CRYSTALLIZATION OF RIBOSOMAL PROTEIN L7/L12 FROM *ESCHERICHIA COLI*

A. LILJAS and C. G. KURLAND

Department of Molecular Biology, Wallenberg Laboratory, Uppsala University, Box 562, S-751 22 Uppsala, Sweden

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1. Introduction

There is one protein in ribosomes from *Escherichia coli* that is present in multiple copies. This is the 50 S protein L7/L12, which has been found in amounts corresponding to between three and four copies per ribosome [1–4]. The protein in solution reflects this oligomeric arrangement by its preference for the dimer state [5–7]. In addition this protein provides an important part of the ribosomal binding site for several factors involved in protein biosynthesis [1]. The fact that the protein is oligomeric in the ribosome suggested to us that it might be more easily crystallized than other ribosomal proteins. This expectation has been realized and we report here a preliminary characterization of a crystal form of L7/L12, which is to our knowledge the first crystal of a purified ribosomal component.

2. Materials and methods

The protein was purified as described previously [7], and fig.1 shows the electrophoretic pattern of the protein on polyacrylamide gels. The purified protein at an approximate concentration of 20 mg/ml in 0.05 M citrate buffer, pH 4.6–6.0, 1.2 M ammonium sulfate was crystallized by the vapour diffusion technique [8] with the protein in X-ray capillaries. After storage for two weeks between 2 and 4°C, crystals of maximum size 1.3 × 0.1 × 0.1 mm appeared (fig.2).

3. Results and discussion

A pecularity of L7/L12 is that it exist in two forms: one (L7) with and the other (L12) without an

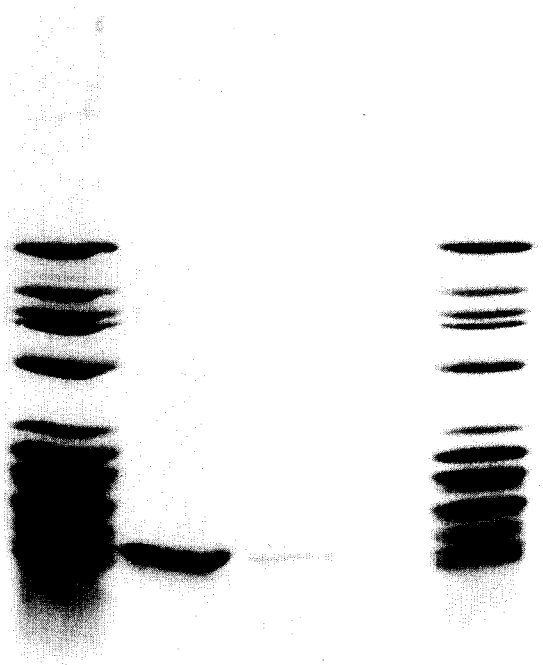

Fig.1. Gel electrophoresis of L7/L12 before crystallization (left) and of crystals that have been dissolved (right). The 50 S total protein pattern is seen on both sides of the samples. The electrophoresis was performed in 15% polyacrylamide gels with SDS buffer. Apparently the present crystals contain both intact L7/L12 and partially degraded protein.

