Interaction of methionine-specific tRNAs from *Escherichia coli* with immobilized elongation factor Tu

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The interaction of three different Met-tRNAs^{Met} from *E. coli* with bacterial elongation factor (EF) Tu · GTP was investigated by affinity chromatography. Met-tRNA^{fMet} which lacks the base pair at the end of the acceptor stem binds only weakly to EF-Tu · GTP, while Met-tRNA^{mMet} has a high affinity for the elongation factor. A modified Met-tRNA^{fMet} which has a C_1–G_{72} base pair binds much more strongly to immobilized EF-Tu · GTP than the native aminoacyl(aa)-tRNA with non-base-paired C_1A_{72} at this position, demonstrating that the base pair including the first nucleotide in the tRNA is one of the essential structural requirements for the aa-tRNA · EF-Tu · GTP ternary complex formation.

Protein biosynthesis *Initiator tRNA* *Elongation factor Tu* *Modified tRNA* *Affinity chromatography* *Protein-nucleic acid interaction*

1. INTRODUCTION

EF-Tu plays a key role in protein biosynthesis in bringing aa-tRNA via an aa-tRNA · EF-Tu · GTP ternary complex to the ribosomal A-site [1–3]. A stable ternary complex is formed with all elongator tRNAs but aminoacylated bacterial initiator tRNA, which is unique among all tRNAs sequenced so far in having a non-base-paired residue at the 5′-terminus [4], is a weak ligand for EF-Tu · GTP [5–7]. Different results were obtained by Tanada et al. [8], who determined a dissociation constant for Met-tRNA^{fMet} similar to those of elongator tRNAs.

By modifying a ligand, its structural requirements for an interaction with protein can be investigated. We modified tRNA^{fMet} by exchanging the A at position 72 for a G so that a base pair with the opposite C at position 1 can be formed. This modified tRNA was tested for its ability to interact with EF-Tu · GTP. Bisulfite modification of Met-tRNA^{fMet} from *Escherichia coli* resulting in a C→U conversion at position 1 and consequently in the formation of a base pair enhances the affinity for EF-Tu · GTP [6]. Thus here we provide evidence that not the nature of the nucleotides in positions 1 and 72, but the presence of the base pair adjacent to the C-C-A terminus is responsible for the observed effects.

For the investigation of the interaction of 3 Met-tRNA species we applied an affinity chromatography with immobilized EF-Tu · GTP, which can be used for the isolation of tRNA isoacceptors and for the qualitative analysis of all the interactions in which the elongation factor is involved [9,10].

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Abbreviations: EF-Tu, elongation factor Tu; aa-tRNA, aminoacyl-tRNA

2. MATERIALS AND METHODS

L-[U-^{14}C]Methionine (280 mCi/mmol) and L-[*methyl*-^{3}H]methionine (15 Ci/mmol) were purchased from Amersham-Buchler (Braunschweig, FRG). Unfractionated tRNA from *E. coli*

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MRE 600 cells came from Boehringer (Mannheim). $tRNA^{fMet}$ was prepared according to [11] and could be aminoacylated with 1170 pmol methionine/A_{260} unit, while $tRNA^{mMet}$ was isolated from *E. coli* bulk tRNA by affinity chromatography [10]. It was free of $tRNA^{fMet}$ as demonstrated by electrophoretic analysis and could not be formylated. Enzymatical and chemical procedures were used to modify $tRNA^{fMet}$. $tRNA^{fMet}$ was treated with nuclease S_1 to remove the 3′-terminal tetranucleotide as described [12] and further treated with periodate [13] to remove the 3′-adenosine. $tRNA^{fMet}$ (C_1–G_{72}) was synthesized by joining the truncated product to pGACCA which was obtained by enzymatic phosphorylation of chemically synthesized G-A-C-C-A as described for the synthesis of a modified $tRNA^{fMet}$ [14]. Methionyl-tRNA synthetase (EC 6.1.1.10, from *E. coli*) was partially purified to a specific activity of 298 units/mg as described [15]. The method of Leberman et al. [16] was utilized for the isolation of EF-Tu·GDP from *E. coli*, which had a specific activity of 21000 units/mg. The affinity column was prepared by covalent attachment of aa-tRNA·EF-Tu·GTP ternary complex to cyanogen bromide-activated Sepharose 4B from Pharmacia (Uppsala). The reaction mixture containing 65 μM aa-tRNA·EF-Tu·GTP ternary complex and 330 μl swollen and equilibrated cyanogen bromide-activated Sepharose 4B in 100 mM sodium borate, pH 8.0, 200 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol and 50 μM GTP in a final volume of 1 ml was rotated end-over-end for 2 h at room temperature. The affinity matrix was then treated as described [10].

