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Thin laminar microcrystals of 70 S ribosomes from *Thermus thermophilus*

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A new crystalline form, thin laminar microcrystals of 70 S ribosomes from *Thermus thermophilus*, have been obtained. Negatively stained ordered sheets derived from these crystals were studied by electron microscopy with subsequent computer image processing. The sheets have two-dimensional unit cell parameters $a=b=480$ Å, $\gamma=90°$ and a resolution limit of about 30 Å. The microcrystals are suitable for three-dimensional image reconstruction.

Ribosome microcrystal; Electron microscopy; Image processing

1. INTRODUCTION

Recently we obtained large well-ordered three-dimensional crystals of 70 S ribosomes from the thermophilic bacterium *Thermus thermophilus* [1]. The crystals have a bipyramidal form. When studied at the LURE synchrotron (France) they appeared to be rather stable in beam at 0°C and diffract up to a 20 Å resolution. The crystals belong to the $P4_12_12$ or $P4_32_12$ space group with unit cell parameters $a = b = 510$ Å, $c = 378$ Å [2]. A crystallographic study of these crystals is now in progress.

In this paper we present a new crystalline form obtained for *Th. thermophilus* 70 S ribosomes. These are thin laminar microcrystals, several ribosome layers thick, and are suitable for electron microscopy investigations aimed at three-dimensional reconstruction of the 70 S ribosome.

2. MATERIALS AND METHODS

2.1. *Crystallization*

Ribosomes from *Th. thermophilus* cells were purified according to [3] with minor modifications. The final preparations were tested for particle homogeneity by sedimentation analysis.

Crystallization was performed by dialysis of the ribosome solution (~10 mg/ml) against 10% (v/v) 2-methyl-2,4-pentanediol (MPD) in buffer A (20 mM Tris-HCl pH 7.5, 25 mM $MgCl_2$, 75 mM NH_4Cl, 200 mM KCl, 1 mM dithiothreitol) at 4°C.

2.2. *Testing the nature of the crystallized material*

The microcrystal pellet was washed several times with 10% MPD in buffer A and then with buffer B (20 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 150 mM NH_4Cl). The microcrystals were dissolved in buffer A at room temperature. The dissolved sample was tested for the sedimentation coefficient, the two-dimensional [4] and SDS-PAGE protein pattern and functional activity in the poly(U)-directed cell-free system [3]. A sample aliquot in buffer C (10 mM Tris-AcOH pH 7.4, 20 mM $Mg(OAc)_2$, 20 mM NH_4OAc) was tested by electron microscopy after negative staining with uranyl acetate.

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2.3. *Electron microscopy*

Microcrystals in a mother solution were dialyzed overnight against buffer B. Crystals were absorbed for 5-30 s on grids coated with formvar film. The grids were washed in buffer D (20 mM Tris-HCl pH 7.5, 50 mM $MgCl_2$, 100 mM NH_4Cl) for 5-10 min, then in buffer C for 1 min and stained with 0.25% uranyl acetate for 30 s.

For thickness testing the microcrystals in a mother solution were fixed with 0.5% glutaraldehyde and embedded in Epon-812 resin. Ultrathin sections about 500 Å thick were made using the Ultratome III (LKB).

Samples were analyzed in a JEM-100C electron microscope (JEOL, Japan) equipped with an anticontamination liquid N_2 trap and a 25 μm objective aperture at 80 kV.

2.4. *Image processing*

Images of negatively stained thin laminar 70 S ribosome microcrystals were enhanced by standard Fourier methods of computer image processing. Electron micrographs were digitized on a Scandig-3M drum densitometer (Joyce Loebl). Crystalline areas were selected in the micrographs by searching for regions with more than 100 unit cells. The digitized images were fast Fourier transformed in a 980 × 980 array. Amplitudes and phases of Fourier components at crystallographic reciprocal lattice points were extracted.

Noise-filtered images of microcrystal were processed by using reverse two-dimensional Fourier transform of the data at the reciprocal lattice points.

