

## Single Crystals of Large Ribosomal Particles from *Halobacterium marismortui* Diffract to 6 Å

Large, well-ordered three-dimensional crystals of 50 S ribosomal subunits from *Halobacterium marismortui* have been obtained by seeding. The crystals have been characterized with synchrotron X-ray radiation as monoclinic, space group  $P2_1$ , with unit cell dimensions of  $a = 182(\pm 5)$  Å,  $b = 584(\pm 10)$  Å,  $c = 186(\pm 5)$  Å,  $\beta = 109^\circ$ . At 4°C, the crystals (0.6 mm × 0.6 mm × 0.1 mm) diffract to 6 Å resolution and are stable in the synchrotron beam for several hours. Compact packing is reflected from the crystallographic unit cell parameters and from electron micrographs of positively stained thin sections of embedded crystals

Within the framework of our efforts to elucidate the structure of ribosomal particles by diffraction methods, we have developed procedures for crystallization of ribosomal particles *in vitro*. We have obtained three-dimensional crystals of whole ribosomes from *Escherichia coli* (Wittmann *et al.*, 1982), as well as from 50 S ribosomal subunits from the wild-type and from a mutant of *Bacillus stearothermophilus* (Yonath *et al.*, 1984, 1986a,b). We have also produced two-dimensional sheets of whole 70 S ribosomes (Piefke *et al.*, unpublished results) and of 50 S subunits (Arad *et al.*, 1984; Yonath *et al.*, unpublished results) both from *B. stearothermophilus*.

Ribosomes from *Halobacterium marismortui* are of special interest, since the knowledge of their structure should contribute to the understanding of the process of protein biosynthesis, and to the understanding of the nature of protein–nucleic acid interactions at high concentrations of salt. The latter is a fundamental problem in molecular biology, for which few data are available. Moreover, since *H. marismortui* is an archaebacterium, comparison of the structure of its ribosome with that from eubacteria may shed light on basic points in evolution.

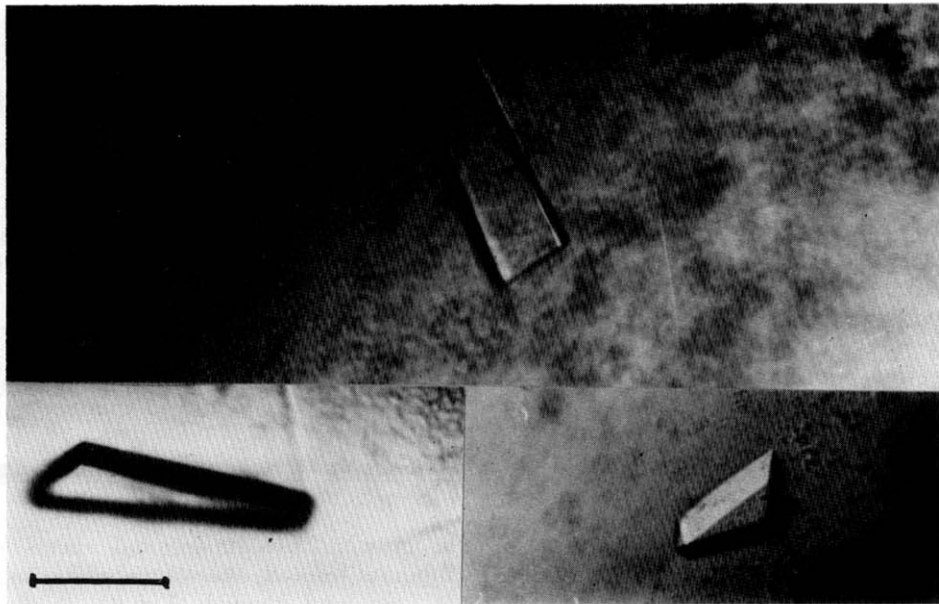
Because ribosomal particles from eubacteria fall apart at high concentrations of salt, three-dimensional crystals of these particles have been obtained exclusively from low molecular weight alcohols (Lake, 1980; Wittmann *et al.*, 1982; Yonath *et al.*, 1980, 1984, 1986a). For this reason, it is almost impossible to handle or transfer these crystals and they have to be grown directly in X-ray capillaries, so that their X-ray diffraction data are collected without removing the original mother liquor (Yonath *et al.*, 1984, 1986a). Many technical difficulties in mounting, data collection and derivatization could be avoided if crystals were grown from less volatile precipitants or from salts or polymers. In this respect, halophilic ribosomes are of particular interest, since they are stable and active in the presence of high concentrations of salt.

