THE CRYSTALLIZATION OF PROTEIN BL17 FROM THE 50 S RIBOSOMAL SUBUNIT OF *BACILLUS STEAROTHERMOPHILUS*

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1. Introduction

At present very little is known about the tertiary structure of ribosomal proteins from *Escherichia coli*. The shapes of ribosomal proteins have been determined by various techniques, which show some ribosomal proteins to be globular while others are more asymmetric [1]. More detailed information on the secondary and tertiary structure of these proteins has been obtained by spectroscopic techniques such as circular dichroism (CD) and proton magnetic resonance [2]. The latter experiments, which were on proteins isolated under very mild conditions [3], have shown that many of the *E. coli* ribosomal proteins have a unique secondary and tertiary structure.

The determination of the three-dimensional structure of ribosomal proteins by X-ray diffraction analysis of protein crystals is not yet possible. Despite many attempts at crystallization of intact ribosomal protein crystals suitable for X-ray analysis have not been obtained, although the crystallization of two fragments of proteins L7/12 has been reported [4].

In view of the largely negative results with *E. coli* proteins we have undertaken the isolation of ribosomal proteins from thermophilic bacteria under mild conditions with a view to their crystallization. Ample evidence exists for the increased resistance of thermophilic proteins against various denaturing conditions [5], thereby increasing the probability of crystallization. From the thermophilic bacteria, the ribosomal proteins of *Bacillus stearothermophilus* have been characterized and correlated to some extent with those of *E. coli* [6–8].

We present here a description of the crystals of protein BL17 from the 50 S subunit of *Bacillus stearothermophilus*. These are the first crystals of an intact ribosomal protein and are suitable for structural analysis by X-ray diffraction.

2. Experimental procedures

2.1. *Purification*

Bacillus stearothermophilus cells (strain NCA 1503) were obtained from the Microbiological Research Establishment, Porton Down. Ribosomes and subunits were prepared as in [3]. 50 S subunits were extracted with 2 M NaCl in the presence of 0.01 M $MgCl_2$ at pH 7.0. The extracted proteins were fractionated on a CM-Sephadex C-25 column with a KCl gradient in 0.01 M potassium phosphate at pH 7.0. Further purification was achieved by gel filtration on a Sephadex G-75 Superfine column (4 × 220 cm) eluted with 0.35 M KCl in phosphate buffer at pH 7.0.

Protein solutions were concentrated by embedding the sample, contained in Spectrapor 3 dialysis tubing (Spectrum Medical Industries Inc., Los Angeles), in dry Sephadex G-150 or Ficoll 400 or by absorption of the protein at low ionic strength onto a 1 ml CM-Sephadex C-25 column and elution with a small volume of 2 M NaCl in phosphate buffer at pH 7.0. All solutions contained in addition 3 mM 2-mercaptoethanol and the protease inhibitors phenylmethane

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sulphonyl fluoride and benzamidine hydrochloride at 100 μM and 20 μM, respectively. All purification steps were performed in a cold room at 3–5°C.

The purified protein was stored at –80°C in 0.35 M NaCl in phosphate buffer (pH 7.0), in which the 2-mercaptoethanol was replaced by 1 mM dithioerythritol.

The identity and purity of the protein were determined by two-dimensional gel electrophoresis [9], using the nomenclature proposed [6] and by slab-gel electrophoresis in the presence of dodecylsulphate [10]. Protein concentrations were determined by amino acid analysis.

2.2. *Crystallization*

Protein BL17 was dialysed, at 2–3 mg/ml, against 0.05 M sodium phosphate (pH 7.0). Saturated ammonium sulphate solution was added so as to give 1.5 M final conc. Crystals were obtained by vapour diffusion of 20 μl protein droplets against 2.2–2.4 M ammonium sulphate in sealed Petri dishes. Crystallization trials were performed at both 4°C and 10°C.

Suitable crystals were washed with 2.4–2.6 M ammonium sulphate in phosphate buffer (pH 7.0) and mounted in thin-walled glass capillaries. Screenless precession photographs were recorded at room temperature on a Nonius-Enraf precession camera, using graphite-monochromatized CuKα radiation produced by a 'Rigaku' rotating anode generator working at $U = 50$ kV, $I = 60$ mA.

