# ROLE OF DIFFERENT REGIONS OF RIBOSOMAL PROTEINS L7 AND L10 IN THEIR COMPLEX FORMATION AND IN THE INTERACTION WITH THE RIBOSOMAL 50 S SUBUNIT

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## 1. Introduction

It has been shown recently that ribosomal proteins L7 and L10 form a stable complex with each other in solution [1,2]. A tertiary and quaternary structure has been proposed for the protein L7 [3]. The stoichiometry of the complex between the proteins L7 and L10 have been determined and some of its physical characteristics studied [4,5].

This paper presents the results of further physicochemical studies of the $(L7)_4 \cdot L10$ complex. The conclusions are drawn that:

(1) The N-terminal regions of the protein L7 which are responsible for its dimerization also participate in the binding with the protein L10;
(2) The C-terminal sequence 71–165 of the protein L10 binds all the 4 copies of the protein L7;
(3) Cys-70 of the protein L10 is easily accessible to modifying agents both in the free protein and in the $(L7)_4 \cdot L10$ complex;
(4) The N-terminal sequence 1–69 of the protein L10 participates in the binding of the $(L7)_4 \cdot L10$ complex with the ribosome.

## 2. Materials and methods

Isolation and purification of the proteins L7 and L10, formation of the $(L7)_4 \cdot L10$ complex and their equilibrium sedimentation were carried out as in [4]. The N-terminal fragment 1–69 (NF) and the C-terminal fragment 70–165 (CF) were obtained by cleavage of the protein L10 at the cysteine residue [6] and separated on a Sephadex G-75 column (1.5 × 160 cm) in phosphate buffer (see below) with 6 M urea. Purity of the fragments was tested by amino acid analysis and SDS–electrophoresis in 12.5% polyacrylamide gel [7]. The complex of the protein L10 fragment 70–165 with the protein L7 was obtained in the same way as with the intact protein L10. The stoichiometry of the complex was determined as in [4].

NMR spectra were recorded on a Bruker WH-360 instrument (BRD) in a 5 mm ampoule at 2–3 mg protein/ml.

Fluorescence spectra were measured on the instrument made in the Institute of Protein Research and were not corrected.

Modification of the thiol group by iodoacetamide or iodoacetylaminorhodamine B was done as in [8] and titration by dithiobisnitrobenzoic acid as in [9].

The factor-dependent poly(U) translation was carried out as in [10]. The EF-G-dependent GTP hydrolysis was determined by following the release of $^{32}P\,P_i$ according to [11]. The factor-dependent binding of [$^3$H]guanylylmethylenediphosphonate (GMPPCP) was measured according to [12].

Proteins L7, L12 and L10 were extracted from ribosomal 50 S subunits by 50% ethanol with 0.5 M $NH_4Cl$ at 37°C [13]. The extraction was checked by two-dimensional gel-electrophoresis [14]. Reconstitution of 50 S subunits was carried out by incubation of the mixture of the subunits deprived of the protein L7, L12 and L10 with different derivatives of the L7·L10 complex for 15 min at 37°C. 50 S subunits with the L7·L10 complex modified by iodoacetylaminorhodamine B were pelleted in the Ti 50 rotor at 50 000 rev./min for 6 h, using the subunit : complex molar ratio of 1:1. The pellet of ribosomal subunits was suspended in the appropriate



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buffer and used for recording fluorescence spectra and for functional tests.

The following buffers were used: (A) 10 mM Tris–HCl (pH 7.4–7.6), 8 mM $MgCl_2$, 300 mM KCl; (B) 20 mM phosphate (pH 6.6), 300 mM KCl in $D_2O$ or $H_2O$; (C) 10 mM Tris–HCl (pH 7.4–7.6), 10 mM $MgCl_2$, 100 mM KCl, 1 mM dithiothreitol.

