THE TERNARY COMPLEX CONSISTING OF RAT LIVER RIBOSOMAL 5 S RNA, 5.8 S RNA AND PROTEIN L5

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1. Introduction

Procedures for extraction of 5 S RNA–protein complex from eukaryotic ribosomes usually yield a complex of the RNA with a single protein [1–4]. For rat liver ribosomes it was identified as protein L5 [4]. However, affinity chromatography of rat ribosomal 60 S subunit proteins on the immobilized 5 S RNA did not reveal this protein in the formed complex [5–7], while it was present in a similar 5.8 S RNA–protein complex [5,6]. It was suggested that the immobilization technique itself (i.e., 3′-end oxidation of 5 S RNA with periodate and its subsequent linkage to hydrazide group) might prevent 5 S RNA–L5 interaction [7].

Closer inspection, however, shows that L5 does bind to the immobilized rat liver 5 S RNA. Here, we demonstrate that both 5.8 S RNA and 5 S RNA bind protein L5 and a ternary complex consisting of 5 S RNA, 5.8 S RNA and protein L5 can be formed.

2. Experimental

Isolation of rat liver ribosomal subunit proteins and 5 S RNA was as in [6]. 5.8 S RNA was isolated according to [8]. To facilitate quantitative estimations, 5′-end labelled ^{32}P 5.8 S RNA, prepared as in [9], was mixed with cold 5.8 S RNA. Preparation of the 5 S RNA–Sepharose gel and affinity chromatography of 60 S subunit proteins was as in [6]. The latter was performed in 10 mM Tris–HCl (pH 7.6) buffer, containing 20 mM $MgCl_2$, 300 mM KCl and 6 mM 2-mercaptoethanol at 3–4°C. The bound proteins were eluted from the column with 10 mM Tris–HCl (pH 7.6) buffer, containing either 1 M KCl, 5 mM EDTA or 8 M urea, 4 M LiCl.

Ribosomal proteins were separated by two-dimensional gel electrophoresis [10], and the proposed universal nomenclature was used [11]. Secondly, a unidimensional sodium dodecyl sulphate (SDS) electrophoresis system [12] with identification given in [13] was employed.

The pre-formed 5 S RNA–L5 complex was prepared as follows: 3.5 ml 60 S subunit total protein (0.2 mg/ml) was passed through the 5 S RNA–Sepharose column (0.12 ml, 200 μg 5 S RNA) and washed with 6 ml (50 vol.) 1 M KCl, 5 mM EDTA buffer. After that, the column was washed with 50 vol. binding buffer and 5.8 S RNA was applied. The column was washed with binding buffer until there was no A_{254}. The urea–LiCl wash of the column was analysed for RNA on urea-containing polyacrylamide gels [9] and for proteins on 15% SDS gels [12].

3. Results

In [5,6] and [7,8] affinity chromatography has been used to identify 5 S RNA and 5.8 S RNA-binding proteins of the rat liver ribosome. According to both experimental protocols, the formed complexes were dissociated and proteins eluted by high salt buffer containing EDTA [5–8]. The absence of L5 in the 5 S RNA–protein complex [5–7], found in [4] as a single 5 S RNA-bound protein, was somehow surprising since a similar prokaryotic 5 S RNA–protein complex obtained by various techniques, including affinity chromatography, contained identical main

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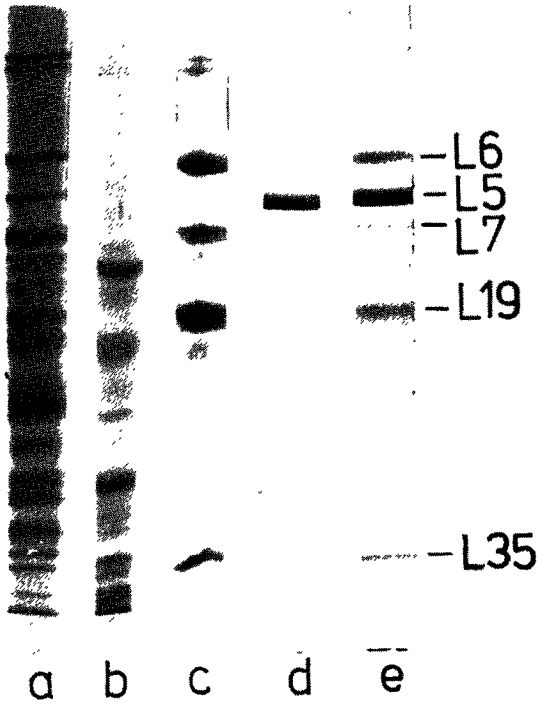

Fig.1. Electrophoresis of rat liver 60 S ribosomal subunit proteins in SDS–15% polyacrylamide gel: (a) total protein of rat liver 60 S ribosomal subunit (TP 60); (b) 60 S subunit proteins unbound to the immobilized 5 S RNA; (c) 1 M KCl, 5 mM EDTA buffer wash of bound proteins. 1 mg of TP60 was applied to the 5 S RNA–Sepharose column; (d) 8 M urea, 4 M LiCl buffer wash of proteins still bound to 5 S RNA after 1 M KCl, 5 mM EDTA wash; (e) 1 mg TP60 was applied to the 5 S RNA–Sepharose column and bound proteins were eluted instantly with 8 M urea, 4 M LiCl buffer.

