RIBOSOMAL PROTEINS CONTACTING WITH DEACYLATED tRNA IN THE S-SITE OF THE TRANSLATING RIBOSOME

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1. Introduction

Amino acid starvation of bacterial cells leads to essential deceleration of protein biosynthesis (reviews [1]). Obviously, deficiency of amino acids should result in the decrease of the tRNA aminoacylation rate and hence the translation rate. But the main reason for the protein synthesis deceleration is suppression of the biosynthesis of ribosomal components and factors [1–6], which is determined by the increase of the guanosine tetra- and pentaphosphate intracell concentrations [1]. Formation of the last compounds by the ribosome-associated protein – a stringent factor is stimulated by codon-dependent binding of deacylated tRNA to translating ribosome [7,8].

It was suggested that induction of guanosine tetra- and pentaphosphates synthesis is determined by the binding of deacylated tRNA to so-called A-like site of the translating ribosome [9]. It remained obscure, however, what the A-like and the real A-site had in common.

Using UV-induced crosslinking, the proteins which contact tRNA molecules inside the complexes of aminoacyl-tRNA · 70 S · template and peptidyl-tRNA · 70 S · template were determined (tRNA located in the A- and P-sites, respectively, see table 1) [10].

Here, using the same method we show that deacylated tRNA, added to the NAcPhe-tRNA^{Phe} · 70 S · poly(U) complex contacts with another set of proteins, namely S7, L5 and L11. Hence, in this system, which imitates the interaction of deacylated tRNA with translating ribosome, deacylated tRNA is located neither in A-, nor in P-site. We name this new site of binding deacylated tRNA S-site, since such an interaction is probably the reason for stringent control induced by amino acid starvation.

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2. Materials and methods

70 S ribosomes of *Escherichia coli* MRE600 and t[^{32}P]RNA were prepared according to [11] and [12], respectively. Total unlabelled tRNA was enzymatically aminoacylated in the presence of 10-fold excess of [^{14}C]phenylalanine as a single amino acid. After removal of the enzyme and of the unreacted amino acid, *N*-acetylation of Phe-tRNA was performed by acetic anhydride under standard conditions [13]. The preparation obtained contains 30 pmol NAc-[^{14}C]Phe-tRNA and <2 pmol [^{14}C]Phe-tRNA/1 *A* unit (per 1600 pmol total tRNA). Complex I (70 S · poly(U) · NAc-[^{14}C]Phe-tRNA) was prepared by incubation of 14 *A* units of the last preparation (~450 pmol NAc-[^{14}C]Phe-tRNA) with 20 *A* units (500 pmol) of 70 S ribosomes and 2 *A* units of poly(U) in 2 ml buffer (10 mM $MgCl_2$, 20 mM Tris–HCl, 200 mM NH_4Cl (pH 7.2)). According to the puromycin test [14] >95% of NAc-[^{14}C]Phe-tRNA is located in the P-site. Complex II was prepared by addition of 14 *A* units (22.4 nm) of total deacylated t[^{32}P]RNA to the solution of complex I and further incubation at +4°C for 40 min [15]. As revealed by retention of radioactivity on the millipore filters 2 ± 0.1 pmol NAc-[^{14}C]Phe-tRNA^{Phe} are complexed with 2.5 pmol 70 S ribosomes. Complex II was purified by sucrose density centrifugation. t[^{32}P]RNA removed from this complex was analyzed by two-dimensional gel-electrophoresis according to [16]. More than 90% of ^{32}P-radioactivity coincided on the gel with tRNA^{Phe}.

Complex II was irradiated in Petri dishes by the full light of the mercury low-pressure lamp under vigorous stirring. Intensity of the incident light ($\lambda = 254$) measured by uridine actinometry [17] was 4×10^{17} quanta . cm^{-2} . s^{-1}. The extent of t[^{32}P]RNA crosslinked to complex II was determined by retention of radioactivity on the millipore filters in the presence of EDTA [10] (fig.1). After absorption of ~50 quanta/nucleotide the ribosomal proteins crosslinked to deacylated t[^{32}P]RNA were determined by two-dimensional electrophoresis according to [18] (fig.2) and by peptide maps (according to [19], not shown) as in [10].

3. Results and discussion

The deacylated tRNA in translating ribosome can exist in one of the two states:

(i) After transpeptidation before translocation, when deacylated tRNA occupies P-site [20] (or, probably, P-like site; in preparation);

(ii) After the binding of deacylated tRNA to the post-translocational 70 S · template · peptidyl-tRNA complex.

The latter imitates the situation, which appears in the bacterial cell as a consequence of amino acid starvation.