## 3. Results and discussion

### 3.1. *N-terminal regions of the protein L7 are responsible for binding with the protein L10*

Earlier the protein L7 with oxidized methionine residues in the N-terminal sequence has been shown to lose the ability for dimerization [15]. This result was later confirmed [16]. Further, it has been demonstrated that the oxidized protein L7 does not form a complex with the protein L10 [17]. Thus, the N-terminal sequence seems to be important both for dimerization of the protein L7 and for the complex formation with the protein L10. This conclusion is corroborated now by the NMR spectroscopy data on the L7 dimer and its complex with the protein L10 as shown below.

In the PMR spectrum of the protein L7 in a dimer form, two separate multiplet signals at 7.31 and 7.15 ppm are revealed (fig.1A). Earlier studies allowed us to attribute the resonance signal in the higher field (7.15 ppm) to the residue Phe 54 and the signal at 7.31 ppm to the residue Phe 30 [18]. In the spectrum of the L7·L10 complex the resonance line of Phe 30 is shifted to the higher field and its width increases (fig.1B). This can be clearly seen also from the difference spectrum (fig.1C). This result can be explained by the participation of Phe 30 in the complex formation which leads to a strong change of its environment.

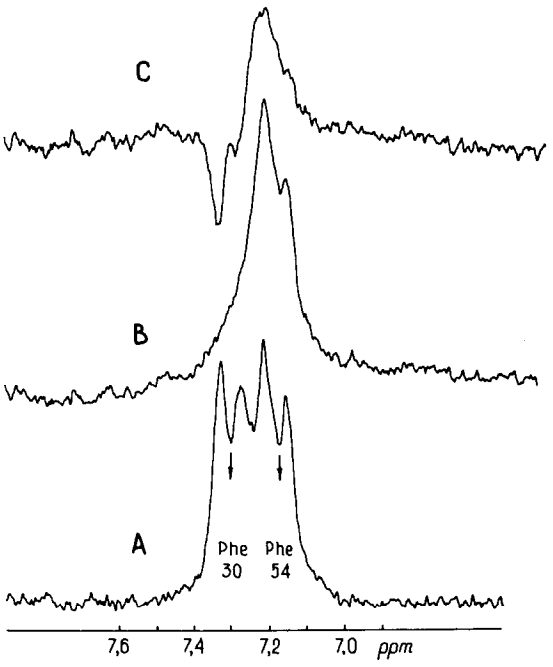


Fig.1. NMR spectra, 360 MHz (low field region) in buffer B of the protein L7 dimer (A), and the L7·L10 complex (B). (C) is the difference spectrum obtained by subtraction of the protein L7 spectrum from that of the complex.

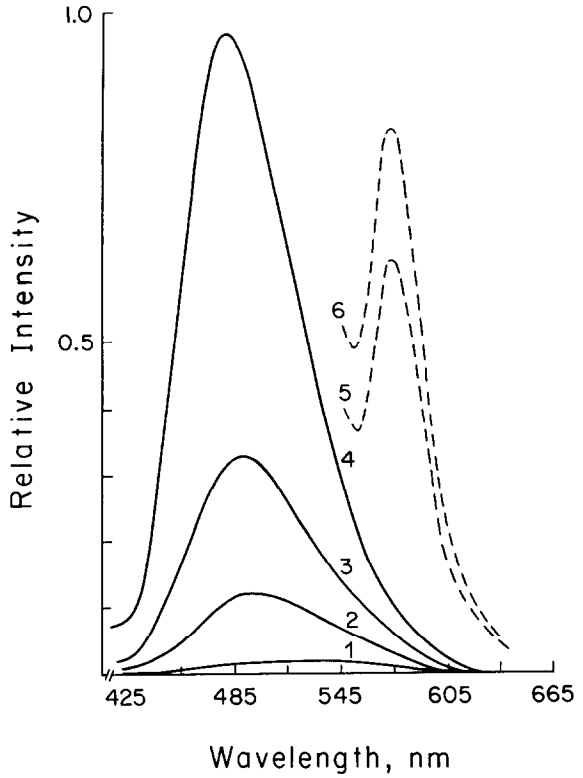


Fig.2. Fluorescence spectra with ANS and iodoacetylaminorhodamine B in buffer C. Solid curves: (1) ANS in water; (2) oxidized protein L7; (3) L7 dimer; (4) L7·L10 complex with the ratio of 4:1. ANS was $10^{-4}$ M, that of proteins 1.72 mg/ml, $\lambda_{ex.}$ = 410 nm. Dashed curves: (5) spectrum of the complex modified by iodoacetylaminorhodamine B; (6) spectrum of the modified complex within the ribosomes. The complex was 0.09 mg/ml, $\lambda_{ex.}$ = 535 nm.

