ISOLATION AND CRYSTALLIZATION OF STABLE DOMAINS OF THE PROTEIN L7/L12 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

The crystallization of the 50 S ribosomal protein L7/L12 from *E. coli* has been reported [1]. At that time we believed that the crystals contained a mixture of intact L7/L12 as well as fragments of the same protein. The latter were detected by electrophoretic analysis of the crystalline material following exposure to X-radiation. Since then, we have had the opportunity to study the fragments of L7/L12 in more detail.

So far it has not proven possible to obtain crystals of pure, intact L7/L12. However, it has been possible to obtain separately crystals containing either an N-terminal or a C-terminal domain L7/L12. The crystals that we described earlier have the characteristics of the N-terminal domain.

2. Materials and methods

2.1. *Purification of protein and fragments*

The protein was purified as in [2]. The fragments can be obtained in several ways, the easiest being incubation for several weeks under the crystallization conditions in [1]. The material can be dialysed against 10 mM acetate buffer pH 5.7 with 0.01% β-mercaptoethanol and subsequently run on a DEAE-cellulose (Whatman) column equilibrated with the same buffer. The column was eluted with a gradient of 0.0–0.1 M NH_4Cl. The most acidic fraction was eluted by 1.5 M NH_4Cl.

2.2. *Crystallization of a C-terminal fragment*

Supernatant from the crystallization of N-terminal fragments [1] in 50 mM citrate buffer, pH 4.6, with 1.2 M $(NH_4)_2SO_4$ and 10 mM $MgSO_4$ was diluted about 5 times by 2 M $(NH_4)_2SO_4$ in 50 mM citrate buffer, pH 5.4. The crystals were obtained by vapour diffusion [3] against saturated $(NH_4)_2SO_4$ at 4°C in 100 μl open glass vials inside a larger sealed glass container.

2.3. *Gel electrophoresis*

For the analysis of ribosomal proteins and various fragments, we found the polyacrylamide gel containing SDS and urea [4] to be very useful. It was applied with the following modifications. The separation gel was 15% polyacrylamide with a ratio bis:acrylamide of 1:20. The gel buffer was 0.2 M Tris–H_3PO_4, pH 6.8. A 10% spacer gel with a bis:acrylamide ratio of 1:10 was used. The spacer buffer was identical with the reservoir solutions: 40 mM Tris–H_3PO_4, pH 6.3. Urea and SDS concentrations were according to the original recipe.

2.4. *X-Ray studies*

X-Ray precession photographs of the crystals were recorded on an Elliott GX6 rotating anode unit operating at 45 kV and 50 mA with a focal spot of 0.2 × 2.0 mm, and equipped with a graphite monochromator.

3. Results and discussion

The starting material for our experiments is intact L7/L12 which is electrophoretically pure (fig.1). However, after L7/L12 has been kept in buffer in the

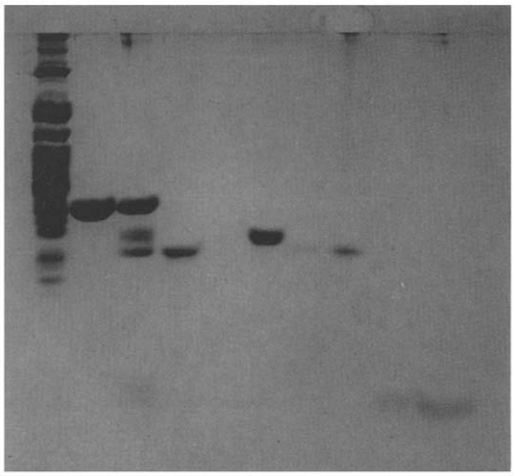

Fig.1. SDS–urea polyacrylamide gel of L7/L12 and fragments of the protein: a, Total 50 S protein; b, Purified L7/L12; c, Partially degraded L7/L12; d, Purified fragment II; e, Purified fragment III; f, Purified fragment II; g, Crystals of the C-terminal fragment; h, Purified fragment IV; i, Crystals of the N-terminal fragment.

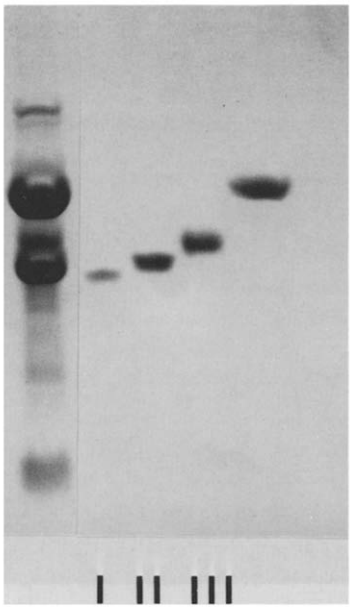

Fig.2b

Fig.2. DEAE chromatography of fragmented L7/L12. (a) Optical density profile. The peaks are numbered from left to right I, II, III, IV. (b) SDS–urea polyacrylamide gel of the peaks I to III. On the left is fragmented L7/L12. On the right is purified L7/L12.

cold for a week or more, it becomes fragmented. The resulting fragments can be purified by chromatography on DEAE-cellulose (fig.2). The electrophoretic characteristics of the fragments are illustrated in fig.1,2 and their respective amino acid compositions are recorded in table 1. The fragments are numbered according to the order of their elution from the DEAE–Sephadex column.

