Interaction of $tRNA^{Phe}$ with donor and acceptor tRNA-binding sites of *Escherichia coli* ribosomes

G.T. Babkina, D.M. Graifer, G.G. Karpova and N.B. Matasova

Institute of Organic Chemistry, Siberian Division of the USSR Academy of Sciences, Novosibirsk, 630090, USSR

Received 11 January 1983

Abstract not received

Photoreactive derivative of tRNA *Affinity labelling* *Ribosome* *tRNA-binding site* *Ribosomal protein* *Photocross-linking*

1. INTRODUCTION

With the use of photoreactive derivative of *Escherichia coli* $tRNA^{Phe}$ (azido-$tRNA^{Phe}$) bearing arylazido-groups scattered statistically over tRNA guanosine residues [1] we have identified the proteins contacting with tRNA bound at the P-site at 20 mM Mg^{2+} and evaluated the effect of poly(U) on the arrangement of $tRNA^{Phe}$ located at the P-site [2]. So azido-$tRNA^{Phe}$ allowed us to detect even small differences in arrangements of tRNA bound at the same ribosomal site in different conditions. It seems attractive to compare arrangements of tRNA located at the A- and P-sites.

Here we repeat studies of photoaffinity labelling of ribosomal A- and P-sites by azido-tRNA in the presence of poly(U) as a template. Phe-azido-$tRNA^{Phe}$ was directed to the A-site at 10 mM Mg^{2+} by EF-Tu and GTP. After UV irradiation of the corresponding complex labelling of both subunits was observed. Proteins S9, S15, S16, S17, S18, S19 and L8/L9, L13, L15, L27 were the only labelled materials (5 S RNA not tested). Deacylated azido-$tRNA^{Phe}$ was bound at the P-site non-enzymatically at 10 mM Mg^{2+} and after irradiation proteins S5, S9, S11, S12, S13, S19, S20, S21 and L14, L24, L27, L31, L33 were labelled (5 S RNA not tested). Some deviations from results obtained at 20 mM Mg^{2+} [1,2] are discussed. Hence the arrangements of internal parts of the tRNA molecule are rather different at A- and P-sites.

2. MATERIALS AND METHODS

Ribosomes were isolated from *E. coli* MRE-600 as in [3] and were then purified by gel-filtration on Sephadex G-200 in buffer (1.5 M NH_4Cl, 10 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.4), 1 mM DTT) to completely remove translation factors. Fifty percent of ribosomes were active in poly(U)-dependent binding of Phe-$tRNA^{Phe}$. Elongation factor Tu–Ts (3000 pmol/mg) was isolated as in [4,5]. 4-(*N*-2-chloroethyl-*N*-methylamino) [^{14}C]-benzylamine (25 mCi/mmol) was synthesized as in [6,7]. 2,4-Dinitro-5-fluorophenylazide was prepared as in [8]. Poly(U) and [^{14}C]phenylalanine (360 mCi/mmol) were from Reanal (Hungary), *E. coli* $tRNA^{Phe}$ from Sigma, RNAs A and T_1 from Sankyo (Japan). Aminoacylation of $tRNA^{Phe}$ was performed as in [1]. Aminoacylation of azido-$tRNA^{Phe}$ was carried out in the same conditions, but Phe-azido-$tRNA^{Phe}$ was isolated from the reaction mixture by ethanol precipitation, not by ion-exchange chromatography. Azido-$tRNA^{Phe}$ was prepared as in [1]. Extent of tRNA modification (n) was 2 mol reagent residues/mol $tRNA^{Phe}$. Ternary complex 70 S ribosome–poly(U)–azido-$tRNA^{Phe}$ (P-site) was obtained by incubation of 9 nmol 70 S ribosomes, 1.5 mg poly(U) and 3.2 nmol azido-$tRNA^{Phe}$ in buffer A (10 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.4), 100 mM NH_4Cl, 1 mM DTT) at 0°C for 3 h. Azido-tRNA and ribosomes in all cases were preincubated