In the complex I (70 S · poly(U) · NAcPhe-tRNA^{Phe}) the NAcPhe-tRNA^{Phe}/70 S ratio was ⩾0.8:1.0 and according to the puromycin test >95% of NAcPhe-tRNA^{Phe} was located in the P-site. Addition of deacylated tRNA to complex I resulted in

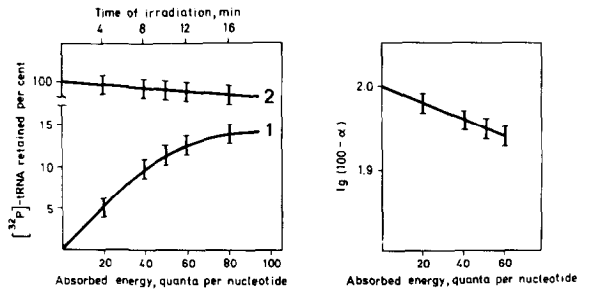

Fig.1. Effect of ultraviolet ($\lambda = 254$ nm) irradiation of complex II (70 S · poly(U) · NAc-[^{14}C]Phe-tRNA · t[^{32}P]RNA on t[^{32}P]RNA retention on nitrocellulose filters in the presence (1) and in the absence (2) of EDTA. Right: semilogarithmic plot of (1) (ordinates = log (100 − α), where α is % retained t[^{32}P]RNA); radioactivity retained on filters in the absence of EDTA was equal to $2–4 \times 10^3$ cpm.

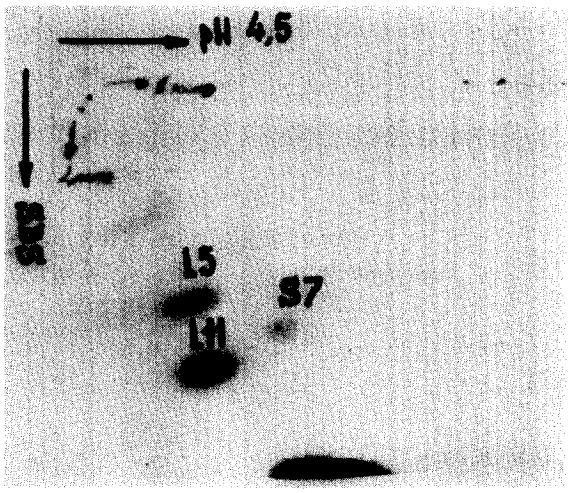

Fig.2. Autoradiograph of the gel after separation of the proteins by two-dimensional electrophoresis [10]. Complex II containing deacylated t[^{32}P]RNA was irradiated, digested with a mixture of RNase A and T_1 and resulting hydrolysate was subjected to electrophoresis. The total radioactivity of the gel $4–5 \times 10^3$ cpm.

formation of complex II with deacylated tRNA to complex I ratio of 0.75:1.0. Most of deacylated tRNA (~90%) in complex II was tRNA^{Phe} according to its electrophoretic mobility [16].

During ultraviolet (254 nm) irradiation of complex II deacylated tRNA is crosslinked to the ribosome exponentially (up to dose 50 quanta/nucleotide, fig.1) with the quantum yield 3×10^{-3}. Even the dose increase up to 150 quanta/nucleotide does not influence the number and quantity of proteins crosslinked to deacylated tRNA (cf. [21]).

After RNase treatment of the irradiated complex II and electrophoresis (cf. [10]) the radioactivity from the crosslinked to the proteins fragments of deacylated t[^{32}P]RNA was found only in 3 spots. According to the positions of these spots (fig.2) and peptide maps these proteins are identified as S7, L5 and L11.

As seen in table 1, the set of proteins contacting deacylated tRNA in complex II strongly differs from those contacting aminoacyl-tRNA in the A-site and peptidyl-tRNA in the P-site. Hence, the site for binding of deacylated tRNA in complex II is different from the A- and P-sites. We call this site S-site, since codon-specific binding of deacylated tRNA to this site is most probably the reason for inducing of the stringent control by the amino acid starvation of bacterial cells.

Table 1
Proteins of 70 S ribosome crosslinked by UV-irradiation to deacylated tRNA, which was added to the complex I (70 S · poly(U) · NAcPhe-tRNA) before irradiation

Ribosomal proteins	Complex I [10] (70 S · poly(U) · NAcPhe-tRNA, tRNA in the P-site)	Complex II (70 S · poly(U) · NAcPhe-tRNA · [^{32}P]-tRNA^{Phe}, tRNA in the S-site)	70 S · poly(U) · Phe-tRNA complex (tRNA in the A-site [10])
S5	–	–	19
S7	2	34 ± 4	3
S9	9	–	16
S10	–	–	15
S11	9	–	–
L2	20	–	13
L4	19	–	–
L5	–	22 ± 6	–
L6	–	–	19
L7/L12	16	–	–
L11	–	44 ± 4	–
L16	–	–	15
L25/S17	4	–	–
L27	21	–	–

Relative radioactivity (mean values of 3 expt) is shown as the % of total radioactivity in the two-dimensional gel portions containing proteins to which fragments of t[^{32}P]RNA are crosslinked. The average total radioactivity present in the gel was equal to 4000–5000 cpm; background ~60 cpm/cm^2 of the gel

This agrees with the finding that mutations of one of the S-site components, namely L11, lead to the so-called relaxed control [22]. For such *rel* mutants, amino acid starvation and relative increase of deacylated tRNA concentration does not result in specific suppression of the biosynthesis of ribosomal components and elongation factors [23].

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