THE INTERACTION OF 5 S RNA AND ITS LARGE FRAGMENTS WITH RIBOSOMAL PROTEINS

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1. Introduction

The ribosomal 5 S RNA–protein complex has been the focus of investigations for ~10 years because this complex is possibly situated in functionally active sites of ribosome and, as a relatively simple model system to study RNA–protein interactions (reviews [1–3]).

The topography of 5 S RNA–protein complex has been examined mostly by partial endonuclease cleavage and chemical modification techniques [4–11], allowing the determination of the 5 S RNA sequences involved in the interaction with ribosomal proteins. In spite of a similarity in methods, the results obtained so far by different authors do not always agree. Some possible reasons for ambiguities, which became apparent in the *Escherichia coli* 5 S RNA–protein complex studies, have been discussed in detail [9].

We employed a different approach using affinity chromatography of ribosomal proteins on the immobilized 5 S RNA and its large fragments. These experiments were complemented by nitrocellulose filtration measurements, giving, in some cases, the possibility for a quantitative interpretation of the contribution of various parts of 5 S RNA in their interaction with proteins.

2. Experimental

50 S Ribosomal subunit proteins (TP50) and 5 S RNA were prepared from *E. coli* MRE600 [12,13]. ^{32}P-Labelled 5 S RNA was isolated from bacteria, grown in [^{32}P]orthophosphoric acid containing low-phosphate medium [14].

Mild conditions for the preparation of 5 S RNA large fragments were similar to those in [15]. Renatured (60°C, 10 min) 5 S RNA was digested with T_1 ribonuclease (Sankyo, EC 3.1.4.8) in 50 mM Tris–HCl (pH 7.6) containing 20 mM $MgCl_2$, 200 mM NaCl, at 0°C for 1 h. A different set of fragments was obtained by digesting 5 S RNA, denatured (60°C, 10 mM EDTA) before enzymatic reaction. 5 S RNA fragments were separated in 15% polyacrylamide gel [16], several individual bands were extracted [17] and reprecipitated 3 times with ethanol. 5 S RNA fragments were identified by standard 2-dimensional cellulose–acetate–DEAE paper fingerprinting [14] and/or, after end-labelling with [γ-^{32}P]ATP (1000 Ci/mmol, Isotop, USSR) as in [17], by direct read-off gel sequencing [18] as in [19].

Individual ribosomal proteins L18 and L25 were isolated from TP50 by combination of affinity chromatography on 5 S RNA–Sepharose gel [13] and subsequent fractionation on CM-cellulose column [20].

Three different polynucleotides – 5 S RNA, fragment U_1–G_{41} (the smaller fragment) and fragment C_{42}–U_{120} (the larger fragment) were immobilized to epoxy-activated Sepharose 2B as in [12,13,21]. The smaller fragment was dephosphorylated [22] before immobilization.

Reassociation of the fragments in the affinity column was achieved by passing a free fragment, either U_1–G_{41} or C_{42}–U_{120}, through the column with the immobilized larger or smaller fragment, respectively. Reassociation, performed at 20°C in 10 mM Tris–HCl buffer (pH 7.5), 115 mM KCl, 30 mM $MgCl_2$ was stoichiometric as judged by comparing the molar amounts of the immobilized fragment and the bound to the column free fragment.

Affinity chromatography of TP50 (0.3 mg/ml) on

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the immobilized to Sepharose polynucleotides was carried out in 10 mM Tris–HCl buffer (pH 7.5), 10 mM $MgCl_2$, 300 mM KCl, 6 mM 2-mercaptoethanol (BA), as in [13].

The values of K_d for the interaction between 5 S RNA or its fragments with individual proteins L18 and L25 in solution were estimated by nitrocellulose filtration technique, titrating a constant amount of ^{32}P-labelled RNA with increasing amounts of a protein in 50 mM Tris–HCl (pH 7.8), 200 mM KCl, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol (BF) in 40 μl–80 μl. The samples were incubated for 30 min at 20°C and filtered through nitrocellulose filter (Chemapol, 0.3 μm pore size). Filters were washed with 100 μl BF and the amount of the complex was taken as radioactivity, retained to filters. The conditions were similar to [23]. Where possible, retention coefficients of various complexes to nitrocellulose filter were estimated as in [23] and, within a range of concentrations used, were found to be ~0.8. K_d of various complexes were estimated from linear transformation of their binding isotherms by plotting the concentration of protein versus concentration of protein divided to the fraction of an RNA in the complex.