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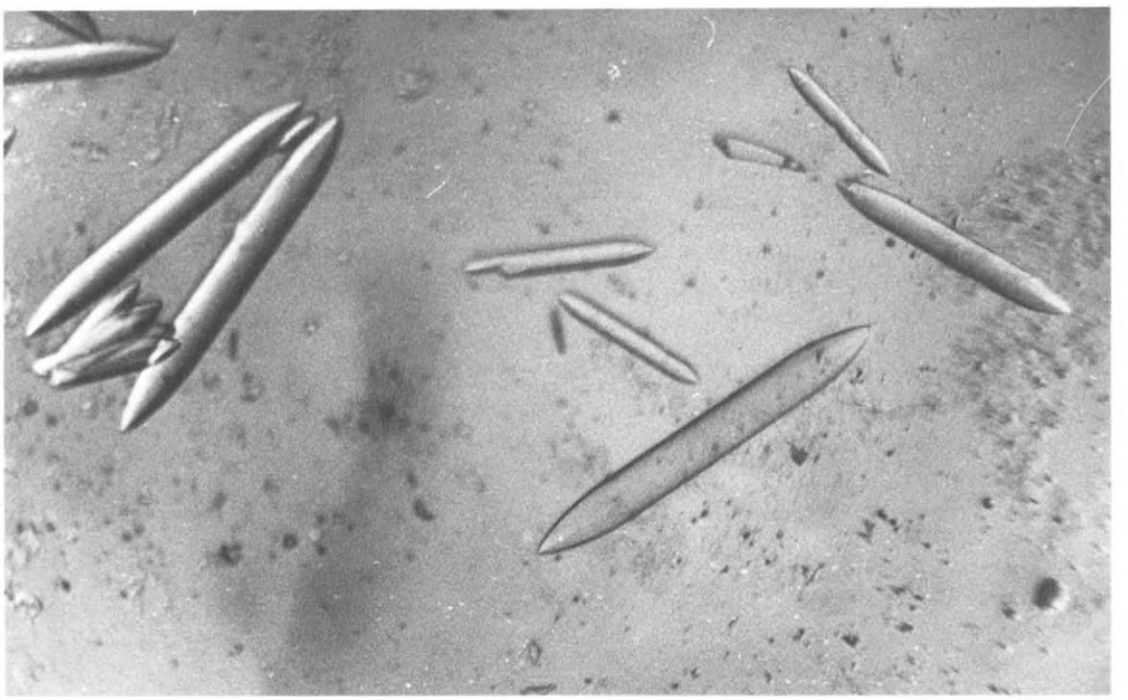

Fig.2. Crystals of L7/L12 at pH 5.4. The six-fold axis is along the longest direction of the crystal. The cross-sectional view of the crystals is hexagonal.

N-terminal acetyl group [9]. We have not accumulated sufficient crystalline material to decide whether the mixture of L7/L12 or L7 or L12 has been crystallized.

Screenless precession and oscillation photographs (fig.3) show that the diffraction pattern has 6/mmm symmetry and 0001 reflexions are absent unless $1 = 6n$. At the present resolution (circa 45 Å) this would imply the space group $P6_122$ (or $P6_522$). The cell dimensions are $a = b = 71$ Å; $c = 138$ Å. The volume of the unit cell is 602 000 Å^3. We assume that we have one dimer (mol. wt 24 400) per asymmetric unit and since there are twelve assymmetric units, we obtain a ratio of the volume of the unit cell to the molecular weight of protein in the cell (V_M) of 2.06 Å^3/dalton. This figure is close to the average V_M for proteins of this size [11].

Since the dimers of L7/L12 in solution have a length of about 180 Å [7] our finding that the c-axis has a length of 138 Å suggests that the dimers may be oriented essentially along the c-axis. The long length of these dimers is unusual when compared to other proteins crystallized previously. That such an asymmetric molecule has been crystallized suggests that other elongated ribosomal proteins may also by crystallized with similar techniques.

One problem is assessing the value of crystallographic data from purified proteins is their relevance to the conformations in the ribosome. However, L7/L12 is as we have indicated above an oligomeric protein in the ribosome and therefore the dimer structure is likely to retain important structural properties of the protein as it is found in the ribosome. In experiments reported elsewhere we have succeeded to recover crosslinked L7/L12 dimers from the ribosome as well as from the isolated protein. Therefore, we are optimistic that the crystallographic elucidation of the structure of the L7/L12 dimer will provide significant information about the corresponding domain within the 50 S ribosomal subunit of *E. coli*.

