Several crystal forms of the *Bacillus stearothermophilus* 50 S ribosomal particles

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Well-ordered three-dimensional crystals of the large subunit of *Bacillus stearothermophilus* have been obtained. Electron micrographs of positively stained sections of these crystals revealed that the ribosomal particles are packed in several modes. Cell dimensions have been determined for 4 crystal forms. Representative electron micrographs, their optical diffraction patterns and their two-dimensional images are shown.

Three-dimensional crystal *Crystal form* *Ribosome* Bacillus stearothermophilus *Diffraction pattern* *Electron microscopy*

1. INTRODUCTION

Ribosomes are multifunctional particles consisting of many proteins and several RNA chains. During protein biosynthesis they not only interact with mRNAs, aminoacyl-tRNAs, initiation, elongation and termination factors, but also dissociate into their subunits, which, in turn, associate after the initiation process. The ribosomal particles possess a number of sites which facilitate the specific interactions with the components mentioned above.

Under special conditions, ribosomes of some species (e.g., lizard, chicken, amoeba and human) associate with each other and form ordered layers in vivo [1–4]. Similar layers have also been obtained in vitro from ribosomal subunits from *Escherichia coli* [5].

We have obtained three-dimensional crystals from the whole ribosome of *E. coli* [6] as well as from the large subunits of *Bacillus stearothermophilus* ribosomes [7,8]. In both cases the crystals contain intact and functionally active subunits. Some of these forms are suitable for structural studies. Here, we describe electron-microscopic studies of several crystal forms and helical arrangements of the large ribosomal subunits from *B. stearothermophilus*.

2. MATERIALS AND METHODS

Ribosomal subunits of *B. stearothermophilus* were obtained as in [9], and the crystallization procedure is detailed in [10]. The crystals were fixed in 0.2% glutaraldehyde and embedded in resin ERL 4206. Thin sections were cut and positively stained with 2% uranyl acetate, or with uranyl acetate and lead citrate [7,8]. Electron microscopy was done in a Jeol 100B at 10000, 30000 and 60000-fold magnifications or in a Philips 400 at 6000, 22000 and 30000-fold magnifications. Diffraction patterns were either computed or obtained in 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 μm.

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Their diffraction patterns and reconstructed filtered images were then computed and displayed on a TV screen [11].

3. RESULTS AND DISCUSSION

Electron micrographs of positively stained thin sections of embedded crystals show regular arrangements of the stain distribution. We concentrated mainly on images of sections of principal unit-cell faces which contained an integral number of asymmetric units along the vertical axis of the section [11]. The unit-cell dimensions of several crystals, as determined from electron micrographs, are given in table 1. Some representative electron micrographs, their optical diffraction patterns and their filtered two-dimensional images are shown in fig.1–3. Since only projected images are recorded on electron micrographs, the observed variations (table 1 and fig.4) may stem either from the existence of different crystal forms or from different projected views of the same form.

The first two forms (table 1) have a projected P_1 symmetry with slightly different cell constants which are consistent with X-ray diffraction data obtained from a 70′ precession pattern of a glutaraldehyde cross-linked crystal that shows

Table 1

Description of some crystal forms and their growth conditions

Form	Unit-cell dimensions	Conditions
1	a = 13.4 nm b = 25.6 nm γ = 96°	Hanging drop 10% ethanediol (pH = 7.0)
2	a = 15.8 nm b = 28.8 nm γ = 97°	Hanging drop 3% ethylhexanediol (pH = 6.6)
3	a = 31.4 nm b = 28.0 nm γ = 105°	Capillaries 30% methanol (pH = 6.3)
4	a = 40.5 nm b = 40.5 nm c = 25.6 nm $\alpha = \beta$ = 90° γ = 120°	Capillaries 30% methanol (pH = 6.9)

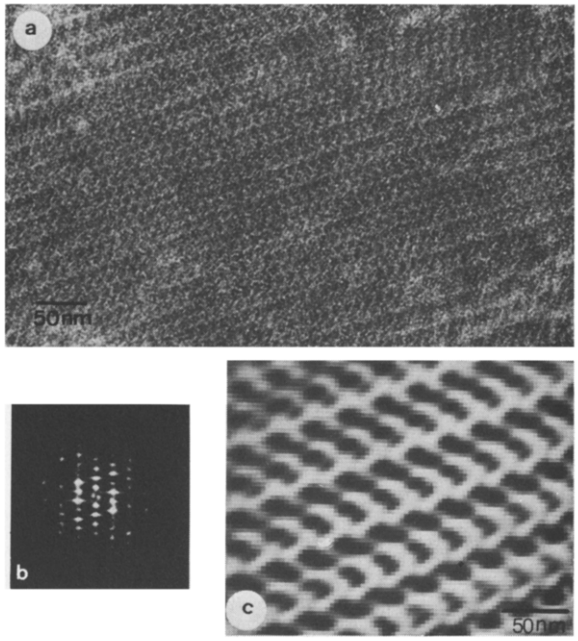

Fig.1. (a) Electron micrograph of a thin section of crystal form 1 (table 1). (b) Optical diffraction pattern of the electron micrograph in (1a). (c) Computed filtered image of (a). The black and white assignments are reversed.