One A_{260} unit of 5 S RNA was taken as 1 nmol and the molar concentrations of the fragments were calculated from the known specific activity of 5 S RNA. For proteins, A_{230} for 1 mg/ml was taken as 5 [24].

3. Results

For the renatured 5 S RNA, a single break at G_{41} largely prevails, yielding two fragments, U_1–G_{41} and C_{42}–U_{120} (fig.1a). From a number of bands, obtained from the denatured 5 S RNA, several were extracted and used for further experiments (fig.1c).

The ability of TP50 proteins to bind to the immobilized 5 S RNA, to the larger (C_{42}–U_{120}) and to the smaller (U_1–G_{41}) fragments was studied in 5 different combinations (table 1). Under the experimental conditions, in agreement with [13,25,26], the immobilized intact 5 S RNA forms a complex with 3 ribosomal proteins: L5, L18 and L25. An identical result was obtained with the immobilized larger and smaller fragment, provided they were complemented with the free smaller or larger fragment, respectively (table 1). Hence, the integrity of the loop around nucleotide 41, depicted in most of the 5 S RNA secondary structure models (for models [1–3]), is unimportant for a

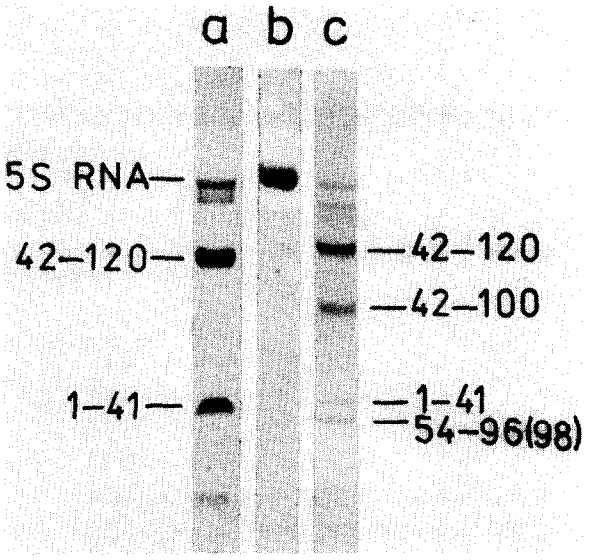

Fig.1. Electrophoretic separation of a mild T_1 ribonuclease digest of *E. coli* 5 S RNA on 15% urea–polyacrylamide slab gel [16]. Fragments, indicated at margins were extracted and used for further experiments: (a) renatured 5 S RNA digest; (b) 5 S RNA marker; (c) denatured 5 S RNA digest.

stable complex formation between 5 S RNA and these proteins. The immobilized larger fragment alone formed a stable complex only with protein L25, whereas the smaller fragment did not bind proteins (table 1).

Here, it would be premature to conclude, that the smaller fragment makes no contribution to the 5 S RNA–L25 interaction. We next compared the values of K_d of the interaction of individual protein L25 with intact 5 S RNA, the smaller and the larger fragment, and, in addition, fragments C_{42}–G_{100}, A_{54}–$G_{96(98)}$ and U_{103}–U_{120}. It turned out that K_d for the intact

Table 1
The binding of *E. coli* 50 S ribosomal subunit proteins to the immobilized 5 S RNA, its large fragments and reassociated 5 S RNA

Immobilized polynucleotide	Free polynucleotide	L5	L18	L25
5 S RNA	–	+	+	+
U_1–G_{41}	–	–	–	–
U_1–G_{41}	C_{42}–U_{120}	+	+	+
C_{42}–U_{120}	–	–	–	+
C_{42}–U_{120}	U_1–G_{41}	+	+	+