3. Results and discussion

3.1. *Purification*

Protein BL17 was extracted from the 50 S subunit with 2 M NaCl in the presence of 0.01 M $MgCl_2$ at pH 7.0. The extract, after dialysis against starting buffer, was fractionated on a CM-Sephadex C-25 column with a 0.07–1.0 M gradient of KCl in 0.05 M K-phosphate (pH 7.0). The protein was eluted at 0.18 M KCl; its peak overlapped considerably with the preceding BL10 peak. Attempts at further purification by gel filtration on Sephadex G-75 or G-50 Superfine were unsuccessful since both proteins were eluted in the same position. The gel filtration served only to remove some aggregates of protein BL17 (fig.1). Consequently, the preparations

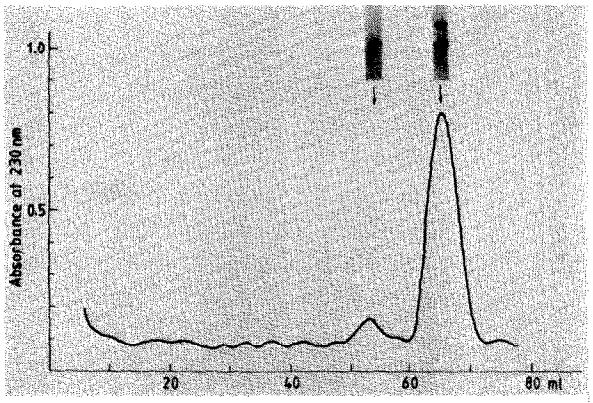

Fig.1. Gel filtration of protein BL17 on Sephadex G-50. BL17, 1 mg in 2 ml 0.35 M KCl, 0.05 M K-phosphate (pH 7.0), was applied to a Sephadex G-50 Superfine column (1 × 140 cm; vol. 110 ml) and eluted at 6 ml/h. Fractions of 1.0 ml were collected and 20 μl samples were analysed by slab-gel electrophoresis in the presence of dodecylsulphate. The inserted tracks show the analysis of peak fractions 53 and 66. Direction of migration was from top to bottom.

of BL17 obtained were somewhat contaminated with BL10, the extent of contamination being dependent on the selection of the peak fractions of the CM-Sephadex column.

The position of the protein on two-dimensional gels according to [9] is shown in fig.2. The intensity of the BL17 spot in a mixture of 50 S proteins was considerably lower than that of others. Since, however, the yield of BL17 during purification was in the expected range it is most likely that the protein stains poorly with amido black.

3.2. *Properties*

The molecular weight of BL17, as determined by gel electrophoresis in the presence of dodecylsulphate, is 17 000. A value of 16 000 was obtained by sedimentation equilibrium centrifugation (J. Georgalis, unpublished results). From gel filtration experiments such as shown in fig.1 a Stokes radius of 19 Å was obtained for BL17. By CD spectroscopy the protein was found to have a rather high α-helix content, ~50%. Protein BL17 resisted digestion with trypsin and chymotrypsin at 0°C. Under those conditions most ribosomal proteins of *E. coli* are digested to smaller fragments. These results suggest that protein BL17 has a compact and tightly-folded structure.

