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Crystals of 70S ribosomes from thermophilic bacteria are suitable for X-ray analysis at low resolution

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Single crystals of 70S ribosomes from *Thermus thermophilus* and from the wild-type and a mutant of *Bacillus stearothermophilus* have been obtained. Using synchrotron X-ray beam, these crystals diffract to 19 Å and 35 Å, respectively, and can be irradiated at temperatures of 110–120 K for days without noticeable decay. Diffraction data sets to 22 Å and to 33 Å with reasonable R scale were collected from the crystals of 70S from *T thermophilus*. The cell dimensions ($a = b = 524$ Å, $c = 315$ Å), the symmetry ($P4_12_12$ or $P4_32_12$) and the density (1.063 g/cm^3) of these crystals indicate that there is one 70S ribosome per asymmetric unit.

1. Introduction

We have previously grown three-dimensional crystals of 70S ribosomes from *E. coli* [1]. At that time the crystals were too small for analysis by X-ray crystallography. However, positively stained thin sections of these crystals could be investigated by electron microscopy and revealed reasonable internal order [2]. This stimulated further efforts aimed at obtaining better crystals of 70S ribosomes from various bacterial sources. Based on our previous experience in growing crystals suitable for X-ray studies from ribosomal subunits of halophilic or thermophilic bacteria, we chose to attempt the crystallization of 70S ribosomes from these sources. These appear to be rather stable during the preparation and the crystallization.

The growth of three-dimensional micro-crystals of ribosomes from two thermophilic bacteria, namely *Bacillus stearothermophilus* [3] and *Thermus thermophilus* [3,4], were reported two years ago. Refinement of the growth conditions resulted in larger crystals which are suitable for X-ray crystallographic analysis at low resolution.

2. Experimental procedures

Intact 70S ribosomes and their 50S and 30S subunits were prepared and their homogeneity and activity were tested as described earlier [3,5]. Hybrid 70S particles were prepared with 30S subunits from *E coli* and 50S subunits from *B stearothermophilus* and kept in H-I buffer [5] with 10mM $MgCl_2$.

All crystals were grown within 6–10 days by vapor diffusion in hanging drops using Linbro dishes at 19°C. Crystals of 70S ribosomes from *B stearothermophilus* and of the hybrid 70S particles grew from solution containing H-I buffer at pH = 7.6, 10mM–15mM magnesium chloride, 0.2M–0.6M potassium chloride, 0.5M ammonium (chloride or sulfate, respectively) and 2.5% (1.5% in the initial drop) polyethylene glycol (average MW 10000 and 6000, respectively). For obtaining large crystals we added 1.5mM of β-octyl glucoside to the crystallization drop of the 70S ribosomes, or 15 mM cadmium acetate and 4mM zinc chloride to the drop containing the "hybrid". Crystals of 70S ribosomes from *T thermophilus*

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grew from solutions containing the same (H-I) buffer at pH = 6.6, 2mM–10mM spermidine, which were equilibrated with a reservoir of 15%–18% MPD (2,5 methylpentanediol)

To establish the nature of the ribosomal particle which had been crystallized, we washed samples of each of the crystals thoroughly in stabilization solutions, which contained the crystallization agent at high concentration, and then dissolved them in the storage buffer of the ribosomes (H-I). These solutions were characterized by sucrose gradients and checked for in vitro biochemical activity with the poly(U) test [3,5].

Crystallographic studies were carried out with synchrotron radiation on beamline X11 of the EMBL outstation at DESY (Hamburg, Germany).

For initial characterization, the crystals were placed in X-ray capillaries and examined at 277 K. Data collection was performed on crystals mounted on glass spatulas [6] and maintained at a temperature of 110–120 K during the entire period of data collection. For data collection we used the Enraff–Nonius rotation camera with crystal-to-film distances of 500–700 mm (through a helium path) and X-ray wave length of about 1.5 Å.

3. Results and discussion

Figs. 1 and 2 show the crystals of the complete 70S ribosomes from *B. Stearothermophilus* and *T thermophilus*, respectively. The conditions of the growth of crystals of 70S ribosomes from wild-type *B stearothermophilus* were found to be suitable for growing crystals of ribosomes obtained from a mutant of these bacteria in which the ribosomal protein BL11 is missing. We have shown earlier that 50S ribosomal subunits from this mutant crystallize isomorphously with those of the wild-type 50S subunit [7,8]. We are using the mutant particles to introduce specifically bound heavy-atom clusters into the crystals of the 50S subunit [9].

