J. Mol. Biol. (1986) 187, 633–636

# Characterization of Single Crystals of the Large Ribosomal Particles from *Bacillus stearothermophilus*

Single, three-dimensional crystals of the 50 S ribosomal subunit from *Bacillus stearothermophilus* (strain NCA) have been characterized using a synchrotron X-ray source. The crystals are orthorhombic with unit cell dimensions: $a = 350$ Å, $b = 670$ Å, $c = 905$ Å, and contain at least one 2-fold screw axis. With cooling to $-2$°C, the large crystals ($1{\cdot}0$ mm $\times$ $0{\cdot}2$ mm $\times$ $0{\cdot}1$ mm) diffract to 15 to 18 Å resolution and are stable in the synchrotron beam for several hours. Despite the large cell dimensions, the reflections are readily resolved when the X-ray diffraction patterns are densitometered with a 25 $\mu$m raster.

Ribosomes are distinct assemblies of proteins and RNA chains that are found in all organisms. Despite thorough characterization by a variety of chemical, physical, immunological and genetic techniques (for reviews, see Chambliss *et al.*, 1980; Wittmann, 1982, 1983), the understanding of the function of ribosomes at the molecular level still awaits elucidation of an accurate three-dimensional structure.

Our efforts are directed in growing three-dimensional crystals suitable for X-ray diffraction studies of ribosomes from prokaryotes. These particles are smaller and have been characterized in much greater detail than those from eukaryotes. Also, they can be produced in high purity and large quantity, and provide a system for crystallization that is independent of events *in vivo*.

We developed a crystallization procedure (Yonath *et al.*, 1982) that has yielded *in vitro* three-dimensional crystals of the whole ribosomes from *Escherichia coli* (Wittmann *et al.*, 1982), and of intact 50 S ribosomal subunits from *Bacillus stearothermophilus* (Yonath *et al.*, 1980, 1983*a*, 1983*b*, 1984; Yonath, 1984), and *Halobacterium marismortui* (Shevack *et al.*, 1985). The large (50 S) ribosomal subunit from *B. stearothermophilus* is similar to that from *E. coli*, which has a molecular weight of $1{\cdot}6 \times 10^6$ to $1{\cdot}8 \times 10^6$ and contains 32 different proteins and two RNA chains (for a review, see Wittmann, 1983). Except for four copies of the protein L7/L12, the 50 S subunit contains no known chemical or structural periodicity, making it the largest supramolecular structure without internal symmetry ever crystallized.

Our crystallization procedure produces three-dimensional crystals under similar conditions, from virtually all preparations of active 50 S subunits. However, owing to the intricate nature of the particles, the exact conditions for growing large, mechanically stable and well-ordered crystals still must be varied for each ribosomal preparation. We screened several strains capable of producing high-quality ribosomes in sufficient amounts needed for refinement of the crystallization conditions as well as for the production of large crystals needed for data collection.

Two strains of *B. stearothermophilus*, 799 and NCA, yield large, three-dimensional crystals of 50 S subunits suitable for X-ray diffraction experiments. In all cases, ribosomal subunits from dissolved crystals comigrated with freshly prepared 50 S subunits on sucrose gradients, maintained 60 to 93% of their original biological activity, and contained all constituent proteins. The 23 S RNA of the crystalline material was slightly more fragmented than in the original preparation. In general, crystals of strain 799 remain intact at room temperature for some time (1 to 2 h), whereas the NCA crystals begin to dissolve at temperatures above 7°C after more than a few minutes. We are not certain whether this property is correlated merely with the bacterial strain, or if it is attributed to other factors such as the age of the crystals. We report here the characterization of the three-dimensional crystals of the NCA strain.