periodic spacings of 15.4 nm and 26 nm [10] as well as from low-angle powder diffraction patterns where the longest detected spacing was 30.0 nm allowing up to 20% contraction during the preparation for electron microscopy. The variations in cell constants (table 1) may either be due to differences in the conditions of crystal growth and in the preparation for electron microscopy or due to the existence of two different crystal forms. It is of interest to note that the unit-cell of form 3 corresponds approximately to two unit-cells of form 1 or 2 joined in the direction of the short axis.

The crystals of form 4 (table 1 and fig.3) are still fairly small (0.03 mm diam.) but survive extremely well during the preparation for electron microscopy so that the whole crystal could be sectioned (fig.3a). We also could section these crystals in two orthogonal directions (fig.3b,c). These crystals contain an approximate 6- (or 3-)fold rotation axis, roughly perpendicular to the projected image shown in fig.3a,b. In order to verify that the images shown in fig.3a,b contain about one vertical repeat of the unit cell, we sectioned the same

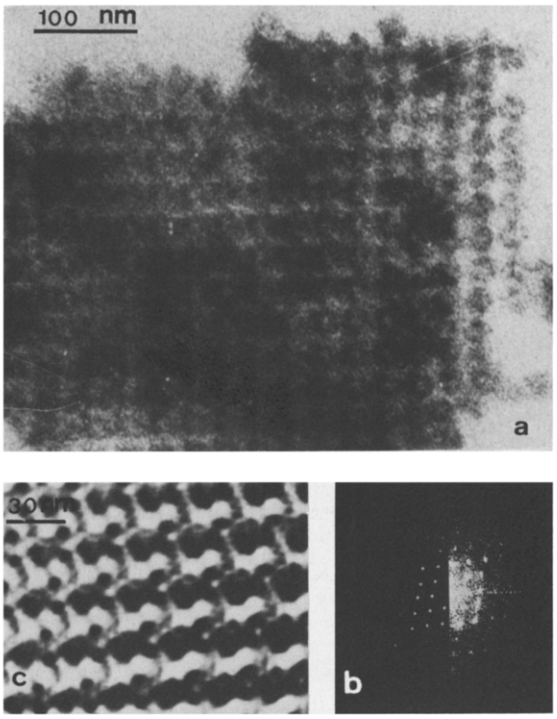

Fig.2. (a) Electron micrograph of a thin section of crystal form 3 (table 1). (b) Optical diffraction pattern of the electron micrograph in (2a). (c) Computed filtered image of (2a). The black and white assignments are reversed.

grid perpendicular to its original sectioning direction (fig.3h). Thus, we could measure directly the thickness of the section (~27 nm) which agrees well with the length of the C axis (table 1).

Although the image in fig.3b looks as if it is hexagonally packed, its diffraction pattern (fig.3d) shows that this symmetry does not hold even for the second diffraction order. The symmetric unit is built of 2 parts which are related by a pseudo 2-fold rotation axis. Each of these parts interacts with its neighbors in 3 different modes (fig.3f). Since the sectioning direction may not be exactly perpendicular to any crystallographic symmetric axis, we searched for the 3- and 6-fold rotation axis by sectioning the same embedded block in several directions and then by tilting those sections which were close to the estimated correct one over a range of ± 50°, with intervals of 5–10° around 4 different axes, 45° apart, in the plane of the section. There was no evidence either in the computed diffraction patterns or in the filtered images of these sections for a true crystallographic 6- (or 3-)fold symmetry axis. A partial loss of the symmetry may be due to the fact that the stain has been applied to sections which were not perfectly perpendicular to the rotation axis. Thus equivalent particles may be contrasted differently by the stain. Three-dimensional image reconstruction studies of this crystal form are currently in progress [11].

The 4 studied crystal forms share a common periodicity of 15 ± 1.5 nm or an integral multiplicity of it. It cannot yet be determined whether this is due to the formation of preferred packing interactions of single particles or merely to a coincidence.

Each of the sections (fig.4a–e) obtained from crystals grown from several ribosomal preparations under slightly different conditions may represent an independent crystal form, or, instead, some (or all) of them may show different views of the same three-dimensional crystal. In general, all the periodic distances observed in these micrographs are fairly long (> 30 nm), and in some cases (fig.4b,d,e) there are variations in the image over several repeats of the unit cell. This may be introduced by a section direction making an oblique angle with the unit cell principal faces.

