Crystallization of *Escherichia coli* ribosomes

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Three-dimensional crystal *Ribosome, of* E. coli *D10* *Image reconstruction study*
Optical diffraction pattern *Poly(U)-directed synthesis* *Polyphenylalanine*

1. INTRODUCTION

The knowledge of the structure of the ribosome is an essential requirement to reveal its role at the molecular level in the process of protein biosynthesis. This information is being obtained by a battery of chemical, physical, immunological and genetic methods (for reviews see [1]).

Important methods for the study of the three-dimensional structure of the ribosomes are X-ray crystallography and image reconstruction. Two-dimensional ordered sheets of ribosomes from a few eukaryotic species are found under special conditions in vivo [2–4] or in cell homogenates [5]. Small two-dimensional arrays of *E. coli* ribosomal subunits were obtained in vitro [6,7].

The first three-dimensional crystals of ribosomal particles were obtained in vitro from the large subunit of the *Bacillus stearothermophilus* ribosome [8,9]. These have been characterized by three-dimensional image reconstruction studies [10].

Here we report the in vitro crystallization of *E. coli* ribosomes. We have obtained ordered three-dimensional microcrystals. After washing and redissolving, the crystalline ribosomes sediment with a coefficient of 70 S in a sucrose gradient and are biologically active in the poly(U) in vitro system. Electron micrographs of thin sections of the crystals show regular arrangement of the ribosomes, and their optical diffraction patterns extend out to about 6.5 nm. Thus three-dimensional image reconstruction studies can be carried out.

2. MATERIALS AND METHODS

2.1. *Biochemical methods*

Ribosomes from *E. coli* strain D10 were isolated as tight couples [11]. Sucrose gradients between 10% to 35% were run in rotor SW60 at 50 000 rev./min for 130 min at 4°C in 10 mM Tris–HCl (pH 7.8), 6 mM $MgCl_2$, 30 mM NH_4Cl. The poly(U)-assays were carried out as described in [12].

2.2. *Crystallization*

The crystals were grown employing a slightly modified version of the vapour diffusion technique in hanging drops, depression slides and in capillaries [9,13]. The crystallization drops which contained the ribosomes and MES, HEPES or glycine buffers (pH 6.0–9.0) were equilibrated with organic solvents (see below) in the presence of 0.5–0.9 M NaCl and 6 mM β-mercaptoethanol in the reservoir, at 3–7°C.

2.3. *Electron microscopy*

Crystals were fixed in 0.6% glutaraldehyde and embedded in resin ERL 4206 [14]. Thin sections (40–50 nm) were positively stained with uranyl acetate and lead citrate for 20 min [15]. Electron microscopy was carried out in a Philips 300 instrument at 19 000, 29 000 and 54 000 magnifications. Crystalline areas were selected in the electron micrographs by searching for regions where the appearance of the lattice was constant over an area of more than 100 unit cells. Selected areas of elec-

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tron micrographs were digitised on an Optronics (P-1000) densitometer, as 512 × 512 points on a square raster of 50 μm. Fourier transforms of the digitised images were computed on a 512 × 512 matrix.

2.4. *Characterization of crystallized ribosomes*

Crystals, together with their growth medium, were placed in 0.7 mm quartz X-ray capillaries and were centrifuged at 800 rev./min for 3 min. The supernatant was removed, and the pellet which contained the crystals was either stored or resuspended in a solution containing the crystallizing agents. The suspension was centrifuged again. Both pellets (after the first and second centrifugation) were dissolved in the storage buffer (containing 10 mM HEPES, 10 mM $MgCl_2$, 60 mM NH_4Cl and 6 mM β-mercaptoethanol), analysed on a sucrose gradient and checked for biological activity in the poly(U)-system (see above).

3. RESULTS AND DISCUSSION

Crystals with typical dimensions of ~0.1 × 0.05 × 0.05 mm were obtained in capillaries and depression slides within 3–8 weeks. Methylpentanediol, methanol, ethylbutanol, ethanol and ethylhexanediol were found to be suitable agents for the growth of crystals which were obtained over a pH range between 6.0 and 9.0. Addition of spermidine did not have any detectable effect on crystal growth. Figure 1 shows the crystals as seen by light microscopy. The crystals are fairly stable at 6°C, but dissolved rapidly at room temperature.