In contrast to our crystals of the large and small ribosomal subunits, which diffract X-rays to 4.5–11 Å [8,10–13], the crystals of 70S ribosomes diffract X-ray to rather low resolution (35 Å for those from *B stearothermophilus* and 18 Å for those from *T thermophilus*, fig. 3). Interestingly, we have observed earlier that the resolution limit of the two-dimensional sheets of 70S ribosomes from *B. stearothermophilus* was significantly lower

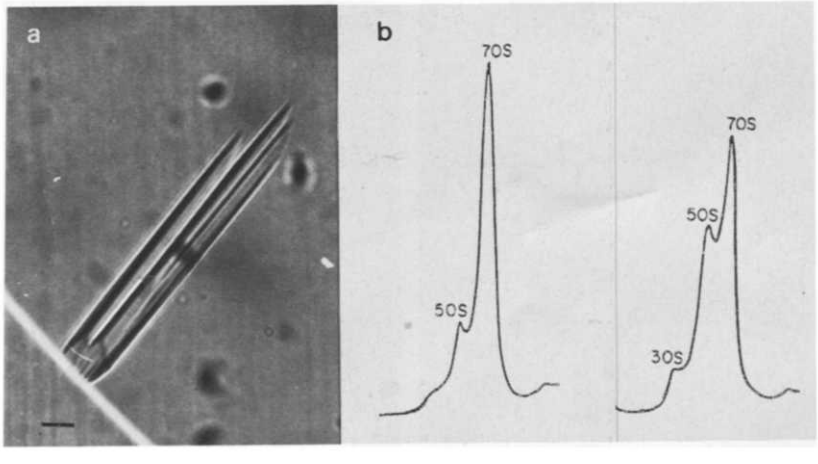

Fig 1. (a) Crystals of 70S ribosomes from *Bacillus stearothermophilus* Bar 0.1 mm. (b) Sucrose gradient profiles of (left) the material used for crystallization, and (right) of the dissolved crystals shown in (a)

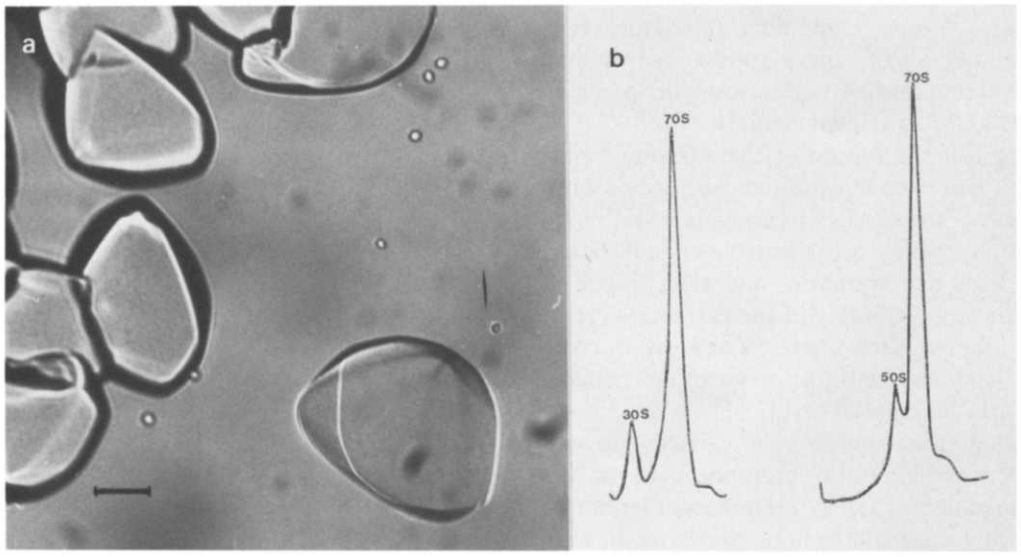

Fig 2 (a) Crystals of 70S ribosomes from *Thermus thermophilus* Bar 0 1 mm (b) Sucrose gradient profiles of (left) the material used for crystallization, and (right) of the dissolved crystals shown in (a)

than that of the sheets of 50S ribosomal subunits from the same source (47 Å and 28 Å, respectively [14,15]).