There are three main fragment recovered (II, III, IV). Fragment IV is identical in size with the fragment recovered from the crystals [1]. The amino acid composition of such crystals matches the first 36

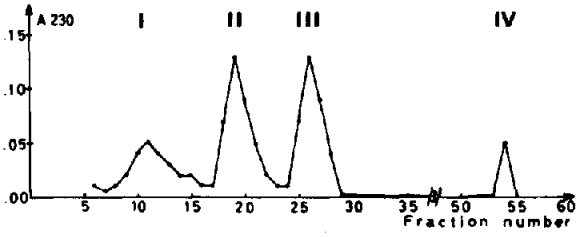

Fig.2a

Table 1
Amino acid composition of L7/L12 fragments compared with the compositions of pieces from the amino acid sequence [5]

	Fragment II		Fragment IV	
	Comp	49–118	Comp	1–36
Lys	9.4	10	2.2	2
Arg	1.1	1	0.0	0
Asp	4.8	5	2.1	2
Thr	2.0	2	1.0	1
Ser	2.4	2	3.9	4
Glu	11.9	11	6.1	5
Pro	1.1	1	0.0	0
Gly	6.0	6	1.2	1
Ala	14.9	15	7.0	7
Val	6.7	7	4.6	5
Met	0.1	0	2.9	3
Ile	1.4	2	2.9	4
Leu	6.9	7	1.1	1
Phe	1.1	1	1.0	1
Sum	69.8	70	36.0	36

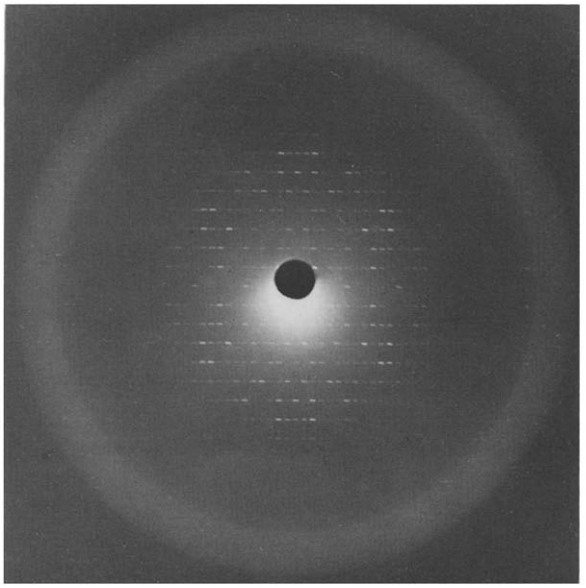

Fig.3. Precession photograph (h01) of the N-terminal fragment. Precession angle 10°, exposure time 43 h.

residues from the N-terminus of L7/L12 (table 1). We refer to it as the N-terminal fragment.

The remaining fragments probably contain overlapping lengths of what appears to be the C-terminus of L7/L12. The alignment of fragment II within the sequence of L7/L12 is given in table 1.

The N-terminal fragment crystallizes first at 1.2 M $(NH_4)_2SO_4$. The mother liquid after a month or more at 4°C contains only fragment II, which can be recovered in crystalline form if the solution is saturated with $(NH_4)_2SO_4$. Its characteristics (table 1) suggest that it contains residues 49–118. We refer to it as the C-terminal fragment.

The previously obtained crystals (fig.3) of the N-terminus belong to space groups $P6_1$ (or $P6_5$). Local 2-fold axis perpendicular to the 6-fold (Bartels, Schwager and A. L., unpublished observations) and occasional twinning of the crystals misled us in the preliminary space group determination. The poor diffraction (about 4 Å) is due to a very low density of the crystals. We used the Ficoll gradient method [6] for its determination and obtained a value of 1.07 g/cm^3. Four N-terminal fragments/asymetric unit would give a calculated density [7] identical with the one observed, and a V_m of 6.9 Å^3/dalton.