The aminoacylation reactions were performed in a buffer containing 50 mM Hepes, pH 7.5, 50 mM KCl, 50 mM NH_4Cl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 4 mM ATP, 1 μM $tRNA^{Met}$, 6 μM [^{14}C]- or [^{3}H]methionine and 0.05 units methionyl-tRNA synthetase in a volume of 60 μl. After incubation at 37°C for 20 min the reaction mixture was deproteinized, the aa-tRNA was ethanol precipitated and isolated by centrifugation. The dried pellet was dissolved in 100 μl buffer A (50 mM Hepes (pH 7.5), 50 mM KCl, 50 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 μM GTP). All affinity chromatographies of Met-tRNAs on immobilized EF-Tu·GTP were performed at 5°C. The elution profiles were monitored by determining the radioactivity in appropriate aliquots precipitable in 5% aqueous trichloroacetic acid. After application of the samples to the affinity column and incubation for 5 min at 5°C, it was washed with buffer A, then with buffer B (50 mM Hepes (pH 7.5), 150 mM KCl, 50 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 μM GTP) followed by buffer C (50 mM Hepes (pH 7.5), 1 M KCl, 50 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 μM GTP). The conditions of the 2D gel electrophoresis were essentially those of Fradin et al. [17]. The tRNA was detected after staining in 'Stains all' (Serva, Heidelberg) [18].

3. RESULTS AND DISCUSSION

The $tRNA^{Met}$ isoacceptors present in *E. coli* $tRNA^{bulk}$ were aminoacylated and isolated by affinity chromatography on immobilized EF-Tu·GTP [10]. Fig.1a shows the 2D electrophoretic pattern of the tRNA fraction which is not bound to the affinity matrix. This is consistent with a typical pattern of bulk tRNA. Neither of the 2 species which were bound to the column and eluted with a buffer of high ionic strength (fig.1b) is identical with native $tRNA^{fMet}$ as demonstrated by different electrophoretic mobility (fig.1c) and the inability of the tRNAs to become formylated. This result indicates that no interaction between the immobilized EF-Tu·GTP and $Met\text{-}tRNA^{fMet}$ takes place under the conditions of the affinity chromatography, i.e. excess of deacylated tRNAs. In a control experiment it was shown that there was no methionyl-tRNA formyltransferase activity in the synthetase preparation, so that formylation of $Met\text{-}tRNA^{fMet}$ as the reason for non-binding to the affinity matrix can be excluded. Corresponding results were obtained when an affinity matrix with immobilized EF-Tu·GTP from *Thermus thermophilus* was utilized for the isolation of $tRNA^{Met}$ isoacceptors (not shown) suggesting that the weak interaction with $Met\text{-}tRNA^{fMet}$ might be a common feature of all bacterial EF-Tu.

The qualitative differences in affinity of the Met-tRNA species to EF-Tu·GTP were also demonstrated in experiments shown in fig.2, when purified $Met\text{-}tRNAs^{Met}$ were used for affinity chromatography. Here the specific interaction of

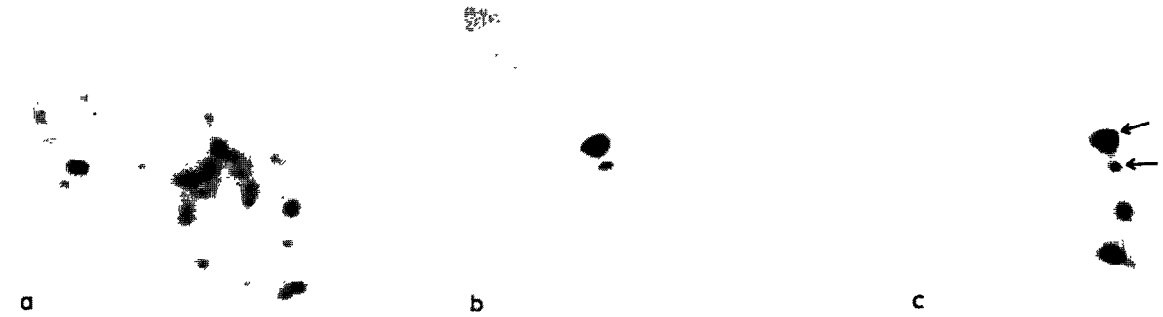

Fig.1. 2D gel electrophoretic analysis of $tRNA^{Met}$ isoacceptors isolated by affinity chromatography. (a) tRNAs not interacting with EF-Tu·GTP in the break-through fraction; (b) Met-tRNAs bound to the column of immobilized EF-Tu·GTP; (c) as b with addition of a sample of authentic $tRNA^{fMet}$. The spots corresponding to tRNA are indicated by arrows.