Hydrophobic interactions in the N-terminal sequences of the protein L7 are very important for the L7 dimer formation [3]. Fluorescence spectra of the oxidized protein L7, the L7 dimer and the $(L7)_4$·L10 complex with 8-aniline-1-naphthalene sulphonic acid (ANS) as a hydrophobic probe testify to the important role of hydrophobic interactions at the complex formation (fig.2). It is seen that the fluorescence intensity increases as a result of the formation of the protein quaternary structure, and the fluorescence maximum shifts towards shorter wavelengths thus indicating lower accessibility of ANS molecules to water.

### 3.2. *C-terminal sequence 71–165 of the protein L10 binds all the four copies of the protein L7*

There is only one cysteine residue in the protein L10. Titration of SH-groups of the protein L10 within the $(L7)_4$·L10 complex by dithiobisnitrobenzoic acid is found to proceed with the same rate both under denaturing and native conditions which indicates easy accessibility of the thiol group of the complex in solution. At the same time the complex modified by iodoacetylaminorhodamine B binds with the ribosome, and 50 S subunits pelleted in the ultracentrifuge are crimson-coloured as they contain the rhodamine in the $(L7)_4$·L10 complex. The increase of iodoacetylaminorhodamine B fluorescence intensity upon addition of ribosomes (fig.2) suggests that the regions of the protein L10 sequence near Cys 70 participate in the binding with the ribosome, and the environment of this residue is changed within the 50 S subunit.

To test the role of N- and C-terminal sequences of the protein L10, we cleaved the protein at the cysteine residue. The obtained fragment 70–165 (CF) binds the protein L7 while the fragment 1–69 (NF) does not. From SDS electrophoresis data (fig.3) and amino acid analysis it follows that the stoichiometric ratio in the complex is 4 mol L7 : 1 mol CF. The molecular weight, according to equilibrium sedimentation, is 60 000 (fig.4), whereas the $M_r$ calculated from the amino acid composition is 59 000.

### 3.3. *N-terminal sequence 1–69 of the protein L10 seems to participate in the binding of the $(L7)_4$·L10 complex with the ribosome*

The complex of the proteins L7 and L10 and its different derivatives were assayed in factor-dependent reactions on ribosomes.

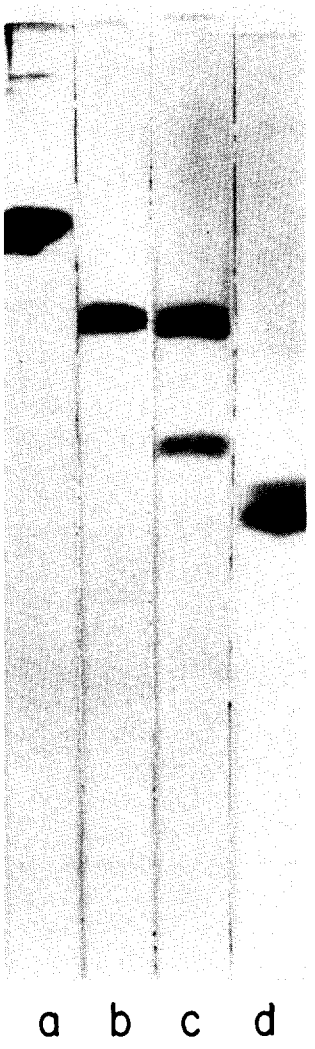


Fig.3. SDS–electrophoresis in 12.5% gel: (a) L10; (b) L7; (c) complex of L7 with the fragment 70–165 of the protein L10; (d) N-fragment of the protein L10.

