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Ribosomal proteins, TL4 and TL5, from *Thermus thermophilus* form hybrid complexes with 5 S ribosomal RNA from different microorganisms

G.M. Gongadze*, S.V. Tishchenko, S.E. Sedelnikova, M.B. Garber

Department of Structure and Function of Ribosomes, Institute of Protein Research, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russian Federation

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Hybrid complexes of the ribosomal proteins, TL4 and TL5, from *Thermus thermophilus* with 5 S ribosomal RNA from *Escherichia coli* and *Bacillus stearothermophilus* have been prepared. There was no competition between the two proteins for the binding sites. Stoichiometry of 5 S RNA binding for both proteins was 1·1 (protein/RNA). The TL4 protein competed with the *E. coli* ribosomal L5 protein, and the TL5 protein competed with the *E. coli* ribosomal proteins, L18 and L25, for binding with 5 S RNA.

5 S RNA; Hybrid complex, *Thermus thermophilus*; Ribosomal protein

1. INTRODUCTION

The 5 S RNA–protein complex provides a convenient system for studying RNA–protein interactions in the ribosome. This complex is a sufficiently autonomous structural domain of the large ribosomal subunit and can easily be isolated and inserted into the ribosomal subparticle [1–3]. The primary structures of ribosomal 5 S RNA [4,5] and of the proteins that it binds [6–10] display high homology when studied in different organisms. It was shown that *Escherichia coli* 5 S RNA binds strongly to the ribosomal proteins L18 and L25, and weakly to L5 in vitro [3,11,12]. The same *E. coli* ribosomal proteins also bind to *Bacillus stearothermophilus* and *Thermus thermophilus* 5 S RNAs [13]. Similarly, the *B. stearothermophilus* 5 S RNA binding proteins, BL5 (corresponding to L5) and BL22 (corresponding to L18), bind not only to *B. stearothermophilus* 5 S RNA but also to the 5 S RNAs from *E. coli* and *T. thermophilus* [13]. Recently two 5 S RNA binding proteins were isolated and partially sequenced from *T. thermophilus* [14]. The gene for the ribosomal protein, L5, from *T. thermophilus* has been cloned and sequenced [10].

The binding sites for proteins L5, L18 and L25, on 5 S RNA from *E. coli* have been identified [15–17] and a hypothetical model of the 5 S RNA–protein complex has been suggested [17]. The most direct way to investigate the structure of such a complex is crystallization and X-ray analysis. Some positive results have been obtained in crystallization trials for 5 S RNA from *T. thermophilus* [18] and from *T. flavus* [19], and for a stable fragment of 5 S RNA from *E. coli* [20]. Erdmann and co-workers have reported in principle the possibility of crystallization of the 5 S RNA–protein complexes from *E. coli* and *B. stearothermophilus* [21].

Our group has been engaged in studies of the proteins and other components of ribosomes from the extreme thermophile, *T. thermophilus*, for many years. We have succeeded in purifying more than half of the ribosomal proteins from this bacterium in non-denaturing conditions, and several of them have been crystallized [22,23]. We consider *T. thermophilus* ribosomal proteins as very promising for structural studies and work has started to investigate 5 S RNA binding proteins from this microorganism. The reconstruction technique has been used to find the *T. thermophilus* ribosomal proteins that bind to the 5 S RNA. We have obtained highly stable hybrid complexes of 5 S RNA from *E. coli* and *B. stearothermophilus* with only the ribosomal proteins, TL4 and TL5. One unexpected result was obtained: the rather large ribosomal protein, TL5, from *T. thermophilus*, ousted the two small ribosomal proteins, L18 and L25, from the *E. coli* 5 S RNA–protein complex.

2. MATERIALS AND METHODS

2.1. *Materials*

Sephadex G-100 was purchased from Pharmacia Fine Chemicals (Sweden). All other reagents were purchased from Fluka (Switzerland) or from Serva (Germany).

