Selection of tRNA by different polynucleotide–ribosomal protein complexes

Tago Sarapuu and Richard Villems*

Laboratory of Molecular Genetics, Institute of Chemical Physics and Biophysics, 14/16 Kingissepa Str., 202400 Tartu, Estonian SSR, USSR

Received 22 December 1982

Abstract not received

Synthetic polynucleotide *Ribosomal protein* *RNA–protein complex* *tRNA selection* E. coli *ribosome*

1. INTRODUCTION

Most of our current knowledge of the structure of the decoding site of the *Escherichia coli* ribosome comes from a number of different mRNA crosslinking and 30 S subunit reconstitution experiments. However, up to now, almost all the proteins from the 30 S subunit, as well as a number of those from the 50 S subunit, have been implicated in decoding (reviews [1–3]). Clearly, other methods are needed for a critical interpretation of the results obtained so far. It was previously shown that different synthetic polynucleotides and MS2 phage RNA form a largely invariable complex with certain *E. coli* ribosomal proteins, which, in turn bind tRNA [4]. We report here that poly(A)–, poly(C)– and poly(U)–50 S ribosomal subunit protein (TP50) complexes bind but do not select tRNA, while the same polynucleotides in the complex with 30 S subunit proteins (TP30) select their cognate tRNAs. We suggest that proteins S4, S9 and S13, found in these complexes, participate in the decoding of mRNA in the *E. coli* ribosome.

2. EXPERIMENTAL

Isolation of *E. coli* MRE600 ribosomal proteins, tRNA and ^{32}P-labelled bulk tRNA was as in [5]. 5′-end-labelling of *E. coli* $tRNA^{Phe}$ and $tRNA_1^{Val}$ (Boehringer, Mannheim; 1.3 nmol and 1.18 nmol/A_{260} unit) with [γ-^{32}P]ATP (Amersham, ~2000 Ci/mmol) was as in [6], and the tRNAs were used as stock solutions at 15 μM (10^6 cpm/nmol).

Synthetic polynucleotides (Reanal) were immobilized on epoxy-activated Sepharose 6B (Pharmacia) gel (25–45 A_{260} units/ml wet gel) [4]. Preformation of polynucleotide–ribosomal protein complexes and binding of ^{32}P-labelled bulk tRNA was as in [4] and was carried out in 10 mM Tris–HCl (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol (buffer A).

In titration experiments, a weighed amount of the complex preformed in the immobilized poly(U)-Sepharose gel column (about 50 mg of wet gel) was transferred into a siliconized 1.5-ml Eppendorf tube. A known molar amount of either [^{32}P]$tRNA^{Phe}$ or [^{32}P]$tRNA_1^{Val}$ in buffer A were added up to a total volume of 400 μl. The contents of the tube were mixed for 20 min and, after brief centrifugation (~5 s), two 40 μl aliquots were withdrawn and their radioactivity was measured. The volume of the sample was again adjusted to 400 μl by adding 80 μl of labelled tRNA. This cycle was repeated by rising the added tRNA concentration from zero to about 2 μM. Since no liberation

* To whom correspondence should be addressed

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

of the poly(U)-bound fraction of ribosomal proteins was observed during complex formation and titration with tRNAs, we took the molar amount of the preformed poly(U)–S4–S9–S13 complex equal to the molar amount of TP30 used for its formation. Other details of similar titration experiments are discussed elsewhere [5].

Selection of tRNA on various polynucleotide–protein complexes was carried out as follows: the polynucleotide–TP50 and –TP30 complexes were preformed using 8-ml immobilized polynucleotide-Sepharose gel columns, and 3 mg of TP50 or 2.4 mg of TP30. Unlabelled *E. coli* bulk tRNA (2 ml, 15 μM) was loaded onto the affinity columns and the bound fractions were eluted with 8 M urea, 4 M LiCl. After phenol treatment and ethanol precipitation these tRNA samples were aminoacylated with [^{3}H]phenylalanine (23.5 Ci/mmol), or [^{3}H]lysine (40 Ci/mmol), or [^{3}H]proline (46 Ci/mmol) (Amersham), with the 19 other cold amino acids present [7]. For 1 A_{260} unit of the initial bulk tRNA, the following levels of amino acid incorporation were obtained: Phe = 44 pmol; Lys = 60 pmol; Pro = 41 pmol.

3. RESULTS AND DISCUSSION

The chromatography of *E. coli* 70 S ribosomal proteins through an affinity column containing polynucleotides immobilized on Sepharose results in the formation of stable polynucleotide–protein complexes containing several 30 S and 50 S subunit proteins [4]. Here we varied the affinity chromatography conditions, so that the complexes of the 3 synthetic polynucleotides (poly(A); poly(U); poly(C)) with ribosomal proteins contained either proteins L2 and L17 (using TP50) or S4, S9 and S13 (with TP30).

