

Some X-ray Diffraction Patterns from Single Crystals of the Large Ribosomal Subunit from *Bacillus stearothermophilus*

X-ray diffraction patterns of three-dimensional crystals of the large ribosomal subunit from *Bacillus stearothermophilus* have been obtained using a synchrotron radiation beam. The patterns contain resolved diffraction spots and indicate packing in relatively small unit cells, the dimensions of which have been tentatively determined. The internal order of the crystals, as reflected in these patterns, has been correlated with the size, shape and conditions of growth of these crystals.

Ribosomes, the organelles at which protein biosynthesis occurs, are defined assemblies of proteins and RNA chains. Although they are currently well characterized (for reviews see Chambliss *et al.*, 1980; Wittmann, 1982, 1983; Liljas, 1982) the understanding of their role in protein biosynthesis still awaits a molecular model. *In vivo* and *in vitro* produced two-dimensional sheets and helices have been studied by electron microscopy and yielded some structural information (Lake & Slayter, 1972; Kühlbrand & Unwin, 1980; Clark *et al.*, 1982; Milligan & Unwin, 1982). We have attempted X-ray crystallographic studies of intact ribosomal particles from bacterial sources. Being of complex structure and enormous size as well as somewhat flexible and unstable, the ribosomes are extremely difficult to crystallize. However, once the crystallization procedure was established (Yonath *et al.*, 1982a), we have been able to obtain three-dimensional crystals both of whole ribosomes from *Escherichia coli* (Wittmann *et al.*, 1982) and of the large ribosomal subunits from *Bacillus stearothermophilus* (Yonath *et al.*, 1980, 1983a, b). In all cases the crystalline material maintains its integrity and is biologically active.

Preliminary X-ray diffraction patterns have been obtained from crystals of the 50 S particles from *B. stearothermophilus*, grown from methanol (type M) or a mixture of methanol and ethylene glycol (type ME). Sharp, resolvable patterns with an enhanced signal to noise ratio have been obtained with synchrotron radiation using a double focusing X-ray camera equipped with a newly designed collimator system (Bartunik *et al.*, 1983):

(1) A screenless 1° precession photograph of a glutaraldehyde cross-linked needle-like thin crystal (type M) with average dimensions of 0.15 mm × 0.05 mm × 0.03 mm (Yonath *et al.*, 1982a), which contains features to 9.5 Å resolution and periodic spacings of 154 Å and 261 Å.

(2) Still photographs of chunky, somewhat larger crystals (type M) with average dimensions of 0.3 mm × 0.15 mm × 0.1 mm (Fig. 1). These diffract to 15 to 18 Å and show periodic spacings of 138 ± 3 Å and 258 ± 3 Å (average of 5 patterns).

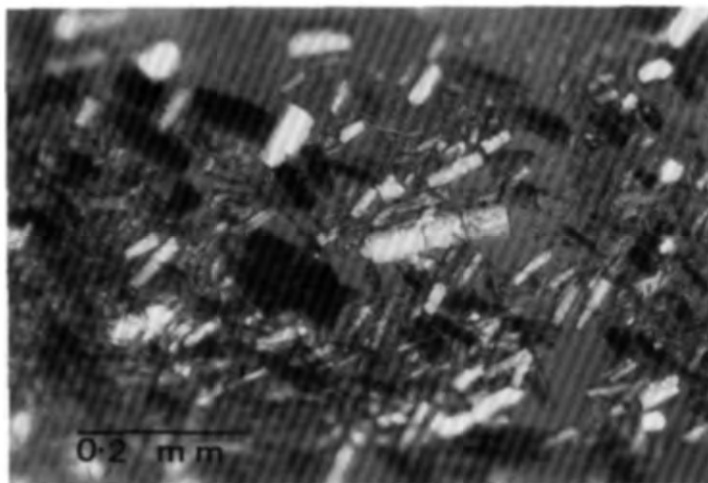


FIG. 1. Crystals, grown from 30% (v/v) methanol at pH 8.7, using a modified version of the crystallization method described by Yonath *et al.* (1982a); i.e. addition of 1% alcohol to the crystallizing solution and seen under polarized light.

(3) Still and 2° oscillation photographs of 0.1 to 0.3 mm long fragments of rather large crystals (types M and ME), with dimensions that can reach 0.9 mm \times 0.25 mm \times 0.15 mm (Fig. 2(a) to (c)). These diffract to 12 to 22 Å (Fig. 3) and contain features with spacings of 139 ± 2 Å and 259 ± 2 Å (observed in 3 patterns of type M crystals), and 259 Å and 336 Å in two patterns of a fragment of a type ME crystal.

(4) High resolution powder diffraction photographs from samples containing large amounts of microcrystals which show weak but sharp rings among them some with spacings (e.g. 10.4 Å; 7.8 Å; 5.6 Å; 4.9 Å and 3.5 Å) similar to those previously reported for gels of ribosomes and extracted ribosomal RNA (Zubay & Wilkins, 1960; Klug *et al.*, 1961). For aligned crystals the patterns are fairly well oriented with an average arc length of $\pm 30^\circ$. Such patterns may arise from partial orientation of the nucleic acid component within the particle.

