The properties of the complex between ribosomal protein L2 and tRNA

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Escherichia coli ribosomal protein L2 interacts with fMet-tRNA$_f^{Met}$ and NacPhe-tRNA^{Phe} in solution, protecting their 3′-ends from enzymatic degradation. At the same time L2 enhances the rate of spontaneous hydrolysis of the ester bonds between terminal riboses and amino acyl moieties of these two peptidyl-tRNA analogues. L2 has, however, only a slight effect on the rate of spontaneous deacylation of aminoacyl-tRNAs. We suggest that the role of L2 is in the fixation of the aminoacyl stem of tRNA to the ribosome at its P-site, and speculate that this protein is directly involved in the peptidyl transferase (PT) reaction.

Peptidyl transferase *Protein L2* *tRNA-protein complex*

1. INTRODUCTION

L2, the largest of the structural proteins of the 50 S ribosomal subunit, interacts directly with 23 S RNA and belongs to the group of proteins essential for the assembly of the subunit [1]. Several lines of evidence suggest that L2 is a component of the ribosomal peptidyl transferase (PT) center. L2 cross-links in situ to unmodified tRNA [2], as well as to almost all affinity and photoaffinity analogues of tRNA (reviews [3,4]). Chemical modification of a few amino acid residues of B-L3 of *B. stearothermophilus* (homologous to *E. coli* L2) inactivates PT activity and affects the binding of substrates to 50 S ribosomal subunit [5]. L2 belongs to a group of proteins essential for the restoration of PT activity during 50 S subunit reconstitution [6].

We have reported that L2 interacts directly with deacylated tRNA in solution [7]. Here we extend these experiments and suggest that L2 may, like L16 [8,9], participate in the interaction between the 3′-terminus of tRNA with the PT center.

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2. EXPERIMENTAL

The isolation of *E. coli* MRE600 individual ribosomal proteins was as outlined in [10]. Three different individual ribosomal protein L2 preparations were used. In addition, a sample of L2 prepared by a different method was kindly provided by Dr A. Gudkov. *E. coli* tRNA^{Phe} (1600 pmol/unit, Boehringer Mannheim) was aminoacylated with [^{3}H]phenylalanine (Amersham, 50 Ci/mmol). *N*-acetylated Phe-tRNA^{Phe} (Nac[^{3}H]Phe-tRNA^{Phe}) was prepared as in [11]. f[^{14}C]Met-tRNA$_f^{Met}$ was prepared from tRNA$_f^{Met}$ (1300 pmol/unit, Boehringer, Mannheim) using [^{14}C]methionine (Amersham, 215 Ci/mol) according to [12]. Bulk *E. coli* tRNA was aminoacylated with a mixture of 20 different amino acids from which 11 were radioactive ([^{3}H]amino acids, 23–40 Ci/mmol). Ribonuclease protection experiments were as in [9]. f[^{14}C]Met-tRNA$_f^{Met}$ (1.2×10^{-7} M) was incubated with or without 3 ng RNase A (Sigma, EC 3.1.4.22) in the

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presence or absence of L2 (10^{-5} M) in 100 μl of 20 mM Tris-HCl (pH 7.4), 150 mM NH_4Cl, 10 mM $MgCl_2$ and 6 mM 2-mercaptoethanol at 0°C. After given time intervals (fig.1) samples were withdrawn, mixed with 1 ml cold 5% trichloroacetic acid, kept at 0°C for 30 min and filtered through Whatman GF/A glass fiber filters. The filters were washed with ice-cold 5% trichloroacetic acid, ethanol, and after drying their radioactivity was counted in a toluene-based scintillation cocktail. Nac[^{14}C]Phe-tRNA^{Phe} was digested under similar conditions, but with lower amounts of RNase A (0.4 ng/100 μl).

Nonenzymatic deacylation of aminoacylated tRNAs was followed in 75 mM Tris-HCl (pH 7.4), 50 mM NH_4Cl, 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol at 25°C. In L2 concentration dependence experiments, 2×10^{-7} M f[^{14}C]Met-tRNA$_f^{Met}$ in 0.1 ml buffer was incubated with various amounts of L2 (fig.2 legend). After given time intervals 15-μl samples were withdrawn and further analysis was as described above for enzymatic degradation experiments. Semilogarithmic plots to describe the process were as in [13], used originally for aa-tRNA · EFTu · GTP complex. The logarithms of concentrations of the intact fMet-tRNA at the abscissa (fig.2) are arbitrary, calculated from the 5% trichloroacetic acid-insoluble radioactivity retained on glass-fiber filters. In experiments with Nac[^{14}C]Phe-tRNA and aminoacylated bulk tRNA, L2 was 10^{-5} M and tRNAs 10^{-7} M; [^{3}H]Phe-tRNA^{Phe} was 5×10^{-8} M. In some experiments deacylated bulk tRNA was added at 2-fold molar excess over L2.

