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The Crystallization of Ribosomal Proteins from the 50 S Subunit of the *Escherichia coli* and *Bacillus stearothermophilus* Ribosome*

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Several individual intact ribosomal proteins purified from bacterial sources under mild conditions have been crystallized. A number of these are suitable candidates for three-dimensional structural studies by x-ray diffraction techniques. Data collection to 3 Å resolution for one of these proteins is in progress.

The process whereby polypeptide chains are manufactured on the ribosome is evidently both complex and universally important in biology. Although the various steps are well understood, it is clear that a complete understanding of the mechanisms involved awaits a detailed description of the ribosome structure at the molecular level. The success achieved in identifying function within the structures of many globular proteins, notably enzymes (1, 2), encourages a similar approach to this more complex system.

Already, much structural information is available on ribosomes, especially those from *Escherichia coli*. The three RNA components are now well characterized at the primary and secondary levels (3–5) and the chemical cross-linking of regions of the molecules which juxtapose in the ribosome will provide information about the tertiary structure. Furthermore, nearly all of the 53 proteins from the *E. coli* ribosome have now been sequenced and predictions about their secondary structure have been made. In addition, by a combination of immune electron microscopy, chemical cross-linking, neutron scattering, and fluorescence spectroscopy, a picture of their relative topology in the organelle is gradually emerging. These studies have been reviewed recently (6).

During the past few years, using mild extraction procedures, ribosomal proteins have been isolated which, on investigation by CD and NMR, exhibit well defined secondary and tertiary structures.[1,2] In this article, we report the crystallization of several proteins prepared in this way and discuss the possibilities for determining their molecular structure by x-ray crystallography. This technique has already been successfully applied to the COOH-terminal fragment of the ribosomal protein L7/L12 from *E. coli* (7).

It was recognized early in the work that the proteins from thermophilic bacteria, being more resistant to the thermal denaturation and generally more robust, might prove better candidates for the extraction/crystallization procedure. Therefore, work has progressed in parallel on the ribosomal proteins from *E. coli* and the thermophilic organism *Bacillus stearothermophilus*. During the couse of these studies, many of the 50 S proteins from *B. stearothermophilus* have been characterized and several correlated with those from *E. coli*.

EXPERIMENTAL PROCEDURES

Individual ribosomal proteins were isolated from the 50 S subunits using the techniques developed by Dijk and Littlechild (8). Extensive crystallization surveys, varying pH, type and concentration of precipitant, and temperature were performed on those proteins obtained in sufficient yield. Vapor diffusion using the hanging drop technique (9) was found to be the most efficient method when dealing with the relatively small quantities of material available (typically 5 to 30 mg from each extraction).

Control of pH was achieved using citrate or acetate (4.0 to 6.0), cacodylate or phosphate (5.5 to 7.8), Hepes[3] (7.0 to 7.8), Bicine (7.8 to 8.8), and tetraborate or glycine (8.8 to 10.0) buffers, all at final concentrations of between 0.1 M and 0.3 M. Many precipitants were tested including $(NH_4)_2SO_4$, Li_2SO_4, $Na_3C_6H_5O_7$ (citrate), and equimolar mixtures of Na_2HPO_4 and KH_2PO_4 as well as the alcohols, 2-methyl-2,4-pentandiol, 2-ethyl-1,2-hexandiol, *t*-butyl alcohol, and 2-propanol.

The crystallization experiments were set up in Linbro dishes (Flow Laboratories, United Kingdom). Reservoir solutions were placed in the depressions and single drops of protein on cover slips were sealed over the depressions with silicone grease. Each drop was prepared on a plastic or siliconized glass cover slip with a particular pH, and a precipitant concentration 10% below that of the reservoir. For initial experiments, drops were made by mixing appropriate amounts of buffer, protein, and precipitant solutions. After conditions for crystallization had been characterized, protein solutions were dialyzed against the buffer and precipitant to avoid protein dilution. The concentration of protein in the drop varied between 2 and 10 mg/ml.

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis, several crystals were washed twice in stabilizing solutions and dissolved in sample buffer. The samples were analyzed on 15% sodium dodecyl sulfate gels (10).

RESULTS

Details on the proteins successfully crystallized are given below, and more specific information is listed in Table I. The proteins are designated either E (*E. coli*) or B (*B. stearothermophilus*) and L refers to their location in the large subunit. The numbering scheme refers to the position of the protein on a two-dimensional gel (11).

