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STOICHIOMETRY AND PROPERTIES OF THE COMPLEX BETWEEN RIBOSOMAL PROTEINS L7 AND L10 IN SOLUTION

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1. Introduction

It is known that the binding of proteins L7/L12 with the 50 S subparticle of *Escherichia coli* ribosomes depends on protein L10 [1]; antibodies against protein L10 block the binding of these proteins [2]. It has also been shown that proteins L7/L12 form a complex with protein L10 in solution [3,4] and small-angle X-ray scattering by the complex has been studied [5].

This paper presents the results of sedimentation, calorimetric, optical and some other studies of the complex between proteins L7 and L10 in solution. The L7·L10 complex was shown to have a stoichiometry of 4 : 1. Complex formation resulted in stabilization of both secondary and tertiary structures of protein L10.

2. Materials and methods

Ribosomal proteins L7 and L12 were prepared by extraction from 50 S subparticles with 50% ethanol [6] and then separated on DEAE-cellulose [7]. Protein L10 was prepared by the subsequent extraction of the same particles with 2 M LiCl and purified by CM-cellulose chromatography [8]. Purity of the isolated proteins was checked by amino acid analysis, sodium dodecyl sulphate (SDS)–gel electrophoresis [9] and two-dimensional electrophoresis [10].

The complex was obtained in the following way: L10 and an excess of L7 were dissolved in 2 M guanidinium chloride and the complex thus formed was separated from excess protein on a G-100 Sephadex column equilibrated with 10 mM ammonium acetate buffer, pH 7.4.

Circular dichroism (CD) spectra were measured in a J-41A spectropolarimeter (JASCO, Japan). The instrument was calibrated with d-10 camphoric acid according to [11]. The results are expressed in units of molar ellipticity per amino acid residue $[\theta]$. The average weight of the residue was taken as 102 for L7, 107.5 for L10 and 103.4 for the L7·L10 complex. The average values of measurements in 0.093 mm 0.194 mm and 0.212 mm cells in the concentration range 0.3–1.66 mg/ml are presented.

Calorimetric measurements were done on a DASM-1M microcalorimeter (USSR) [12].

Equilibrium sedimentation was done in an MOM ultracentrifuge (Hungary) according to Yphantis [13] and high-speed sedimentation in a Beckman E centrifuge in a double-sector synthetic boundary cell. The sedimentation coefficient was measured at 42 000 rev./min and the diffusion coefficient at 12 500 rev./min.

The following buffers were used: (A) 20 mM phosphate, pH 6.8, with 0.15 M KCl; (B) 20 mM phosphate, pH 6.8, with 0.3 M KCl and 1 mM β-mercaptoethanol; (C) 20 mM glycine–KOH, pH 8.5, with 0.3 M KCl and 8 mM $MgCl_2$. Protein concentrations were determined from the nitrogen content measured in a Hitachi O-26 C, H, N-analyzer (Japan).

3. Results and discussion

3.1. *Stoichiometry of the L7·L10 complex*

Proteins L7 and L10 strongly differ in their con-

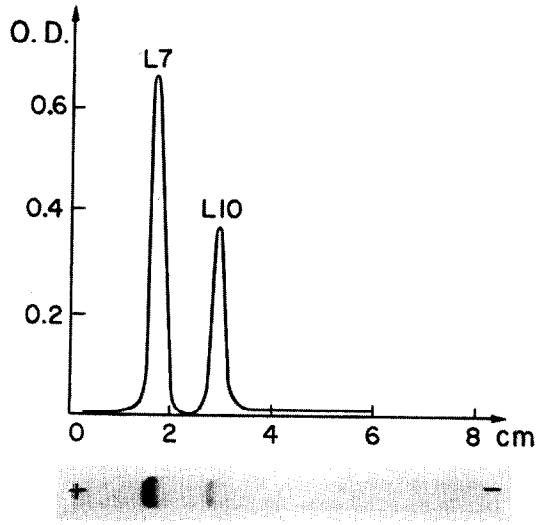

Fig.1. SDS electrophoresis of the L7·L10 complex: (a) gel scanning; (b) gel photo.

tents of tyrosine (3 residues in L10, and none in L7) and arginine (1 residue in L7 and 13 residues in L10) [14,15]. To determine the stoichiometry we used the ratio of Phe/Tyr and Lys/Arg in the hydrolyzate of the L7·L10 complex. We found Phe/Tyr to be 5.0 and Lys/Arg to be 3.8; calculated values for the 4 mol L7 to 1 mol L10 are 4.7 and 3.8, respectively.