Acknowledgements

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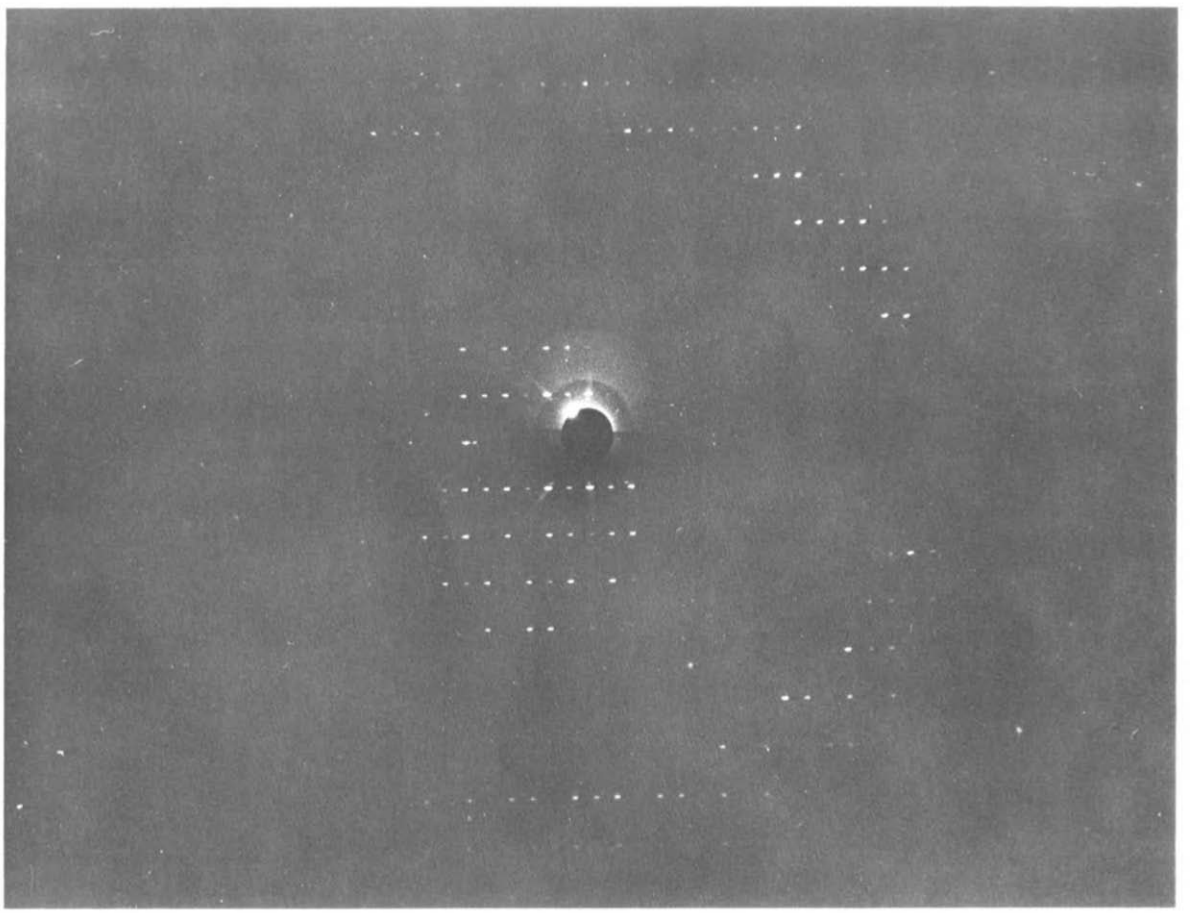

Fig.3. X-ray diffraction pattern from crystals of L7/L12 at pH 5.4. An oscillation photograph (4.5°, F=100 mm) with the c*-axis horizontal. The exposure time was 36 h. The crystals had a lifetime greater than 48 h using double focusing mirrors [10] with CuKα radiation from an Elliot GX20 rotating anode operated at 45 kV and 50 mA with a focal spot of 0.2 × 2.0 mm.

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