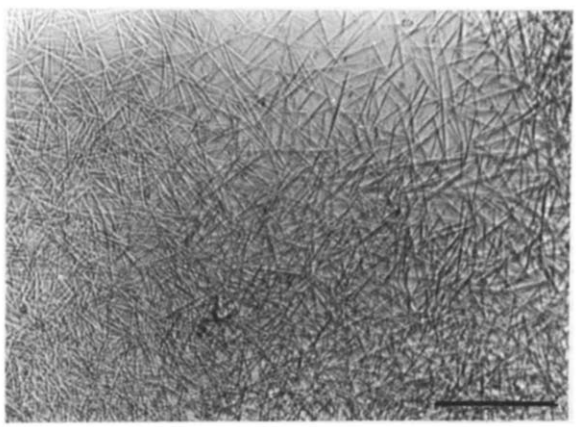

Fig.1. Crystals of *E. coli* ribosomes grown in 12% methylpentanediol at pH 8.7 and 6°C. Further growth conditions are given in Materials and Methods. Bar length corresponds to 0.2 mm.

Table 1

Biological activity of ribosomes from washed and dissolved crystals in the poly(U)-assay

Sample	A_{260}	cpm	activity
Ribosomes from crystals	0.2	520	59%
Control ribosomes	0.2	880	100%
Background (minus 70 S)	–	30	

The background value has been subtracted. The control ribosomes were stored at −70°C during the crystallization experiment

An electron micrograph of a thin section through several crystals that were grown in 12% methylpentanediol at pH 8.7 is shown in fig.2. Optical diffraction patterns are inserted into the figure. Two orthogonal views can be seen. *A*: a side view, parallel to the long face of the crystal; *B*: a cross-section through the crystal. The apparent unit cell constants are $a = b = 34 \pm 3$ nm, $\gamma = 120°$ as derived from the diffraction patterns of *A* and of *B*, and $C = 59 \pm 3$ nm as derived from the diffraction pattern of *A* with observed minimum spacing of about 6.5 nm.

The systematic absences in the diffraction pattern of area *A* along the C^*-axis together with the hexagonal symmetry observed for area B suggest that the ribosomes are arranged along a 6-(or 2-) fold screw axis. This axis is parallel to the long faces of the crystals. Thus, the unit-cell packing mode corresponds to the outer morphology of the crystals (hexagonal prisms).

In order to substantiate that the crystalline material consists of ribosomes, the isolated crystals were dissolved in their storage buffer (see above) and analysed by sucrose gradient centrifugation (fig.3). Since the dissolved crystalline material comigrated with standard ribosomes it was concluded that the crystals contain intact 70 S ribosomes, and that the crystallization conditions do not cause gross morphological changes.