2.2. *Isolation and purification of ribosomal 50 S subunits, ribosomal proteins and 5 S ribosomal RNA*

Ribosomal 50 S subunits were obtained from *T. thermophilus* as described in [22] and from *E. coli* as in [24]. Total ribosomal protein was prepared by acetic acid extraction as in [25]. Individual ribosomal

Corresponding author. E-mail: PROTRES@GLAS.APC.ORG

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proteins from *T thermophilus* were purified by CM–Sepharose CL-6B column chromatography under non-denaturing conditions as in [22]. 5 S ribosomal RNA from *E. coli* and *B. stearothermophilus* was isolated by Sephadex G-100 gel-filtration of the total RNA preparation obtained by standard phenol extraction as in [24].

2.3. *Isolation and reconstruction of the 5 S RNA–protein complexes*

The 5 S RNA–protein complex from *E. coli* was isolated by EDTA treatment of 50 S ribosomal subunits as in [1] All the types of hybrid 5 S RNA–protein complexes were reconstituted according to [3]. These complexes were obtained using proteins of the ribosomal 50 S subunits from *T. thermophilus* and 5 S RNA from *E. coli* or *B stearothermophilus* in 20 mM Tris-HCl buffer, pH 7.5, with 20 mM $MgCl_2$, 300 mM KCl and 5 mM 2-mercaptoethanol. The 5 S RNA–protein complexes were isolated by centrifugation in a linear 5–20% sucrose gradient in the same buffer. To investigate the competition between ribosomal proteins in binding to 5 S RNA, the *E. coli* 5 S RNA–protein complex was mixed with the proteins, TL4 or TL5, at an RNA-to-protein molar ratio of 1:1 or 1:1.5. The mixture was centrifuged in a linear sucrose gradient. The buffer and sucrose gradient were the same as in the experiment described above. The protein content of the complexes was analysed by PAGE. SDS-PAGE was performed according to the method of Laemmli [26]. Two-dimensional PAGE was performed according to [27]. The amount of RNA and protein in the complexes was quantitated by co-migrating known amounts of the pure RNA and proteins as described in [12].

3. RESULTS AND DISCUSSION

The first experiments to obtain hybrid complexes were performed using *E. coli* 5 S RNA and preparations of total ribosomal protein from *T. thermophilus*. These experiments showed that only two ribosomal proteins formed a stable complex with the RNA at a stoichiometry of about 1:1. Two-dimensional PAGE of the proteins extracted from this complex showed two major spots corresponding to the ribosomal proteins, TL4 and TL5 (Fig. 1B). The same complex was obtained using 5 S RNA from *B. stearothermophilus* (data not shown). These proteins bind to 5 S RNA both separately and together. As is seen in Fig. 2, TL4 and TL5 bind tighter to 5 S RNA than the *E. coli* ribosomal proteins under the same conditions. The molar ratio of TL4 and TL5 to 5 S RNA in the hybrid complexes was about 1:1. It has been reported previously that in the native *E. coli* complex the molar ratio of the proteins, L5, L18 and L25, to 5 S RNA is 0.15:0.8:0.4:1.0, respectively [11,12].

The ribosomal proteins, TL4 and TL5, have been purified and characterised [22]. Both are comparatively large among ribosomal proteins, with molecular masses of about 20 kDa (Fig. 2, lane B). Two-dimensional electrophoresis shows that the TL5 protein is one of the most acidic proteins of the 50 S ribosomal subunit from *T. thermophilus* (Fig. 1A). The TL5 spot is located near that of TL11 (an analog of the most acidic ribosomal protein L7/L12). There is no similar spot on the electrophoretogram of ribosomal proteins from the *E. coli* 50 S ribosomal subunit. However, TL5 can be considered as an intrinsic ribosomal protein because it can be extracted from the 50 S subunit only after treatment with 3.5 M LiCl or with a mixture of 1 M NH_4Cl and 50% ethanol at 60°C [22].

