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# A new crystal form of large ribosomal subunits from *Halobacterium marismortui*

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A new form of three-dimensional crystals of the 50 S ribosomal subunits from *Halobacterium marismortui* has been obtained at 19°C, using polyethylene glycol in the presence of 1.2–1.7 M KCl in the crystallization mixture. The crystals diffract X-rays to 13 Å and are stable in the synchrotron radiation beam for 4–8 h. Being aggregates of thin plates, the dimensions of only two unit cell edges, 147 × 181 Å, with an angle of 95°, could so far be determined by both X-ray crystallography and electron microscopy. Attempts to produce thicker crystals by sophisticated seeding are in progress.

Ribosomes (Halobacteria) Crystallization Synchrotron radiation

## 1. INTRODUCTION

Within our efforts to determine the structure of ribosomes, we have grown ordered two-dimensional sheets and three-dimensional crystals of several ribosomal particles [1–9]. Due to the unique properties of *Halobacterium marismortui* from the Dead Sea, we have undertaken structural studies on ribosomal particles from this organism as well. These ribosomes are especially appropriate for crystallization since they are stable and active at high salt concentration.

Initially, pure and homogeneous ribosomal particles have been isolated from a large scale preparation of cells [4]. The biological activity of these particles in different salt concentrations was determined, and microcrystals (form I) of the 50 S ribosomal particles were obtained [4]. Samples containing a large number of these microcrystals were examined using synchrotron radiation, but only few reflections to 25 Å resolution could be detected. Thus, these crystals had still to be improved in order to provide a suitable system for crystallographic analysis.

It has been observed, for these as well as for

other crystals of ribosomal particles [7] that the internal order of the crystals is inversely related to the rate of their growth. In order to slow down the crystallization process, we have decreased the concentration of KCl in the crystallization mixture. Our efforts to improve the internal order and to increase the size of crystals from *H. marismortui* resulted in obtaining significantly more ordered and larger crystals of a different crystal form (form II). Using synchrotron radiation, single crystals of this form have been investigated crystallographically. Here we report these studies.

## 2. MATERIALS AND METHODS

### 2.1. Ribosomal particles

Ribosomal particles were isolated and their integrity and biological activity were determined according to [4].

### 2.2. Crystals

Crystals were obtained at 19°C by vapor diffusion in Linbro tissue culture dishes. The crystallization mixture contained 5% polyethylene glycol (PEG 6000–20000) in the presence of 1.2 M