BL17—This was the first intact ribosomal protein to be crystallized, and crystal data have been published elsewhere (12). We have now optimized the crystallization conditions and reproducibly obtain good quality crystals from $(NH_4)_2SO_4$ with dimensions up to 1.0 mm (Fig. 1*E*). The complete native

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[1] Dijk, J., Littlechild, J., Freund, A. M., Pouyet, J., Daune, M. & Provencher, S. W. (1981) *Eur. J. Biochem.*, submitted for publication.

[2] Littlechild, J., Malcolm, A. L., Paterakis, K., Ackerman, I. & Dijk, J. (1981) *Eur. J. Biochem.*, submitted for publication.

[3] The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Bicine, *N,N'*-bis(2-hydroxyethyl)glycine.

TABLE I

Summary of crystal parameters for the four ribosomal proteins

	Crystallization conditions			Crystal parameters
	Concentration of precipitant	Buffer	Approximate temperature	
			°C	
BL17 (M_r = 16,068)	2.4 M $(NH_4)_2SO_4$	Tris, pH 7.8	~20	Orthorhombic a = 37.5, b = 48.9, c = 135.3 Å
BL34 (M_r = 6,500)	3.2 M $(NH_4)_2SO_4$	Bicine, pH 8.4	~20	Orthorhombic a = 43.7, b = 44.0, c = 64.4 Å
	3.2 M $(NH_4)_2SO_4$	Bicine, pH 8.4	~20	Tetragonal a = 44, b = 44, c = 129 Å
BL10 (M_r = 19,168)				
Type I	1.5 M $(NH_4)_2SO_4$	Tetraborate, pH 8.8	~20	Not determined
Type II	2.4 M Na-K-phosphate	Tetraborate, pH 9.4	10	Hexagonal a = 62, b = 62, c = 124 Å
EL29 (M_r = 7,262)	1.2 M $(NH_4)_2SO_4$	Acetate, pH 5.9	~5	Monoclinic a = 52, b = 119, c = 90 Å β = 71°

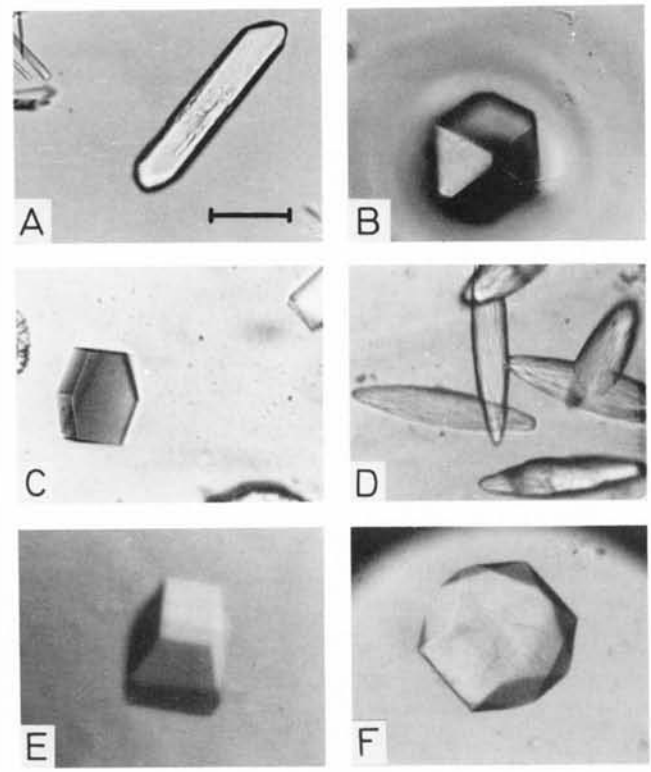

FIG. 1. **Crystals of the proteins described in the text and in Table I.** The *bar* indicates a length of 0.2 mm. *A*, BL34, orthorhombic form; *B*, BL34, tetragonal form; *C*, BL10, type I; *D*, BL10, type II; *E*, BL17; *F*, EL29.