The purified individual ribosomal proteins, TL4 and TL5, were examined for competition with the ribosomal proteins, L5, L18 and L25, from *E. coli*, in binding to 5 S RNA, as described in section 2. It was found that TL4 competed for binding to 5 S RNA with L5, while TL5 competed with L18 and L25 (Fig. 2, lanes C,D). One can see that the TL4 and TL5 proteins ousted the L5, L18 and L25 proteins from the *E. coli* 5 S RNA–protein complex. These results suggest that the TL4 and L5 proteins, as well as TL5, and L18 and L25, have similar binding sites on the 5 S RNA molecule. It is known that the binding sites for L18 and L25 are arranged near each other on the 5 S RNA molecule, and so the larger protein, TL5, can cover the binding sites of both the small proteins, L18 and L25. We suggest that ribosomal protein TL4 is homologous to ribosomal

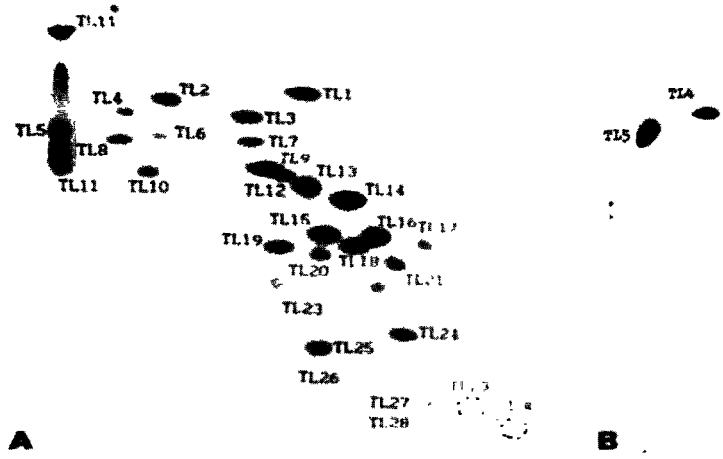

Fig. 1. Two-dimensional electrophoregrams of ribosomal proteins from *T. thermophilus*. (A) Proteins from the 50 S ribosomal subunits, and (B) from the hybrid 5 S RNA–protein complex. We have used the procedure proposed in [27].

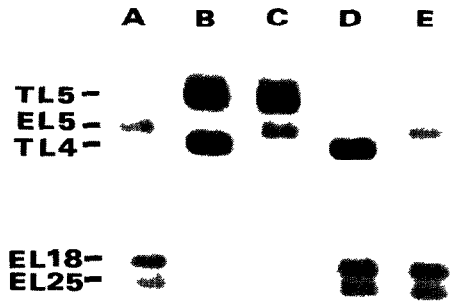

Fig. 2. SDS-PAGE of proteins from 5 S RNA–protein complexes. Proteins (lanes A,E) from the 5 S RNA–protein complex of *E coli*; (lane B) from the hybrid 5 S RNA–protein complex; and (lanes C,D) bound to 5 S RNA after gradient centrifugation of the mixture of the 5 S RNA–protein complex from *E coli* with the TL5 protein (C), or with the TL4 protein (D). 0 5–0 7 A_{260} units of a complex were loaded on a slot.

protein L5. N-Terminal sequencing will be done in the near future to check this assumption. As for the ribosomal protein, TL5, its complete amino acid sequence is needed for a thorough comparison with proteins L18 and L25.

In conclusion we can say that two main new results have been obtained in this work. The first: very stable and homogeneous hybrid 5 S RNA protein complexes have been prepared. In our opinion, these complexes are promising for crystallization. The second: a large ribosomal protein, TL5, from *T. thermophilus*, displays a high affinity to 5 S RNA. This protein competes with two small ribosomal proteins, L18 and L25, from *E. coli* for binding to 5 S RNA.

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