A difference between the projected image of form 4 and the other forms concerns the density of the material within the crystals. Forms 1–3 and those shown in fig.4a–e are fairly compact, whereas sections parallel to the pseudo 6-fold axis of form 4 show 'empty' space (fig.3b). A similar 'empty' arrangement has been observed for large crystalline sheets of lizard ribosomes [12]. However, the three-dimensional packing of crystal form 4 is fairly compact, as indicated by the orthogonal sections (fig.3c).

The 50 S subunits aggregate also into various regular structures, such as helices (fig.4f–h). A high degree of polymorphism is an inherent characteristic of crystals of the ribosomal subunits from *B. stearothermophilus* and was also detected in the early stages of crystal growth [8]. Thus, our results suggest that the surface of these particles contains a number of inter-particle attachment sites, some of which have the potential to produce periodic structures. It is hoped that further three-dimensional structure analysis of several of these

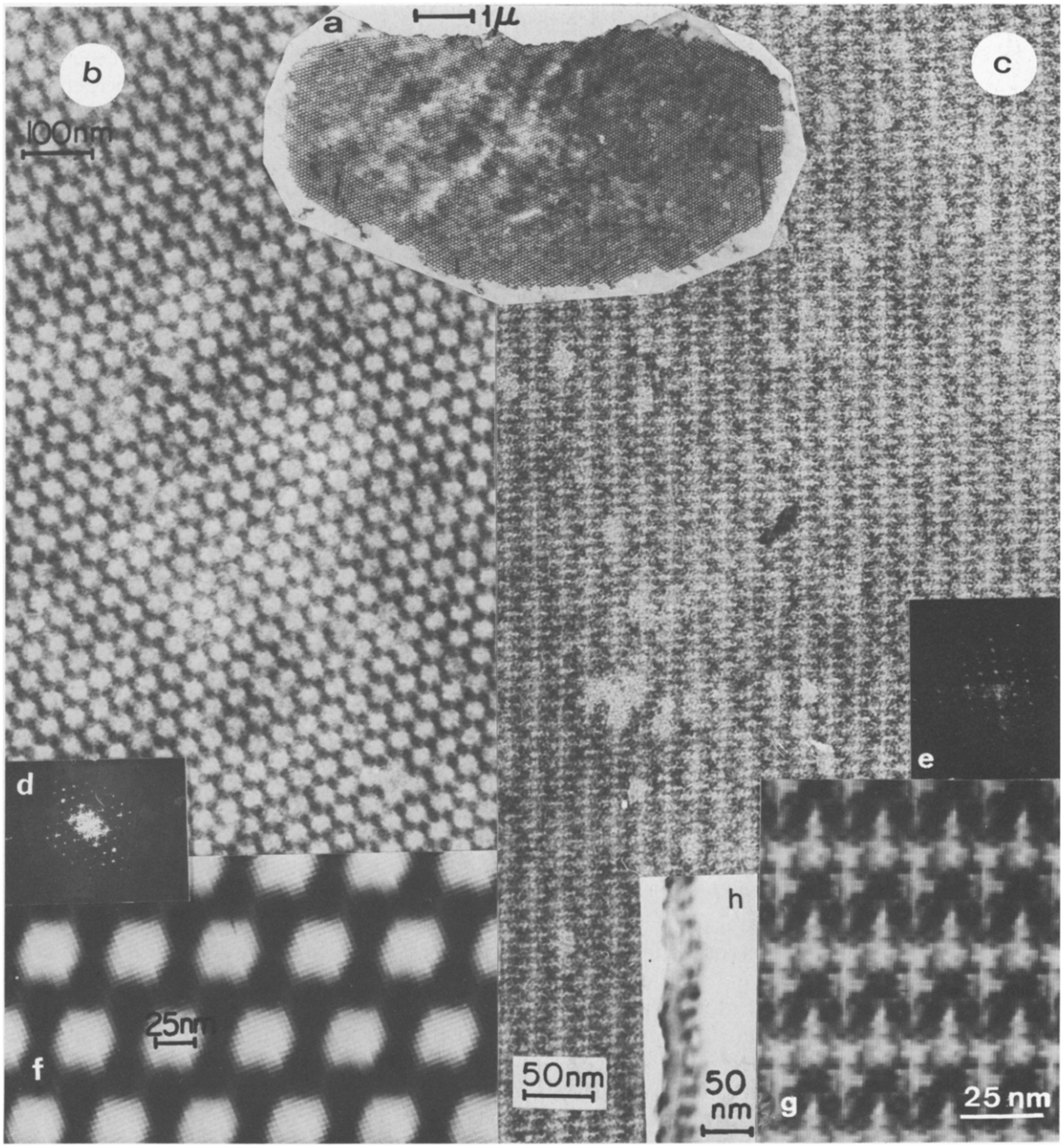

Fig.3. (a) Electron micrograph of a thin section of crystal form 4 (table 1). (b) An enlarged view of the section in (3a). (c) An enlarged view of a section orthogonal to that in (3a,b). (d) Optical diffraction pattern of the electron micrograph in (3b). (e) Optical diffraction pattern of the electron micrograph shown in (3c). (f) Computed filtered image of (3b). (g) Computed filtered image of (3c). (h) Sideways on section of the grid shown in (3a,b).