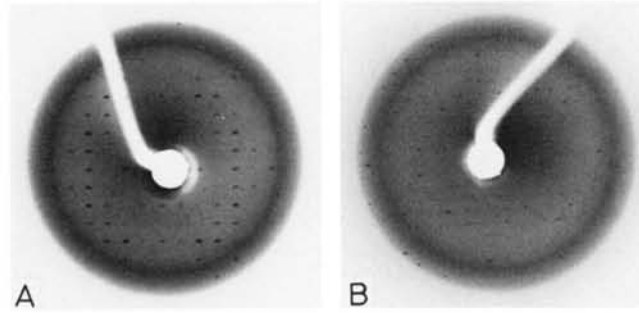

FIG. 2. **7° precession photographs of (*A*) an orthorhombic crystal of BL34 and (*B*) a hexagonal crystal of BL10.** The photographs were recorded on a Nonius precession camera with CuK_α radiation from a fine-focus tube run at 1000 watts. Exposure times were 12 h.

data have been collected with an oscillation camera to a resolution better than 3 Å, and data from two isomorphous heavy atom derivatives are currently being measured. BL17 crystals are stable in the x-ray beam for up to 250 h. However, because it is necessary to work with a slow rotation speed (15 h/deg) on the rotation camera in order to obtain a resolution better than 3 Å, only ~15° of data can be collected from each crystal.

BL34—This protein crystallizes reproducibly at room temperature from $(NH_4)_2SO_4$, and two distinct crystal forms have been identified under the same conditions. The more common form has an orthorhombic unit cell and extended plate-like morphology (Fig. 1*A*). The crystals grow up to 1 mm in length, diffract to 3 Å resolution, and are stable in the x-ray beam for at least 30 h. The unit cell dimensions estimated from precession photographs (one of which is shown in Fig. 2*A*) are 43.7 × 44.0 × 64.4 Å. The second type of crystal shows a tetragonal unit cell and a regular polyhedral shape (Fig. 1*B*). This form is obtained less often and the crystals are considerably smaller (typically 0.2 mm across the diagonal). The unit cell dimensions have been estimated from weak precession photographs as 44 × 44 × 129 Å and, interestingly, this appears to correspond to two unit cells of the orthorhombic form joined in the direction of the long cell axis.

BL10—Two types of crystal have been obtained. Type I grows from $(NH_4)_2SO_4$ solution, is stable at room temperature, and has a very distinctive morphology (Fig. 1*C*). However, the crystal size is limited to a maximum dimension of 0.2 mm, and no useful diffraction is observed. The crystal form hence remains undetermined at present. Crystals of type II appear using a variety of precipitants including phosphate, $(NH_4)_2SO_4$, Li_2SO_4, and 2-methyl-2,4-pentandiol. They have an unusual oval morphology (Fig. 1*D*), diffract to a resolution of 4 Å, and grow up to 0.4 mm in length. Precession photographs have been recorded from the type II crystals (Fig. 2*B* shows an example) and the unit cell appears to be hexagonal with dimensions approximately $a = b$ = 62 Å, c = 124 Å.

EL29—Crystals of this protein grow at 4–6 °C using a whole spectrum of organic and inorganic precipitants, although the best are from $(NH_4)_2SO_4$. The crystals have a polyhedral shape (Fig. 1*F*) and measure between 0.1 mm and 0.5 mm across the diagonal. They are stable only between 4 °C and 8 °C, are somewhat fragile, and their diffraction pattern is presently limited to 6 Å. The crystals display a degree of disorder, but we estimate that the unit cell is monoclinic with dimensions roughly a = 52 Å, b = 119 Å, c = 90 Å, and β = 71°. We are investigating the use of low temperatures to improve the diffracting properties and stability of the crystals.

To confirm that the crystals contain intact protein and not proteolytic fragments, we analyzed crystalline material by