As a result of a systematic survey of a large variety of crystallization agents, we have succeeded

in growing two-dimensional sheets of the 50 S subunits from *B. stearothermophilus* using mixtures of salts and alcohol (Yonath *et al.*, 1986a and unpublished results) and three forms of three-dimensional crystals of the large ribosomal subunits from *H. marismortui* using mixtures of salts and polyethylene glycol (Yonath *et al.*, 1986a; Shevack *et al.*, 1985; Shoham *et al.*, unpublished results). All three crystal forms from *H. marismortui* grow by vapor diffusion as fragile thin plates and tend to form multi-layer aggregates. All have relatively small unit cells and are compactly packed, in contrast to the large unit cell dimensions, 360 Å × 680 Å × 920 Å (Yonath *et al.*, 1986a) and loose packing of the large crystals of *B. stearothermophilus*. For all forms, the dissolved crystalline particles are biologically active in a poly(U) system (Shevack *et al.*, 1985; Shoham *et al.*, unpublished results) and comigrate with standard 50 S subunits on sucrose gradients.

Here, we describe the crystals of form (III) from 50 S subunits of ribosomes from *H. marismortui* and compare them to those of the other two forms. The three forms differ mainly in the degree of internal order and in their morphology. The crystals of form (I) are extremely thin (<0.01 mm) and disordered (Shevack *et al.*, 1985). Crystals of form (II) are larger, thicker (0.4 mm × 0.4 mm × 0.1 mm) and significantly more ordered, with diffraction patterns extending to 13 Å resolution (Yonath *et al.*, 1986a; Shoham *et al.*, unpublished results). They are, however, composites of several layers. Consequently “still” X-ray patterns of their flat face ( $hk0$  zone) appear as “precession” photographs, and those taken with the X-ray beam perpendicular to their thin edge ( $h0l$  or  $0kl$  zones) appear as “fiber” diffraction patterns (Shoham *et al.*, unpublished results; Yonath & Wittmann, 1986).

Internal order and the rate of crystal growth have been found to be inversely related in all systems of crystalline ribosomal particles studied so far. This correlation is especially pronounced for crystals of forms (I) and (II) of halophilic ribosomes, since these grow very quickly, occasionally within a few hours. We attempted to



**Figure 1.** Crystals of the 50S ribosomal subunits from *H. marismortui* obtained as a result of seeding by vapor diffusion at 19°C. The crystallization drop contained 5% polyethylene glycol 6000, 1.2 M-KCl, 0.5 M-NH<sub>4</sub>Cl, 0.05 M-MgCl<sub>2</sub>, at pH 5.6, and was equilibrated with 7% polyethylene glycol, 1.7 M-KCl, 0.5 M-NH<sub>4</sub>Cl, 0.03 M-MgCl<sub>2</sub>, pH 5.6. The bar represents 0.2 mm. Ribosomal particles were prepared as described by Shevack *et al.* (1985). Seeds were small, well-shaped crystals that grew spontaneously at 19°C from 4 to 5% polyethylene glycol in the presence of 1.2 M-KCl, 0.5 M-NH<sub>4</sub>Cl, 0.05 to 0.06 M-MgCl<sub>2</sub>, 10 mM-spermidine in the crystallization mixture (pH 5.6 to 5.8), and were equilibrated against 1.7 to 1.8 M-KCl, 6 to 7% polyethylene glycol, 0.5 M-NH<sub>4</sub>Cl, 0.05 to 0.06 M-MgCl<sub>2</sub>. For transfer, we used a stabilization solution of 7% polyethylene glycol in 1.7 M-KCl, 0.5 M-NH<sub>4</sub>Cl, 0.05 M-MgCl<sub>2</sub>, at pH 5.6.