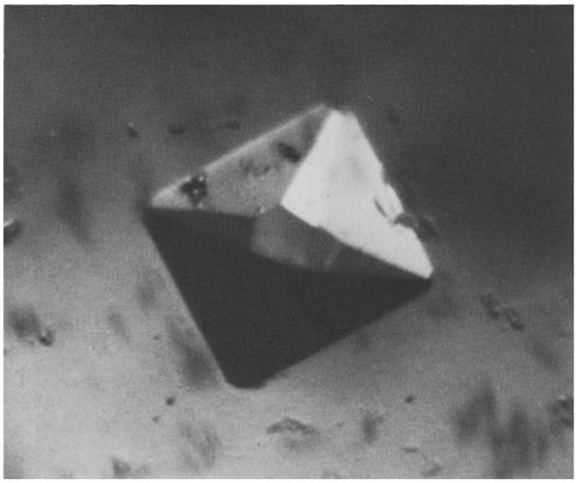

Fig.4. A crystal of the C-terminal fragment of L7/L12.

Crystallographic data are given in table 2. Three dimensional data of the native peptide and of heavy atom derivatives have been collected and are being processed.

The crystals of the C-terminal fragment are tetragonal bipyramids with a longest dimension of 0.3 mm (fig.4). They show no birefringence when viewed along the tetragonal axis. Precession photographs (fig.5)

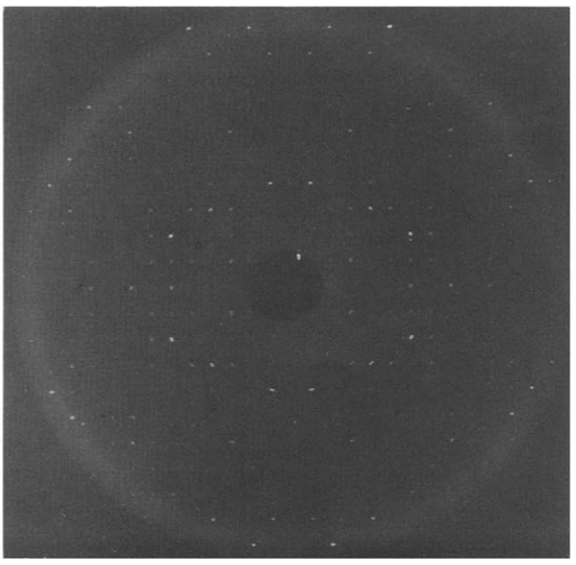

Fig.5. Precession photograph (h01) of the C-terminal fragment. Precession angle 11°, exposure time 5 h.

Table 2
Crystallographic data

Fragment	Probable M_W	Space group	Asym. unit	Cell dimensions (Å) a	b	c	Vol. unit cell (Å^3)	Density (g/cm^3)	Molecules/ asym. unit	V_m (Å^3/dalton)	Approx. diffrac. power (Å)
N-terminal	3750	$P6_1$ ($P6_5$)	6	72.2	72.2	137.4	620 000	1.07	4	6.9	4
C-terminal	7350	$P4_12_12$ ($P4_32_12$)	8	54.2	54.2	41.6	122 000	–	1	2.08	2.5

indicate that the crystals belong to the space group $P4_1\ 2_1\ 2$ or its enantiomorph $P4_3\ 2_1\ 2$. The diffraction pattern on oscillation photographs extends to about 2.5 Å and the crystals last in the X-ray beam for more than 100 h. The asymetric unit very likely contains one C-terminal fragment leading to a V_m of 2.1 $Å^3$/dalton which is close to the average for proteins of this size.

The spontaneous degradation of L7/L12 in the region of residues 35–50 with no obvious further breakdown of the molecule suggests that this region is flexible and accessible for proteolysis. The remaining parts of the molecule behave as stable protein domains and can be crystallized.

The high quality of the crystals of the C-terminal fragment certainly indicates that this domain is structurally very stable and might be directly related to the structure in the ribosome. The low density of the crystals of the N-terminus probably is due to a very elongated shape of this fragment which limits the density of its packing.

We believe that these crystalline fragments represent two stable domains in L7/L12 which we have fortuitously obtained in our experiments. Similarly, one possible way to crystallize other ribosomal components and their complexes might be to begin by degrading them to structurally stable domains, and analyzing the structures of these.

Acknowledgements

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References

[1] Liljas, A. and Kurland, C. G. (1976) FEBS Lett. 71, 130–132.
[2] Liljas, A., Eriksson, S. and Sköld, S. E. (1978) submitted.
[3] Hampel, A., Labanauskas, M., Connors, P. G., Kirkegaard, L., Raj Bhandary, V. L., Siegler, P. B. and Bock, R. M. (1968) Science 162, 1384–1387.
[4] Swank, R. T. and Munkres, K. D. (1971) Anal. Biochem. 39, 462–477.
[5] Terhorst, C., Möller, W., Laursen, R. and Wittmann-Liebold, B. (1973) Eur. J. Biochem. 34, 138–152.
[6] Westbrook, E. M. (1976) J. Mol. Biol. 103, 659–664.
[7] Matthews, B. W. (1974) J. Mol. Biol. 82, 513–526.