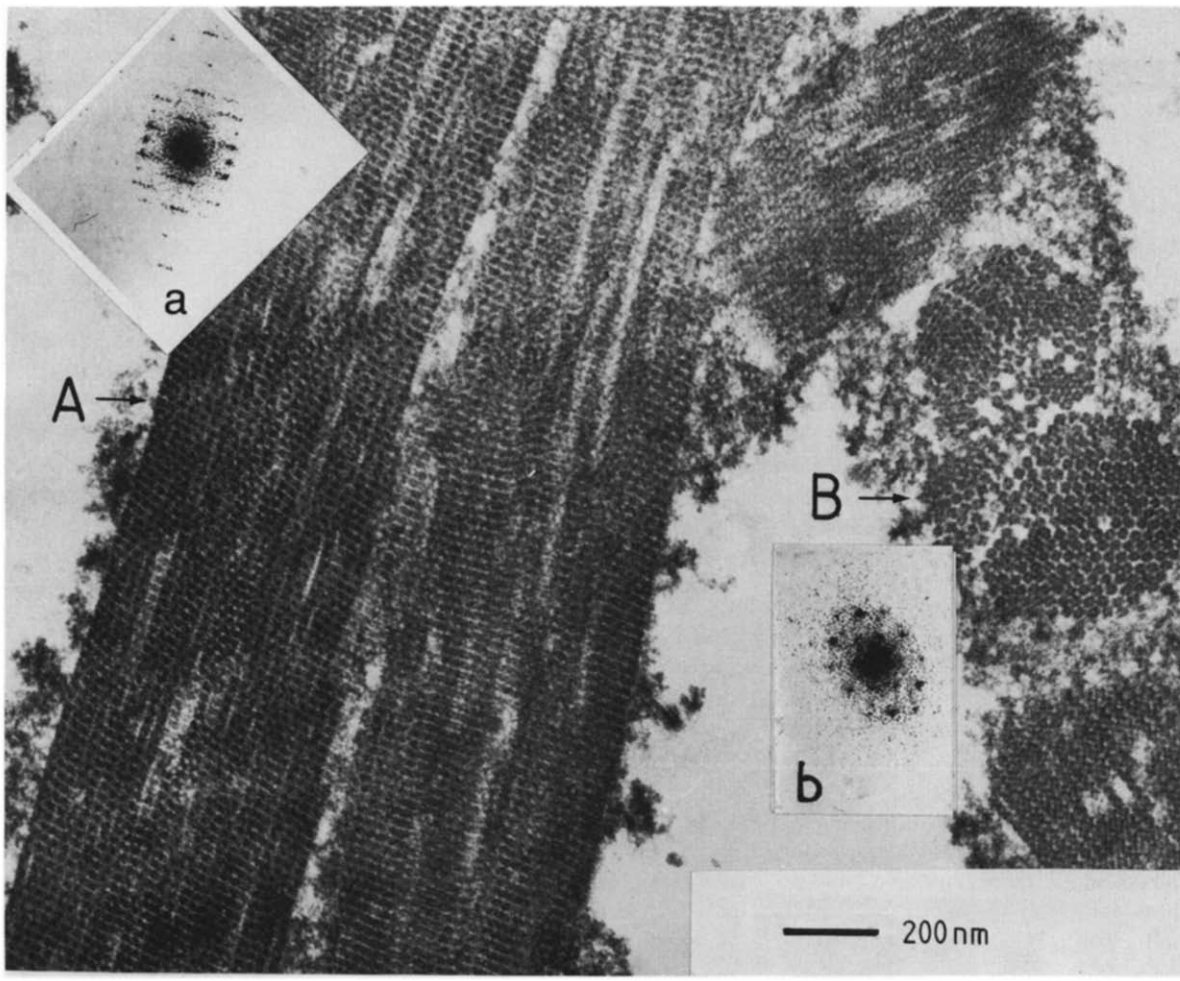

Fig.2. Electron micrograph of a thin section (~50 nm) through embedded crystals which were grown under the same conditions as in fig.1. Inserts a and b are the optical diffraction patterns of areas *A* and *B*, respectively.

The crystallized material was also tested for its biological activity (e.g., table 1). It was found that the crystallized ribosomes retained 50–60% of the biological activity in a poly(U)-directed polyphenylalanine synthesizing system as compared to ribosomes stored at −70°C for the same length of time. The preservation of the biological activity shows that the crystalline ribosomes are intact.

The crystals are still not suitable for X-ray three-dimensional structure analysis. However, important information on their internal arrangement, unit cell constants and symmetry may be obtained from image reconstruction studies. We also expect to obtain a low resolution model of the stain distribution within the ribosome. Since in this case it is possible to study the same crystal form from two or more independent views and orientations, we hope that a comparison of the resulting images obtained will yield an isotropically reliable model which will be well characterized along its three dimensions. Work along these lines is in progress.

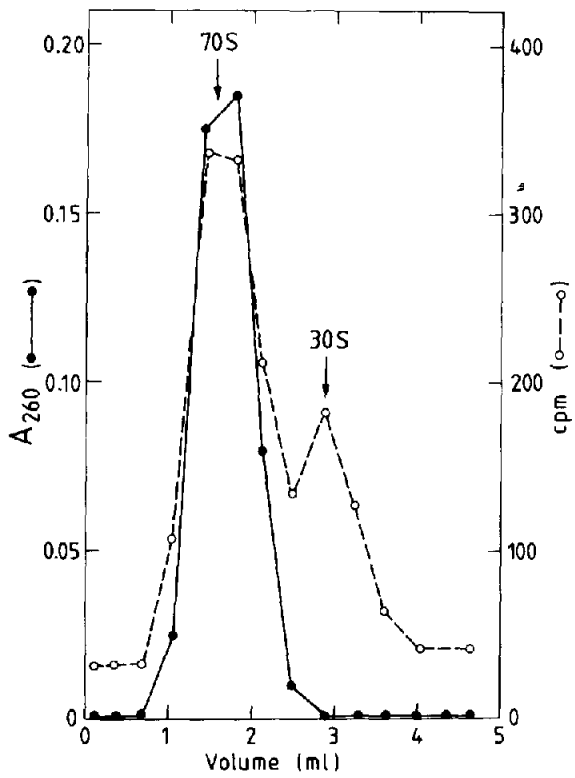

Fig.3. Sucrose gradient centrifugation of ribosomes from pelleted and dissolved crystals (•—•) together with a small amount of radioactive ribosomal markers (○- - -○) in 10 mM Tris–HCl (pH 7.8), 6 mM $MgCl_2$ and 30 mM NH_4Cl. Centrifugation was carried out as described in Materials and Methods.

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