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5 S RNA–Protein complex is involved in ribosomal subunit association

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Received 4 January 1983

The immobilized tRNA–50 S ribosomal subunit protein (TP50) complex binds the smaller ribosomal subunit. We constructed tRNA·TP50·5 S [^{32}P] RNA and tRNA·TP50·t [^{32}P] RNA complexes and investigated the accessibility of the ^{32}P-labelled tRNAs to ribonuclease T_1. It was found that in this complex both 5 S RNA and tRNA are attacked by T_1 RNase. In sharp contrast, the addition of 30 S subunit protects 5 S RNA as well as tRNA from degradation. We suggest that 5 S RNA–TP50 complex is exposed to the ribosomal interface and is involved in subunit interaction.

E. coli *ribosome* *5 S RNA–Protein complex* *Subunit association* *Affinity chromatography*

1. INTRODUCTION

Cyclic association and dissociation of ribosomal subunits is needed for protein biosynthesis in the cell [1]. The structural organization of the ribosomal interface region and, in particular, the question which ribosomal components participate in subunit association are of special interest since this region of the ribosome is believed to play an important role in its functioning [2]. These problems have been studied by various techniques (reviewed [3,4]), among which crosslinking studies [5–10] have been particularly useful to establish ribosomal proteins possibly involved in over-interface interactions.

We showed earlier [11,12] that the tRNA- and 5 S RNA–50 S ribosomal subunit protein (TP50) complexes can bind 30 S ribosomal subunit. Here we specify and extend this finding.

2. EXPERIMENTAL

Preparation of *E. coli* MRE600 ribosomal subunits, ribosomal proteins, tRNA, 5 S RNA and ^{32}P-labelled RNAs was as in [11,12]. Bulk tRNA was immobilized to Sepharose [13] and the preformed tRNA·TP50 complex, containing ribosomal proteins as specified in [12,14], was prepared in 10 mM Tris–HCl (pH 7.5) containing 10 mM $MgCl_2$, 100 mM KCl and 6 mM 2-mercaptoethanol. This buffer was used in all experiments, except for removing the material bound to the affinity column, where 1 M KCl and 10 mM EDTA containing buffer was employed. About 20% of the TP50-binding capacity of tRNA–Sepharose gel columns was used.

^{32}P-Labelled tRNA or 5 S RNA (~2×10^5 dpm/A_{260} unit) were bound to the preformed tRNA·TP50 complex [12], giving tRNA·TP50·tRNA* and tRNA·TP50·5 S RNA* complexes (see below, asterisk specifies non-covalently bound RNAs). These two complexes, prepared in affinity columns (0.4 ml wet gel), were used for 30 S ribosomal subunit binding and ribonuclease digestion studies.

T_1 ribonuclease (Sankyo, EC 3.1.4.8) digestion of tRNA·TP50·tRNA* and tRNA·TP50·5 S RNA* complexes, with and without a saturating amount of 30 S subunits present, was carried out in 1.5 ml Eppendorf tubes using ~30 mg wet gel containing ~70 pmol 5 S RNA* or tRNA* and, in some experiments, equal amount of 30 S subunits.

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Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00 © Federation of European Biochemical Societies

Samples were incubated with T_1 ribonuclease (50 units, 0°C, 30 min), treated with phenol containing 10 mM $ZnCl_2$, RNA was recovered from the water phase by ethanol precipitation. Digestion products were separated on 15% polyacrylamide–7 M urea slab gel and visualized by autoradiography. In a control experiment we confirmed that the possible effect of 30 S subunits cannot be attributed to the simple increase of the ribonuclease substrate concentration: a 2-fold excess by weight of cold 5 S RNA compared with 30 S subunits did not alter the digestion results for the tRNA·TP50·tRNA* complex in the absence of 30 S subunits.

3. RESULTS AND DISCUSSION

Differently from [11,12], where we investigated the binding of 30 S ribosomal subunit to the preformed 5 S RNA·TP50 or tRNA·TP50 complexes, here the tRNA·TP50 complex was supplemented by noncovalently bound tRNA or 5 S RNA. As shown in [12], the immobilized tRNA alone does not bind 30 S ribosomal subunit, showing that a codon-independent interaction of tRNA with 30 S subunits [15] is not sufficient for the formation of a stable complex. Here, also, the amount of bound 30 S subunits was much less than the amount of immobilized tRNA. We found that, at saturation (fig.1), molar amounts of the bound tRNA* or 5 S RNA* and 30 S subunit are nearly equal (1:0.85–0.92). Since from [12] we know that the preformed tRNA·TP50 complex binds one mole of either tRNA or 5 S RNA per mole of the complex, we conclude that the tRNA·TP50 complex binds a stoichiometric amount of 30 S ribosomal subunits.

