A QUATERNARY COMPLEX CONSISTING OF TWO MOLECULES OF tRNA AND RIBOSOMAL PROTEINS L2 AND L17

Ene METSPALU, Toivo MAIMETS, Mart USTAV and Richard VILLEMS*

Laboratory of Molecular Biology, Tartu University and **Laboratory of Molecular Genetics, Institute of Chemical Physics and Biophysics, 14/16 Kingissepa Street, 202400 Tartu, Estonian SSR, USSR*

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1. Introduction

Experiments with various affinity and photo-affinity analogues of tRNA have revealed its proximity to a number of ribosomal proteins (reviews [1–3]). More recently, UV-induced in situ crosslinking of tRNA has allowed the establishment of a group of proteins in the close vicinity of tRNA [4,5]. Direct evidence for the interaction of ribosomal proteins with tRNA was obtained by affinity chromatography of ribosomal proteins on immobilized tRNA [6–10]. The complex of 50 S subunit proteins with tRNA, covalently bound to the epoxy-activated Sepharose is described in [9] and consists of proteins L2, L15, L16, L17, L18, L22, L33 and L34.

We have been able to show that this tRNA–protein complex binds another molecule of tRNA and, independently from the latter, a molecule of 5 S RNA [11]. Here we show that two 50 S ribosomal subunit proteins, L2 and L17, bind directly to the immobilized tRNA. Neither of these proteins in separate dimeric complex with tRNA form a binding site for the second tRNA. However, these two proteins, when taken together, form a complex with immobilized tRNA which possesses a binding site for the second molecule of tRNA.

2. Experimental

Escherichia coli tRNA was isolated from MRE600 strain, purified on a Sephadex G-100 column (5 × 90 cm) and its purity was checked by urea–polyacrylamide gel electrophoresis according to [12].

* To whom correspondence should be addressed

E. coli ^{32}P-labelled tRNA was isolated from MRE600 strain, grown on low-phosphate medium containing ^{32}P orthophosphoric acid [13]. Hot phenol–SDS-extracted RNAs [14] were fractionated by polyacrylamide gel electrophoresis [12], eluted from the gel [15] and reprecipitated 3 times with alcohol. *E. coli* 50 S ribosomal subunits were prepared according to [16]. In the isolation and fractionation of 50 S ribosomal subunit proteins we followed the procedure in [17], modified by pre-fractionation of 50 S subunit proteins on a preparative tRNA–Sepharose gel column followed by Sephadex gel filtration and CM-cellulose chromatography at neutral pH. Details of the procedure will be published elsewhere. Individual proteins were identified by two-dimensional polyacrylamide [18] and SDS–15% polyacrylamide gel electrophoresis [19].

The binding of the individual proteins, protein mixtures, and ^{32}P-labelled deacylated tRNA to the tRNA–Sepharose gel (53 A_{260} units/ml) was carried out in 10 mM Tris–HCl binding buffer (BB) (pH 7.5) containing 10 mM $MgCl_2$, 100 mM KCl and 6 mM 2-mercaptoethanol at 4°C. Proteins were loaded onto 0.5 ml tRNA–Sepharose gel column at ~10^{-6} M. The column was washed with 10 column vol. BB, and t[^{32}P]RNA was loaded onto it at 2 × 10^{-5} M. After that the affinity column was again washed with BB, until there was no radioactivity in the eluate (about 10 column vol.) and the bound t[^{32}P]RNA with proteins were finally eluted with an elution buffer (EB) containing 1 M KCl and 10 mM EDTA. Radioactivity was measured in an LKB Ultrobeta 1210 scintillation spectrometer with an efficiency of ~40% on ^{3}H-channel for ^{32}P-induced Čerenkov radiation. One-third volume of 30% trichloroacetic acid was added to all affinity column fractions (loading of proteins, loading

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of tRNA and elution with 1 M KCl). The precipitated proteins were analyzed on SDS–polyacrylamide slab gels [19]. Control experiments were those in [8–10]. Other experimental details are given in legends to figures.

In the nitrocellulose filter assay ^{32}P-labelled tRNA ($1–6 \times 10^{-7}$ M) was incubated with increasing amounts of individual proteins L2 or L17, at 25°C in 50 μl for 15 min and thereafter filtered through a nitrocellulose membrane (Schleicher and Schüll, BA85), washed with 100 μl BB and the amount of the bound tRNA–protein complex was estimated as radioactivity retained on the nitrocellulose filter.

3. Results and discussion

The experiments with individual proteins L2 and L17 show that both of them can bind independently to the immobilized tRNA (fig.1). Neither the tRNA–L2 nor the tRNA–L17 complex are able to bind a second molecule of tRNA.