Like all other crystals of ribosomal particles grown so far in our laboratory, the crystals and the two-dimensional sheets of the 70S ribosomes were obtained from functionally active particles. In fact, the 70S ribosomes used for crystallization have been isolated from logarithmically growing cells as "tight couples" and have never been dissociated in vitro It would be reasonable to expect that some of the particles in the preparation re-

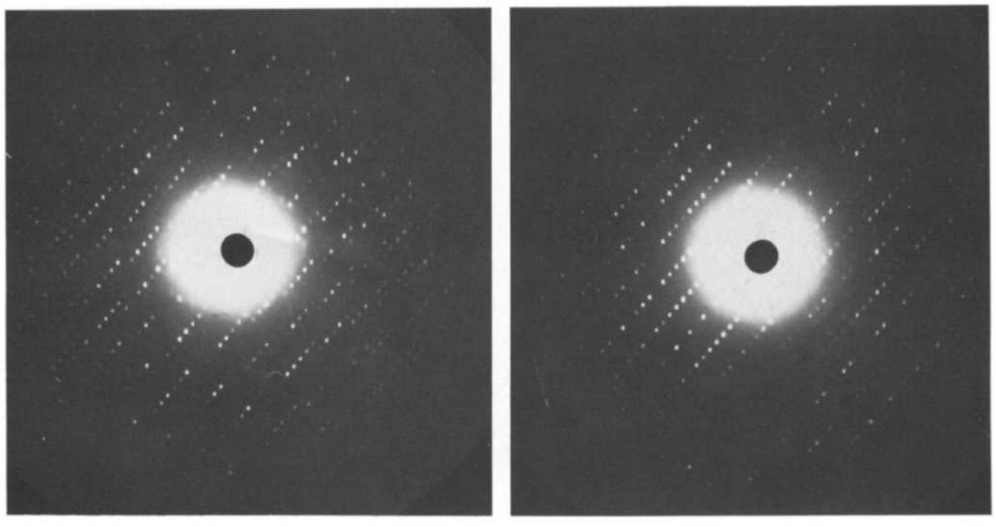

Fig 3 Diffraction patterns recorded for a 3° rotation of the crystals shown in fig 2, under the conditions described in section 2

main bound to other components of protein biosynthesis, such as tRNA molecules, initiation, elongation or termination factors, fragments of mRNA or of the growing protein chain. Therefore, the preparations used for crystallization were probably rather heterogeneous. This might be the case for the low resolution of the diffraction of these crystals.

To obtain a more homogeneous population, we attempted to crystallize ribosomal particles which had been assembled from purified large and small subunits. We chose to concentrate our efforts on 70S assemblies which show high biological activity, including hybrid 70S particles composed of 50S subunits from *B stearothermophilus* and 30S subunits from *E coli*. Crystallization of the hybrid particles was carried out under conditions close to those used for testing their biological activity but with a higher Mg^{2+} concentration (up to 15mM). These experiments yielded large and well-shaped crystals (fig. 4). However, in contrast to crystals of 70S ribosomes grown from "tight couple" preparations, the dissolved crystals of "hybrid" particles migrated as 50S subunits (and their dimers) on sucrose gradients, with no sign of 70S or 30S particles (fig. 4b). These results are consistent with our earlier observation that 50S subunits exhibit high tendency to crystallize [13,16] and show that the crystallization of 50S subunits can take place even in the presence of 30S subunits. These observations may indicate that the interparticle interactions within the 50S crystals are stronger than the affinity between heterologous large and small subunits in the absence of other components of protein synthesis. Studies of natural aggregates of eukaryotic ribosomes [17,18] have suggested that the large subunits of these ribosomes also interact strongly under some physiological conditions.

Crystals of the 70S particles from *T thermophilus* appear as bipyramids with smooth edges and reach a size of $0.3 \times 0.3 \times 0.2$ mm^3 (fig. 2a). Crystallographic data have been collected photographically using a well collimated synchrotron X-ray beam (fig. 3). The crystals have $P4_12_12$ or $P4_32_12$ symmetry, with unit cell dimensions of $a = b = 524$ Å, $c = 315$ Å.

A common feature in the diffraction patterns of crystals of ribosomal subunits is that the reflections of Bragg resolution higher than 15–20 Å decay within a few minutes of irradiation of 0 19° C. In contrast, reflections with the lower resolution can be detected even after 20–30 exposures.

