Requirement of modified residue m1A9 for EF-Tu binding to nematode mitochondrial tRNA lacking the T arm

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ABSTRACT

Most of nematode mitochondrial (mt) tRNAs lacking the T arm have 1-methyladenosine (m' A) at position 9. To investigate the effect of m' A, we constructed a nematode Ascaris suum mt tRNAMet containing only m' A9 as the modified nucleoside by means of molecular surgery. Although the unmodified A. suum mt Met-tRNAMet did not bind to nematode mt EF-Tu, the m' A9-containing tRNA bound to the EF-Tu, suggesting that m' A at position 9 is necessary for binding of nematode mt tRNAs lacking the T arm to the EF-Tu, probably because of maintenance of the L-shape-like structure or interaction with the C-terminal amino acid residues of the EF-Tu.

INTRODUCTION

Bacterial elongation factor Tu (EF-Tu) binds mainly to two regions within tRNA; the terminal region of the acceptor stem and the T stem (1), whereas most nematode mitochondrial (mt) tRNAs lack the T stem (2,3,4). We previously found that a nematode mt EF-Tu, which has unusual C-terminal extension, recognized the T arm-lacking tRNA of nematode mitochondria (5). To elucidate its detailed recognition mechanism, we prepared unmodified Ascaris suum mt tRNAMet by chemical synthesis and enzymatic ligation (molecular surgery) as described previously (6). However, the unmodified Met-tRNAMet had no binding activity toward the EF-Tu (Fig. 2b). Conserved modified nucleoside in all the known T arm-lacking tRNAs of nematode mitochondria (3,4) is 1-methyladenosine (m' A) at position 9, and almost all of nematode mt tRNAs lacking the T arm have A9 at DNA level (7). Therefore, in this work, we focused on m' A9, and constructed A. suum mt tRNAMet containing only m' A9 as the modified nucleoside by molecular surgery. The binding activity of the tRNAMet to the EF-Tu was analyzed in order to estimate the effect of m' A9.

MATERIALS AND METHODS

Construction of tRNA containing m' A

A. suum mt tRNAMet was synthesized according to the scheme shown in Fig. 1. 3'-,5'-diphosphorylation of m' A (SIGMA) was performed as described (8) and purified on a DEAE-sephadex A25 column (Pharmacia). The purified nucleotide was confirmed by mass spectrometry. tRNA fragments were chemically synthesized and purified as described (6). Treatments of 3'- or 5'-ends of RNA with T4 polynucleotide kinase, NalC and bacterial alkaline phosphatase (BAP) necessary for aimed ligation reaction, were performed as described (6, 9). Fragment 1 (AAUAAGAU) (90 uM) and pm'Ap (210 uM) were incubated at 11 °C for 15 hour in a buffer consisting of 50 mM Tris-HCl (pH7.5), 15 mM MgCl2, 3.5mM dithiothreitol, 15 µg/ml BSA, 300 uM ATP, 5 % PEG and 2.5 units/µl T4 RNA ligase. Incorporation of m' A into Fragment 1A was confirmed by thin layer chromatography. Fragments 1A2 and 3 (UACCCUUGUGGUUUUCUCUCUUAUGCCAC) (70 uM each) were incubated under the same conditions as ligation of Fragment 1 and pm'Ap except that the concentration of ligase was 3.75 units/µl. Fragments 1A2 and 3 were ligated and purified as described (6).

Hydrolysis protection assay

Binding of EF-Tu to aminoacyl-tRNA was analyzed by hydrolysis protection assay (8). Recombinant EF-Tu of Caenorhabditis elegans mitochondria was prepared as described (3). A. suum mt tRNAMet was aminoacylated using [15S]Met and purified as described (5). The hydrolysis protection assay was performed basically according to Ohtsuki...
et al. (5). The EF-Tu was preincubated without $^{[35]S}$Met-tRNA$_{Mcl}$ at 30°C for 5 min to form GTP-form. After that, $^{[35]S}$Met-tRNA$_{MeI}$ was added and ternary complex was formed. Deacylation reaction was carried out at 30°C.

RESULTS AND DISCUSSION

A. suum mt tRNA$_{Met}^m$ containing m$^1$A9 as the sole modified nucleoside was constructed as shown in Fig. 1. Each ligation step was confirmed by 10% denatured polyacrylamide gel electrophoresis analysis. The overall yield of tRNA was about 5% (molar ratio) of Fragment 1 used at the first step. It is difficult to isolate large amount of cognate tRNA from nematode mitochondria. This tRNA-preparation method helps us to perform experiments to study the interaction between nematode mt EF-Tu and tRNAs.

In order to examine the effect of m$^1$A9 in nematode mt tRNAs for EF-Tu binding, we performed hydrolysis protection assay (10). The Met-tRNA$_{Met}^m$ containing m$^1$A9 was protected by the EF-Tu (Fig. 2a). On the other hand, the protection of the unmodified Met-tRNA$_{MeI}$ was not detected (Fig. 2b). Although the difference between these two Met-tRNAs$_{MeI}$ was only m$^1$A9, the EF-Tu binding efficiency differed considerably. The results presented here strongly suggest that nematode mt tRNAs lacking the T arm requires m$^1$A9 for EF-Tu binding. We previously reported that unmodified A. suum mt tRNA$_{Met}^m$ had low efficiency of aminoacylation compared with native one (6). Probably m$^1$A9 contributes to maintain L-shape-like structure of T arm-lacking tRNAs, like the case of human mt tRNA$^{Lys}$ (11). Although the difference of the overall structures between the unmodified and m$^1$A9-containing tRNAs was small (6), the difference seemed to be important for biological activity, especially for EF-Tu binding. Otherwise the C-terminal extension of the EF-Tu might interact around m$^1$A9, because it was suggested from the crystal structure of the bacterial EF-Tu-aminoacyl-tRNA complex (1) that the C-terminus of bovine mt EF-Tu might touch connector region of the tRNA (12).

REFERENCES