PROPERTIES OF ALANYL-OLIGONUCLEOTIDE, PUROMYCIN, AND *STAPHYLOCOCCUS EPIDERMIDIS* GLYCYL-tRNA IN INTERACTION WITH ELONGATION FACTOR Tu:GTP COMPLEX

Makoto KAWAKAMI, Shozo TANADA and Shosuke TAKEMURA
Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusaku, Nagoya, Japan 464

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1. Introduction

Aminoacyl-tRNA (aa-tRNA), elongation factor EF-Tu, and GTP forms a ternary complex which plays a key role in protein biosynthesis [1–3] Structural requirements of tRNA for recognition of EF-Tu would provide a good system for studying nucleic acid:protein interaction. Formation of the ternary complex with various aa-tRNAs was studied by Skoultchi et al. [4] using the gel filtration method. Tarragó et al. [5] showed that the ternary complex flowed out of a column of Sephadex G-100 faster than EF-Tu or aa-tRNA.

We have investigated to ascertain whether the ternary complexes are formed between *Escherichia coli* EF-Tu:GTP complex and aa-tRNA analogues which lack GG (in the dihydrouridine loop) and GTψC sequences commonly found in tRNA [6]. These common sequences may be the candidates of the sites recognized by EF-Tu:GTP, since various aa-tRNAs are able to form the ternary complex with EF-Tu:GTP. Alanyl-oligonucleotide (UCCACCA-Ala), puromycin, and *Staphylococcus epidermidis* Gly-tRNAs [6] have been used for this study. The results presented here show that none of the above aa-tRNA analogues and *S. epidermidis* Gly-$tRNA^{Gly}_{I}$ form a stable ternary complex as assayed by the gel filtration method.

2. Materials and methods

L-[U-^{14}C]Alanine (130 mCi/mmol) and [2-^{3}H]glycerine (2000 mCi/mmol) were purchased from the Radiochemical Centre, England, and [U-^{14}C]glycine (83 mCi/mmol) was obtained from Dai-ichi Pure Chemicals, Co., Japan. [2-^{3}H]Glysine was diluted with cold glycine to 500 mCi/mmol. [methoxy-^{3}H]Puromycin (3.82 Ci/mmol) was obtained from New England Nuclear, U.S.A. Pyruvate kinase and GTP were obtained from Boehringer Mannheim, Germany.

Torulopsis utilis tRNA was acylated with [^{14}C]alanine using *T. utilis* aminoacyl-tRNA synthetase as described previously [7]. [^{14}C]Ala-oligonucleotide was prepared by RNase T_1 digestion of *T. utilis* Ala-tRNA by a slight modification of the method of Lessard and Pestka [8]. The sequence must be UCCACCA-Ala as judged by the established primary structure of *T. utilis* $tRNA^{Ala}$ [9].

S. epidermidis $tRNA^{Gly}_{I}$ (mixture of $tRNA^{Gly}_{I}$ and $tRNA^{Gly}_{IB}$) and $tRNA^{Gly}_{III}$ were prepared from *S. epidermidis* IFO 12993 cells by the method of Stewart et al. [10]. The preparation of $tRNA^{Gly}_{I}$ was contaminated with a small amount of $tRNA^{Gly}_{II\ or\ III}$, and used in the following experiments without further purification. These preparations were acylated with [^{14}C]glycine using *S. epidermidis* aminoacyl-tRNA synthetase.

A EF-Tu:GDP complex was supplied by Drs Arai and Kajiro who prepared and purified from *E. coli* Q 13 cells as described previously [11]. This complex was converted to EF-Tu:GTP as follows; the reaction mixture contained 50 μmol Tris–HCl (pH 7.4), 50 μmol NH_4Cl, 10 μmol $MgCl_2$, 5 μmol dithiothreitol, 0.1 μmol GTP, 20 μg pyruvate kinase, 5 μmol phosphoenolpyruvate, and EF-Tu:GDP (100 μg, as the amount of protein estimated by the method of Lowry et al. [12]) in a final volume of 1.0 ml. The

reaction mixture was incubated at 30°C for 10 min, and then chilled to 0°C.