Large crystals ($1{\cdot}0$ mm $\times$ $0{\cdot}2$ mm $\times$ $0{\cdot}1$ mm; Fig. 1) grow as long, pointed needles directly in X-ray capillaries by vapor diffusion of alcohol mixtures (Yonath *et al.*, 1984). Since most of them grow with one of their faces adhering to the walls of the capillaries, it is possible to irradiate them without removing the original growth solution. Experiments have been done to prove that crystals kept in growth solution last significantly longer in the X-ray beam and rarely develop as many cracks as crystals that have been slightly dried. Consequently, the X-ray diffraction patterns extend to higher resolution, and data of better quality may be obtained. Although most of the crystals grow so that their long axis lies parallel to the capillary axis, a fair number of them grow in different directions. Thus, despite the natural overcrowding of crystals, which often imposes difficulties in finding solitary crystals in proper orientations, we were able to obtain diffraction patterns from all of the crystallographic zones without manipulating the crystals.

In their natural growth medium, at 4 to 7°C, the



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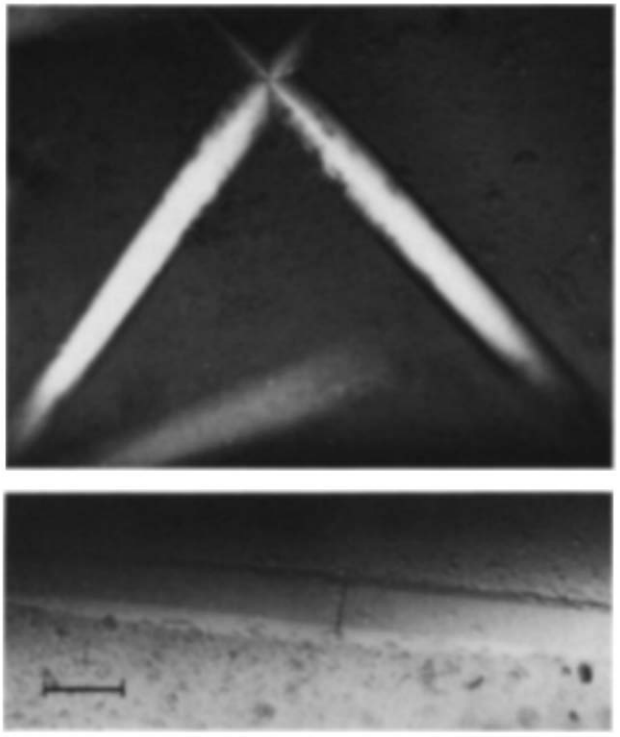

**Figure 1.** Crystals of the 50 S ribosomal subunits from *B. stearothermophilus* (strain NCA) grown in X-ray capillaries by vapor diffusion at 4°C (Yonath *et al.*, 1982). The bar represents 0·2 mm. Reservoir: 12 to 17% (v/v) methanol, 12 to 17% ethylenediol, 0·5 M-NaCl in H-I buffer (60 mM-$NH_4Cl$, 10 mM-$MgCl_2$, 10 mM-Hepes-HCl (pH 7·6) at 25°C). Crystallization mixture (20 μl in 0·5 to 1·0 mm diameter X-ray capillaries): 50 S ribosomal subunits (10 to 20 mg/ml) in H-I buffer, 10 mM-spermine, 1% methanol (optional), 100 mM-Hepes or glycine buffer (pH 8·4 to 9·2).

crystals are adequately stable for six to eight weeks. Any handling of the crystals, such as removing or reorienting them, or replacing the growth medium with a different solution, is extremely difficult in our system. Once handled, the crystals develop cracks within the first two hours and often separate from the capillary walls and dissolve. Even a slight intervention, such as sealing the X-ray capillaries in which the crystals grow, shortens their lifetime drastically (to 6 to 8 h).

Since the crystals grow from mixtures of methanol and ethyleneglycol, cooling to temperatures below 0°C is possible and yields patterns of better resolution than those obtained at 4°C. Furthermore, the sharp decay of the diffraction intensities in their diffraction patterns at about 20 Å resolution is not as severe as at higher temperatures.