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

(20 min, 37°C) in buffer A. EF-Tu-dependent binding of Phe-azido-tRNA^{Phe} to ribosomes was performed by incubation of complex (I) 70 S–poly(U)–tRNA with complex (II) Phe-azido-tRNA^{Phe}–EF-Tu–GTP at 0°C for 15 min. In some experiments complex (I) was preincubated with 4×10^{-5} M tetracycline at 0°C for 30 min. Complex (I) was obtained by incubation of 10 nmol 70 S ribosomes, 840 nmol tRNA (unfractionated) and 3 mg poly(U) in 3 ml buffer A at 37°C for 15 min. To obtain complex (II) the mixture of 12 nmol Phe-azido-tRNA^{Phe}, 78 nmol EF-Tu–Ts was incubated in 4 ml buffer A containing 3×10^{-4} M GTP at 0°C for 15 min. Complex (III) 70 S–poly(U)–tRNA (P-site)–Phe-azido-tRNA^{Phe} (A-site) was isolated by centrifugation through 10% sucrose in buffer A at 4°C, 100000 × *g* for 4 h. UV irradiation ($\lambda > 350$ nm) of the complexes and following analysis of the labelled ribosomal components was done as in [2].

3. RESULTS AND DISCUSSION

Affinity labelling of the ribosomal P-site was done with deacylated azido-tRNA^{Phe} [1,2]. For the labelling of the ribosomal A-site it is reasonable to use Phe-azido-tRNA^{Phe} which may be directed to this site by EF-Tu and GTP. So acceptor activity of azido-tRNA was tested. Aminoacylation of modified tRNA^{Phe} was carried out in the conditions optimal for unmodified one [1]. Modification of tRNA^{Phe} resulted in reduction of acceptor activity which was found to be 55% for $n = 1$ mol reagent residues/mol tRNA^{Phe}, 40% for $n = 2$ and 30% for $n = 3$ assuming acceptor activity of unmodified tRNA^{Phe} to be 100%.

In the experiments on the labelling of the ribosomal A-site [^{12}C]Phe-[^{14}C]azido-tRNA^{Phe} ($n = 2$) was used. It is seen from table 1 that binding of this derivative to ribosomes is EF-Tu-dependent and proceeds at the A-site as revealed by the inhibi-

Table 1

Binding of azido-tRNA to ribosomes at the A- and P-sites in the presence of poly(U)

tRNA^{Phe} derivative	2-Fold excess of tRNA^{Phe} towards azido-tRNA	EF-Tu	Tetracyline	Binding, mol of azido-tRNA/mol ribosomes
Phe-azido-tRNA^{Phe}	–	+	–	0.4
Phe-azido-tRNA^{Phe}	–	–	–	0.05
Phe-azido-tRNA^{Phe}	–	+	+	0.02
Azido-tRNA^{Phe}	–	–	–	0.25[a]
Azido-tRNA^{Phe}	+	–	–	0.08[a]

[a] Binding analysis was performed by gel-filtration on Sephadex G-200 in buffer A. 100% binding should correspond to a value of 0.3 because ribosomes were taken in 3-fold excess towards azido-tRNA^{Phe} (see section 2)

Table 2

Distribution of the label between the ribosomal subunits after irradiation of the complexes of azido-tRNA with ribosomes and poly(U) at the A- and P-sites

Complex irradiated		Tetracycline	Mol azido-tRNA covalently linked to mol subunit	
A-site	P-site		30 S	50 S
–	[^{14}C]azido-tRNA^{Phe}	–	0.035	0.018
Phe-[^{14}C]azido-tRNA^{Phe}	tRNA	–	0.055	0.044
Phe-[^{14}C]azido-tRNA^{Phe}	tRNA	+	0.002	0.002

tion of the binding with 2×10^{-5} M tetracycline [9]. Complex 70 S ribosome–poly(U)–azido-tRNA^{Phe} (P-site) was obtained non-enzymatically in the same buffer A (10 mM Mg^{2+}). In this case the location of modified tRNA^{Phe} was proved by the competition test with an unmodified one (see table 1), which is known to bind only at the P-site under conditions used [9]. Both complexes were irradiated in the same conditions and then centrifuged in sucrose density gradient in dissociating conditions (0.5 mM Mg^{2+}). One can see from table 2 that location of the tRNA derivative at the A-site results in labelling of both subunits to about an equal extent. P-site location of azido-tRNA^{Phe} leads to labelling of both subunits too but labelling of the small subunit was 2-times that for the large subunit. The lack of unspecific photo-crosslinking of azido-tRNA^{Phe} to ribosome when the tRNA derivative is located at the P-site was shown in [1,2]. The same is true for the A-site photoreaction which is completely inhibited by 2×10^{-5} M tetracycline (see table 2). The only ribosomal proteins (5 S rRNA not tested) were labelled by tRNA^{Phe}-derivatives located at the A-site as well as at the P-site as analysed by sucrose density gradient centrifugation in the presence of SDS and EDTA [1].