3. RESULTS AND DISCUSSION

Microcrystals of 70 S ribosomes from *Th. thermophilus* were formed within 1-2 days under the conditions described. They were visible in a light microscope as lamellae of 2-5 μm capable of rotating the polarization.

To identify the microcrystal nature we used several tests as described in section 2. It was shown that the microcrystals consist of ribosomes posessing the same sedimentation coefficient and protein pattern as the

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Fig.1. Electron micrographs of thin laminar microcrystal of 70 S ribosomes from *Thermus thermophilus*. The embedded microcrystal cross-section positively stained with uranyl acetate.

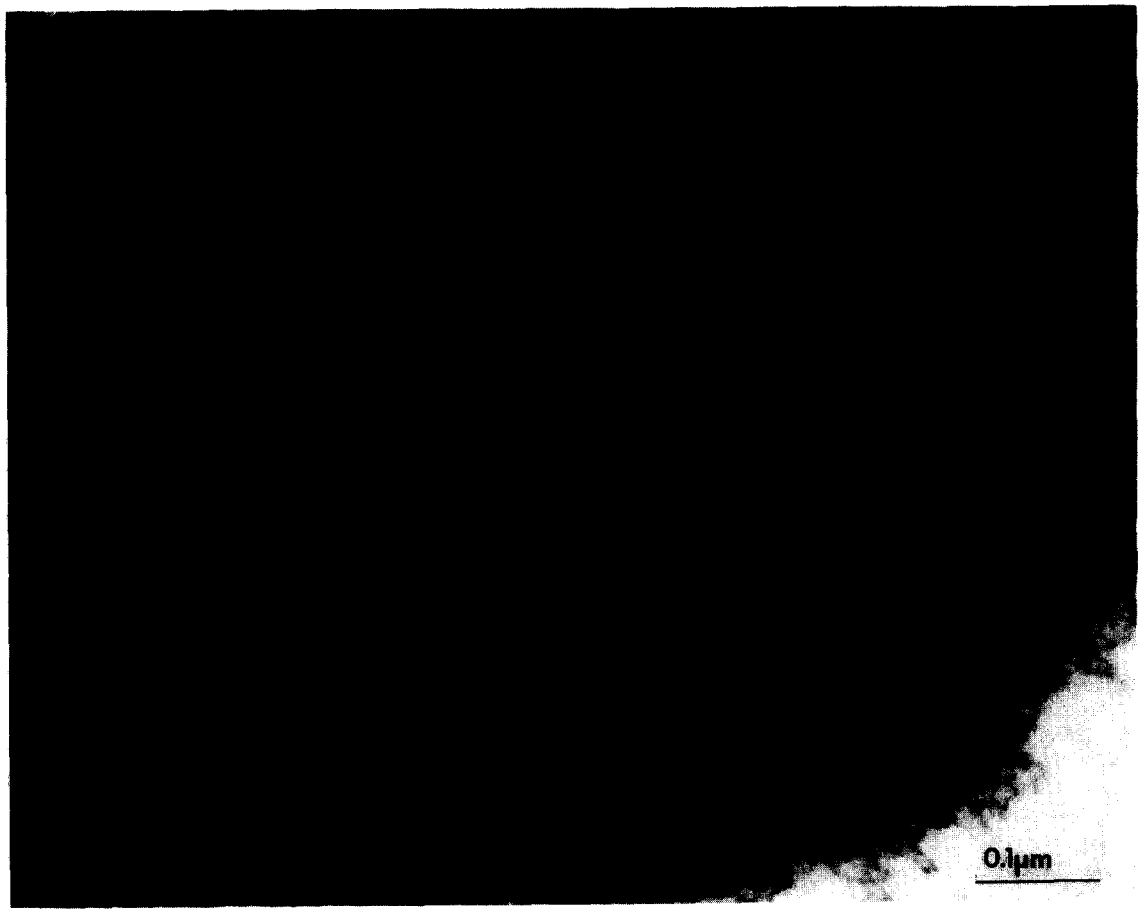

Fig.2A.

original ribosomes. Their electron microscopy images do not differ from those of native ribosomes. They also retain the translational activity in a poly(U)-dependent cell-free system.