Scanning of SDS electrophoresis gels showed the weight ratio of L7 to L10 in the complex to be

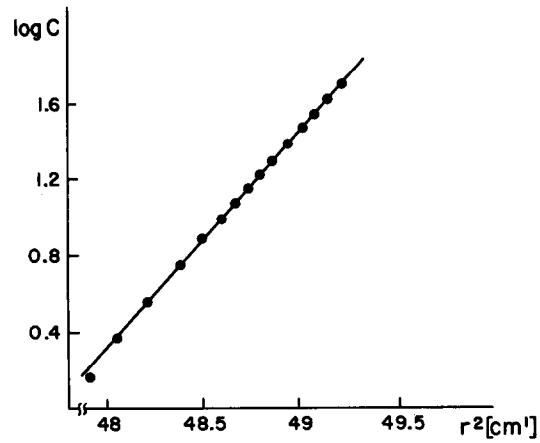

Fig.2. Dependence of log C on r^2 of the L7·L10 complex. c_0 = 0.35 mg/ml, n = 26 540 rev./min.

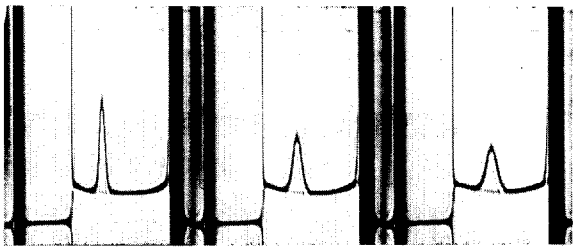

Fig.3. Schlieren sedimentation diagrams of L7 and L10 mixture (concentration ratio 2.7). The photos were taken 8 min, 24 min and 40 min after reaching the speed.

between 2.5 and 3 (fig.1). The calculated weight ratio for the 4:1 complex is 2.74 (M_r of L7 is 12 200 [14] and of L10 is 17 800 [15]).

Equilibrium sedimentation analysis gave molecular weight values for the L7·L10 complex from 65 000 to 71 000 in different experiments ($\bar{v}$ = 0.757, as calculated from the amino acid composition [16]) (fig.2). Sedimentation (fig.3) and diffusion coefficient values for the complex were 3.5 and 5.4, respectively. The molecular weight calculated from s and D was 67 700.

Thus, from all the above data it follows that the stoichiometry of the complex is 4 mol L7 to 1 mol of L10.

3.2. *Secondary structure*

Figure 4 shows the CD spectra of L7 and L10. It is seen that both the proteins are characterized by high helical content (~50–60%). At the same time the shape of their CD curves differ. The greatest differences being in the region of the negative maximum (221 nm) and the positive maximum (191–193 nm). In addition, for L10 the point of transition of the curve through the zero line is shifted towards shorter wavelengths. Such differences in CD curves can be explained by differences in lengths and/or in deformation of α-helices within these proteins.

Figure 5 shows the experimental CD spectrum of the L7·L10 complex, as compared with the curve obtained by summation of the L7 and L10 spectra, assuming additivity of their optical properties. The ellipticity value for the complex proved to be higher than that derived from the additive curve. The corresponding difference curve (fig.5, curve 3) has the typical shape for a polypeptide chain in the α-helical

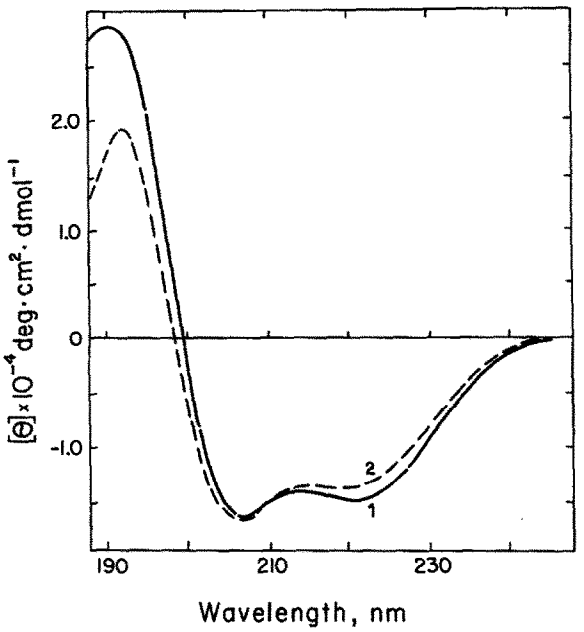

Fig.4. CD spectra of L7 (curve 1) and L10 (curve 2) in buffer solutions A and B.