The experiments described above compare binding efficiency of various tRNAs in the total tRNA population (bulk tRNA) to different polynucleotide–protein complexes, in order to registrate the enrichment of the bound fraction of tRNA in certain tRNA species. Fig.1 shows that both TP30– and TP50–polynucleotide complexes bind [^{32}P]tRNA while by themselves the immobilized polynucleotides do not.

Nevertheless, there is an apparent difference in the binding of tRNA to polynucleotide–TP50 and polynucleotide–TP30 complexes (fig.1). When low

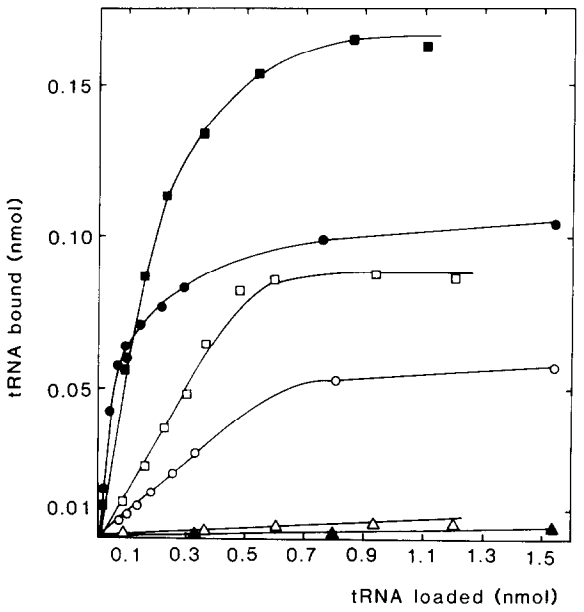

Fig.1. The binding of ^{32}P-labelled deacylated *E. coli* bulk tRNA to immobilized poly(A) (▲—▲); to poly(A)–TP50 complex (■—■); to poly(A)–TP30 complex (●—●), and to immobilized poly(U) (△—△); to poly(U)–TP50 complex (□—□); to poly(U)–TP30 complex (○—○) in 20 mM Tris–HCl (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$ and 6 mM 2-mercaptoethanol at 20°C (buffer A). 0.26 mg of TP50 or 0.2 mg of TP30 were loaded onto polynucleotide-gel columns (1 ml, 25–45 A_{260} units/ml). [^{32}P]tRNA (~10^5 cpm/μg) was loaded onto preformed mRNA–protein complex columns in buffer A at a concentration 15 μM. The bound fraction of tRNA, together with proteins, was eluted (8 M urea, 4 M LiCl) and its radioactivity was measured.

amounts of tRNA are added to the polynucleotide–TP50 complexes, most of the tRNA binds until all binding sites are filled. Therefore, it is obvious that the polynucleotide–TP50 complexes do not select a cognate tRNA, neither under conditions where the tRNA binding sites are not fully filled, nor at saturation. The results presented in table 1 were obtained using saturating amounts of tRNA (about 1 nmol of tRNA); they clearly reveal that none of the 3 polynucleotide–TP50 complexes select their cognate tRNA.

In sharp contrast, when tRNA is applied to the affinity columns at amounts below the plateau binding level, the poly(A)–TP30 complex binds only about one tenth, and the poly(U)–TP30 com-

Table 1

Amounts of $tRNA^{Phe}$, $tRNA^{Lys}$ and $tRNA^{Pro}$ found/1 A_{260} unit of the tRNA bound to various polynucleotide–TP50 complexes

	Initial amounts	Immobilized polynucleotide		
		poly(U)	poly(A)	poly(C)
$tRNA^{Phe}$	44	38	28	30
$tRNA^{Lys}$	60	51	50	56
$tRNA^{Pro}$	41	37	42	53

For details see section 2

Table 2

Amounts of $tRNA^{Phe}$, $tRNA^{Lys}$ and $tRNA^{Pro}$ found/1 A_{260} unit of the tRNA bound to various polynucleotide–TP30 complexes

	Immobilized polynucleotide		
	poly(U)	poly(A)	poly(C)
$tRNA^{Phe}$	325.0	4.5	67.7
$tRNA^{Lys}$	1.2	383.0	4.2
$tRNA^{Pro}$	58.0	2.7	433.0

Initial amounts are given in table 1. For details see section 2

plex one sixth of the bulk tRNA applied (fig.1); i.e., the complex seems to select tRNA. Indeed, the data in table 2 show that all polynucleotide–TP30 complexes select their cognate tRNAs: the poly(U) complex selectively binds $tRNA^{Phe}$ (codons UUU and UUC); the poly(A) complex selectively binds $tRNA^{Lys}$ (codons AAA and AAG) and the poly(C) complex selectively binds $tRNA^{Pro}$ (codons CCC, CCA, CCG and CCU).

