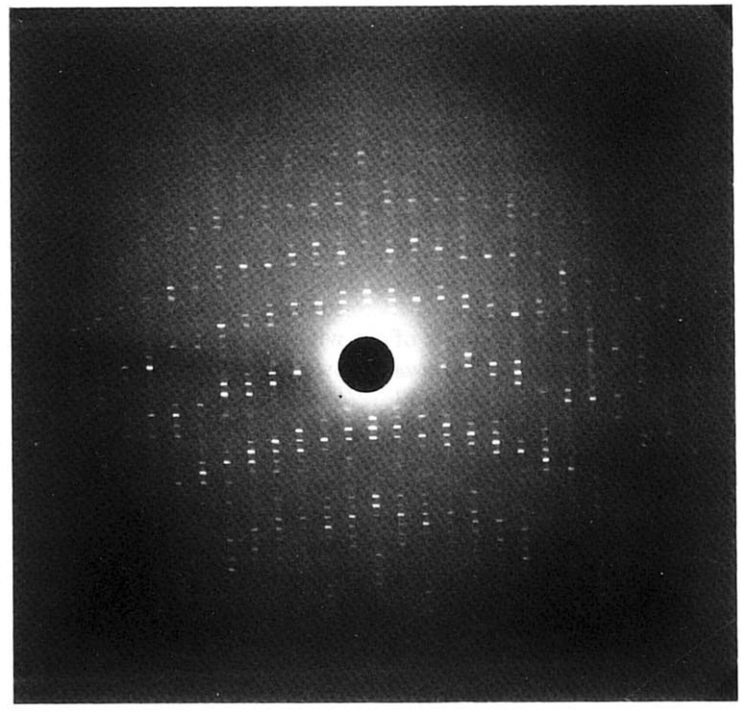

Figure 2. Rotation photograph (3°) of a crystal similar to those shown in Fig. 1. The pattern was obtained at 90 K at Station A1/CHESS, operating at about 5·3 Gev and 50 to 80 mA. The crystal-to-film distance of 400 mm was partially filled by helium. Diameter of the collimator = 0·3 mm; wavelength = 1·588 Å (15·58 nm).

sional sheets (Yonath *et al.*, 1987). On the basis of this information, as well as on electron micrographs of positively stained thin sections of the crystals of the 50 S subunits of *T. thermophilus*, we conclude that about 50% of the volume of these crystals is occupied by the solvent and that each asymmetric unit contains one particle.

It is of interest that all ribosomal particles from *T. thermophilus* are packed in crystals with tetragonal symmetry. Moreover, the symmetry of the crystals of the 70 S ribosomes, $P4_12_12$ is identical to that of the 50 S subunits. The *a* and *b* unit cell dimensions of the 70 S ribosomes and the 50 S subunits are also rather similar: 524 Å × 524 Å × 315 Å and 495 Å × 495 Å × 196 Å, respectively. This may be a mere coincidence, but owing to the restrictions of the packing imposed by the space group symmetry, and considering the size of the 30 S particle (Wittmann, 1983; Hardesty & Kramer, 1986; Yonath & Wittmann, 1989; Berkovitch-Yellin *et al.*, 1990*b*), it is conceivable that the 50 S and 70 S particles are packed in a similar motif and that the 70 S ribosomes are arranged so that the 30 S subunits are aligned roughly perpendicular to the crystallographic *c* axis, contributing to the difference in the lengths of the *c* axes of the two unit cells.

At present we are conducting crystallographic studies on other ribosomal particles from these bacteria: namely, small (30 S) ribosomal subunits (Glotz *et al.*, 1987; Yonath *et al.*, 1988*b*), isolated 70 S ribosomes (Berkovitch-Yellin *et al.*, 1990*a*) and complexes mimicking defined stages in protein biosynthesis from the same bacteria: namely 70 S ribosomes together with (1) an oligomer of 35 uridines, and (2) the same oligonucleotide together with 1·5 to 1·8 Phe-tRNA^{Phe} molecules (Hansen *et al.*, 1990). This series of crystals should permit the investigation of the conformational changes that take place upon the association of ribosomes from their subunits, as well as upon binding of mRNA and tRNA. Results of functional and biophysical experiments indicate such conformational changes. Structural elements that may be interpreted along these lines were also observed by close inspection of our image-reconstructed models (Yonath & Wittmann, 1989; Berkovitch-Yellin *et al.*, 1990*b*).

In parallel, we have crystallized the large ribosomal subunits from other bacterial sources, *B. stearothermophilus* (Yonath *et al.*, 1986*a*,*b*; Müssig *et al.*, 1989) and *H. marismortui* (Makowski *et al.*, 1987; Yonath & Wittmann, 1989). The addition of the crystals of the large subunits from *T. thermophilus* should facilitate a thorough comparison of these particles from radically different sources, including moderate and extreme thermophiles, both belonging to eubacteria, as well as extreme halophiles, which are classified as archaebacteria.

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