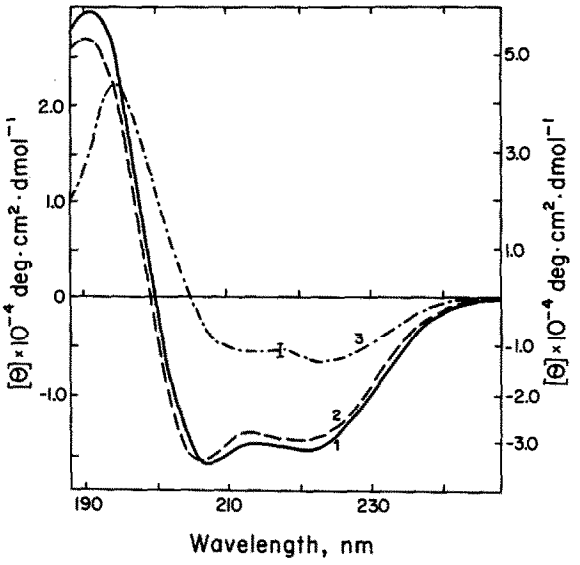

Fig.5. CD spectra of L7·L10 complex obtained by mixing equal volumes of solutions of L7 (in buffer A) and L10 (in buffer B) to a weight concentration ratio of 2.7. 1, The spectrum of the complex isolated on a column with G-100 (not shown, see section 2); 2, the additive curve of protein L7 and L10 CD spectra; 3, their difference curve (scale expanded 5 times). The vertical bar on curve 3 shows standard deviation.

conformation. The difference found in the spectra suggests that the number of amino acid residues in the helical conformation is somewhat more (by 20–25 residues) in the complex than their sum in the free proteins.

At the present stage of the studies it is difficult to say definitely which protein is changed as a result of complex formation. However, taking into consideration the difference in the CD spectra of free proteins (fig.4), it can be assumed that the changes pertain more to protein L10 and are the result of a decrease of deformation and/or some increase of length of the helical regions.

3.3. *Stability of the tertiary structure*

The difference between the properties of the L7·L10 complex and its constituent proteins is substantiated by the results of heat denaturation

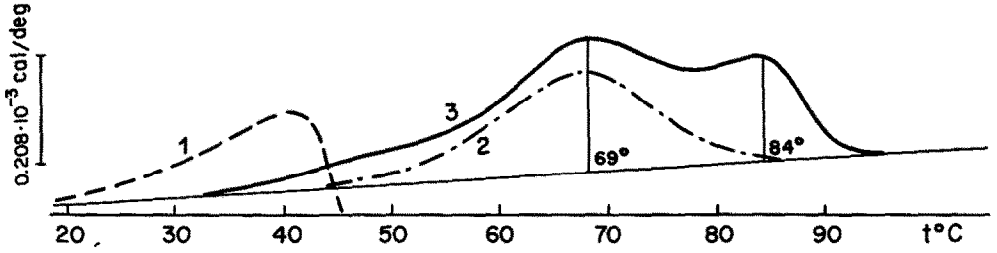

Fig.6. Melting curves of L10 (curve 1, c = 1 mg/ml in buffer B), L7 (curve 2, c = 1.7 mg/ml in buffer C) and of the L7·L10 complex (curve 3, c = 1.4 mg/ml in buffer C). Vertical bar corresponds to 0.208 × 10^{-3} cal/deg.

studies. Microcalorimetric measurements have shown that L10 has a comparatively low thermal stability in the free state; at 42°C aggregation takes place with the formation of precipitate (fig.6, curve 1). The temperature of denaturation transition for L7 is 68–70°C (fig.6, curve 2; see also [17,18]). The melting profile of the L7·L10 complex has been found not to be the sum of the two melting profiles: it lacks the low temperature transition. At the same time it is complicated, extended and has two distinct maxima at 69°C and 84°C.

The heat of denaturation calculated from the area under the curve of the L7·L10 complex is 373 kcal/mol. The enthalpy of denaturation of the L7 dimer is 136 kcal/mol [18]. Unfortunately, it is impossible to determine the exact enthalpy value for protein L10 due to its aggregation. The minimal enthalpy value for protein L10 can be calculated assuming that aggregation proceeds beyond the transition point; it is about 55 kcal/mol. The maximal enthalpy value for protein L10 within the complex (the value can include the energy of interaction between the proteins), taking into account the stoichiometry of the complex, is equal to the difference between the heat denaturation of the complex and the two L7 dimers; it is 101 kcal/mol.

The main qualitative features of the melting profile of the L7·L10 complex, in comparison with those of the free proteins, are that the peak corresponding to the melting of free L10 is absent in the melting curve of the complex while a new high-temperature peak is present (fig.6). This suggests that a stabilization of L10 takes place within the complex. The stabilization of L10 in the complex is consistent with the changes in the secondary structure revealed by CD spectra measurements (fig.4).

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