SMALL-ANGLE X-RAY SCATTERING AND CROSSLINKING STUDY OF THE PROTEINS L7/L12 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

In *E. coli* ribosomes there is only one protein that occurs in more than one copy [1]. This is the protein L12 and its α-aminoacetylated form, L7 [2]. The total sum of these two components appears to be at least three and possibly four copies per ribosome [1,3,4] and the fraction of L12 found varies from 25% to 85% during the growth cycle of the bacteria [5]. The L7/L12 molecules play an important role in the ribosome, since they are involved in the binding of a number of soluble factors involved in the protein biosynthesis (see [6] for a review). Whether the four L7/L12 molecules bind to the ribosome as a tetramer, as two dimers, or as four monomers is not known. Ultracentrifugation of L7/L12 indicates that this protein molecule exists in the form of dimers in vitro [7–9]; however, under certain conditions, tetramers also occur [7]. Furthermore, gel filtration studies of L7/L12 reveal a surprisingly small elution volume, which may indicate that L7/L12 is in the form of tetramers, or alternatively, in the form of highly hydrated or elongated dimers [10]. In order to explore this ambiguity, and in order to characterize the conformation of the L7/L12 ribosomal protein in solution, we have studied L7/L12 using low angle X-ray scattering and chemical crosslinking methods; the protein was prepared by a gentle procedure [10]. The results indicate that L7/L12 exists in vitro as highly elongated dimers.

2. Materials and methods

2.1. *Preparation of the L7/L12 proteins*

50S subunits of ribosomes from *E. coli* MRE 600 were prepared according to Hardy et al. [11]. Protein was extracted from 50S subunits at 0°C according to Hamel et al. [12]. The supernatant containing L7/L12 was dialysed against a buffer consisting of 20 mM Tris-HCl pH 7.6, 0.36 M NaCl, and 1.5 mM β-mercaptoethanol and then concentrated. The protein sample intended for low angle X-ray scattering experiments was chromatographed on a Sephadex G-100 column. After concentration, the major fraction [10] was dialysed against fresh buffer and then stored frozen at −80°C. The protein concentration was determined via nitrogen and carbon analyses [13].

The proteins L7 and L12 were separated from each other by DEAE-cellulose chromatography of the supernatant from the extraction procedure, using 6 M urea in acetate buffer as eluent [8]. The samples were dialysed against the chromatography buffer without any precipitation in acetone.

Proteins were identified and checked for purity by SDS polyacrylamide slab gels and two-dimensional gel electrophoresis [14,15].

2.2. *X-ray measurements*

The X-ray small-angle scattering data were recorded with a camera developed by Kratky and

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Skala [16]. 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 [17]; the Lupolen sample had been previously calibrated at the Graz Institut für Physikalische Chemie.

2.3. Crosslinking experiments

The crosslinking experiments were performed according to Lutter et al. [18] using 10 mM dimethyl-suberimidate (DMS). The protein concentration was 0.1–0.2 mg/ml in a buffer, pH 7.5–8.5, consisting of 10 mM triethanolamine-HCl (or 40 mM triethanol-amine-HCl, pH 9) 0.35 M KCl, and 6 mM β-mercapto-ethanol. The crosslinking reactions were performed at 25° for about an hour.

3. Results

The small-angle X-ray scattering data were recorded for concentrations (c) equal to 11.5, 7.87, and 3.9 mg/ml of the L7/L12 protein. When the normalized intensity ($\widetilde{I}/c$) was plotted against the scattering angle, no significant concentration dependence was observed (fig.1). After slit correction (desmearing) of the data [19], the radius of gyration was determined to be 41.0 ± 2.0 Å. When the X-ray scattering data were compared with theoretical curves, calculated for different triaxial bodies, the experimental data rather than their desmeared counterparts were used; the set of theoretical curves were smeared using a computer program [19]. Fig.1 illustrates the comparison between the experimental data and three different theoretical curves; the best fit is obtained for an ellipsoid having the semiaxes $A = 90$, $B = 16$, $C = 6$ Å. It should be noted that theoretical curves calculated for other triaxial bodies, such as elliptic cylinders, did not give as good agreement with the experimental data as that calculated for the above mentioned ellipsoid.

The partial specific volume, $\bar{v}$ of the L7/L12 protein was analysed by using a densitometer [20]; the results was $\bar{v} = 0.729$ cm^3/g. Using this value and the formula described by Kratky [21], the mol. wt. of L7/L12 was calculated to be 23 400. Since this
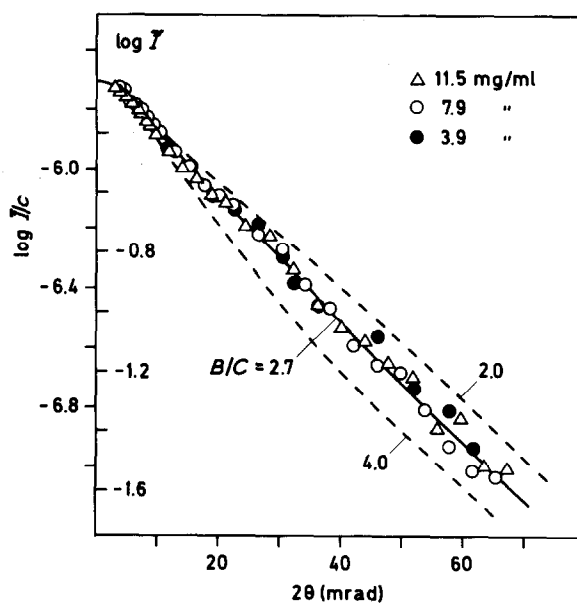