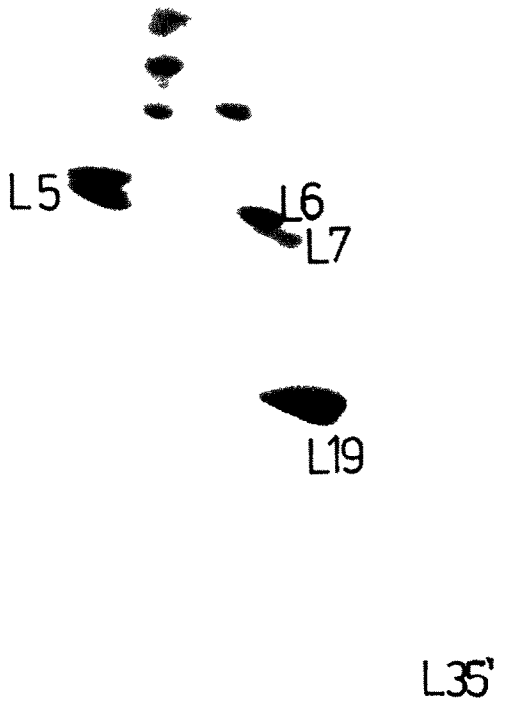

Fig.2. Two-dimensional electrophoresis of rat liver 60 S subunit proteins bound to the immobilized rat liver 5 S RNA in 10 mM Tris–HCl buffer (pH 7.6) containing 20 mM $MgCl_2$, 300 mM KCl and 6 mM 2-mercaptoethanol at 4°C. 5 ml 60 S subunit proteins (0.2 mg/ml) was applied to 0.2 ml RNA–Sepharose column containing 7 A_{260} units 5 S RNA. Bound proteins were eluted with 8 M urea, 4 M LiCl containing Tris–HCl buffer. Protein identification is according to [10].

proteins [14–16]. We also observed that the unbound fraction of 60 S subunit proteins contained a far smaller amount of L5 compared with the initial sample (fig.1a,b). These circumstances led us to the idea that protein L5 might be bound to the immobilized 5 S RNA in a way not elutable by high salt–EDTA buffer, resembling the complex of *Escherichia coli* protein S1 with polynucleotides [17].

Fig.1c shows that 1 M KCl–EDTA eluate of the 5 S RNA–protein complex contains two major proteins L6 and L19 and several minor components. The next eluate of the same column by 8 M urea–4 M LiCl, as shown on (d), contains L5 as a single protein. A single-step elution by urea–LiCl (e) is a simple sum of (c + d). Two-dimensional identification of the bound proteins is shown on fig.2. Affinity chromatography at room temperature gives identical results. Neither 4 M LiCl nor 8 M urea alone were able to dissociate efficiently the 5 S RNA–L5 complex. Thus, these results show that L5 does interact with the immobilized 5 S RNA and the earlier controversy is therefore resolved.

Protein L5 was also found in the complex of 60 S subunit proteins with the immobilized 5.8 S RNA [5,6]. This complex, however, was readily dissociable by high salt–EDTA buffer [6]. Therefore, the next question to ask was whether both of these interactions can take place simultaneously. The stability of the 5 S RNA–L5 complex in 1 M KCl–5 mM EDTA buffer allows selective removal of all proteins except L5 from the complex of 5 S RNA with 60 S subunit proteins (fig.1).