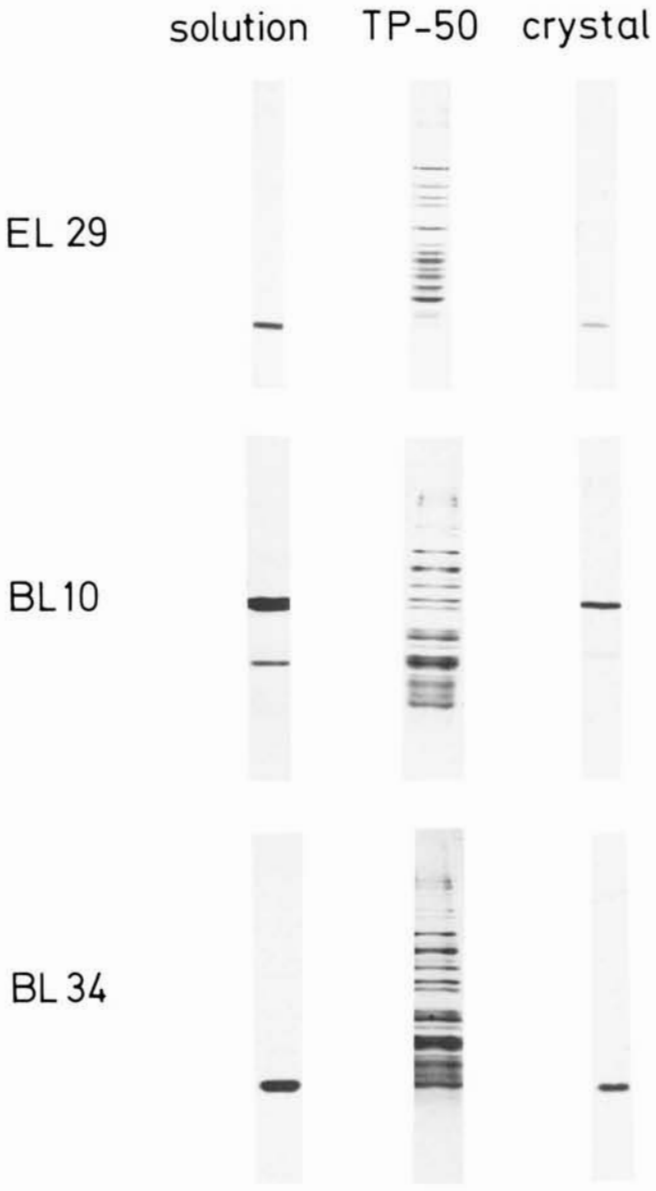

FIG. 3. **Gel electrophoresis of the dissolved protein crystals.** The protein crystals were collected by centrifugation, washed extensively with stabilizing solution, and applied to a 15% sodium dodecyl sulfate-polyacrylamide gel (13). For comparative purposes, the original protein and a mixture of the total 50 S proteins (TP-50) were co-electrophoresed. The BL10 preparation was contaminated with a small amount of BL17. Although the crystals were washed thoroughly, this contamination also shows up in the crystal sample.

sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The analysis was carried out on the plate-like BL34 crystals (Fig. 1*A*), the oval BL10 crystals (Fig. 1*D*), and EL29 (Fig. 1*F*). The results are shown in Fig. 3 and, clearly, the dissolved crystalline material corresponds to the intact protein in solution, as was found for BL17 (12).

Further studies of the four proteins are proceeding in the Max-Planck-Institute für Biochemie, Martinsried (BL17), the Max-Planck-Institut für Molekulare Genetik, Berlin (BL10 and BL34), and the Weizmann Institute (EL29).

DISCUSSION

Based on considerations of amino acid composition, in particular the ratio of hydrophobic to charged residues, it has been suggested (13) that the ribosomal proteins are unlikely to have a well defined fold and are therefore poor candidates for crystallization. Similar doubts have been expressed (2) for those ribosomal proteins which are elongated because of the extensive protein-nucleic acid associations which almost certainly occur within the ribosome. We have shown, however, that at least some appear to behave as well structured proteins in that they form ordered crystals from which we confidently expect detailed structural information.

It is evident from our results that the proteins from the thermophilic bacterium crystallize more readily than those from *E. coli*, therefore justifying our original decision to work also on the less well characterized *Bacillus* ribosome. During this work, the results from several types of experiments have consistently indicated a greater stability and degree of compactness of the *B. stearothermophilus* proteins. For example, the proteins are more resistant to limited proteolysis and refold more easily after thermal denaturation. It is likely, therefore, that our future crystallographic studies will be more concerned with the *B. stearothermophilus* system, although *E. coli* proteins that do yield suitable crystals will provide useful comparisons between the two ribosomes.

The small size of these crystals and the difficulties involved in extracting large quantities of protein will call for the most efficient methods of data collection. Consequently, we plan to use the very high intensity x-rays available from synchrotron radiation in conjunction with the oscillation camera to increase the effective resolution and crystal lifetime. BL17 data are currently being collected with a rotating anode x-ray generator although synchrotron radiation may prove invaluable for higher resolution work.

How confident are we that the structural information gained from x-ray crystallography on isolated ribosomal proteins will be relevant to the structure of the ribosome? Based on the accumulated knowledge of protein structure obtained by x-ray crystallography it is now generally accepted that the structure of a protein in the crystalline state closely resembles that of the protein in solution. This leaves the question as to whether the structures of isolated ribosomal proteins are similar to those which exist in the intact ribosome. It has been shown that ribosomal proteins, when isolated under mild conditions, have a stable and well defined spatial structure.[1,2] In view of this, it seems very unlikely that there are gross conformational differences between the proteins in solution and in the ribosome.

The information obtained from the studies described in this paper will be a valuable contribution to the current investigation on the spatial structure of the ribosome by chemical, physical, and immunological techniques.

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