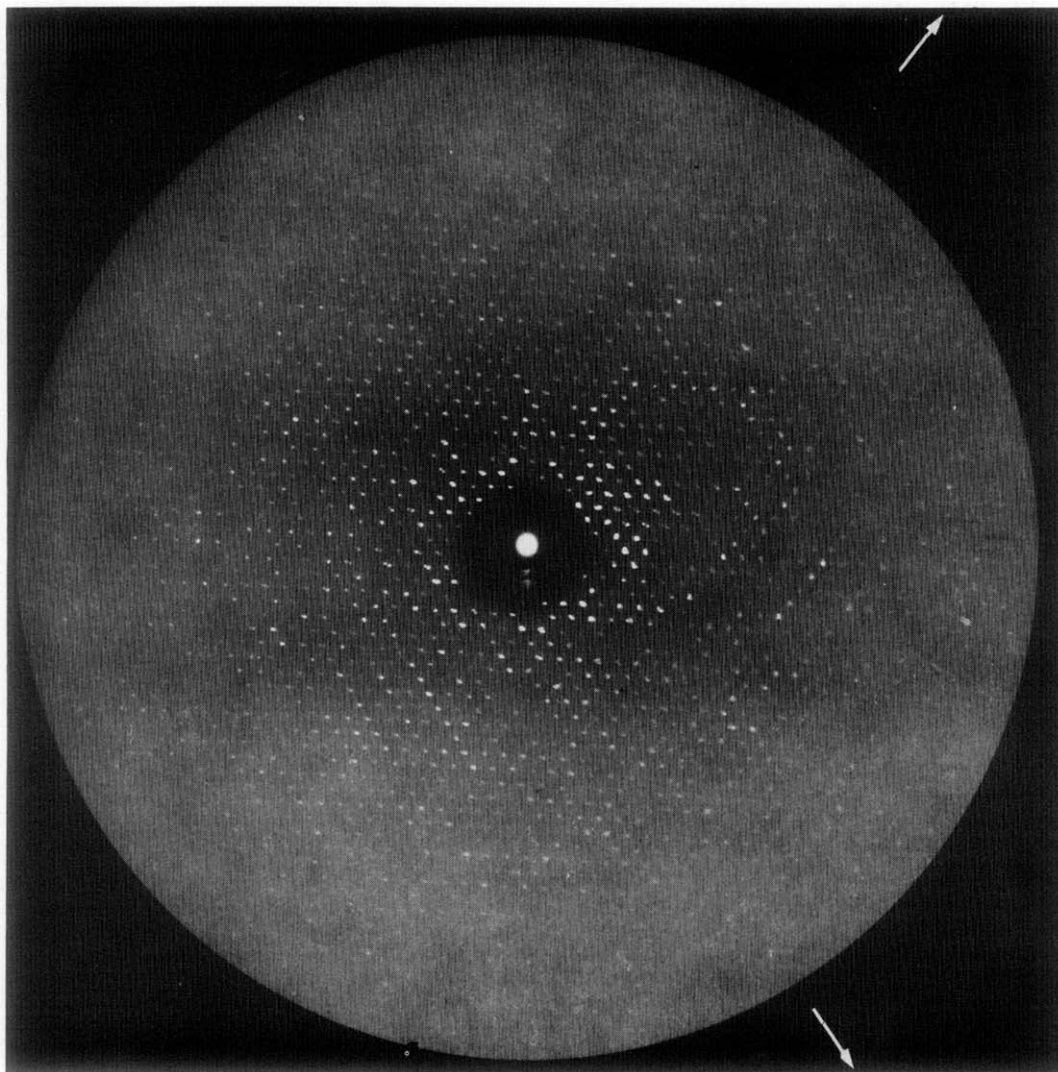
slow down the crystallization process by applying a special seeding procedure (details are given below and in the legend to Fig. 1), as well as by drastically reducing the concentration of KCl in the crystallization mixture (from 3 M to 1.2 M) and in the reservoir (from 3 M to 1.7 M), while keeping the concentration of NH<sub>4</sub>Cl constant. As shown earlier (Shevack *et al.*, 1985), ribosomal particles from *H. marismortui* stored under these conditions maintain their full biological activity.

