SMALL-ANGLE X-RAY SCATTERING STUDY OF THE PROTEIN COMPLEX OF L7/L12 AND L10 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

The protein L12 from *Escherichia coli* ribosomes and its α-aminoacetylated form L7 play a key role in protein biosynthesis [1]: they are necessary for the binding of most soluble factors, such as the initiation factor 2 (IF-2) [2], the elongation factor Tu (EF-Tu), the elongation factor G (EF-G) [3] and the release factors 1 and 2 (RF-1, RF-2) [4] to the *E. coli* ribosomal surface. The protein L7/L12 is probably present in four copies per ribosome [5–7], and previous studies of various L7/L12 preparations indicate presence of dimers in solution [8–10]. Using the small-angle X-ray scattering method, we recently reported that highly elongated dimers are the only species of L7/L12 that can be detected in solutions of the native protein [11].

In this study we have extended the previous small-angle X-ray scattering investigation on L7/L12 [11] to include the complex formed between L7/L12 and L10. This L7/L12-L10 complex is normally extracted from ribosomes as a single species, which does not dissociate under the conditions of two-dimensional electrophoresis; it migrates to the position denoted L8 [12]. The complex very probably exists within the ribosome. This is indicated by the fact that L7/L12 does not rebind properly to 50 S particles when L6 and L10 have been removed or blocked by specific antibodies [13–16]. The present study deals with the molecular weight and volume of the L7/L12–L10 complex as well as its size and shape in solution. The complex appears to consist of two L7/L12 dimers and one L10 molecule; this agrees with stoichiometric studies on ribosomes [5–7]. The shape of the complex appears to be a flattened ellipsoid having a thickness of only 12 Å.

2. Experimental

The complex of L7/L12 and L10 comigrates with the L8 protein [12] according to the nomenclature of Kaltschmidt and Wittmann [17,18]; it was prepared from 50 S subunits of *E. coli* MRE 600 ribosomes as described elsewhere [19]. The L7/L12–L10 complex moves as a single symmetrical peak in chromatography on both Sephadex G-100 and DEAE-Cellulose. The complex was split into its components by DEAE chromatography in acetate buffer and 6.0 M urea [9], and the proteins L7, L12 and L10 were identified by electrophoresis in SDS polyacrylamide slab-gels and by two-dimensional gel electrophoresis [17,18]. Before the protein samples were subjected to the X-ray beam, they were dialysed to equilibrium against a buffer of pH 7.60 consisting of 0.02 M Tris–HCl, 0.36 M NaCl and 6 mM mercaptoethanol. The final protein concentration of the solutions used for recording the X-ray data was determined by nitrogen and carbon analyses [20].

The X-ray small-angle scattering data were

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recorded with a camera developed by Kratky and Skala [21]. The scattering angle was set by an on-line Hewlett-Packard computer 2100S, which also received and recorded the intensity data (Wingren, B. G., Sjöberg, B. and Österberg, R., unpublished data). Monochromatization was achieved with a nickel β-filter and a pulse height discriminator in conjunction with a proportional counter.

All measurements were made at 21°C. The absolute scattered intensities were obtained using a standard Lupolen sample [22]; the Lupolen sample had been previously calibrated at the Graz Institut für Physikalische Chemie.

3. Results

The X-ray scattering curve recorded for the L7/L12–L10 complex is shown in fig.1; the curve is slit-corrected [23] and extrapolated to zero concentration from data recorded at 10.6, 7.1 and 3.6 mg/ml of the complex. The gyration radius was determined to be 45 ± 2 Å. When this scattering curve was compared with theoretical curves calculated for various different triaxial bodies of uniform electron density, it was found that the data could be explained by the curve calculated for an ellipsoid having the semiaxes A = 90.0, B = 45.0 and C = 6 Å. Such an ellipsoid yields a volume, V, of 101 800 Å^3 which is in agreement with that calculated from the data via Porod's invariant, cf. [24], V = 97 000 Å. The partial specific volume, $\bar{v}$, was analysed by using a densitometer [25] and the result was $\bar{v}$ = 0.726 cm^3/g. Using this value as well as the formula described by Kratky [24], the molecular weight was determined to be 60 000. Within the experimental errors, this value agrees with the molecular weight of two L7/L12 dimers [26] and one L10 molecule [27], 66 200.

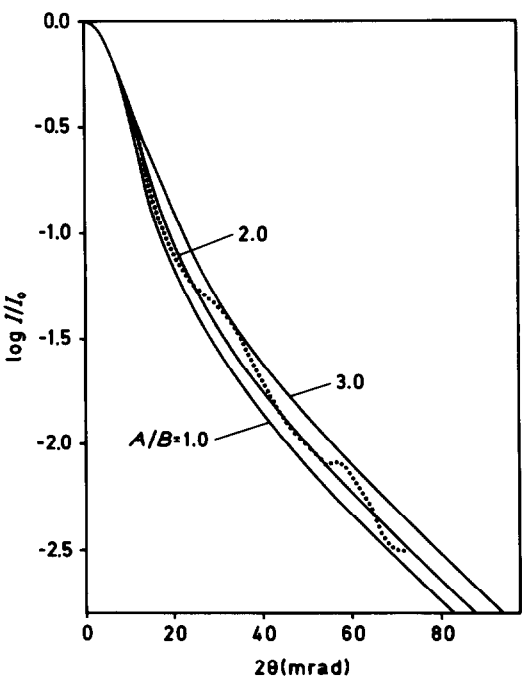

Fig.1. Experimentally observed X-ray scattering curve, normalized and extrapolated to zero concentration and after elimination of the collimation effect (dotted curve) compared with the theoretical scattering curves calculated for ellipsoids with the gyration radius of 45 Å, the short semiaxis, C = 6 Å, and the A/B axial ratio of 1.0, 2.0 and 3.0; 2θ = scattering angle.

4. Discussion

Our previous small-angle X-ray scattering study on L7/L12 indicated that, in the solution, L7/L12 exists in the form of dimers; no monomers or higher aggregates could be detected [11]. The ellipsoidal semiaxes of the L7/L12 dimers were found to be A = 90, B = 16 and C = 6 Å [11]. Thus, the model of the present complex, which has the semiaxes A = 90, B = 45 and C = 6 Å, seems to have two axes in common with those of the L7/L12 dimer; only the B axis is different, 45 Å, in the complex compared to 16 Å in L7/L12. These data, as well as the molecular weight and volume of the complex are consistent with results showing that the complex is made up of 4 copies of L7/L12 and one copy of L10 [28].

The L7/L12 dimers and the L7/L12–L10 complex both have the shapes of flattened ellipsoids with one dimension being no more than 12 Å. This dimension is fairly close to the average diameter of an α-helix, which is about 11 Å. It is interesting to note that these proteins appear to be mainly helical according to circular dichroism measurements [29,30] and secondary structure prediction methods (Argos and Liljas, unpublished results). Thus, it seems probable that the complex is formed by one layer

of helices. In an attempt to analyse the secondary structure of the complex we are presently combining the small-angle X-ray scattering data and the results obtained by prediction methods for the individual proteins. As far as L7/L12 is concerned, the tertiary structure is also being analysed using X-ray diffraction data obtained from L7/L12 single crystals [31].

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