Fig.1. Experimental X-ray scattering data for the L7/L12 protein compared with the theoretical scattering curves calculated for three ellipsoids, with a gyration radius of 41.0 Å and the axial ratio B/C as indicated; the A- and C-semiaxes are equal to 90 and 6 Å, respectively. The logarithm of absolute intensity, log $\widetilde{I}/c$ (uncorrected for slit effect), is plotted against the scattering angle, 2θ (in milliradians). Please note that the theoretical curves, log $\widetilde{I}$ versus 2θ, are smeared; they are normalized so that their intensity (not smeared) at zero angle, $I(0)$, is equal to 1.0. The upper dashed curve is shifted downwards (0.03 units) and the lower dashed curve is shifted upwards (0.04 units).

value is very close to twice the mol. wt. of L7/L12, as calculated from the primary structure, 24 400 [22], these small-angle X-ray scattering data indicate that, for this preparation of L7/L12, the protein molecules exist in solution as dimers.

The volume of L7/L12 was calculated via Porod's invariant, cf. [21]; the result was 42 000 Å^3. Although this volume should be considered only as a very first approximation, it does compare with the volume of the ellipsoid model, which is 36 000 Å^3. These data yield a water content of 0.28 g H$_2$O per g protein (dry wt.).

Dimethylsuberimidate (DMS) is a bifunctional reagent that can form a covalently bound bridge between two lysyl residues. As a result, protein molecules associated into an oligomer can be covalently
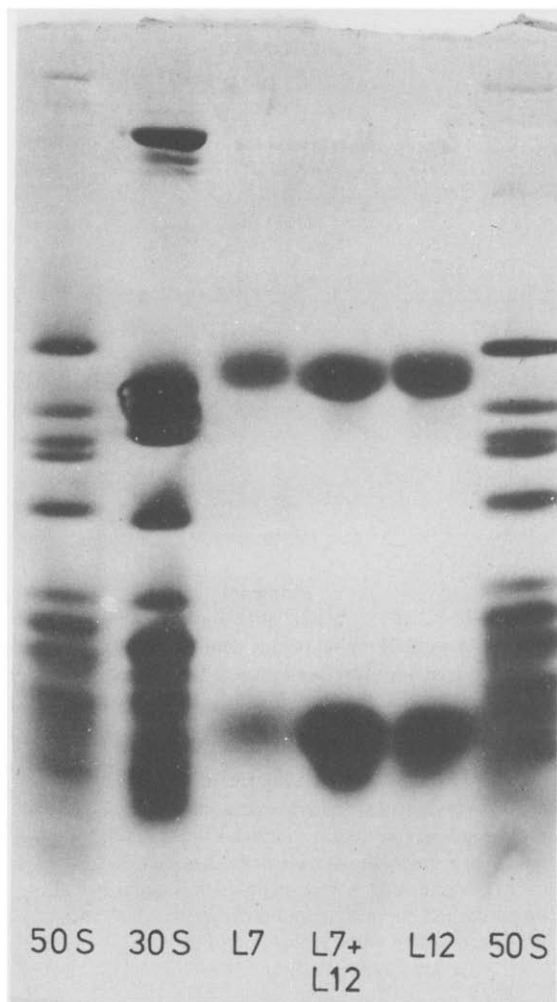
Fig.2. Gel electrophoresis of DMS crosslinked L7, L7 + L12, and L12 obtained from DEAE-cellulose columns [8]. The crosslinked proteins are surrounded by the other 50S and 30S proteins. The upper band migrates like a protein with a mol. wt. that is twice as large as that of L7/L12.

linked to each other and this can give information about the structure of the oligomer. After reaction of DMS with L7/L12, either with that obtained from Sephadex G-100 columns or with that resolved in discrete species of L7 and L12 from a DEAE column, aggregates having a mol. wt. corresponding to dimers were observed (fig.2). Since the yield of crosslinked dimers was very high, and no larger aggregates were observed, these crosslinking data support the results

obtained by the small-angle X-ray scattering method that the L7/L12 molecules exist in solution in the form of dimers.

4. Discussion

The results described in the previous section indicate that the ribosomal protein L7/L12 exists in vitro as a dimer. The methods used, X-ray scattering and chemical crosslinking, have one advantage over ultracentrifugation; they do not apply a strong force to the sample. Nevertheless, no species larger than dimers were found, which is in good agreement with previous results [7–9].

The X-ray scattering data indicate that the shape of the L7/L12 dimer is a highly elongated ellipsoid with a length of 180 Å and an axial ratio of 15:2.7:1. Similar, but less elongated, shapes have recently been reported for the L18 and L25 proteins of the E. coli ribosomes [23]. These latter proteins were prepared by a method which is markedly different from that used for L7/L12. Thus, the L7/L12 protein was prepared via a gentle technique which is expected to preserve the native structure; the L18 and L25 proteins were prepared using 6 M urea. In spite of these differences in preparation, L7/L12 is found to be more elongated than the other two proteins, and this may be used as an indirect argument that the native structures were well preserved in the study of L18 and L25 [23]. It should be noted that elongated ribosomal proteins have also been indicated from other studies on ribosome proteins using immune electron microscopy [24,25] and neutron scattering [26].

Electron microscopy studies using antibody markers indicate a continuous and extended region of L7/L12 on the ribosome [25], which suggests that the four L7/L12 proteins are close together. However, whether L7/L12 really exists as a tetramer in vivo is not known, but an indication for the existence of such a tetramer is that a minor fraction of L7/L12 elutes with the void volume on the Sephadex G-100 column [10]. However, as follows from the present data, the dimer is a very stable form of the L7/L12 protein, and it appears likely that the dimeric state, at least partly, reflects the situation within the ribosome.

Acknowledgements

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