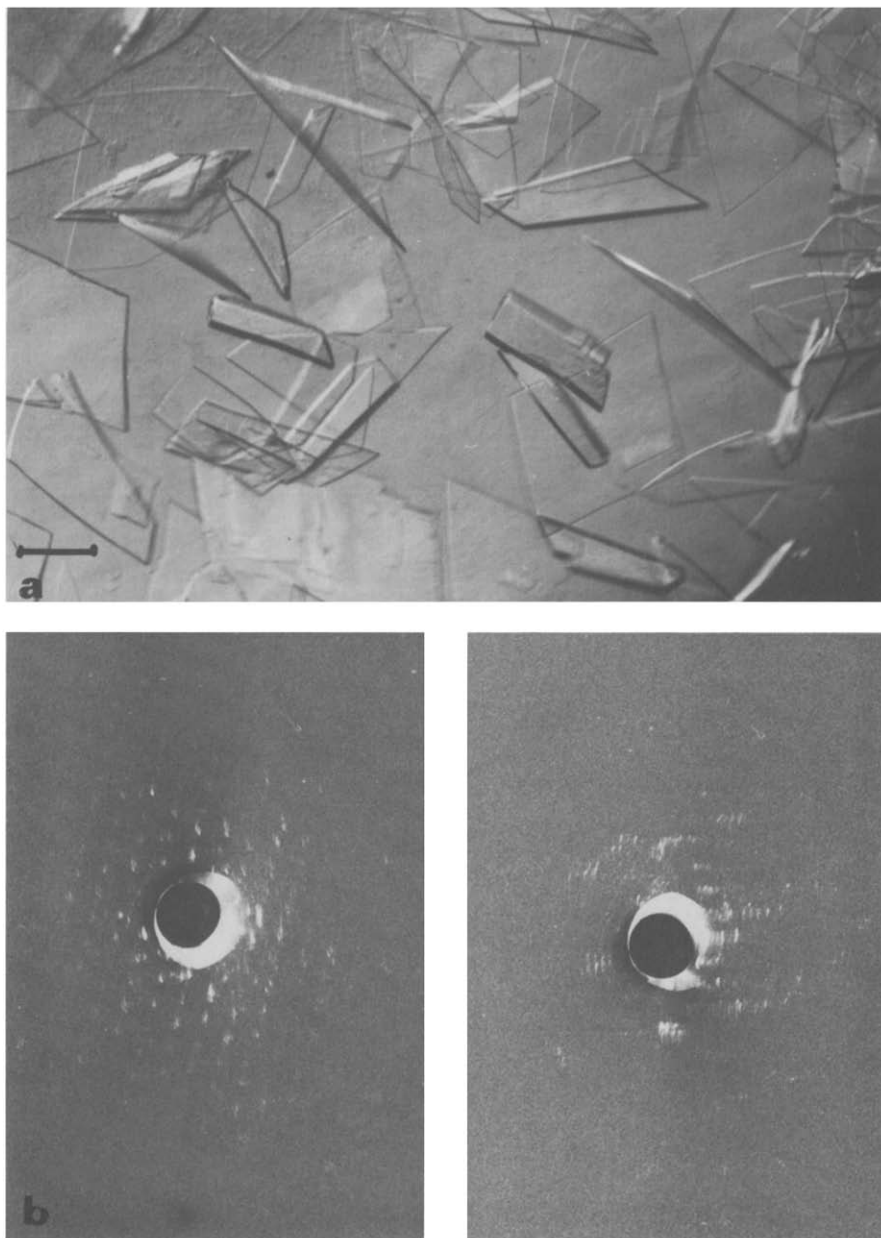


Fig.1. (a) Crystals of the 50 S ribosomal particles from *H. marismortui* grown at 19°C from 5% PEG (8000) in the presence of 1.2 M potassium chloride, 0.5 M ammonium chloride, 0.1 M magnesium chloride, 20 mM acetate buffer, pH 5.2, equilibrated against 3 M KCl, 9% PEG, 0.5 M  $\text{NH}_4\text{Cl}$  and 0.1 M  $\text{MgCl}_2$ . Bar length = 0.2 mm. (b) 0.8° rotation X-ray diffraction patterns of a fragment of a crystal shown in (a) obtained within 5 min at 2°C, with synchrotron beam at CHESS/Cornell University, operated at about 5 GeV and 20 mA, with 0.20 mm aperture, crystal to film distance was 185 mm. Left: beam perpendicular to the flat face of the crystal. Right: beam parallel to the thin edge of the crystal.

KCl, 0.5 M  $\text{NH}_4\text{Cl}$  and 0.1 M  $\text{MgCl}_2$ , at pH 5.2, and was equilibrated against a reservoir of 9% PEG in 3 M KCl, 0.5 M  $\text{NH}_4\text{Cl}$  and 0.1 M  $\text{MgCl}_2$ , pH 5.2.

### 2.3. X-ray photographs

X-ray photographs were taken at  $-2^\circ\text{C}$  using synchrotron radiation, at the A1 station at CHESS/Cornell University, operated at about 5 GeV and 20 mA. A Nonius rotation camera equipped with a He path and a collimator of 0.20 mm was used.

### 2.4. Electron microscopical and optical diffraction

Electron microscopical and optical diffraction studies were performed according to [4].

### 2.5. Characterization of the crystallized particles

Single crystals were selected from their original crystallization drop, washed in the reservoir solution, and dissolved in their storage buffer [4]. The biological activity and integrity of the crystallized ribosomal particles were determined by poly(U) assay and migration on sucrose gradients, respectively, as described in [4].

## 3. RESULTS AND DISCUSSION

Plate-shaped crystals with dimensions of  $0.4 \times 0.4 \times 0.08$  mm, were obtained at  $19^\circ\text{C}$  within 1–2 days and reached their final size within 4–5 days. The dissolved crystalline particles comigrate on sucrose gradients with standard 50 S subunits, and are biologically active (not shown).

At  $-2^\circ\text{C}$  these crystals are stable in the synchrotron X-ray beam for 4–8 h. Their X-ray diffraction patterns extend to about 13 Å resolution. Because the crystals grow as thin plates, they tend to form multi-layer aggregates. As a result, 'still' X-ray diffraction patterns of their flat faces (presumably the  $hk0$  zones) appear as 'precession' photographs. Furthermore, X-ray patterns taken with the beam perpendicular to the thin dimension of the crystals (presumably the  $h0l$  or  $0kl$  zones) appear as diffraction patterns of fibers or films indicating that these crystals are built of several

layers. Being rather thin, the crystals are somewhat fragile. However, the mechanical strength of these crystals can be greatly improved by mild crosslinking with glutaraldehyde.

X-ray diffraction patterns with the beam perpendicular to the large face of these crystals show periodic spacings of 147 and 181 Å, with an angle of  $95^\circ$  between them. These spacings were assigned as unit cell dimensions since similar spacings have been derived from optical diffraction patterns of electron micrographs of positively stained thin sections of these crystals.

The crystals are compactly packed and the dimensions of their unit cells resemble those of forms I and II of the 50 S ribosomal subunits from *Bacillus stearothermophilus* [7]. It should be mentioned that the 50 S particles from *B. stearothermophilus* have been crystallized in six crystal forms, with a variety of packing modes and unit cell dimensions [7]. However, the crystals of only one form, with the largest unit cell dimensions ( $360 \times 680 \times 920$  Å) and loose packing, are of a size suitable for crystallographic studies [5–7].

As a result of decreasing the salt concentration in the crystallization mixture, the growth rate of crystals of the 50 S ribosomal subunits from *H. marismortui* was slowed down. Consequently, larger and better shaped crystals with improved internal order have been obtained. Although these crystals are still not suitable for crystallographic analysis, encouraging results have been obtained from preliminary experiments in seeding. Somewhat better ordered and thicker crystals have already been produced. More sophisticated seeding techniques are currently being developed in order to further improve and increase the thickness of the crystals.

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