$Met\text{-}tRNA^{fMet}$ (C_1A_{72}) with immobilized EF-Tu·GTP takes place. However, the elution of the bound $Met\text{-}tRNA^{fMet}$ could be achieved by a slight increase of the salt concentration of the binding buffer (buffer B). A quantitative elution of the remaining $Met\text{-}tRNA^{fMet}$ was obtained immediately after application of the high salt buffer. In contrast, binding of almost all of the $Met\text{-}tRNA^{mMet}$

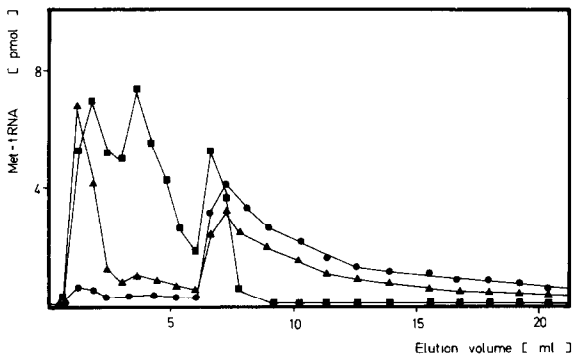

Fig.2. Interaction of the Met-tRNA species with immobilized EF-Tu·GTP from *E. coli* (220 μl gel matrix with about 1.6 mg covalently bound protein). About 60 pmol of each of the Met-tRNA species were applied to the column in double-labelling experiments. Hereby combinations of 2 particular species, one labelled with [^{3}H]- and the other with [^{14}C]methionine were used (—■—, $Met\text{-}tRNA^{fMet}$ C_1A_{72}; —▲—, $Met\text{-}tRNA^{fMet}$ C_1–G_{72}; —●—, $Met\text{-}tRNA^{mMet}$). The profiles correspond to average values of several experiments with all combinations of the Met-tRNA species. The column was washed with 2.5 ml buffer A followed by 3 ml buffer B and 15 ml buffer C. The flow rate was 80 μl/ml.

to the affinity matrix occurred. It was not eluted with the buffer of intermediate ionic strength (buffer B) and the elution with buffer C resulted not in a sharp, but elongated peak. We conclude from this experiment that elongator $Met\text{-}tRNA^{mMet}$ binds significantly stronger to immobilized EF-Tu·GTP than $Met\text{-}tRNA^{fMet}$. This finding is at variance with the report of Tanada et al. [8] and supports the observations made in [5–7]. A significantly better binding of the modified $Met\text{-}tRNA^{fMet}$ (C_1–G_{72}) as compared to the native species (C_1A_{72}) was observed. Modified $Met\text{-}tRNA^{fMet}$ (C_1–G_{72}) was partially eluted in the exclusion volume of the column, but about 62% of this aa-tRNA remained bound to the column. The bound substance was then eluted in a way similar to that of $Met\text{-}tRNA^{mMet}$ demonstrating the high affinity of the bound, modified $Met\text{-}tRNA^{fMet}$ (C_1–G_{72}) to immobilized EF-Tu·GTP. From this experiment it can be concluded that there must be 2 different components in the modified $Met\text{-}tRNA^{fMet}$ (C_1–G_{72}) preparation, one with a high affinity for EF-Tu·GTP similar to that of elongator tRNAs and the other showing no detectable interaction with the affinity matrix. This inactive component is probably due to the modification procedure in which inactive side products can be formed by attachment of additional nucleotide residues to the 5′-end of tRNA.

Clear qualitative differences between modified and native $Met\text{-}tRNA^{fMet}$ were demonstrated by affinity chromatography reflecting the important structural role of the modified part of the tRNA in ternary complex formation. Taking the results of

Schulman et al. [6] into account it can be concluded that the presence of the base pair at the end of the acceptor stem is one of the most important structural requirements for aa-tRNA·EF-Tu·GTP ternary complex formation.

Other authors have already demonstrated the involvement of the acceptor stem of aa-tRNA in the ternary complex with EF-Tu·GTP [19–23]. However, other structural features of the tRNA besides the terminal base pair must also be recognized by the elongation factor as indicated by following results. Gly-tRNA$_I^{Gly}$ from *Staphylococcus epidermis*, whose 5′-terminus is base-paired, does not take part in ternary complex formation [24]. On the other hand, a tyrosine-specific suppressor tRNA, which lacks this base pair, is brought to the ribosome by EF-Tu·GTP to take part in translation and to act as a suppressor [25]. Schulman et al. [6] observed that the ternary complex between bisulfite-modified Met-tRNA^{fMet} (U_1–A_{72}) and EF-Tu·GTP is less stable than complexes formed with elongator aa-tRNAs [6]. However, in the case of Met-tRNA^{fMet} (C_1–G_{72}) we did not observe a diminished affinity to EF-Tu·GTP as compared to Met-tRNA^{mMet}. This discrepancy might be a consequence of side reactions in the case of bisulfide modification. Therefore we conclude that a single base mutation in the position 72 of tRNA^{fMet} converts this tRNA to a ligand in respect to EF-Tu·GTP binding, which is indistinguishable from other elongator tRNAs.

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