Nucleotide sequence of non-initiator methionine tRNA from Bacillus subtilis

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Received 12 August 1980

ABSTRACT

Non-initiator methionine tRNA ($tRNA^{Met}_m$) was purified from *Bacillus subtilis* W 168 by a consecutive use of several column chromatographic systems. The nucleotide sequence was determined to be pG-G-C-G-G-U-G-U-A-G-C-U-C-A-G-C-G-G-C-D-A-G-A-G-C-G-U-A-C-G-G-U-U-C-A-U-m^6A-C-C-C-G-U-G-A-G-G(m^7G)-U(D)-C-G-G-G-G-G-T-Ψ-C-G-A-U-C-C-C-C-U-C-C-G-C-C-G-C-U-A-C-C-A_{OH}. The nucleosides of G_{46} and U_{47} were partially modified to m^7G and D, respectively. The nucleotide sequence shows a unique feature that the position adjacent to 3'-end of the anticodon C-A-U is occupied by m^6A, not by t^6A, although the $tRNA^{Met}_m$ belongs to a group of tRNAs which recognize codons starting with A.

INTRODUCTION

We have already reported the primary structure of *B. subtilis* initiator $tRNA^{Met}_f$ (1,2). *B. subtilis* $tRNA^{Met}_f$ shows a high degree of resemblance to *E. coli* $tRNA^{Met}_f$, although it contains a smaller number of modified nucleotides than the latter. In order to know whether *B. subtilis* $tRNA^{Met}_m$ is similar to *E. coli* $tRNA^{Met}_m$, as seen in the case of initiator tRNAs, we purified it and determined its nucleotide sequence. The tRNAs which recognize codons starting with A contain *N*-[(9-β-D-ribofuranosylpurin-6-yl) carbamoyl]threonine (t^6A) or its derivatives at the position next to 3'-end of anticodon except prokaryotic initiator tRNAs. Unexpectedly, *B. subtilis* $tRNA^{Met}_m$ was found to contain 6-methyladenosine instead of t^6A or its derivatives at the position following the anticodon.

MATERIALS AND METHODS

Purification of $tRNA^{Met}_m$. *B. subtilis* W 168 grown in Penassay medium was harvested in a late logarithmic stage. In the process of purification of $tRNA^{Met}_f$ from *B. subtilis* previously described (2), two peaks of methionine acceptor activity were observed in BD-cellulose column chromatography. The first peak was formylatable and $tRNA^{Met}_f$ was purified from the

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fraction eluted in the first peak. The second peak was not formylatable, and $tRNA^{Met}_{m}$ was further purified to homogeneity on Sepharose 4B (3) and DE-23 columns.

Sequence analysis. Sequencing method of oligonucleotides obtained by complete digestion with RNase T_1 or pancreatic RNase was essentially as described in references 2 and 4. Overlapping sequences were constructed by post labeling techniques. The 3'-end of purified $tRNA^{Met}_{m}$ was labeled with RNA ligase (P-L Biochemical Inc.) and [5'-^{32}P]pCp (2000-3000 Ci/mmol). ^{32}P-3'-End labeled $tRNA^{Met}_{m}$ was partially hydrolyzed in 0.05 M Na-carbonate buffer (pH 9.0) at 90°C for 15 min. ^{32}P-3'-End labeled hydrolyzate was separated by two dimensional electrophoresis on polyacrylamide gel according to De Wachter and Fiers (5) except that 1 mm thin slab gel for first dimension, 2 mm thin for second, and urea was omitted from the electrophoresis buffer. In addition, the method of Stanley and Vassilenko (6) was used, but the ^{32}P-5'-end labeled RNA fragments were separated by two dimensional polyacrylamide gel electrophoresis under the same condition as described above instead of the usual one dimensional separation. After recovery from the gel, each fragment was completely digested with nuclease P_1 and 5'-terminal nucleotide was identified by thin layer electrophoresis in 5 % acetic acid adjusted with aqueous ammonia to pH 3.5.

RESULTS

Complete digestion with RNase T_1 or pancreatic RNase. The content of m^7G ranged from 0 to 0.4 mole per mole of tRNA. The sum of G-A-G-m^7G-U- and G-A-G-G-U- was almost 1 mole per mole of tRNA in pancreatic RNase digests of $tRNA^{Met}_{m}$. About 40 % of U_{47} was found to be modified to D.