The above reaction mixture contains the EF-Tu:GTP complex thus formed. To this mixture was added 0.1 ml of the labeled aa-tRNAs or aa-tRNA analogues as indicated in the legends to the figures. The solution was kept at 0°C for 10 min to form the ternary complex, and immediately applied to a Sephadex G-100 column (1.0 × 41 cm) preequilibrated with a solution containing 50 mM Tris–HCl (pH 7.0), 100 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 1 mM EDTA. The same solution was used for elution at the rate of 9 ml/hr at 4°C. Fractions (1.0 ml each) were collected and their aliquots were counted for radioactivity in a liquid scintillation counter as described previously [13].

3. Results

In the absence of EF-Tu:GTP, the radioactive peak of *T. utilis* Ala-tRNA was eluted at fraction number 30, but after incubation with EF-Tu:GTP, the radioactive peak shifted to fraction number 24 as shown in fig.1A. The elution profile of EF-Tu revealed a shoulder at fraction number 24, indicating that the ternary complex was formed. In cases of UCCACCA-[^{14}C]Ala and [^{3}H]puromycin used as aa-tRNA analogues, no such shift of radioactive peak was observed after incubation with EF-Tu:GTP (fig.1B and 1C).

Possible interaction between EF-Tu:GTP and *S. epidermidis* Gly-tRNA$^{Gly}_{I}$ was also tested. This tRNA lacks both GG and GTψC sequences and does not participate in protein synthesis [10]. As indicated by a shoulder at fraction number 24 in fig.2A, the [^{14}C]Gly-tRNA$^{Gly}_{I}$ preparation formed a small amount of the ternary complex with EF-Tu:GTP probably due to the contamination of tRNA$^{Gly}_{II\ or\ III}$. However, the possibility still remained that some of Gly-tRNA$^{Gly}_{I}$ itself may also interact with EF-Tu:GTP. Hence, [^{14}C]Gly-tRNA recovered from pooled fractions 28–35 in fig.2B was incubated again with EF-Tu:GTP, but the radioactive shoulder was no longer appeared. On the other hand, all the added [^{14}C]Gly-tRNA$^{Gly}_{III}$ complexed with EF-Tu:GTP and was eluted in the first peak (fraction number 24) as shown in fig.2C. In a double labeling experiment