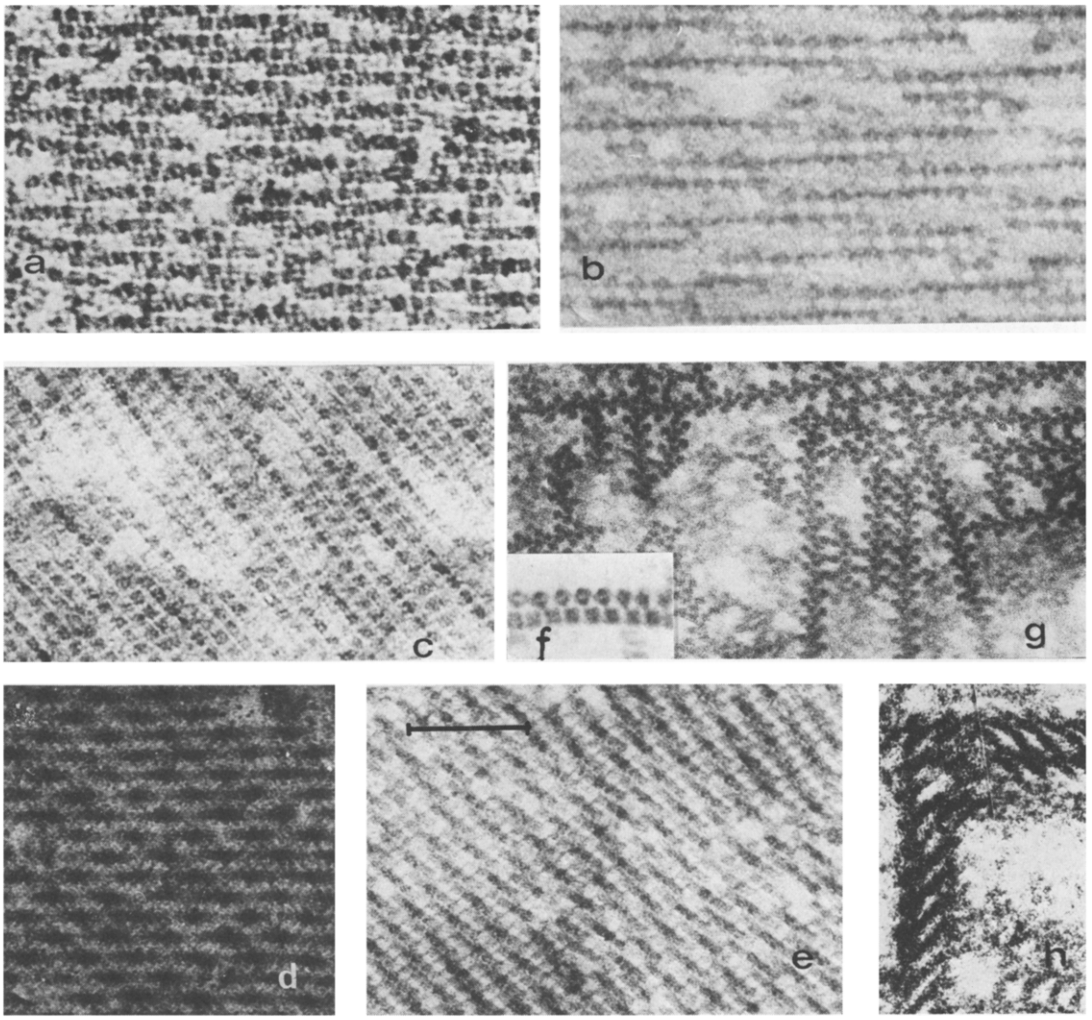

Fig.4. Electron micrographs of various crystal forms and helical objects which were obtained in capillaries in the presence of 5 mM spermidine. Growth conditions: (a) 30% methanol (pH 6.9); (b) 30% methanol (pH 7.2); (c) 30% methanol (pH 6.9); (d) 30% methanol (pH 6.9); (e) 30% methanol (pH 7.5); (f) 10% ethylhexanediol (pH 6.9); (g) 10% ethylhexanediol (pH 6.9); (h) 30% methanol (pH 7.5); bar length = 100 nm.

crystals will lead to a reliable molecular model of the large subunit of the *B. stearothermophilus* ribosomes.

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