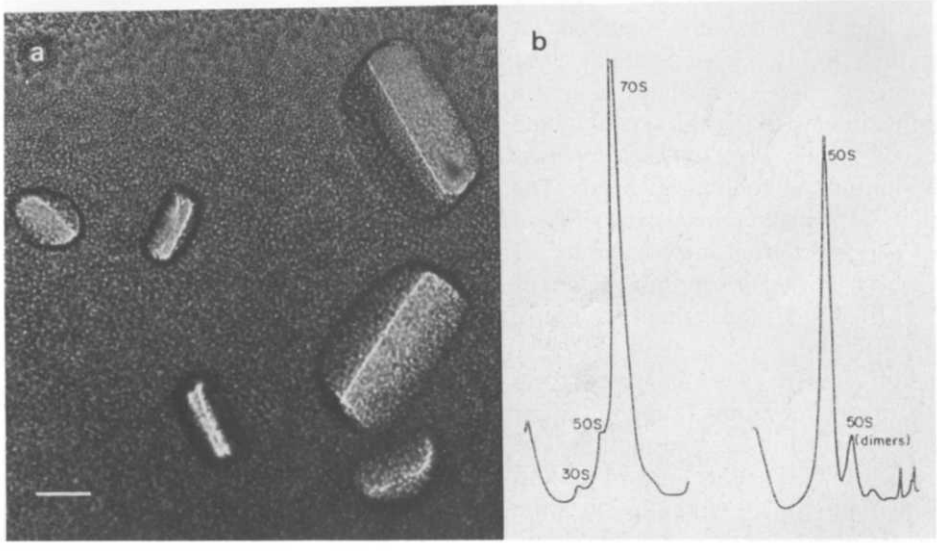

Fig 4 (a) Crystals of 50S subunits from *Bacillus stearothermophilus* grown from preparations hybrid 70S particles as described in the text Bar 0 1 mm (b) Sucrose gradients profiles of (left) the material used for crystallization, and (right) of the dissolved crystals shown in (a)

The crystals of the 70S ribosomes showed no exception, and in this respect they behave similarly to the crystals of the 30S and the 50S ribosomal subunits [6,7,19,20]. Therefore at the current resolution of about 19 Å, the crystallographic data could have been collected in capillaries at ambient temperature. However, it was found to be more convenient to collect the data at cryogenic temperatures, since under these conditions a complete diffraction data set could be obtained from a single crystal. In addition, it is possible to preserve irradiated crystals in solid propane, at the temperature of liquid nitrogen, for long periods (months) for further investigation [21].

For the cryogenic pre-cooling treatment of crystals of the 70S ribosomes, it was essential to modify slightly the procedures used by us for crystals of other ribosomal particles [6,8,11]. Before cooling, the crystals of the 70S ribosomes were transferred to a solution which contained in addition to the components of the stabilizing solution, 30% MPD which served as an "anti freeze" and also caused a slight increase in the viscosity of the solution. Unlike all other crystals of ribosomal particles, those of the 70S ribosome from *T. thermophilus* are not thin enough to fit into double-layer ("sandwich") spatula [11]. Therefore, after immersion in the viscous stabilization solution for a few minutes, the crystals were mounted on single-layer spatulas [6,11]. These Spatulas were constructed from very thin glass plates of a size comparable to the dimensions of the crystal, glued to short glass rods, which were soldered to brass pins used for mounting on goniometer heads. The mounted crystals were instantaneously cooled in liquid propane, and transferred in propane to the X-ray camera, where they were kept in a stream of nitrogen gas (at 110–120 K) throughout the entire period of data collection.

Two diffraction datasets were collected from crystals of 70S ribosomes from *T. thermophilus*. One to 33 Å and the other to 22 Å resolution. The effective angular width of the reflections was found to be 1.6°. The data to 33 Å resolution were collected as a series of 9° rotations, the 22 Å data were collected with 3° rotations. There were 2820 fully recorded significant observations (intensities greater than 2σ) in the former data set and 8000 in the latter. These were reduced to 580 unique reflections, or 80% of the possible reflections to 33 Å and 1800 reflections comprising 80% of the set to 22 Å, with merging R-factors (on I) of 12.5% (to 33 Å) and 6.9% (to 22 Å).

The approximate shape of the 70S ribosome has been recently reconstructed at very low resolution (about 47 Å) from Fourier transformation of electron micrographs of several tilt series of negatively stained two-dimensional crystalline sheets [13,15]. Using this information, together with that obtained by other physical methods [22,23] and from the measured density of the crystals (1.063 g/cm^3), we can conclude that there is probably one ribosome in each asymmetric unit, and that about 50% of the volume of the unit cell is solvent. Similar results have been obtained by electron-microscopy investigations of positively stained thin sections of Epon-embedded crystals [3]. It is noteworthy that these sections show rather regular packing, hinting that the internal order of these crystals may actually be somewhat better than currently expressed in the X-ray patterns.

This paper demonstrates that crystals of 70S ribosomes of *T. thermophilus* are suitable for data collection at about 20 Å resolution. At this resolution a reliable model of the overall shape of the ribosomes should be revealed.

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