Ribosomal proteins were extracted from the modified subunits by 2 M LiCl in 4 M urea. tRNA linked covalently to proteins was removed by hydrolysis with RNAs A and T_1. Distribution of the label among the proteins was analysed by 2-dimensional polyacrylamide gel electrophoresis [2] and is presented in fig.1. It is seen that the A-site location of the tRNA derivative results in labelling of the proteins S9, S15, S16, S17, S18, S19 and L8/L9, L13, L15, L27 whereas the P-site location gives another set of the labelled proteins S5, S9, S11, S12, S13, S19, S20, S21 and L14, L24, L27, L31 and L33. Earlier we identified a somewhat different set of the ribosomal proteins labelled by azido-tRNA^{Phe} located at the P-site at 20 mM Mg^{2+} [1,2]. So, protein S20 is labelled only at 10 mM Mg^{2+}. The more pronounced differences are observed for the 50 S proteins labelled by azido-tRNA^{Phe} located at the P-site at 20 mM Mg^{2+} (L11, L13, L14, L27 [1]) and at 10 mM Mg^{2+} (L14, L24, L27, L31, L33).

It is known that the association constant of tRNA with ribosomal P-site is decreased considerably by reducing the [Mg^{2+}] [10]. Therefore the differences between ribosomal components labelled at different [Mg^{2+}] may be explained by:

(i) The greater conformational flexibility of tRNA bound to ribosome at lower [Mg^{2+}];

(ii) Some conformational changes of the ribosome induced by decreasing of [Mg^{2+}].

It is seen from fig.1 that the sets of proteins labelled by azido-tRNA strongly depend on the location of the tRNA derivative indicating different arrangements of tRNA^{Phe} bound at the A- and P-site. Only a few data are known concerning the differences in the structural organization of the A- and P-sites. Authors in [11] used tRNA^{Phe} derivative bearing arylazido-group on 8-S^{4}U (I) or 47-X (II) residues. The derivative (I) labelled proteins of the 30 S subunit (preferentially protein S19) having been located at the A-site. After EF-G-promoted translocation of this derivative to P-site covalent reaction was not observed. However, the derivative (II) labelled proteins of both subunits and 23 S rRNA when located at the P-site, but the

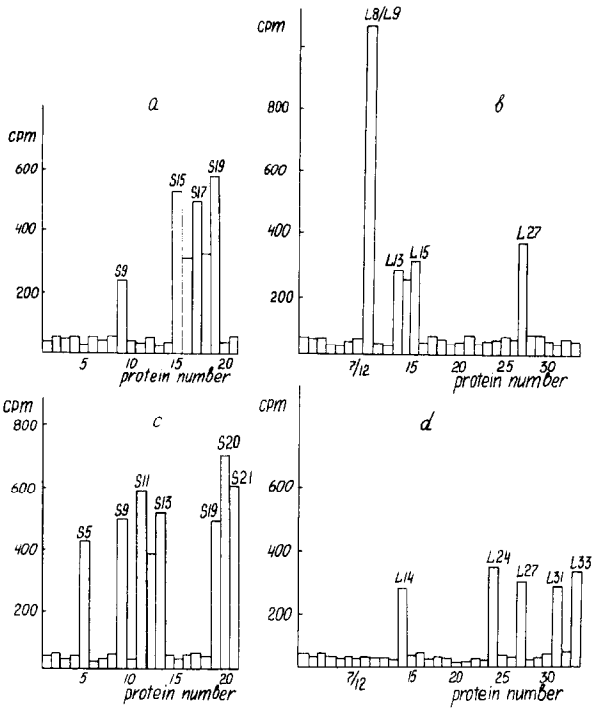

Fig.1. Distribution of the label among 30 S (a,c) and 50 S (b,d) proteins after irradiation of the complexes contained Phe-[^{14}C]azido-tRNA^{Phe} at the A-site (a,b) or [^{14}C]azido-tRNA^{Phe} at the P-site (c,d).