All X-ray diffraction patterns were obtained with synchrotron radiation. Sealed capillaries containing crystals were mounted on a special goniometer (Bartunik & Schubert, 1982) for cooling to −2°C. Still photographs of crystals aligned with their long axis parallel or perpendicular to the incident beam displayed easily interpretable Laue circles (Fig. 2(a)). The major zones were readily aligned and small-angle (0·8 to 2·0°) rotation photographs were taken from each zone (Fig. 2(b) and (c)). Owing to the very close spacings between diffraction spots, the reciprocal lattice dimensions were measured under a low-power microscope. The unit cell dimensions are: $a = 350 \pm 10$ Å, $b = 670 \pm 10$ Å and $c = 905 \pm 10$ Å, $\alpha = \beta = \gamma = 90°$. The $a$ axis always corresponds to the long axis of the crystal needle. The extinctions along the $00l$ reciprocal lattice line (clearly seen in Fig. 2(b)) suggest at least one screw axis in the orthorhombic cell (space group $P222_1$, $P2_12_12$ or $P2_12_12_1$). Though we propose that the ribosomes are packed in a primitive lattice, the $h0l$ zone (Fig. 2(c)) does show extinctions, at least at low ressolution, characteristic of a centered lattice ($h+l = 2n+1$). The unit cell dimensions determined by X-ray diffraction, the packing observed in electron micrographs of thin sections (data not shown), and the results of image reconstruction of the related crystal form no. 4 of *B. stearothermophilus*, strain 799 (Leonard *et al.*, 1982), suggest that each of the asymmetric units contains at least two 50 S particles related by a pseudo-2-fold axis. The current crystals diffract to 13 Å resolution at best (typically 15 to 18 Å). They often last about three to five hours in the synchrotron beam, permitting about 10 to 15 photographs to be taken from an individual crystal (under optimal conditions, still photographs require about 8 min of exposure, and a 1·5° rotation about 12 to 15 min). This is conducive to efficient data collection and should simplify data reduction.

Densitometering the films with a 25 μm raster completely resolved each reflection despite their close spacings (about 0·2 mm along the $c^*$ axis). With a 50 μm raster the digitized optical density between reflections in the $c^*$ direction did not return to background levels. Compressing the digitized film image conserved computer disk storage. Only three "strips" of data were saved: left and right fiducial regions, and the central portion containing the diffraction pattern. This file format occupied 60% less disk space than a normal digitized film. At present we are using conventional film processing programs and the cell dimensions calculated above to index the reflections and refine the crystal mis-setting angles on typical still and rotation diffraction patterns. This is an essential step before diffraction intensities can be successfully evaluated from the films.

This letter demonstrates that our present crystals are suitable for data collection at 15 to 18 Å resolution, which is now in progress. However, the way to full structure determination is still hampered by the absolute dependence on a stable synchrotron beam at all stages of crystallographic studies, including test experiments, which normally are done on conventional X-ray sources. This explains why even the determination of cell constants, which is a routine step in protein