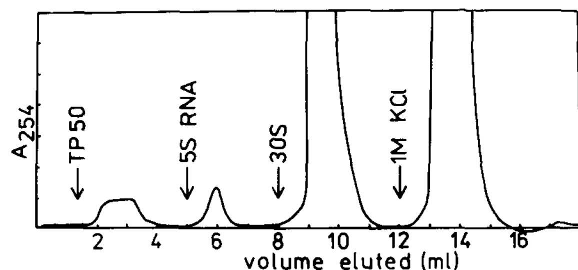

Fig.1. Affinity chromatography of 30 S ribosomal subunits on a preformed tRNA·TP50·5 S RNA* gel column in 10 mM Tris–HCl buffer (pH 7.5), 10 mM $MgCl_2$, 100 mM KCl, 6 mM 2-mercaptoethanol: 0.5 ml TP50 (2 mg/ml) was applied to the immobilized tRNA–Sepharose gel column (2.1 mg tRNA/ml, 0.4 ml) for preformation of tRNA·TP50 complex. The tRNA·TP50·5 S RNA complex was formed with 1.3 A_{260} units of 5 S RNA from which 0.96 units was bound. After that 30 S subunits (3 mg, 200 μl) was passed through the column and the flowthrough and the bound fraction of 30 S subunits was estimated by absorbance. Other details are given in section 2 and [12].

Next we investigated accessibility of tRNA* and 5 S RNA* to ribonuclease T_1 with and without 30 S subunits present. It would have been virtually impossible to carry out enzymatic digestion experiments by following the intactness of the covalently bound tRNA in the tRNA·TP50 complex, because only ~15% of this tRNA is able to bind TP50, although the remaining 85% of tRNA is still largely accessible to ribonuclease (unpublished). Therefore, we studied the accessibility of tRNA* and 5 S RNA*, which can be done easily using radioactive RNAs.

We found that in tRNA·TP50·5 S RNA* and tRNA·TP50·tRNA* complexes both these noncovalently bound RNAs are accessible (fig.2, slots 2 and 5). The molecule of 5 S RNA is mainly cut into two typical fragments [16] about 80 and 40 nucleotides long (slot 2), whereas tRNA yields about 35 nucleotides long fragments (slot 5), most likely as a result of a covalent break in the anticodon loop.

In the 50 S subunit, 5 S RNA is not accessible to ribonuclease [17], although it can be modified by low-M_r reagents like kethoxal [19]. It seems, therefore, that the structure of the tRNA·TP50·5 S RNA* complex is less compact than that of the 50 S subunit.

We are not aware of investigations of the availability of tRNA in its complex with the 50 S subunit; it seems likely that at least its anticodon loop should be exposed. The result presented above agrees with this prediction.

In contrast, after addition of the 30 S subunit, both 5 S RNA* and tRNA* became almost completely protected against ribonuclease (fig.2, slots 3 and 6), tallying with the situation in 70 S ribosome where both tRNA [19] and 5 S RNA are unavailable for ribonuclease.

Our results also show that 23 S RNA is not a prerequisite for subunit association, in an indirect

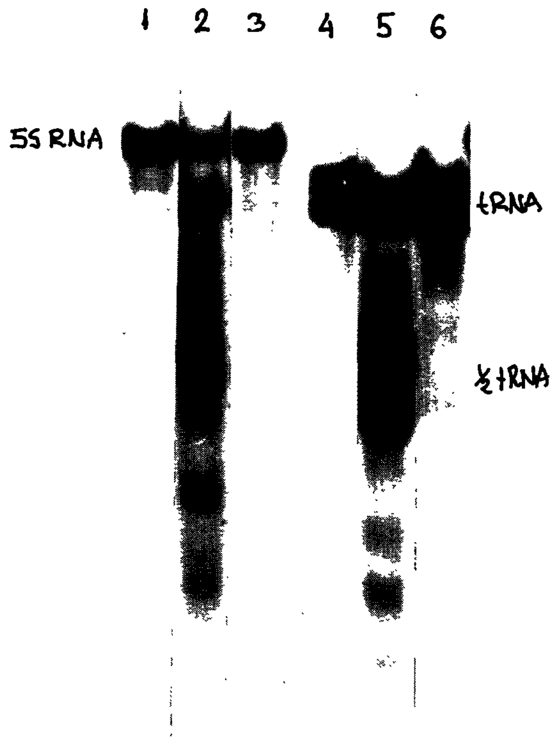

Fig.2. 15% polyacrylamide–7 M urea slab gel electrophoresis of T_1 ribonuclease digest of ^{32}P-labelled 5 S RNA (slots 1–3) and tRNA (slots 4–6), bound to the preformed tRNA·TP50 complex: (1) initial 5 S RNA; (2) tRNA·TP50·5 S RNA*; (3) as (2), but 30 S subunit present; (4) initial tRNA; (5) tRNA·TP50·tRNA*; (6) as (5), but 30 S subunits present. For details see section 2.

agreement with a suggestion that the protein component of the larger subunit is more important in this process [20].

We note that a large number of the components of our 5 S RNA·TP50 complex [11]: L2, L5, L16, L17, L18, L21 and L33 can be crosslinked to various 30 S subunit proteins [5–7] while L2, L5, L16 and L17 crosslink to 16 S RNA in situ [8–10]. Our results might also explain why the 30 S subunit binding capacity of 50 S subunits lacking 5 S RNA and, partially, proteins L5, L16, L18 and L25 is impaired [21].

In summary, we suggest that the 5 S RNA–protein complex region of 50 S ribosomal subunit is directly involved in subunit association.

ACKNOWLEDGEMENT

We wish to thank Dr Mart Saarma for useful suggestions for ribonuclease protection experiments.

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