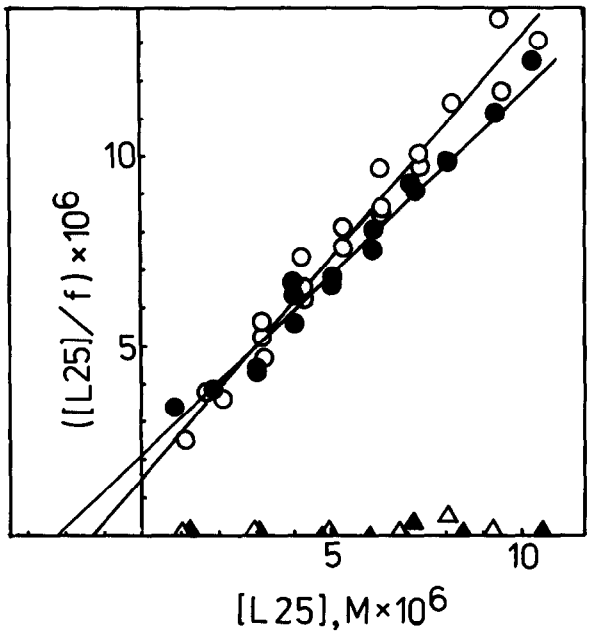

Fig.2. The interaction of ribosomal protein L25 with 5 S RNA and its various fragments, measured by nitrocellulose filtration technique. Conditions are those described in [23] and are specified in section 2: (–○–) 5 S RNA; (–●–) C_{42}–U_{120}; (–△–) U_{103}–U_{120}; (–▲–) U_1–G_{41}. [L25] is molar concentration of protein L25 and f is the fraction of an RNA in the complex.

5 S RNA and the larger fragment are almost identical: 1.4×10^{-6} M and 2.1×10^{-6} M. No interaction was found between L25 and U_1–G_{41} (fig.2). Thus, the nitrocellulose filtration results agree well with affinity chromatography data, and, in addition, allow the conclusion that the smaller fragment of 5 S RNA is not involved in L25 binding. Neither did we detect any complex between U_{103}–U_{120} and L25 (fig.2). Two other fragments, C_{42}–G_{100} and A_{54}–$G_{96(98)}$ both bind L25 with $K_d \sim 5 \times 10^{-6}$ M, which can be considered as very close to that found for the larger fragment.

There is less to say about protein L18. This protein did not bind to the immobilized 5 S RNA fragments (table 1). It seems, therefore, that its binding site is shared by both halves of 5 S RNA. We have been unsuccessful in measuring correct dissociation constants of the interaction between L18 and the fragments C_1–G_{41} and C_{42}–U_{120}. Not that the fragments failed to bind to the nitrocellulose filters in the presence of L18: they did, but the binding curve did not follow an isotherm, analogous to those in the case of L25.

Like L18, protein L5 binds only to the immobilized intact 5 S RNA or to its reassociated halves (table 1). Again, its binding site is possibly distributed between these 2 fragments.

4. Discussion

The combination of 2 methods allowed us to conclude that protein L25 not only interacts with the larger 5 S RNA fragment, but also that its binding is restricted to this fragment. Hence, the double helical stem between the 3′- and 5′-ends of the 5 S RNA (models [1–3]), proposed to interact with L25 [5], is certainly unimportant. An identical conclusion was drawn in [9]. Here, however, we also found that fragments C_{42}–G_{100} and A_{54}–$G_{96(98)}$ interact with L25 almost as strongly as 5 S RNA itself. This result contradicts the identification of sequence A_{101}–G_{116} as a primary L25 binding region, suggested in [8]. Quite the opposite, our quantitative data and the fact that no complex formation between L25 and U_{103}–U_{120} was observed suggests that the nucleotides adjacent to G_{98} have, if any, only a marginal effect in 5 S RNA–L25 interaction.

The other relevant study places the L25 binding site on 5 S RNA within nucleotides 69–110 [9]. The present results allow to narrow the 3′-side of this sequence to nucleotide 96 or 98.

Earlier, Zimmermann and Erdmann identified the L18 binding site on 5 S RNA within nucleotides 58–100 [8]. This result has been disputed in [9] by showing that after covalent cuts between nucleotides 42–58, the L18–5 S RNA complex possibly does not exist any longer. In addition, kethoxal modification of L18–5 S RNA complex clearly indicated the involvement of nucleotide G_{24} in this interaction [10]. Our results support this interpretation, according to which certain structural elements of the smaller fragment are involved in L18 binding. An identical conclusion can be drawn also for protein L5, whose binding area was identified by others to lie between nucleotides 19–56 [8].

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