The modification of Cys in the $(L7)_4$·L10 complex by iodoacetamide does not lead practically to a loss of the ribosome function in all factor-dependent reactions (table 1). However, the modification of the complex by a more bulky reagent, such as iodoacetylaminorhodamine B, results in incomplete restoration of the factor-dependent GTP hydrolysis and GMPPCP binding capabilities of the ribosome. At the same time, this modification does not affect poly(U) translation.

The modification by iodoacetylaminorhodamine B

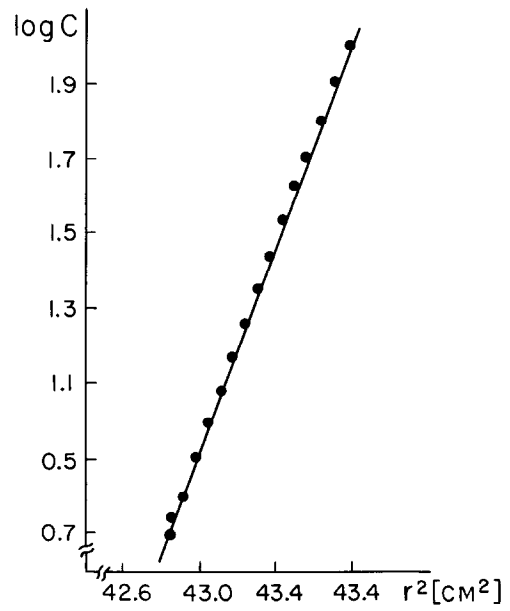


Fig.4. Dependence of the concentration logarithm on the square of the radius for the complex of the protein L7 with the C-fragment of the protein L10 obtained on a Sephadex G-100 column. $n$ = 30,490; $\bar{\nu}$ = 0.754; $c_0$ = 0.2 mg/ml in buffer A.

seems to hinder somehow the correct binding of the $(L7)_4 \cdot L10$ complex with the ribosome. The complex of L7 with the C-terminal fragment of the protein L10 twice increases the polypeptide synthesis activity as compared with the intact complex and practically does not restore the functions of GTP hydrolysis and GMPPCP binding. It is likely that the binding of this $(L7)_4 \cdot CF$ complex with the ribosome is weak. Therefore we can presume that the N-terminal region of the protein L10 sequence and/or regions around the Cys 70 residue are important for stable binding of the complex with the ribosome.

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Table 1
Effect of different derivatives of the L7·L10 complex on EF-G-dependent functions of ribosomes

| Expt. | Components of the mixture | EF-G-dependent poly(U) translation in the presence of GTP (%) | EF-G-dependent GTP hydrolysis (%) | EF-G-dependent [$^3$H]GMPPCP binding (%) |
|---|---|---|---|---|
| 1 | 50 S + 30 S | 100 | 100 | 100 |
| 2 | 50 S (-L7,L12,L10) + 30 S | 30 | 31 | 19 |
| 3 | 50 S (-L7,L12,L10) + 30 S + L7·L10 complex | 98 | 93 | 97 |
| 4 | 50 S (-L7,L12,L10) + 30 S + L7·L10 complex modified by iodoacetamide at Cys | 104 | 90 | 96 |
| 5 | 50 S (-L7,L12,L10) + 30 S + L7·L10 complex modified by iodoacetylaminorhodamine at Cys | 100 | 60 | 67 |
| 6 | 50 S (-L7,L12,L10) + 30 S + L7·$CF_{L10}$ complex | 64 | 36 | 24 |

The amount of [$^{14}$C]phenylalanine incorporated into trichloroacetic acid-insoluble polypeptide after 40 min incubation at 37°C by intact ribosomes was taken as 100% which corresponds to 30–32 pmol [$^{14}$C]phenylalanine incorporated into peptide. The amount of [$\gamma$-$^{32}$P]GTP hydrolyzed by intact ribosomes for 15 min incubation at 37°C was taken as 100% which corresponds to 4.7 nmol inorganic [$^{32}$P]orthophosphate. The amount of [$^3$H]GMPPCP bound with intact ribosomes for 20 min at 37°C was taken as 100% which corresponds to 14 pmol

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