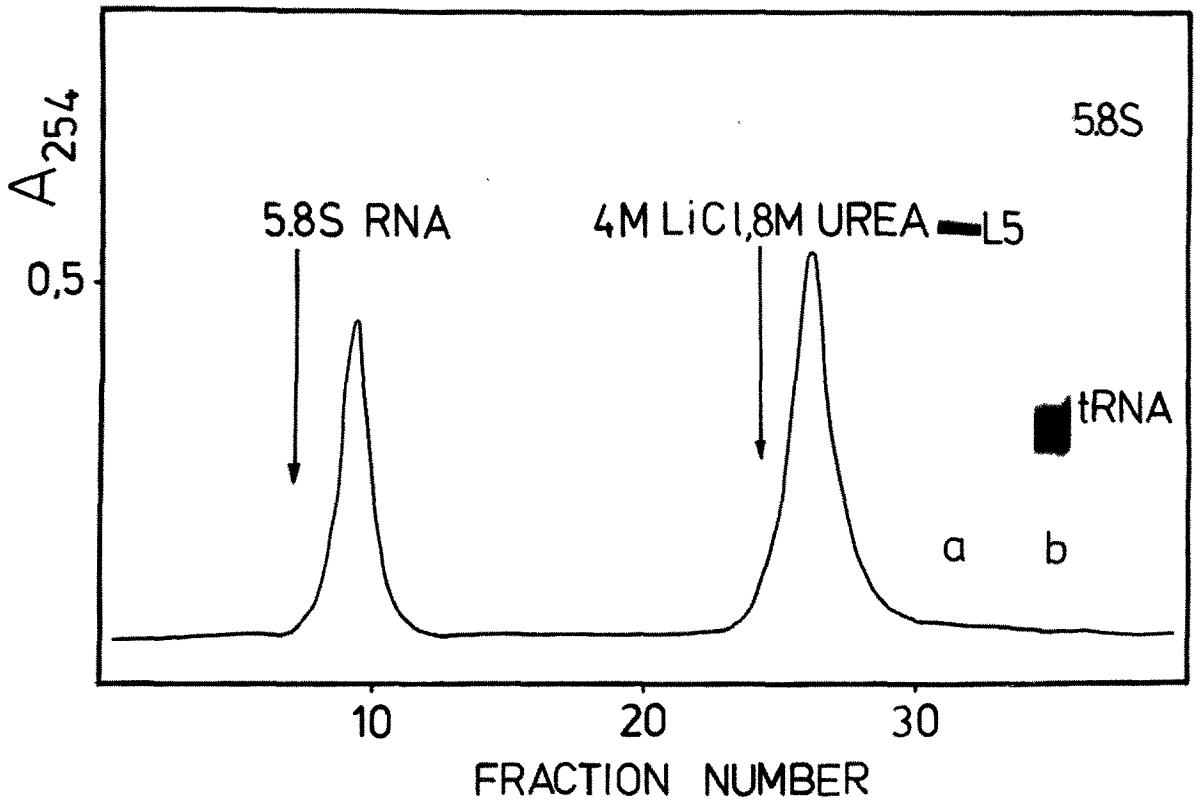

Fig.3. Affinity chromatography of rat liver 5.8 S RNA on the pre-formed rat liver 5 S RNA–protein complex. About 2-fold molar excess of 5.8 S RNA (0.75 A_{260} units, 8800 cpm) over the pre-formed complex in 0.1 ml binding buffer was applied to the column. The bound 5.8 S RNA and protein L5 were eluted with 8 M urea, 4 M LiCl buffer. First peak contained 4300 cpm unbound 5.8 S RNA and the second one 4500 cpm bound 5.8 S RNA (0.38 A_{260} units). One half of the eluate was precipitated with 10% Trichloroacetic acid and analysed on SDS–15% polyacrylamide gel (a). Carrier tRNA was added to the second half of the eluate and RNA was precipitated with 2 vol. ethanol. Separation was carried out on urea–10% polyacrylamide gel (b).

Conditions of the experiment are given in section 2 and legend to fig.3. The latter shows that 5.8 S RNA indeed binds to the pre-formed 5 S RNA–protein complex. No detectable binding of 5.8 S RNA to the immobilized 5 S RNA was observed, nor was the free 5 S RNA able to bind to the immobilized 5 S RNA–protein L5 complex (not shown).

Taking the M_r of rat liver protein L5 as 32 500 [18], that of 5.8 S RNA as 54 000 [19] and the number of individual proteins in TP60 as 45, it was possible to calculate that at saturation conditions (fig.3) ~0.8 copy of 5.8 S RNA binds to 1 copy of the 5 S RNA–L5 complex.

Several conclusions can be drawn from these experiments:

(1) Protein L5 binds to the immobilized 5 S RNA and this binding does not need the presence of other proteins.

(2) The nature of binding of L5 to 5 S RNA and 5.8 S RNA is different – the first interaction is stable in conditions unfavourable for electrostatic interactions.

(3) Both of these interactions can take place simultaneously, resulting in a ternary complex 5 S RNA–L5–5.8 S RNA. It seems that protein L5 has two independent RNA binding sites.

4. Discussion

Only a few ribosomal proteins are known to interact with two different ribosomal RNAs. A relevant example is the *E. coli* 5 S RNA protein L18, which also gives a complex with 23 S RNA [18]. Moreover, this protein in combination with either L5 or L25 is able to stabilize a quarternary complex consisting of 5 S RNA, 23 S RNA and two proteins [15].

The yeast 5 S RNA protein YL3 is structurally homologous with *E. coli* 5 S RNA protein L18 [21]. In [21] the C-terminal half of YL3 interacted with yeast 5.8 S RNA. Although the specificity of the latter interaction is unclear [21] it is interesting to speculate that a certain single eukaryotic 5 S RNA protein as well as the combination of two corresponding *E. coli* 5 S RNA proteins mediate 5 S RNA interactions with other ribosomal RNAs.

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