Proteins of the *Bacillus stearothermophilus* ribosome

Crystallization of protein L6

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Crystals of ribosomal protein L6 from *Bacillus stearothermophilus* suitable for high resolution structural studies have been obtained. Crystals are hexagonal with space group $P6_122$ (or the enantiomorph $P6_522$) and cell dimensions $a = b = 72.7$ Å, $c = 124.9$ Å. A search for heavy atom derivatives is in progress.

Three-dimensional crystal *Ribosomal protein* Bacillus stearothermophilus *X-ray diffraction*

1. INTRODUCTION

Protein L6 is a component of the *Bacillus stearothermophilus* 50 S ribosomal subunit. The protein was originally described as BL10 from its position on 2-D electrophoresis gels [1], but is now labelled L6 to indicate its homology with L6 from *Escherichia coli*. The homology has been established by a comparison of the amino acid sequences of both proteins [2]. The proteins from *B. stearothermophilus* and *E. coli* are 177 and 176 amino acids long, respectively, and have identical residues at 85 (48%) positions in the sequence. It is thus reasonable to assume a closely similar tertiary structure and an equivalent function for the proteins in the ribosomes of the two organisms.

In [3], we reported on the preparation of small crystals of several ribosomal proteins, including L6 from *B. stearothermophilus*. Here, we describe crystals of sufficient quality for a high resolution structural analysis.

2. MATERIALS AND METHODS

2.1. *Protein preparation*

L6 was isolated from *B. stearothermophilus* strain NCA1503 ribosomes as in [4]. The only difference in procedure was the use of 70 S ribosomes as starting material in place of 50 S subunits. Briefly, the ribosomes were washed for 12 h with a solution of 2 M NaCl, 10 mM Hepes, 10 mM $MgCl_2$ (pH 7.5) and the extract, after centrifugation, passed through a CM-Sepharose CL-6B column in 0.07 M NaCl, 10 mM sodium phosphate (pH 7.0). Bound proteins were eluted sequentially with a 0.07–0.7 M gradient of NaCl, L6 appearing at 0.24 M NaCl in the elution. The protein was passed through a Sephadex G-50 column and was then judged to be pure by SDS gel electrophoresis [5]. The protein was concentrated as in [4] and stored at −78°C.

2.2. *Crystallisation*

L6 was crystallised by the hanging-drop vapour diffusion techniques, exactly as in [3].

2.3. *X-ray diffraction*

Diffraction patterns were recorded on a Nonius precession camera, using CuK_α radiation produced by a Seifert stationary anode operating with a fine-focus tube at 40 kV and 30 mA.

3. RESULTS AND DISCUSSION

Crystals of L6 grow reproducibly in 3–5 days from 1.8 M phosphate (NaH_2PO_4/K_2HPO_4) and

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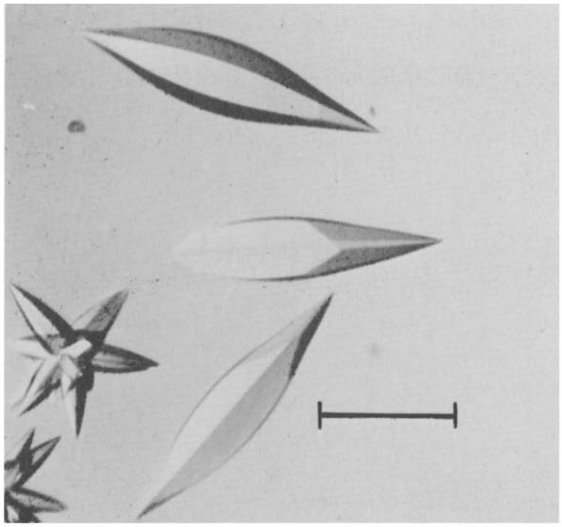

Fig.1. Crystals of protein L6. The bar represents a length of 0.5 mm.

5% dioxane (pH 7.6–8.2) at 8–12 mg protein/ml. Large single crystals grow optimally at pH 7.8, and tend to aggregate at higher pH. The crystals have

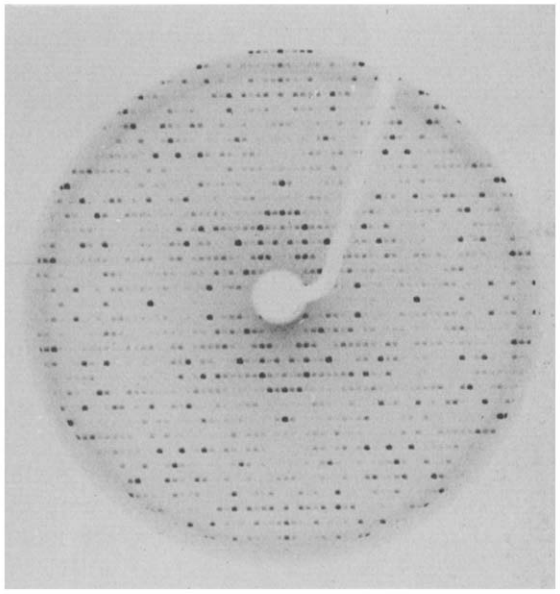

Fig.2. 12° precession photograph of the *h0l* zone of a crystal of protein L6.

Table 1

Properties of the crystals of protein L6

Hexagonal crystals
Space group: $P6_122$ ($P6_522$)
Lattice constants: $a = b = 72.7$ Å $c = 124.9$ Å
Volume of the unit cell: $V = 571692$ Å^3
Volume per unit protein mass: $V_m = 2.4$ Å^3/dalton (M_r 19168 and assuming 1 molecule/asymmetric unit)

a hexagonal, cigar-like, morphology, and grow up to 1 mm long and 0.4 mm wide (fig.1). Crystals can be conveniently stored in a stabilising solution of 2 M NaH_2PO_4/K_2HPO_4 (pH 7.8).

Crystals diffract well, to at least 2.5 Å resolution, and survive continuous irradiation at room temperature for about 100 h. Fig.2 shows a precession photograph of the *h0l* zone. The systematic absences of the 001 reflections for $l \neq 6n$ and the 6-fold symmetry of the *hkn* upper levels identify the space group as $P6_122$ or its enantiomorph $P6_522$. The unit cell dimensions and volume are given in table 1. From the known M_r of L6 (19168 [2]), the assumption of the presence of one molecule in the asymmetric unit gives a value of V_m (volume of the asymmetric unit/M_r-value) of 2.4 Å^3/dalton, which is equal to the mean value found for a range of protein crystals [6].

Diffraction data to better than 3.0 Å resolution have been collected from a single crystal of native protein using an Arndt-Wonacott oscillation camera. A search for heavy-atom derivatives is in progress. Three potential derivatives have been identified; $(NH_4)_2PtCl_4$, $K_2Pt(NO_2)_4$ and $BaPt(CN)_4$, which give significant intensity changes on precession photographs. Data collection and evaluation of these complexes is in hand. We expect to calculate a medium resolution structure of L6 in the near future.

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