3. RESULTS AND DISCUSSION

One of the current views about the nature of the ribosomal PT reaction suggests that no special catalytic mechanism is needed: proper steric orientation of the 3′-ends of aa-tRNA and peptidyl-tRNA leads, ipso facto, to the transfer of the peptidyl moiety (e.g. [14]). Regardless of the actual mechanism, the elucidation of the components of the ribosome interacting with 3′-ends of tRNAs would be an important step towards the understanding of the PT reaction.

Besides L16 [8], L2 is another 50 S ribosomal subunit protein whose interaction with tRNA in solution has been characterized quantitatively [7].

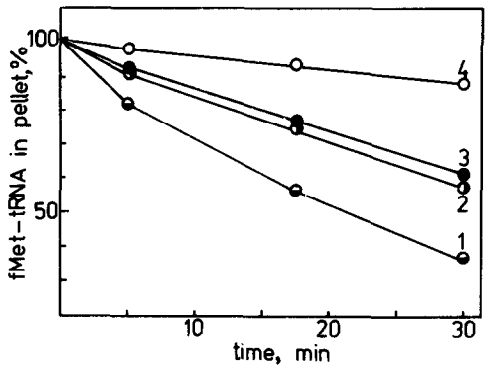

Fig.1. Hydrolysis of f[^{14}C]Met-tRNA$_f^{Met}$ and its protein complex with RNase A. fMet-tRNA$_f^{Met}$ (1.2×10^{-7} M) was incubated with RNase A (1); RNase A + L2 (10^{-5} M) (2); with L2 (3); without L2 or RNase (4) in 100 μl buffer at 0°C. After the given time intervals 15-μl samples were pipetted into 1 ml cold 5% trichloroacetic acid and after 30 min the precipitate was collected onto GF/A filters and its radioactivity measured. For other details see section 2.

The questions we asked here were the following: (i) does L2 protect the 3′-end nucleotides (invariant CCA sequence) of an aminoacylated tRNA against pancreatic ribonuclease attack? (ii) could we see any protective effect of L2 on the ester bond linking tRNA and amino acid, like that observed for the ternary aa-tRNA · EFTu · GTP complex [13, 15,16]?

Indeed, we found that L2 shields the 3′-terminal nucleotides, possibly phosphodiester bonds at C-74 and C-75 of fMet-tRNA$_f^{Met}$, from pancreatic ribonuclease (fig.1). The rationale of this test is identical to that in [9], and is based on the observation that the aminoacylated trinucleotide CCA-fMet, a product of ribonuclease U_2, is already precipitable in 5% trichloroacetic acid. Therefore, the radioactivity in the supernatant is a measure of the amount of liberated A-fMet or CA-fMet ([9] for further discussion). Qualitatively similar results have been obtained with Phe-tRNA^{Phe} and NacPhe-tRNA^{Phe} (not shown).

It is well known that EFTu · GTP not only protects certain regions of aa-tRNA from enzymatic or chemical probes [17,18], but also increases the half-life of aminoacylated tRNA in solution, i.e. protects it from spontaneous hydrolysis [13,15,16, 19,20]. For the aa-tRNA · L16 complex we did not find any effect of this sort [9]. In contrast, here, L2

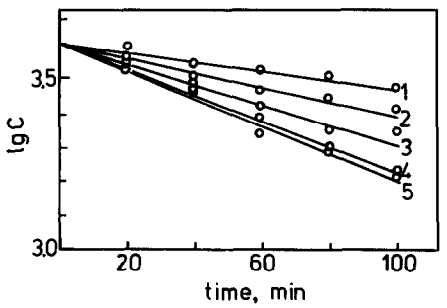

Fig.2. The influence of protein L2 on the rate of the spontaneous deacylation of f[^{14}C]Met-tRNA$_f^{Met}$ at 25°C in 100 μl containing 75 mM Tris-HCl (pH 7.4), 50 mM NH_4Cl, 10 mM $MgCl_2$ and 5 mM 2-mercaptoethanol. L2 was present at: zero (1); 5×10^{-7} M (2); 8×10^{-7} M (3); 10^{-6} M (4) and 4×10^{-6} M (5). 15-μl samples were treated as described in the legend to fig.1. For other details see section 2. IgC, logarithm of acid-insoluble radioactivity.

was found not to decrease, but to accelerate the rate of deacylation of fMet-tRNA$_f^{Met}$ (fig.2). The acid-soluble radioactive product was confirmed to be formulated methionine by paper electrophoresis (not shown). Therefore, the protective effect of L2 on the CCA-terminal sequence of tRNA is even more profound than it appears in fig.1: it is partially masked by the increase of the rate of deacylation, which, due to the particular method used by us, results in the increase of radioactivity of the acid-soluble fraction, exactly as in the case of ribonuclease treatment. In agreement with the K_d value for L2·tRNA complex ($\sim6\times10^{-7}$ M) [8], the increase of the concentration of L2 over 10^{-6} M had only minimal increasing effect on the rate of deacylation of fMet-tRNA$_f^{Met}$ (fig.2).

