

# A compact three-dimensional crystal form of the large ribosomal subunit from *Bacillus stearothermophilus*

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A new form of well-ordered three-dimensional crystals of intact 50 S ribosomal subunits from *Bacillus stearothermophilus* have been obtained. Electron micrographs of positively stained sections of these crystals revealed that the ribosomal particles are packed closely. The cell parameters have been determined. Representative electron micrographs and their computed contoured filtered images are shown.

<i>Three-dimensional crystal</i>	<i>New crystal form</i>	<i>Ribosomal particle</i>	<i>Unit cell dimension</i>
<i>Electron microscopy</i>		<i>Computed contoured image</i>	

## 1. INTRODUCTION

We have reported the in vitro growth of several forms of three-dimensional crystals, two-dimensional sheets and helical organizations of bacterial ribosomes and their subunits. These include 70 S ribosomes from *Escherichia coli* [1] and the large ribosomal particles from *Bacillus stearothermophilus* [2–6].

We have obtained a new three-dimensional crystal form of the large ribosomal subunit of *B. stearothermophilus*. This was achieved by employing a slightly modified version of the vapour diffusion technique in hanging drops and in capillaries. The crystals of this form (no.5 in our nomenclature) are almost suitable for crystallographic studies. They are packed compactly and in a well-ordered form so that their cell parameters could be derived from electron micrographs. Washed and dissolved crystalline ribosomal particles were found to be intact and functionally active.

## 2. MATERIALS AND METHODS

Ribosomal subunits of *B. stearothermophilus* were obtained as in [2]. The crystals were obtained from 10% ethanol or 10% ethylenglycol over pH 7.8–8.7 using the crystallization procedure in [7]. The crystals were fixed in 0.6% glutaraldehyde and embedded in resin ERL 4206. Thin sections were cut and positively stained with 4% phosphotungstic acid followed by 6% uranyl acetate. Electron microscopy was carried out on a Philips 400 at magnifications of 6000, 17000, 28000 and 36000.

Diffraction patterns were either computed or obtained with an optical diffractometer. Areas suitable for further studies were selected on the basis of their optical diffraction patterns. Those electron micrographs which showed the best resolution were digitized on an Optronics (P-1000) densitometer, raster 50 mm. Their diffraction patterns and reconstructed filtered images were then computed and displayed on a TV screen. Equi-contour maps of the filtered images were displayed on a Tektronix screen [3].

The sucrose gradient (10–30%) was run for 110 min at 50000 rev./min in a SW60 rotor and

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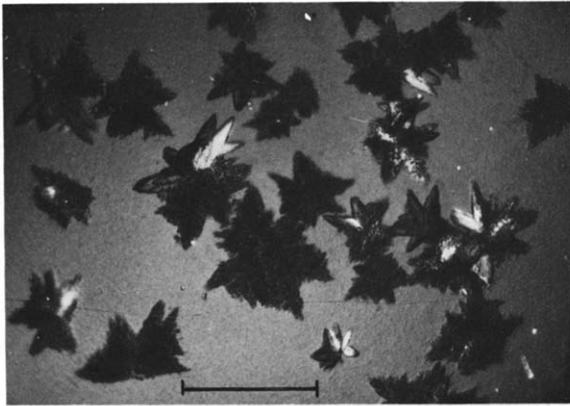


Fig.1. Crystals of form no.5, as seen in polarized light; scale bar, 0.2 mm.

pumped through an Uvicord (LKB model 2138; absorption range, 0.1; cuvette pathlength, 3 mm). The UV-profile was monitored by a LKB recorder (model 2210; 100 mV range).