We employed the nitrocellulose filtration technique

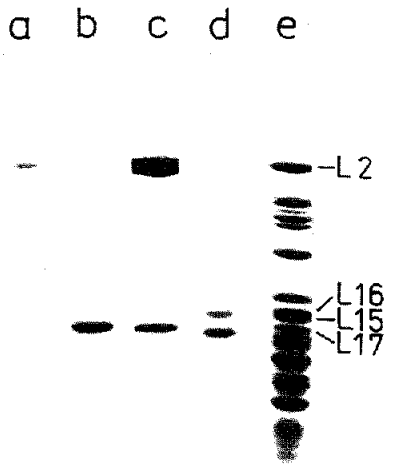

Fig.1. SDS–15% polyacrylamide gel electrophoresis of ribosomal proteins bound to immobilized tRNA in 10 mM Tris–HCl buffer (BB) (pH 7.5), 10 mM $MgCl_2$, 100 mM KCl, 6 mM 2-mercaptoethanol. Individual proteins L2 (a), L17 (b) and protein pairs L2–L17 (c), L15–L17 (d) were applied to the immobilized tRNA–Sepharose gel column (0.5 ml, 26 A_{260} units). The bound proteins were eluted with buffer (EB) containing 1 M KCl and 10 mM EDTA, precipitated with 10% trichloroacetic acid and analyzed [19].

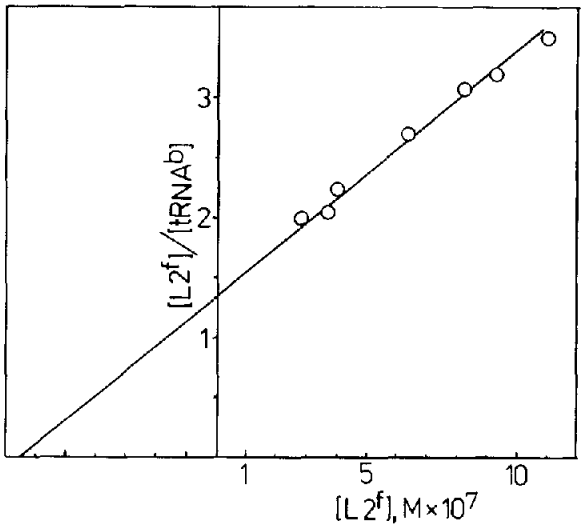

Fig.2. Determination of the complex between protein L2 and tRNA on nitrocellulose filters. ^{32}P-Labelled deacylated tRNA (5.6×10^{-7} M) was titrated with increasing amounts of protein L2 in BB. Samples of 50 μl were incubated for 15 min at 25°C and filtered through nitrocellulose filters. For 1 mg protein/ml, A_{230} was taken as equal to 5 [22]; [$tRNA^b$], concentration of bound tRNA; [$L2^f$], concentration of free protein L2. The latter was calculated from the known amount of protein L2 in the assay and the known amount of tRNA bound onto the nitrocellulose, assuming a 1:1 complex between L2 and tRNA (section 3).

for further characterization of these complexes. For the tRNA–L2 complex we found that equilibrium complex formation as well as exchange between the bound (cold) tRNA and the added (radioactive) tRNA is complete within 2 min (not shown). Therefore, the interaction between tRNA and protein L2 takes place at equilibrium and can be characterized by a corresponding constant. Fig.2 shows a linear transformation of the binding isotherm, giving K_d 6.5×10^{-7} M for the tRNA–L2 complex. The intercept at the ordinate is equal to 1.34, which is in a reasonable agreement with a number one gets dividing K_d by the concentration of tRNA in this experiment (1.18). Hence, each molecule of L2 seems to bind one molecule of tRNA.

We have been unable to measure K_d for the tRNA–L17 complex, since, in the conditions used, its formation was accompanied by aggregation and precipitation of tRNA and protein L17. Thus, the only evidence for a direct tRNA–L17 interaction comes from the affinity chromatography experiment (see above), in which, since the molecules of tRNA are fixed to the solid matrix, the complex cannot aggregate.

Although the complex of 50 S subunit proteins with the immobilized tRNA was described in [8], these results are the first showing that two individual ribosomal proteins, L2 and L17, can bind directly to tRNA. Of these proteins L2 is one of the most frequently labelled by various tRNA affinity analogues (reviews [1,2]), and it can be crosslinked in situ to tRNA by UV-irradiation [4,5]. It should be emphasized here that protein L2 crosslinks to tRNA situated in both ribosomal A and P sites [1,2,5]. There is less information available on protein L17. However, it crosslinks to a photo-affinity analogue of puromycin [20] and is highly stimulating in restoring peptidyl transferase activity [21].