The relative effect of L2 on the rate of spontaneous deacylation of NacPhe-tRNA^{Phe} was even more profound, increasing it by a factor of 10 (table 1). On the other hand, the much longer half-life of this analogue of peptidyl-tRNA increased in that case the relative effect; the half-lives of both tRNAs in the presence of saturating amounts of L2 were nearly equal (table 1).

Table 1 summarizes data on the half-lives of fMet-tRNA$_f^{Met}$, NacPhe-tRNA^{Phe}, Phe-tRNA^{Phe} and aminoacylated bulk tRNA. We wish to emphasize the following. First, deacylated tRnA, which forms a strong complex with L2 [8], taken in stoichiometric amounts, quenches the effect of L2 upon fMet-tRNA$_f^{Met}$. Second, a number of individual proteins (table 1) found to be essential for the restoration of the PT activity [6], have no effect on the stability of fMet-tRNA$_f^{Met}$. Third, L2 has also some effect, although much smaller, on the rate of the deacylation of aminoacylated tRNAs, increasing it by a factor of approx. 1.3, compared with an approx. 3–11-fold effect on the 2 peptidyl-tRNA analogues studied (table 1). It shows that the activity found here for L2 is not strictly specific for N-blocked aminoacyl moieties.

On the one hand, the effect of L2 on the stability of these 2 peptidyl moieties is additional proof for the conclusion that this protein interacts with the 3′-terminal part of tRNA. On the other, it is also clear that this particular ester bond should be hydrolysed before the peptidyl transfer may take

Table 1

The effect of ribosomal protein L2 on the half-lives of various tRNAs (time in min, average error ±6%)

Addition	fMet-tRNA$_f^{Met}$	NacPhe-tRNA^{Phe}	Phe-tRNA^{Phe}	aa-tRNA
None	187	820	48	80
L2	73	70	35	55
Other proteins[a]	187	–	48	–
L2 and deacylated tRNA	183	–	48	–

[a]Individual ribosomal proteins S4, S7, L3, L15, L16, L17, L18 or L25

Conditions were as described in section 2

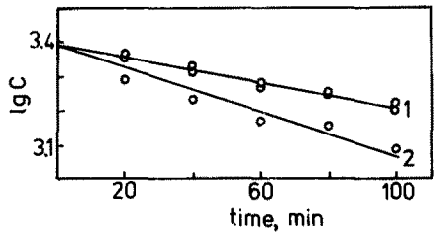

Fig.3. The effect of 50 S ribosomal subunits on the stability of fMet-tRNA$_f^{Met}$. f[^{14}C]Met-tRNA$_f^{Met}$ (1.4×10^{-7} M) was incubated at 25°C without (1) or with (2) 50 S subunits (4×10^{-6} M) in 100 μl of 20 mM Tris-HCl (pH 7.4), 80 mM NH_4Cl, 10 mM $MgCl_2$ and 5 mM 2-mercaptoethanol. 15-μl samples were treated as described in the legend to fig.1. lgC, logarithm of acid-insoluble radioactivity.

place. The fact that L2 also enhances to some extent the deacylation of aminoacylated tRNAs does not contradict this suggestion since the ribosome can promote peptide bond formation between 2 aminoacylated tRNAs as well.

It is, however, known that in 70 S ribosomes both aminoacyl- and peptidyl-tRNAs are protected from nonenzymatic hydrolysis of aminoacyl ester linkage [20,21]. Therefore, we undertook a special study to investigate the stability of fMet-tRNA$_f^{Met}$ in the 50 S ribosomal subunit under conditions where the latter is able to catalyse peptide bond formation. In contrast to 70 S ribosomes, in its 50 S subunit fMet-tRNA$_f^{Met}$ is readily hydrolysed (fig.3). It can be suggested that in 70 S ribosomes, but not in 50 S subunits or in the complex with L2, fMet-tRNA$_f^{Met}$ is covered by a 'hydrophobic screen', protecting its reaction with water.

The destabilizing effect of L2 upon the peptidyl moiety, although clearly pronounced, is obviously too small to allow any definite conclusions about its relevance to the ribosomal PT reaction; at best it gives a hint as to what might be the function of L2 in the mechanism of PT.

The proximity of L2 to the PT center is well documented [3,4]. Recent elegant in situ UV-crosslinking experiments by Budowsky's group (Abdurashidova, personal communication and [22]) show that pre-translocated tRNA in the ribosomal A-site crosslinks to a significant extent with L16 and not at all with L2, whereas the P-site bound tRNA crosslinks in an exactly opposite manner. The results presented in this and in our earlier papers [7,8], together with the evidence cited above, suggest that L2 and L16 are involved in the fixation of the 3'-end(s) of tRNA to the PT center of the *E. coli* ribosome.

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