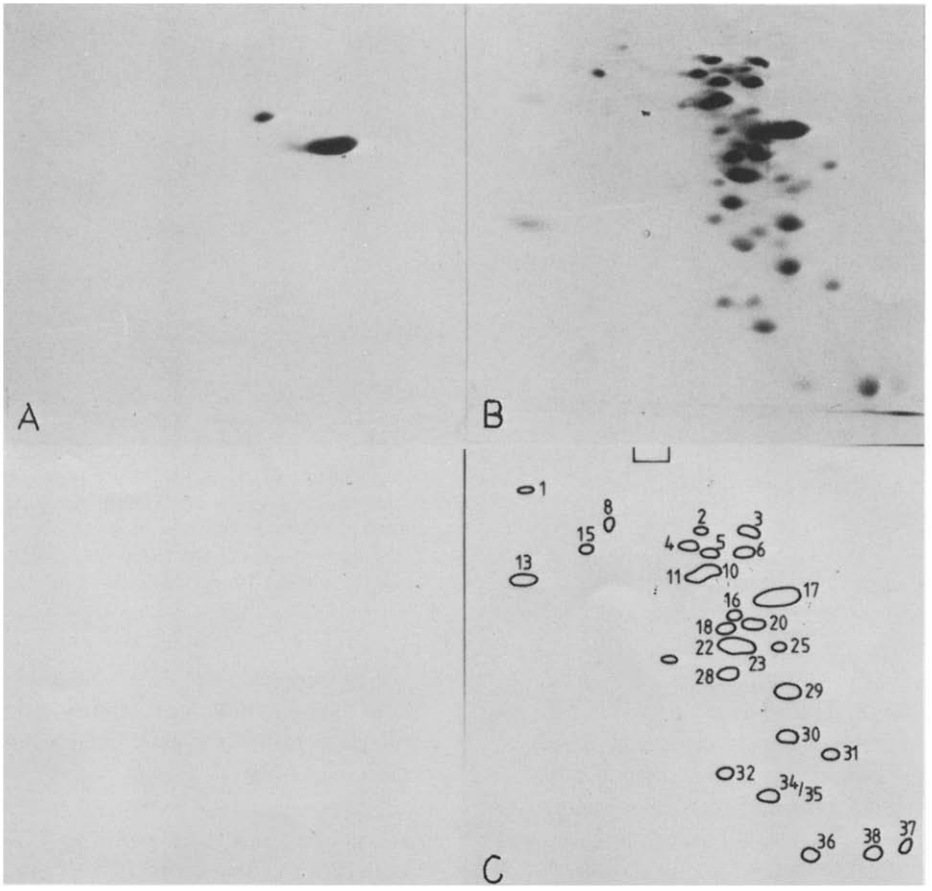

Fig.2. Two-dimensional gel electrophoresis of protein BL17. (A) BL17 (contaminated with BL10). (B) BL17 with a background of 50 S ribosomal proteins from *B. stearothermophilus*. (C) Nomenclature of the 50 S ribosomal proteins from *B. stearothermophilus*, according to [6].

The amino terminal sequence of BL17 was determined by a new micromethod [11], which required only 150 μg protein. The sequence was:

Met–Lys–Val–Ile–Phe–Leu–Lys–Asp–Val–

(J. Y. Chang, unpublished results). This information is, however, insufficient to establish a homology with an *E. coli* ribosomal protein. Only very small amounts of contaminating amino acids were found in this analysis, suggesting that the contamination with BL10 is $<<10\%$.

3.3. *Crystallization*

Crystals of BL17 were easily and reproducibly obtained by vapour diffusion against ammonium sulphate at both 4°C and 10°C. Small crystals usually appeared after 3–5 days and were fully grown in 2–3 weeks. Their shape is shown in fig.3. The larger crystals were 0.3–0.4 mm long. Regardless of the level of contamination of BL17 with BL10 the same crystals were always obtained. Several crystals were collected, washed repeatedly and analysed by gel electrophoresis in the presence of dodecylsulphate (fig.4). The results show that no protein degradation

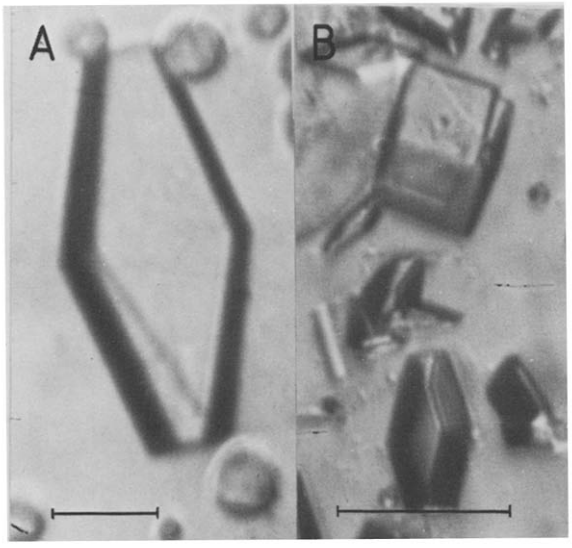

Fig.3. Crystals of protein BL17 obtained at different temperatures. (A) Grown at 4°C very slowly over 3 weeks from 2.20 M ammonium sulphate. (B) Grown at 10°C over 5 days from 2.36 M ammonium sulphate. Bar length: 0.1 mm.

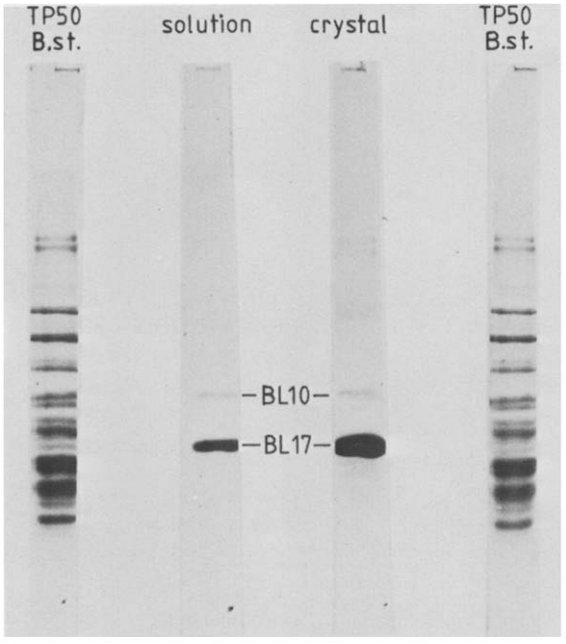

Fig.4. A comparison of the crystallized protein with protein in solution by slab gel electrophoresis in the presence of dodecylsulphate. Several crystals were collected, washed with 2.4 M ammonium sulphate in phosphate buffer and applied to the slab gel. The direction of electrophoresis was from top to bottom.

has occurred during the crystallization. The contamination level of BL10 in the crystal is considerably lower than that in solution. By densitometry of the bands (assuming equal staining for both proteins) 5–6% contamination with BL10 was found for the BL17 solution, in good agreement with the estimate from the sequence data. The crystal has a 2.5-fold lower contamination level, i.e., most likely 2–2.5% and certainly <4%.