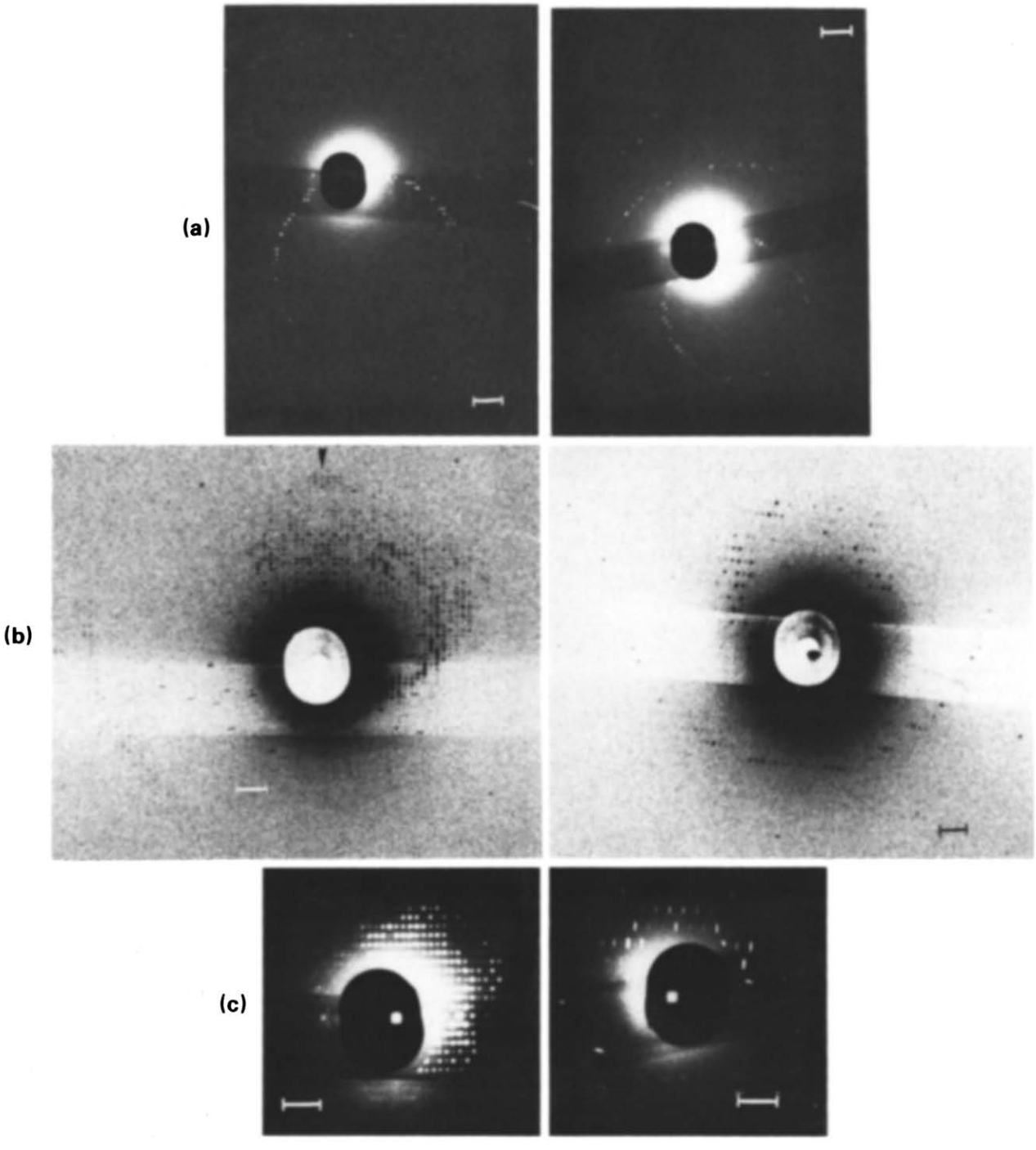


**Figure 2.** X-ray difraction patterns from single crystals shown in Fig. 1. All were obtained with synchrotron radiation (X11 station at EMBL/DESY operating at 5 GeV, initial current 30 to 40 mA). The doubly-focused X-ray beam ($\lambda$ = 1·47 Å) was collimated to 0·2 mm by vertical and horizontal slits. All capillaries were cooled to −2°C. The bar represents ~1 mm. (a) Still patterns of crystals aligned along major crystallographic axes. Exposure time, 15 min; crystal-to-film distance, 144 mm. Left, 0*kl* zone; right *hk*0 zone. (b) Rotation diffraction patterns of 2 major zones. Exposure time, 12 min; crystal-to-film distance, 135 mm. Left, 2° rotation photograph of 0*kl* zone, 670 Å × 905 Å. Systematic absences along the 00*l* line (see arrow) extend for at least 30 orders of diffraction and indicate a 2-fold screw axis. Right, 0·4° rotation photograph of *hk*0 zone, 350 Å × 670 Å. Two different spot shapes can easily be detected in the patterns. This results from exposure of a 2nd crystal in the same capillary oriented approximately perpendicular to the 1st. (c) Rotation (0·8°) patterns, 90° apart, of an aligned crystal. Exposure time, 30 min; crystal-to-film distance, 144 mm. Left, 0*kl* zone, 670 Å × 905 Å. Right, *h*0*l* zone, 350 Å × 905 Å. Note the extinctions in the *h*0*l* zone reminiscent of a centered lattice.