For obtaining thicker crystals, advantage has been taken of the major role that the Mg<sup>2+</sup> concentration plays in crystallization of the 50S ribosomal subunit from *B. stearothermophilus* (Yonath *et al.*, 1986a; Arad *et al.*, 1984). For this system, it was shown that three-dimensional crystals grow in a relatively low concentration of Mg<sup>2+</sup>, whereas the production of two-dimensional sheets requires Mg<sup>2+</sup> in concentrations high enough to prohibit growth of three-dimensional crystals. Thus, although spontaneous crystallization of thin crystals of 50S subunits from *H. marismortui* occurs in the presence of 50 to 200 mM-Mg<sup>2+</sup>, relatively thick crystals grow when the formers are seeded in mixtures containing only 30 mM-Mg<sup>2+</sup>. Under these conditions, the transferred crystals dissolve but, after several days, new microcrystals can be observed. These reach their maximum size (0.6 mm × 0.6 mm × 0.2 mm; Fig. 1) after three to four weeks and have slightly larger cell dimensions than those of form (II) (Yonath *et al.*, 1986a; Shevack *et al.*, 1985; Shoham *et al.*, unpublished results).

Although fragile, the crystals of form (III) can be mounted in X-ray capillaries by conventional methods. Using synchrotron radiation, their unit cell parameters (space group  $P2_1$ ,  $a = 182(\pm 5)$  Å,  $b = 584(\pm 10)$  Å,  $c = 186(\pm 5)$  Å,  $\beta = 109^\circ$ ) have been determined. The crystals diffract to 6 Å resolution at best (typically 9 to 10 Å; Fig. 2). Between -2°C and 4°C they often last about three to seven hours in the beam, permitting over 15 photographs to be taken from an individual crystal.

Two of the unit cell edges ( $a = 182$  Å,  $c = 186$  Å) are approximately the size previously reported for 50S ribosomal subunits from eubacteria (for a review, see Wittmann, 1983). The third unit cell edge ( $b = 584$  Å) is parallel to a 2-fold screw axis and corresponds to the thin dimension of the crystals. This resembles the relation between the long unit cell axis and the shortest dimension of the crystals from the 50S subunits of *B. stearothermophilus*. Based on the approximately known size of the 50S particles and the dimensions of the unit cell, we conclude that each asymmetric unit contains two particles. Assuming a molecular weight of  $1.6 \times 10^6$  and two particles per asymmetric unit,  $V_m$  is 2.87 Å<sup>3</sup>/dalton. This value is within the range reported by Matthews (1968) for proteins, and slightly higher than the values calculated for crystals of other large nucleoprotein structures: 2.64 Å<sup>3</sup>/dalton for polio virus (Hogle, 1982) and 2.68 Å<sup>3</sup>/dalton for nucleosomes (Richmond *et al.*, 1984).

This letter demonstrates that our present crystals of 50S ribosomal subunits from *H. marismortui* are



**Figure 2.** A  $1.0^\circ$  rotation pattern of a crystal similar to that shown in Fig. 1. The pattern was obtained using a rotation camera equipped with a helium tube, with synchrotron radiation (X11 station at EMBL/DESY operating at 5 GeV, initial current 34 mA). The X-ray beam, of wave-length  $1.51 \text{ \AA}$ , was collimated by vertical and horizontal slits ( $0.3 \text{ mm} \times 0.3 \text{ mm}$ ). The capillary was cooled to  $4^\circ\text{C}$ . Exposure time, 3 min; crystal to film distance, 235 mm. The full film is shown. The  $a^*$  and  $c^*$  axis are marked. The high mosaic spread of the crystals is the reason for an apparent "precession" pattern.

suitable for crystallographic analysis at a resolution of 6 to  $10 \text{ \AA}$ . Experiments aimed at establishing the conditions for data collection and for obtaining heavy-atom derivatives are in progress.

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