X-ray diffraction photographs were taken of the crystals at 4°C and at 20°C. Some crystals showed internal disorder causing multiple reflections. A comparison of diffraction patterns at both temperatures showed that the crystals did not suffer any damage from the exposure to room temperature. The crystals were quite stable in the X-ray beam and showed no radiation damage after ~80 h exposure.

The crystal parameters determined from the screenless precession photographs are presented in table 1. The value of V_M obtained when one protein molecule/asymmetric unit is assumed is rather high [12]. The relatively poor diffraction pattern, however, indicates a low protein content in the unit cell.

The crystal form of protein BL17 described here is suitable for a structural analysis by X-ray diffrac-

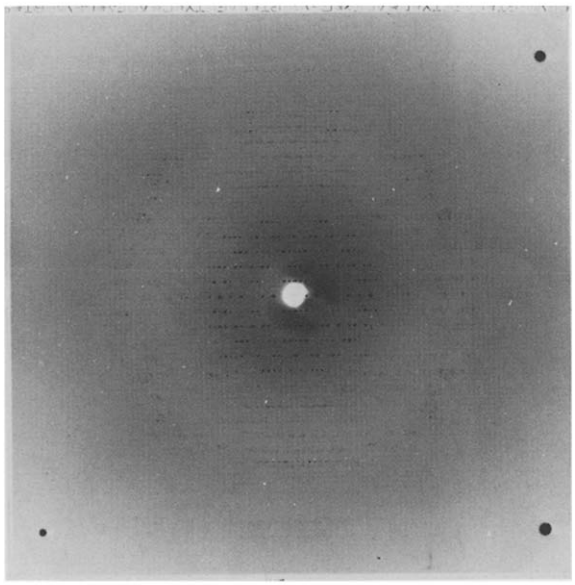

Fig.5. Screenless precession photograph of a crystal of protein BL17 (μ = 5°; crystal-to-film distance, 100 mm).

Table 1
Properties of the crystals of ribosomal protein BL17

Orthorhombic crystals
Space group: $P2_12_12$
Lattice constants: $a = 135.3$ Å (±0.5)
$b = 37.5$ Å (±0.2)
$c = 48.9$ Å (±0.2)
$\alpha = \beta = \gamma = 90°$
Volume of the unit cell: $V = 248\,100$ Å^3
Volume per unit protein mass: $V_M = 3.65$ Å^3/dalton (assuming mol. wt 17 000 and 1 molecule/asymmetric unit)
Resolution: 3.5 Å

tion. However, experiments to improve the quality of the crystals are in progress.

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References

[1] Brimacombe, R., Stöffler, G. and Wittmann, H. G. (1978) Ann. Rev. Biochem. 47, 217–249.
[2] Morrison, C. A., Bradbury, E. M., Littlechild, J. and Dijk, J. (1977) FEBS Lett. 83, 348–352.
[3] Dijk, J. and Littlechild, J. (1979) Methods Enzymol. 59, 481–502.
[4] Liljas, A., Eriksson, S., Donner, D. and Kurland, C. G. (1978) FEBS Lett. 88, 300–304.
[5] Singleton, R., jr and Amelunxen, R. E. (1973) Bacteriol. Rev. 37, 320–342.
[6] Horne, J. R. and Erdmann, V. A. (1972) Mol. Gen. Genet. 119, 337–344.
[7] Isoni, K., Isono, S., Stöffler, G., Visentin, L. P., Yaguchi, M. and Matheson, A. T. (1973) Mol. Gen. Genet. 127, 191–195.
[8] Cohlberg, J. A. and Nomura, M. (1976) J. Biol. Chem. 251, 209–221.
[9] Kaltschmidt, E. and Wittmann, H. G. (1970) Anal. Biochem. 36, 401–412.
[10] Laemmli, U. K. and Favre, M. (1973) J. Mol. Biol. 80, 575–599.
[11] Chang, J. Y., Brauer, D. and Wittmann-Liebold, B. (1978) FEBS Lett. 93, 205–214.
[12] Matthews, B. W. (1968) J. Mol. Biol. 33, 491–497.