How does this primitive tRNA selection mechanism work? Using an experimental approach described in detail elsewhere [5], we found that the binding of cognate $tRNA^{Phe}$ to the poly(U)–TP30 complex can be characterized by a dissociation constant (K_d) equal to 1.4×10^{-7} M (fig.2). On the other hand, under identical experimental conditions non-cognate $tRNA_I^{Val}$ hardly interacts with the poly(U)–S4–S9–S13 complex (fig.2). Since the K_d of the polyuridylic acid–$tRNA^{Phe}$ complex is about 3–4 orders of magnitude lower than the value given above [8], we

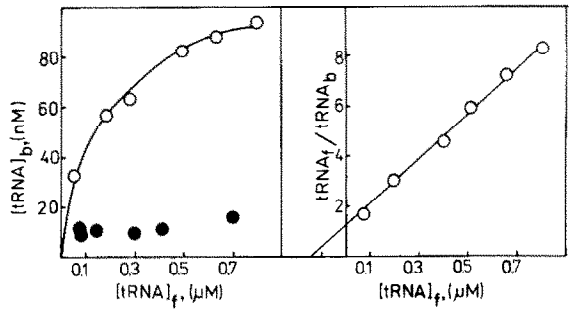

Fig.2. Binding of $tRNA^{Phe}$ (○—○) and $tRNA_I^{Val}$ (●—●) to the preformed poly(U)–S4–S9–S13 complex. Left: binding isotherms; right: linear transformation of $tRNA^{Phe}$ binding isotherm. 5′-end-labelled [^{32}P]tRNAs were prepared from *E. coli* $tRNA^{Phe}$ and $tRNA_I^{Val}$ (Boehringer, Mannheim; 1.3 nmol and 1.18 nmol/A_{260} unit). A constant amount of the preformed poly(U)–protein complex was titrated with increasing amounts of either $tRNA^{Phe}$ or $tRNA_I^{Val}$. K_d of the binding of $tRNA^{Phe}$ to preformed poly(U)–protein complex was found graphically as the intercept with the abscissa by plotting $tRNA_f/tRNA_b$ *vs* $[tRNA]_f$, where $tRNA_f$ and $tRNA_b$ are the free and bound $tRNA^{Phe}$, respectively. For other details see section 2.

suggest that the set of 30 S subunit proteins S4, S9 and S13 (or a subset thereof), are directly involved in the mRNA decoding on the *E. coli* ribosome.

Therefore, as in intact ribosomes, the selection of a cognate tRNA can be demonstrated with either the counterparts of 30 S subunit– or 70 S ribosome–mRNA complexes; i.e., in our experiments with TP30– or TP70–mRNA complexes. Again, as with the ribosomes, no selection was found mediated by 50 S subunits (here, by our TP50–mRNA complexes).

ACKNOWLEDGEMENTS

We are grateful to Dr P. Jelenc for critically reading the manuscript, and to Drs C.G. Kurland and M. Ustav for discussions.

REFERENCES

[1] Kuechler, E. and Ofengand, J. (1979) in: Transfer RNA: Structure, Properties and Recognition (Schimmel, P.R., Söll, D. and Abelson, J.N. eds) pp.413–444, Cold Spring Harbor Laboratory.

[2] Cooperman, B.S. (1980) in: Ribosomes: Structure, Function and Genetics (Chambliss, G. et al. eds) pp.531–553, University Park Press, Baltimore.
[3] Ustav, M. and Villems, R. (1981) in: Structure and Function of Ribosomes (Bogdanov, A.A. and Kretovich, V.L. eds) pp.116–174, Viniti Press, Moscow.
[4] Sarapuu, T. and Villems, R. (1982) Eur. J. Biochem. 124, 275–281.
[5] Metspalu, E., Ustav, M., Maimets, T. and Villems, R. (1982) Eur. J. Biochem. 121, 383–389.
[6] Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Nucl. Acids Res. 4, 2527–2538.
[7] Stanley, W.M. (1974) in: Methods in Enzymology (Moldave, K. and Grossmann, L. eds) vol.29 E, 530–547.
[8] Eisinger, J., Feuer, B. and Yamane, T. (1970) Proc. Natl. Acad. Sci. USA 65, 638–644.