crystallography, in this case required sophistication, hard work and a longer time than usual. Nevertheless, we believe that in spite of the limitations and difficulties we shall be able to collect high-quality crystallographic data. New approaches are now being developed to label specific sites of the particles with large heavy-atom clusters for obtaining isomorphous derivatives. A reliable three-dimensional model from crystallographic data of the large ribosomal subunit, together with the immense amount of biochemical knowledge available, should unambiguously locate specific components such as proteins, antibiotic- and immunoglobulin-binding sites, and functionally active groups. Subsequent work will combine this intermediate model with crystallographic studies of isolated individual ribosomal components at high resolution for phase determination by molecular replacement methods.

We thank Drs K. Leonard, M. Koch, J. L. Sussman, F. L. Hirshfeld and W. Traub for continuing interest and for stimulating discussions; B. Shaanan and M. Shoham for their help in the crystallographic work; and H. S. Gewitz, B. Henneman and T. Arad for skilful technical assistance. M.A.S. is a Sir Charles Clore Post-doctoral Fellow and recipient of a short-term EMBO grant.

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Received 22 June 1985, and in revised form
17 September 1985

## References

Bartunik, H. D. & Schubert, P. (1982). *J. Appl. Crystallogr.* **15**, 227–231.

Chambliss, G., Craven, G. R., Davies, J., Davies, K., Kahan, L. & Nomura, M. (eds) (1980). *Ribosomes: Structure, Function and Genetics*, University Park Press, Baltimore, MD.

Leonard, K. R., Arad, T., Tesche, B., Erdmann, V. A., Wittmann, H. G. & Yonath, A. E. (1982). In *Electron Microscopy 1982*, vol. 3, pp. 9–15, Deutsche Gesellschaft fuer Elevtronenmikroskopie, Frankfurt.

Shevack, A., Gewitz, H. S., Henneman, B., Yonath, A. & Wittmann, H. G. (1985). *FEBS Letters*, **184**, 68–71.

Wittmann, H. G. (1982). *Annu. Rev. Biochem.* **51**, 155–183.

Wittmann, H. G. (1983). *Annu. Rev. Biochem.* **52**, 35–65.

Wittmann, H. G., Müssig, J., Gewitz, H. S., Piefke, J., Rheinberger, H. J. & Yonath, A. (1982). *FEBS Letters*, **146**, 217–220.

Yonath, A. (1984). *Trends Biochem. Sci.* **9**, 227–230.

Yonath, A., Müssig, J., Tesche, B., Lorenz, S., Erdmann, V. A. & Wittmann, H. G. (1980). *Biochem. Int.* **1**, 428–435.

Yonath, A., Müssig, J. & Wittmann, H. G. (1982). *J. Cell. Biochem.* **19**, 629–639.

Yonath, A., Piefke, J., Müssig, J., Gewitz, H. S. & Wittmann, H. G. (1983*a*). *FEBS Letters*, **163**, 69–78.

Yonath, A., Tesche, B., Lorenz, S., Müssig, J., Erdmann, V. A. & Wittmann, H. G. (1983*b*). *FEBS Letters*, **154**, 15–20.

Yonath, A., Bartunik, H. D., Bartels, K. S. & Wittmann, H. G. (1984). *J. Mol. Biol.* **177**, 201–206.

*Edited by A. Klug*