### 3. RESULTS AND DISCUSSION

The crystals as seen by light microscopy are shown in fig.1. Embedded crystals were sectioned in different directions, and two sections with major unit-cell faces are shown in fig.2,3. In each sec-

tion there is an integral number of asymmetric units along the vertical axes and in the reconstructed stain-distribution contour-maps. The cell parameters, as determined from optical diffraction patterns (to a resolution of 6 nm) are:  $a = 24 \pm 2$  nm,  $b = 25 \pm 2$  nm and  $\gamma = 127^\circ$  for face C (fig.2) and  $b = 23 \pm 2$  nm,  $c = 31 \pm 3$  nm and  $\alpha = 111^\circ$  for face A (fig.3). These sections were chosen because they show the shortest repeats and are roughly perpendicular to each other. We arbitrarily related the axis of  $23 \pm 2$  nm in face A to the  $b$  axis in face C, assuming that the slight differences in their dimensions are due either to sectioning in a direction that does not absolutely coincide with the cell face, or to a contraction of the sample during the preparation for electron microscopy. The same argument could also be applied to the other alternative, namely relating  $23 \pm 2$  nm to the  $a$  axis in face C. However, despite this ambiguity we can preliminarily characterize our unit cell as having P1 symmetry with  $a = 24 \pm 2$  nm,  $b = 23 \pm 2$  nm,  $c = 31 \pm 3$  nm,  $\alpha = 111^\circ$ ,  $\beta \sim 90^\circ$ ,  $\gamma = 127^\circ$ .

It is interesting to note that although we had made extensive attempts to use ethanol for crystallization of ribosomes, this is the first time that three-dimensional crystals have grown from this solvent. However, ethanol is not the only

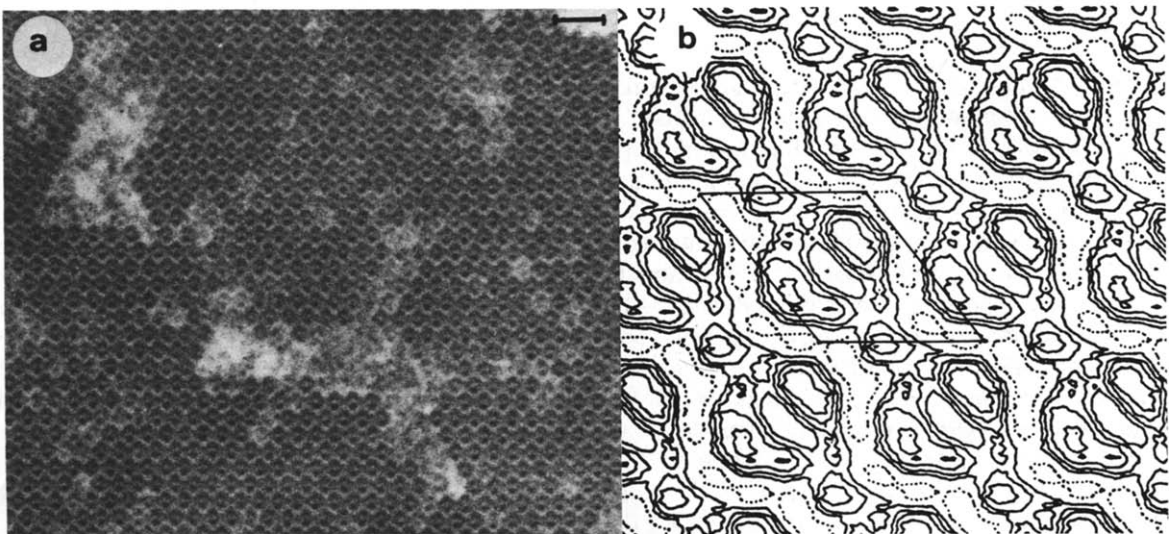


Fig.2. (a) Electron micrograph of a thin section which shows face C of crystal form no.5. (b) Contour map of the computed filtered image of fig.1(a). The unit cell is depicted; scale bar, 100 nm.