Fig.1 shows the ultrathin cross-section of the embedded microcrystal. Such a microcrystal is at least 5–6 layers of ribosomes thick. However, we found that microcrystal thickness can be reduced by slowly dissolving the upper layers of the microcrystal absorbed onto the specimen grid. Such treatment was optimized to get ribosome sheets 2–3 layers thick (the thickness was tested by the shadow casting technique, data not presented). The sheets obtained in this way are well ordered and of a reasonably large size. The eletron micrograph, the computer diffraction pattern and the filtered image of the sheet are shown in fig.2. The two-dimensional unit cell of the microcrystal images belongs to the P_4 plane group with parameters $a = b = 480$ Å, $\gamma = 90°$. The computer diffraction patterns show reflections to the 10th order and extend to about 30 Å.

Two-dimensional ordered sheets have been obtained before for 70 S ribosomes from *B. stearothermophilus* [5]. A special technique was developed utilizing immediate cyrstallization on a specimen grid from a salt-alcohol mixture [6]. The parameters of such obtained 2D crystals were $a = 200$ Å, $b = 400$ Å, $\gamma = 90°$. The resolution limit of these crystals was 42 Å, and a 3D reconstruction with 47 Å resolution was done [7].

The thin laminar microcrystals described in this paper have an even higher orderedness. Diffraction patterns of electron micrographs of these microcrystals stained with uranyl acetate contain features to 30 Å resolution. Such microcrystals are promising for 3D reconstruction.

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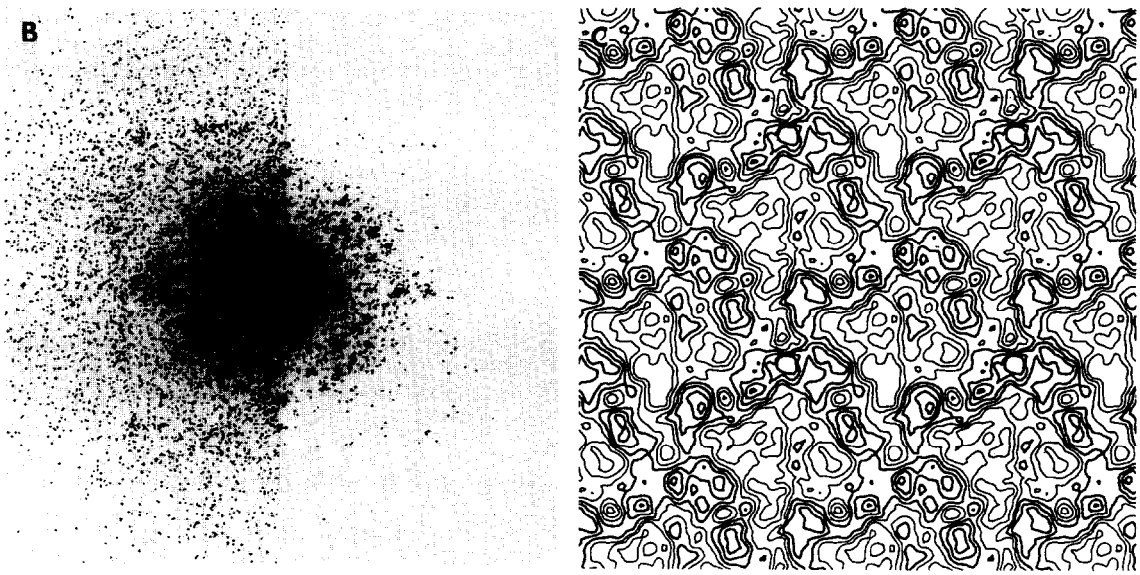

Fig.2. Electron micrograph (A), computer diffraction pattern from area containing about 100 unit cells (B) and computer-filtered image (C) of the microcrystal treated as described in section 2.