Thus, there is some evidence for inverse correlation between size and internal order of the crystals, as reflected by the resolution of their diffraction patterns. Due to technical limitations, all these patterns have been obtained at 4 to 15°C, but since the best crystals grow from methanol, cooling to much lower temperatures is potentially feasible and is expected to yield patterns of higher resolution. Attempts in this direction are currently underway. In parallel we are also trying to increase the size simultaneously with the order of the crystals. The "conventional" procedure of slowing down the crystallization process has, so far, failed, probably owing to the deterioration of the ribosomes before they could form aggregates: the initial step in the crystallization of ribosomal particles (Yonath *et al.*, 1982b). Introducing small amounts of alcohols to the crystallizing solution prior to crystallization led to the growth of larger (e.g. Fig. 1), but somewhat less ordered crystals. This may be due to the too rapid formation of large initial aggregates. We also aim to produce crystals from different mixtures of organic solvents as well as to search for better crystallization conditions. Both

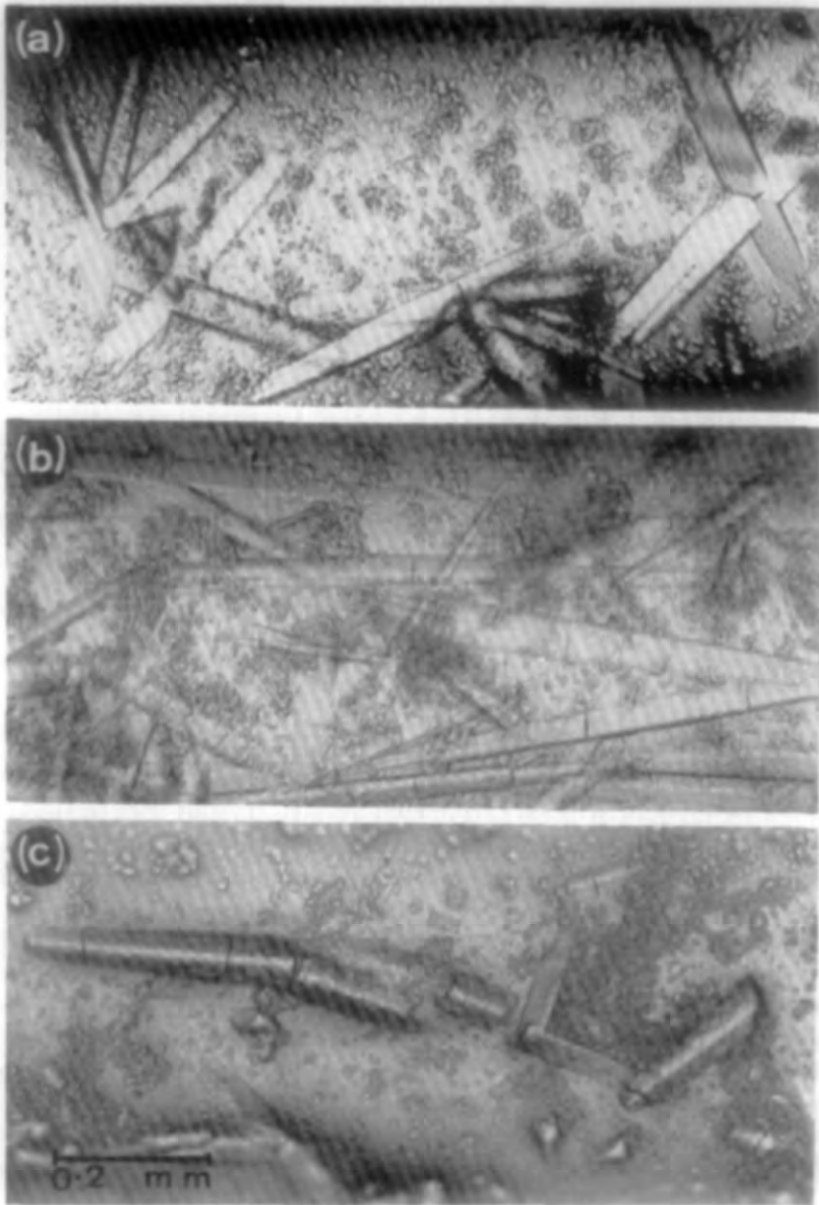


FIG. 2. Development of cracks with crystal growth. Crystals have been obtained as described by Yonath *et al.* (1982a) from 30% methanol at pH 8.4, and are shown after; (a) 25 and (b) 40 days of growth. (c) Cracked crystals which have been grown from a mixture of 17% (v/v) ethylene glycol and 17% methanol (pH 8.4).