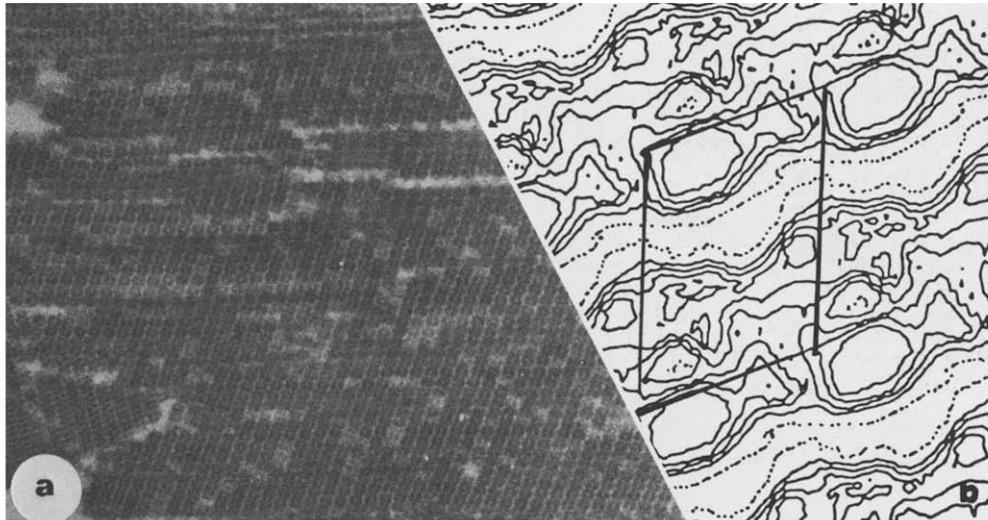


Fig.3. (a) Electron micrograph of an orthogonal thin section to that shown in fig.2(a). (b) Contour map of the computed filtered image of (a). The unit cell is depicted; scale bar, 100 nm.

alcohol in which these crystals could be grown. In fact, ethylenglycol was found to be even more suitable. Recently, ethanol was also successfully used for the production of two-dimensional crystalline sheets [6].

Comparison of the packing of the new crystal form to those obtained in [5] shows that form no.5 shares a common axis (25–28 nm) with forms no.1–3, and it exhibits one face (C in form no.5, A in form no.3) that resembles the dimensions but not the mode of packing of form no.3. The packing is pseudo-orthogonal for form no.3 and pseudo-hexagonal for form no.5. The only other pseudo-hexagonal form that has been obtained for the large ribosomal subunit of *B. stearothermophilus* is form no.4. However, there is a major difference in the density of the material within the crystals of these two forms: form no.5 shows a compact close packing whereas no.4 is rather 'empty'.

The crystals were sedimented, washed and dissolved. Sucrose gradient centrifugation of the dissolved material gave a symmetric 50 S peak comigrating with radioactive 50 S particles added as a marker (fig.4). Therefore, the 50 S particles remained intact under the conditions used for their crystallization.

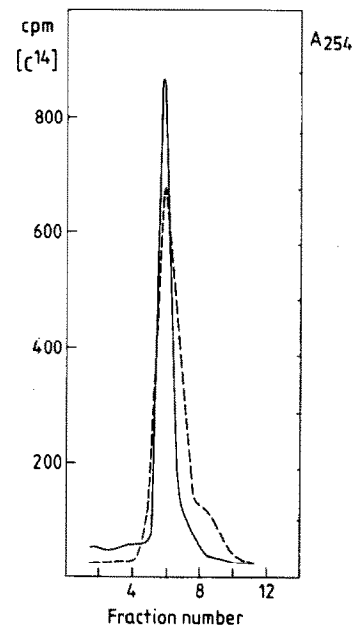


Fig.4. Sucrose gradient centrifugation. After pelleting, washing and dissolving the crystals, the sample was applied to a sucrose gradient. For details see section 2. Radioactive 50 S particles were added as a marker (---).

## ACKNOWLEDGEMENT

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