The growth of ordered two-dimensional sheets of 70 S ribosomes from *Bacillus stearothermophilus*

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Well ordered two-dimensional sheets of intact 70 S ribosomes from *Bacillus stearothermophilus* have been obtained in vitro using salt-alcohol mixtures. These sheets consist of relatively small unit cells with dimensions of 200 ± 20 Å and 400 ± 30 Å. Diffraction patterns of electron micrographs of these sheets stained with uranyl acetate contain features to 42 Å resolution.

Ribosome *Two-dimensional sheet* *70 S ribosomal particle* (Bacillus stearothermophilus)

1. INTRODUCTION

To understand better the mechanism of protein biosynthesis a detailed model for the ribosome is an essential requirement. Diffraction methods provide the most reliable structural information. Thus, we have developed procedures for in vitro growth of three-dimensional crystals and two-dimensional sheets of intact ribosomal particles. Using these procedures, crystals and sheets suitable for structural analysis have been obtained from the large ribosomal subunits of *Bacillus stearothermophilus* [1–7] and *Halobacterium marismortui* [8].

In spite of substantial attempts to grow diffracting three-dimensional crystals or large two-dimensional sheets from whole ribosomes, until recently only micro three-dimensional crystals of 70 S ribosomes from *E. coli* have been obtained [9]. These crystals are very well ordered, but are too small for crystallographic studies.

Recently we have developed a new method for production of large, highly ordered two-dimensional sheets of ribosomal particles [7]. Employing this method, we were able to obtain two-dimensional sheets from the large ribosomal subunits from *B. stearothermophilus* of quality suitable for high-resolution three-dimensional image-reconstruction studies. These studies have already led to elucidation of a model for the large ribosomal subunit in which several striking features, such as a long tunnel, have been revealed [5].

Using this method we were also able to grow two-dimensional sheets of mutated 50 S ribosomal subunits which lack protein L11 and of the 70 S ribosomes from *B. stearothermophilus*. The latter are the subject of this communication.

2. MATERIALS AND METHODS

Ribosomes were prepared and their integrity was checked as described in [7].

Two-dimensional sheets have been grown and applied to electron microscopy grids in the same manner described in [7]. The grids were examined using a 400T Philips electron microscope, operating at 80 kV, at an electron optical magnification of $\times 17000$–96000. Micrographs were checked by optical diffraction for focus and astigmatism correction. Optical diffractograms were used for the determination of unit cell dimensions and resolution limits.

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3. RESULTS AND DISCUSSION

The current two-dimensional sheets of whole ribosomes from *B. stearothermophilus* consist of relatively small unit cells with dimensions of $a = 200 \pm 20$ Å, $b = 400 \pm 30$ Å, $\gamma = 90 \pm 5°$. When stained with uranyl acetate, optical diffraction patterns of their micrographs show reflections to the seventh order and extend to about 42 Å. Although being well ordered (fig.1), a typical sheet contains only about 45–70 particles, thus they are still too small for three-dimensional image reconstruction. However, occasionally long sheets (of about 15 × 10 particles) can be detected, indicating the possibility of producing larger sheets.

Potentially, these two-dimensional sheets provide an attractive system for subsequent structure analysis, since it should be possible to crystallize them together with various functional ligands. Some of these ligands (e.g. tRNA) are of a size large enough to be visualized by electron microscopy. Higher resolution data could be obtained, if the sheets could be investigated frozen and unstained or negatively stained with inert materials such as gold-thioglucose. Unlike uranyl acetate, which may interact chemically with the negatively charged components of the ribosomal particles (most likely the rRNA), gold-thioglucose is an inert stain and is expected to reveal the outer contour of the particles. Unfortunately, attempts to stain these sheets with gold-thioglucose have so far been unsuccessful, since the application of this stain to the grids causes deterioration of the sheets. Mild crosslinking with glutaraldehyde is not effective.

As with other salt-grown two-dimensional sheets [7], the two-dimensional sheets of the whole ribosomes cannot be examined unstained and frozen, since as a result of cooling, the salt crystallizes on the grids, thus preventing visualization of the ribosomal particles. Attempts to grow larger two-dimensional sheets, to develop pro-

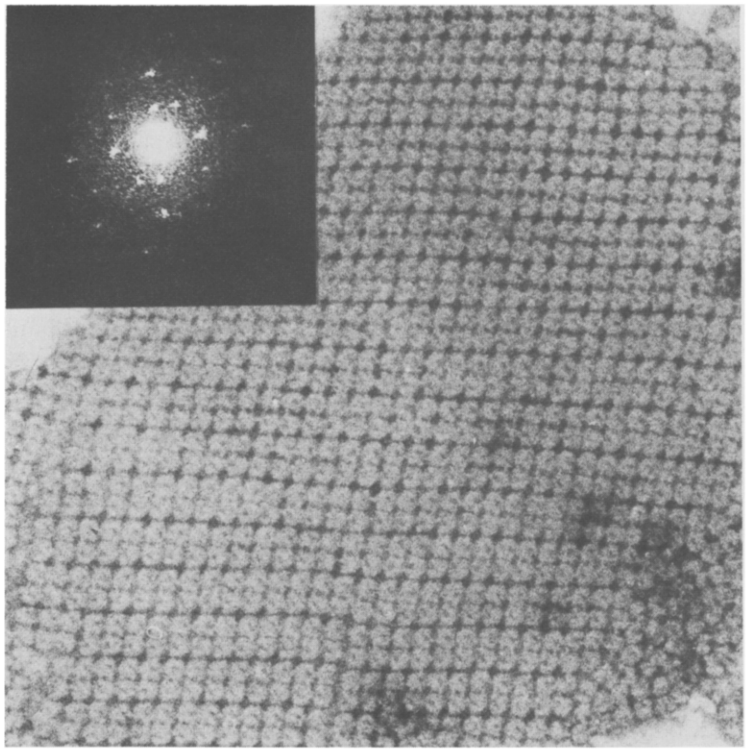

Fig.1. An electron micrograph of two-dimensional sheets (× 28000), stained by 0.5% uranyl acetate, and an optical diffraction pattern from an area containing about 20 × 15 unit cells. Crystallization conditions: 1.5 M $(NH_4)_2SO_4$, 0.15 M $MgCl_2$, 4% methanol.

cedures for staining with gold-thioglucose, and for investigating of unstained frozen specimens are in progress.

The exact conditions for obtaining two-dimensional sheets from 70 S ribosomes depend on the specific ribosomal preparation in an as yet undefined manner. A similar behavior has been observed in the production of two-dimensional sheets from the 50 S ribosomal particles [7]. Based on our success in growing large sheets of the latter [5], we expect that we will also be able to define the right conditions for the growth of large two-dimensional sheets of whole ribosomes. Since the ribosomes are harvested in the early log phase [7], they should contain the nascent polypeptide chain. Polypeptides of variable length may be attached to individual ribosomes. This may inhibit the growth of large two-dimensional sheets. Thus, in parallel to our attempts to obtain larger two-dimensional sheets from the available native ribosomes, we are currently developing a procedure for obtaining 70 S ribosomes which does not include a nascent polypeptide chain.

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