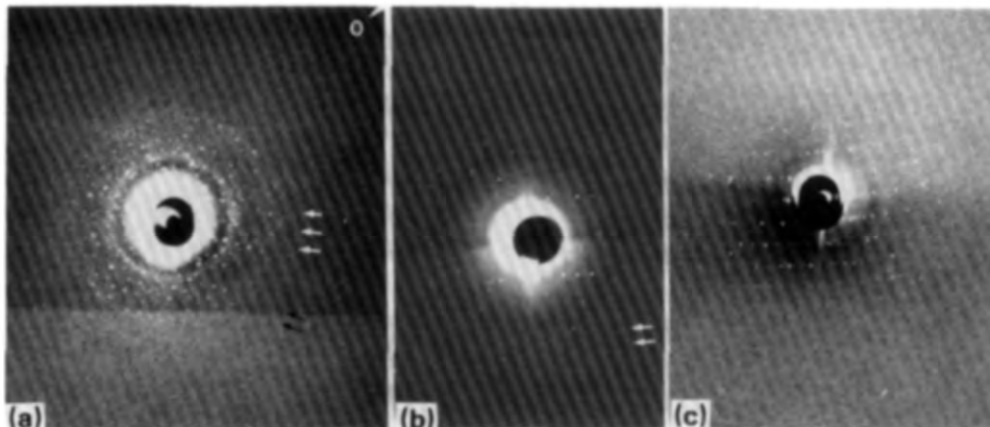


FIG. 3. Still diffraction patterns, obtained with synchrotron radiation at X11/DORIS under parasitic conditions at about 5 GeV and 22 to 28 mA, with 0.12 mm aperture, at 12 to 15°C for about 1 h. From:

(a) A fragmented crystal, grown from 30% methanol (pH 8.7). The contributions of 2 components are marked by white and black arrows. The "white" fragment diffracts with spacings of 259 Å along the layer-lines marked by the arrows and 138 Å between them, and the "black" 260 Å along the direction of the arrows and 207 Å between them. The marked reflection is at 12.7 Å.

(b) Crystals shown in Fig. 2(c). These contain features of 336 Å along the lines marked by arrows and 259 Å between them.

(c) Crystals grown as described (Yonath *et al.*, 1982a) from a mixture of 15% (v/v) ethylene glycol and 17% methanol (pH 8.1). Inter- and intralayer spacings are 129 Å and 316 Å, respectively.

approaches have, so far, yielded encouraging results: crystals such as those shown in Figure 2(c) and a new crystal form with different morphology (Fig. 4), which has been characterized recently by electron microscopy (Yonath *et al.*, 1983b).

The observed periodic spacings agree well with the unit cell dimensions which have been determined from electron micrographs of positively stained sections of similar crystals (Yonath, 1980, 1983a,b) as well as with those of the two-dimensional sheets of the same particles (Arad *et al.*, 1984). The variations in cell constants among the different crystal forms may arise from the differences in the crystallization conditions as well as from the treatment in preparation for electron microscopy. Since the crystals of type M tend to appear with their larger face absorbed to the walls of the capillaries, and since they are fairly fragile and thus difficult to handle, we have obtained most of our X-ray diffraction patterns with the X-ray beam perpendicular to the large faces of the crystals. Thus, the spacings observed may show principal directions of the internal packing parallel to these faces. A common denominator observed in most of the diffraction patterns is 259 ± 4 Å. This is accompanied by 138 ± 3 Å for type M crystals, hence the unit cell dimensions parallel to the large faces of the methanol crystals may well be 138 ± 3 Å \times 259 ± 4 Å. A different projection is shown in the patterns of the ME crystal. This is most likely due to the specific orientation of the exposed ME

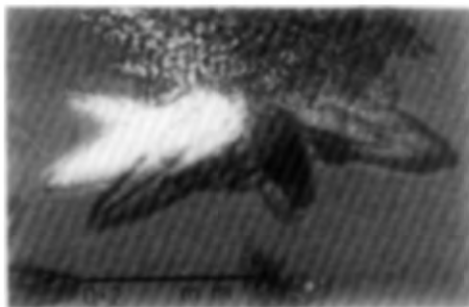


Fig. 4. Crystals grown in hanging drops as described by Yonath *et al.* (1982*a*) from 10% ethylene glycol at pH 7.8 and seen under polarized light.

crystal (Fig. 2(c)), which is different from that of most of the M-type crystals and may stem from its particular morphology.

Regularly, the three-dimensional crystals reach their final length and width in the initial stages of crystal growth. Thus, their large face is almost completely developed within two to three weeks. In this period the crystals are long, very thin and morphologically intact (Fig. 2(a)). Only in later stages do they become thicker and heavier, sink to the bottom of the crystallization drop, and develop cracks perpendicular to their long axes (Fig. 2(b) and (c)). Similar fracturing occurs in cross-linked crystals, no matter how thick they are (Yonath *et al.*, 1982*a*). We therefore assume that the cracking and fracturing of the crystals result from mechanical stress, which originates either from accumulating weight or as a result of chemical forces induced by the cross-linking. Obviously, for recording X-ray patterns, we aimed to expose single separated fragments. However, judging from the nature of the diffraction patterns (Fig. 3) it appears that we could not detect every crack visually.

In spite of the progress in obtaining X-ray diffraction patterns, it is clear that for efficient data collection further improvements in the quality of the crystals as well as in the data collection facilities are needed. Nevertheless, the possibility to resolve the diffraction patterns, the relatively small unit cells and the stability of the crystals in the X-ray beam, encourage us to continue our efforts along these lines.

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