Identification of m^6A was done by comparison with authentic m^6A for UV-absorption spectra at several pHs and its Rf values of thin layer chromatographies on Avicel SF plate. Applied solvent systems were isobutyric acid : 0.5 M NH_4OH (5:3) and 2-propanol : conc. HCl : H_2O (70:15:15).

Overlapping and total nucleotide sequence. ^{32}P-3'-End labeled $tRNA^{Met}_{m}$ was partially hydrolyzed at pH 9.0, and the hydrolyzate was applied on two dimensional gel electrophoresis. The mobility of each spot depends on removal of nucleotide in order, then nucleotide sequence can read from 3'-end part of tRNA toward 5'-end. A reading of nucleotide sequence from $^{3'}U_{72}$-C-G- --- to --- A-G-A-$D_{20.1}$-$^{5'}$ was possible. An ambiguous sequence of RNase T_1 digests, A-U-C-(C-C-C-, U-C-)C-G-, was determined to be A-U-C-C-C-C-U-C-C-G- by this method. Nucleotide sequence of further 5'-end region was

determined by 5'-nucleotide analysis after ^{32}P-5'-end labeled of formamide hydrolyzate of $tRNA_m^{Met}$. ^{32}P-5'-End labeled hydrolyzate was applied on two dimensional polyacrylamide gel electrophoresis, then spots of main stream of autoradiograph were eluted and digested with nuclease P_1. 5'-Nucleotide analysis permitted determination of most of nucleotide sequence from $^{5'}G_4$-U- --- to --- G-G-C_{19}-$^{3'}$. Ambiguities in this sequence were removed by two dimensional gel electrophoresis of partial hydrolyzate of ^{32}P-5'-end labeled $tRNA_m^{Met}$. The established total sequence of $tRNA_m^{Met}$ are shown in Fig. 1.

DISCUSSION

The $tRNA_m^{Met}$ from *B. subtilis* is composed of 76 nucleotides and $D_{20.1}$, m^6A_{37}, T_{54}, Ψ_{55} are contained as modified nucleosides. In addition, G_{46} and U_{47} are partially modified to m^7G_{46} and D_{47} respectively. When the $tRNA_m^{Met}$ is compared with $tRNA_m^{Met}$ from *E. coli* (7), base differences are seen in 27 positions. Also the same number of 27 base differences are observed, when the comparison was made by initiator methionine tRNA from *B. subtilis*. All these tRNAs are chargeable with methionine by *E. coli* methionyl tRNA synthetase.

Fig. 1 Clover-leaf model of *B. subtilis* $tRNA_m^{Met}$.

It is well-known all tRNAs which recognize codons starting with A contain t^6A or its derivatives at the position adjacent to 3'-end of the anticodon, except prokaryotic initiator tRNAs (9). In *B. subtilis* $tRNA_m^{Met}$, however, the site is occupied by m^6A instead of t^6A. At the position adjacent to 3'-end of the anticodon in *B. subtilis* tRNAs, $tRNA^{Thr}$ contains t^6A (8) and $tRNA_1^{Lys}$ contains ms^2t^6A (Yamada, Y., Ishikura, H. and McCloskey, J. A., unpublished data) which was initially thought as a G derivative (4). This means that *B. subtilis* cells do contain enzyme(s) which modifies A to t^6A. Therefore, modification to m^6A cannot be ascribed to the lack of such enzyme(s). From sequential point of view, the sequence around t^6A is always U-t^6A-A (11), i. e., t^6A is preceded by U and followed by A without exception. In order for adenosine to be modified to t^6A, it seems necessary to be situated at an appropriate position in the anticodon loop and preceded by U and followed by A. We think that the sequence of U-A-A is essential for the modification to t^6A. In *B. subtilis* $tRNA_m^{Met}$, the corresponding sequence is U-A-C, i. e., A is preceded by U, but followed by C, not by A. Therefore, we suppose that the A was modified not to t^6A but to m^6A for this reason. The same kind of situation was found in non-initiator methionine tRNA from green alga *Scenedesmus obliquus* chloroplast (10). In that tRNA, the corresponding sequence is also U-A-C as in $tRNA_m^{Met}$ from *B. subtilis* and the A is not modified to t^6A.

ABBREVIATIONS

ms^2t^6A : *N*-[(9-β-D-ribofranosly-2-methylthio-6-yl)carbamoyl]threonine

This work was partly supported by a grant from the Ministry of Education, Science and Culture of Japan.

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