On the other hand, these two proteins form a stable complex in solution, as well as showing weak 'assembly interaction' [21]. Indeed, we also found that these two proteins migrate together in one chromatographic peak containing unequal amounts of proteins L2 and L17, possibly as free L2 and its complex with protein L17 (fig.1c). The molar ratio of L2 and L17 in this particular fraction was 4.5:1 according to densitometric scanning of a Coomassie R-250-stained SDS–polyacrylamide gel.

After forming the tRNA–protein complex with this mixture of proteins we found that free tRNA binds to it. The dependence of the amount of bound ^{32}P-labelled tRNA upon the amount of the preformed tRNA–L2–L17 complex is shown on fig.3. The molar amount of the bound tRNA is almost equal to that of L17 present in the preformed complex and ~4-times lower than the amount of protein L2 present. We found that the column flowthroughs did not contain proteins L2 and L17 and a 3-fold increase of the amount of free tRNA loaded to the column did not lead to further binding of tRNA to it. We also tested another mixture of proteins, L15 and L17, which during separation of ribosomal proteins on Sephadex G-100 column elute from it as a single peak and possibly interact with each other (fig.1d; see also [17]). These proteins, when applied to the immobilized tRNA column, do not create a binding site for a second molecule of tRNA. Since the immobilized tRNA–L2 and tRNA–L17 complexes were also unable to bind free tRNA we conclude that two proteins, L2 and L17, are needed for the formation of a binding site for the second tRNA. This conclusion is supported by finding that a tRNA–protein complex formed from the mixture of L2 and L17 (a different chromatographic fraction) with an opposite molar ratio of proteins (1:2.9)

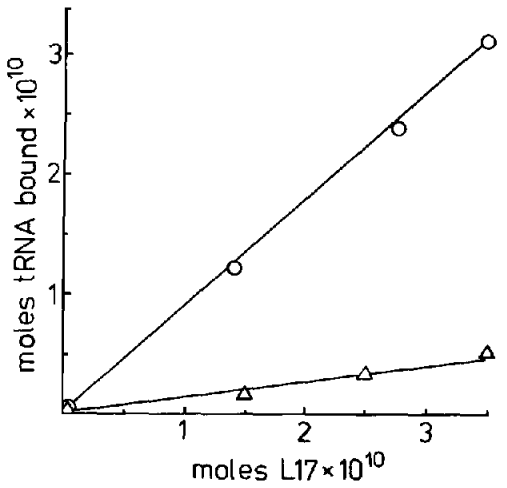

Fig.3. The dependence of the binding of the second molecule of tRNA to the pre-formed tRNA–L2–L17 complex upon the molar amount of the latter: (○) 'native' complex of protein L2 and L17 having molar ratio 4.5:1; (△), equimolar mixture of individual proteins L2 and L17. An indicated amount of the 'native' complex or the mixture of proteins L2 and L17 in BB was loaded onto a tRNA–Sepharose gel column (0.5 ml, 26 A_{260} units/ml) and washed with 10 column vol. BB. A 3-fold molar excess of ^{32}P-labelled tRNA was thereafter applied to the pre-formed immobilized tRNA–protein complex and the column was again washed with BB until there was no radioactivity in the eluate. The bound tRNA was eluted with EB.

bound an amount of tRNA close to the amount of protein L2 (not shown).

We were not entirely successful in restoring, in tRNA–Sepharose gel, the binding site for the second molecule of tRNA with a simple mixture of individual proteins L2 and L17; only ~10–15% of the expected amount of tRNA was bound to such a column (fig.3). It might be that the L2–L17 complex, once dissociated, does not reassociate readily.

Two different models can be proposed:

(i) The free tRNA binds to either protein L17 or L2 which in turn is held in the complex with the immobilized tRNA via protein–protein interaction as a L2–L17 pair.

(ii) The binding site for the second tRNA is formed on both of these proteins, or induced on one of them by a cooperative mechanism.

So far we have been unable to choose between these tow models.

Like the ribosome, the above complex can hold simultaneously two molecules of tRNA. Taken alone,

the 50 S subunits seems to interact strongly with only one molecule of tRNA [23]. However, it may well be that in this much simpler association some steric hinderances present in the 50 S subunit do not exist.

We believe that complexes like the above can be useful to a study of molecular interactions in the peptidyl transferase center of ribosomes.

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