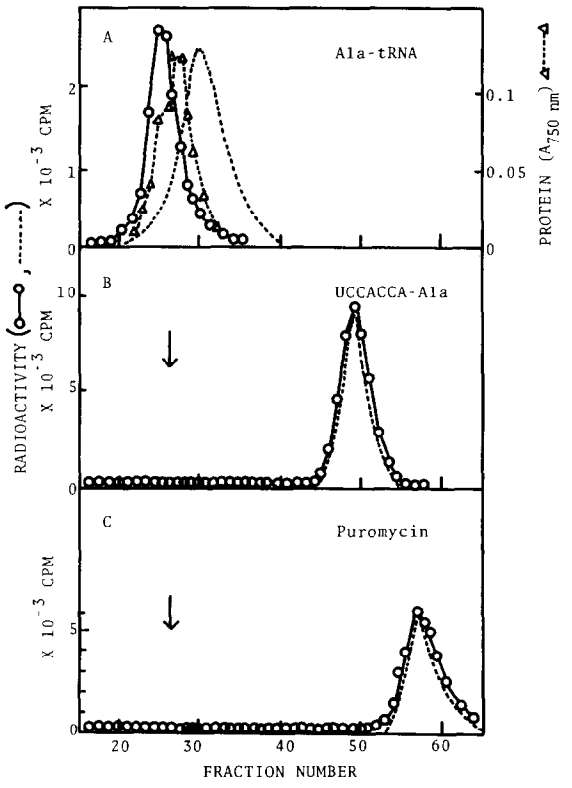

Fig.1. Elution profiles from Sephadex G-100 indicating that UCCACCA-[^{14}]Ala and [^{3}H]puromycin do not form a complex with *E. coli* EF-Tu:GTP, while [^{14}C]Ala-tRNA does. The reaction mixture containing EF-Tu:GTP was incubated A) with [^{14}C]Ala-tRNA (121 pmol, 2.1 × 10^{4} cpm) from *T. utilis*; B) with UCCACCA-[^{14}C]Ala (121 pmol, 2.1 × 10^{4} cpm); and C) with [^{3}H]puromycin (52 pmol, 4.4 × 10^{4} cpm). Gel filtration was performed as in Materials and methods. The arrows indicate the position of EF-Tu in the effluent. (o—o) EF-Tu:GTP was added. (------) control (without EF-Tu:GTP). (^——^) Estimation of protein.

(fig.2D) using [^{14}C]Gly-tRNA$^{Gly}_{I}$ and [^{3}H]Gly-tRNA$^{Gly}_{III}$, all the added Gly-tRNA$^{Gly}_{III}$ and a small portion of the Gly-tRNA$^{Gly}_{I}$ preparation formed the ternary complex. These results show that *S. epidermidis* Gly-tRNA$^{Gly}_{I}$ itself did not form the ternary complex with EF-Tu:GTP under the present conditions.