A-site location of this derivative did not result in covalent reaction. Hence at least two regions of $tRNA^{Phe}$ (8-S^4U and 47-X) have different arrangements at the A- and P-sites. Marked difference between the proteins of A- and P-sites was observed by direct UV-induced tRNA-ribosome crosslinks [12]. tRNA located at the A-site was linked to proteins S5, S9/S11, S10 and L2, L6, L10. P-site location of tRNA resulted in crosslinks with proteins S9/S11, S14, L2, L4, L7/L12, and L27/S13. These data are not in agreement with our results. This may be due to:

(i) Destruction of native structure of ribosome by UV irradiation at $\lambda = 254$ nm [13];

(ii) Length and flexibility of arylazido-groups which may result in labelling of proteins which are not in close contact with tRNA and therefore are not labelled by direct crosslinking;

(iii) Some proteins which are in close contact with tRNA which may give labile photoreaction products [14] and may be lost.

Our data demonstrate in detail the differences in arrangements of tRNA bound at the ribosomal A- and P-sites. Only proteins S9, S19 and L27 are labelled by tRNA derivatives at both A- and P-sites. L27 is known to be the common protein of acceptor and donor sites of peptidyl-transferase center [15].

By comparison of the proteins labelled by alkylating derivatives of oligonucleotides (mRNA-analogs) bearing the reactive group at the 3′-end [16] with the proteins labelled with Phe-azido-$tRNA^{Phe}$ at the A-site one may draw some conclusions concerning the decoding region of the A-site. So proteins S9, S15 and S18 are labelled both by mRNA analogs and the tRNA derivative located at the A-site. These proteins probably form the decoding region of the A-site. We consider proteins S5, S9, S11, S13, S19 as components of the decoding region of P-site from comparison of the proteins labelled by derivatives of oligonucleotides bearing an alkylating group at the 5′-end [16,17] with proteins labelled by azido-tRNA located at the P-site. Hence we may conclude that the decoding regions of the A- and P-sites differ significantly. Only protein S9 is common for both decoding regions.

REFERENCES

[1] Vladimirov, S.N., Graifer, D.M. and Karpova, G.G. (1981) FEBS Lett. 135, 155–158.
[2] Vladimirov, S.N., Graifer, D.M. and Karpova, G.G. (1982) FEBS Lett. 144, 332–336.
[3] Nirenberg, M.W., Matthaei, H. (1961) Proc. Natl. Acad. Sci. USA 47, 1588–1602.
[4] Arai, K.-I., Kawakita, M. and Kaziro, Y. (1972) J. Biol. Chem. 247, 7029–7037.
[5] Murphy, J.B. and Kies, M. (1960) Biochim. Biophys. Acta 45, 382–384.
[6] Belikova, A.M., Vakhrusheva, T.E., Vlassov, V.V., Grineva, N.I., Knorre, D.G. and Kurbatov, V.A. (1969) Molekul. Biol. 3, 210–220.
[7] Bogachev, V.S., Veniaminova, A.G., Grineva, N.I. and Lomakina, T.S. (1970) Izv. Sib. Otd. Akad. Nauk. SSSR, Ser. Khim. Nauk 6, 110–116.
[8] Wilson, D.F., Miyata, Y., Frecinska, M. and Varderkooi, J.M. (1975) Arch. Biochem. Biophys. 171, 104–107.
[9] Kirillov, S.V., Kemkhadze, K.Sh., Makarov, E.M., Makhno, V.I., Odintsov, V.B. and Semenkov, Yu.P. (1980) FEBS Lett. 120, 221–224.
[10] Kirillov, S.V., Katunin, V.I. and Semenkov, Yu.P. (1980) FEBS Lett. 125, 15–19.
[11] Ofengand, J., Lin, F.-L., Hsu, L., Keren-Zur, M. and Boublik, M. (1980) Ann. N-Y Acad. Sci. 346, 324–354.
[12] Abdurashidova, G.G., Turchinsky, M.F., Salikhov, T.A., Aslanov, Kh.A. and Budovsky, E.I. (1977) Bioorg. Khim. 3, 982–983.
[13] Yasuda, K. and Fukutome, H. (1970) Biochim. Biophys. Acta 217, 142–147.
[14] Ehresmann, B., Backendore, C., Ehresmann, Ch., Millon, R. and Ebel, J.-P. (1980) Eur. J. Biochem. 104, 255–262.
[15] Johnson, A.E. and Cantor, C.R. (1980) J. Mol. Biol. 138, 273–280.
[16] Gimautdinova, O.I., Karpova, G.G., Knorre, D.G. and Kobetz, N.D. (1981) Nucleic Acids Res. 9, 3465–3481.
[17] Gimautdinova, O.I., Karpova, G.G. and Kozyreva, N.A. (1982) Molekul. Biol. 16, 752–761.