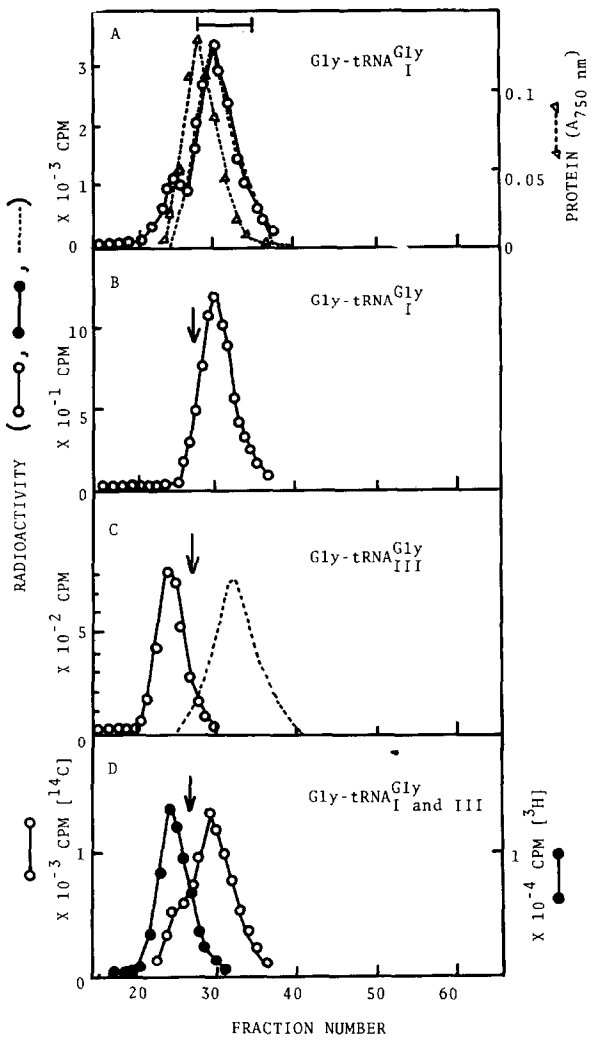

Fig.2. Elution profiles from Sephadex G-100 indicating that *S. epidermidis* Gly-tRNA$^{Gly}_{I}$ does not form a complex with EF-Tu:GTP, while Gly-tRNA$^{Gly}_{III}$ does. The reaction mixture containing EF-Tu:GTP was incubated A) with *S. epidermidis* [^{14}C]Gly-tRNA$^{Gly}_{I}$ (396 pmol, 4.4 × 10^{4} cpm); B) with *S. epidermidis* [^{14}C]Gly-tRNA$^{Gly}_{I}$ recovered from fraction number 28–35 in A); C) with *S. epidermidis* [^{14}C]Gly-tRNA$^{Gly}_{III}$ (378 pmol, 4.2 × 10^{4} cpm); and D) with both *S. epidermidis* [^{14}C]Gly-tRNA$^{Gly}_{I}$ (168 pmol, 1.87 × 10^{4} cpm) and *S. epidermidis* [^{3}H]Gly-tRNA$^{Gly}_{III}$ (540 pmol, 1.2 × 10^{5} cpm), and applied on the column as described in Materials and methods. (o——o) and (•——•) EF-Tu:GTP was added. (———) control (without EF-Tu:GTP). (△——△) Estimation of protein.

4. Discussion

Various aa-tRNAs engage in the ternary complex formation [2]. Uncharged tRNA [14–16], acetyl aa-tRNA [17], formyl Met-tRNA [2], Met-tRNA$^{Met}_{F}$ [2], denatured Leu-tRNA [18], aa-tRNA in which the 3′-terminal ribose is cleaved at the 2′, 3′-carbon-carbon bond [19], and aa-tRNA of which 3′-terminus has an additional cytidine in the form of -CCCA [20] do not form the stable ternary complex. Deamination of aa-tRNA [21], modification of nucleotide in the anticodon loop [22,23], cross-linking between positions 8 and 13 from the 5′-terminus [22], base changes at positions 15 and 31 in *E. coli* S^{+}_{III}tRNA^{Tyr} [24], and replacement of T, ψ, and uridine derivatives in tRNA by 5-fluorouridine [25] do not affect the complex formation. Furthermore, aminoacyl-viral RNA can form the complex [26]. Some of these observations suggest that the anticodon loop is not involved in the interaction with EF-Tu:GTP, and amino acid stem containing aminoacylated CCA is important in the complex formation [27,28].

Ono et al. suggested that the regions near the 3′- and 5′-termini of tRNA are the sites of interaction between aa-tRNA and EF-Tu, since *E. coli* tRNA$^{Met}_{F}$, which is unable to form the ternary complex, is not base paired between the 5′-terminus (C) and the fifth base (A) from the 3′-terminus [2]. Replacement of C to U at the 5′-terminus of tRNA results in the formation of the U·A base pair. Schulman and Her showed that this modified tRNA became able to form the ternary complex with EF-Tu:GTP [29]. However the present data show that the ternary complex cannot be formed with *S. epidermidis* Gly-tRNA$^{Gly}_{I}$ whose 5′-terminus is base paired as in all the other usual tRNAs [6]. Hence the 5′-terminal base pair (in tRNA) is not the only site for binding with EF-Tu:GTP.

T. utilis Ala-tRNA which contained CCA, GTψC, and GG sequences commonly found in tRNA was able to form the ternary complex. *S. epidermidis* Gly-tRNA$^{Gly}_{III}$ participating in protein synthesis [10] formed the complex with EF-Tu:GTP. Although the total nucleotide sequence of tRNA$^{Gly}_{III}$ is unknown, it probably contains the common sequences CCA, GTψC, and GG. On the other hand, UCCACCA-Ala, puromycin, and *S. epidermidis* Gly-tRNA$^{Gly}_{I}$, which cannot form the ternary complex, do not contain

the GTψC and GG sequences. Accordingly the GG and/or GTψC sequences, in addition to the amino acid stem (including the CCA sequence), seem to be of considerable importance for the recognition of EF-Tu:GTP complex.

Although *S. epidermidis* Gly-tRNA$^{Gly}_{I}$ has the anticodon sequence (UCC) complementary to the glycine codons [6], it cannot bind to ribosome [10]. The present data indicate that the reason of the inability of this tRNA in in vivo protein synthesis may be ascribed to the failure of the ternary complex formation rather than that of the binding to ribosome.

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