NUCLEOTIDE SEQUENCE OF INITIATOR tRNA FROM *BACILLUS SUBTILIS*

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1. Introduction

Primary structures of several eukaryotic initiator tRNAs have been recently elucidated [1–3]. They commonly lack T-ψ-sequence in loop IV (so-called T-ψ loop) of the clover-leaf structure, and this position is replaced by A-U-sequence. This feature is considered a likely prerequisite for the initiation of cytoplasmic polypeptide synthesis in eukaryotes. On the other hand, $tRNA_f^{Met}$ of *Escherichia coli*, the only prokaryotic initiator tRNA of known sequence so far [4], possesses T-ψ-sequence in loop IV equally to the majority of tRNAs of which the primary structures were reported. It is quite important to determine whether the presence of T-ψ-sequence in loop IV is a common characteristic to all initiator tRNAs of prokaryotic origin or not. To elucidate this problem was one of our motives to initiate the structural study of tRNAs from *Bacillus subtilis*.

Here we report the total nucleotide sequence of $tRNA_f^{Met}$ of *B. subtilis*. The sequence of 77 nucleotides in length bears fair resemblance on the whole to that of *E. coli* $tRNA_f^{Met}$, containing T-ψ-sequence in loop IV. We observe, however, the following two kinds of difference: (1) Nucleotide replacements in six positions; three in loop I (D-loop) and three in the stem part of loop IV, (2) a lower degree of modifications of nucleosides. A ribothymidine, a pseudouridine and a dihydrouridine are the only modified nucleoside-constituents in the initiator tRNA of *B. subtilis*.

2. Materials and methods

Bacillus subtilis W 168 was grown in Penassay medium under an aerated condition. Cells were harvested at a late logarithmic stage. The method of Zubay [5] was applied for the isolation of total tRNA. The $tRNA_f^{Met}$ was purified with the use of two column chromatographic systems. A single peak of methionine-acceptor activity observed in DEAE-Sephadex A-50 (pH 7.5) column chromatography [6] was separated into two peaks in subsequent BD-cellulose column chromatography [6]. The assay of formate-acceptor activity in the presence of [^{14}C]tetrahydrofolate and crude *B. subtilis* transformylase preparation [7] revealed that the $tRNA^{Met}$ of the first peak is formylatable whereas that of the second peak is not. The first peak of methionine-acceptor activity showed complete accordance with the peak of A_{260} absorbance. $tRNA_f^{Met}$ fraction of the first peak was collected and used as the starting material for sequence determination. The sequential study showed the purity of this $tRNA_f^{Met}$ preparation to be more than 90%. The initiator tRNA thus purified can accept methione in almost equal efficiency by methionyl-tRNA synthetase from *B. subtilis* or *E. coli*. Formylation of this $tRNA_f^{Met}$ is achieved also by transformylase from *E. coli* [7]. The products of complete digestion with RNase T_1 and pancreatic RNase were separated by DEAE-Sephadex A-25 column chromatography in neutral and acid conditions in the presence of 7 M urea, if necessary, by thin-layer chromatography on Avicel SF plates. The nucleotide sequences of oligonucleotides were determined by further enzymic digestion with complementary enzyme, nuclease P_1 from *Penicillium citrinum* [8], silkworm nuclease [9], *E. coli* polynucleotide phosphorylase, RNase U_2, RNase T_2 and/or *E. coli* alkaline phosphatase, followed by column, paper and/or thin-layer chromatography.

Overlapping sequences were constructed by isola-

ting the products of limited digestion with RNase T_1 or pancratic RNase. After partial digestion with each RNase, the oligonucleotides were separated with the use of two column chromatographic systems on DEAE-cellulose and DEAE-Sephadex A-25. Analysis of oligonucleotides was carried out, after extensive hydrolysis with RNase T_1 or pancreatic RNase, by two dimensional fingerprinting technique developed for analysis of ^{32}P-labeled RNA by Sanger et al. [10], the same method as we have used for the sequence determination of *E. coli* serine tRNAs [11,12]. Spots on DEAE-cellulose paper were detected by ultraviolet absorption.

Details of the procedures mentioned above will be reported separately.

Table 1
Products found by complete degradation of *B. subtilis* $tRNA_f^{Met}$

With RNase T_1	With pancreatic RNase
9 Gp	A_{OH}, 15 Cp, 4 Up, 6 p
CGp	4 GCp
AGp	AUp
UGp	GUp
$CAACCA_{OH}$	2 AACp
2 CAGp	AGCp
AAGp	AGUp
2 UCGp	GGDp
DAGp	pCp
CUCGp	AAAUp
UUCGp	GGGCp
pCGp	AGGTp
CCCCCGp	GGAGCp
CUCAUAACCCGp	GGGGUp
TψCAAAUCCUGp	GAAGGUp

3. Results

The oligonucleotides obtained by complete enzymic digestion of $tRNA_f^{Met}$ are listed in table 1. Only three nucleotides, a thymidine, a pseudouridine and a dihydrouridine were detected as the modified constituents. The sequences established by combining the data obtained from complete digestion with both RNases, the nucleotide sequences of large fragments isolated by partial RNase digestion, and the deduced total primary sequence are given in fig.1. In this sequence, we have insufficient confirmatory evidence for the sequence constituting from 9th to 18th nucleotides from 5′-terminus without making use of analogies with other tRNAs. From the analytical data of overlapping sequences, two possibilities were left for that sequence: GGAGCAGUUC and UCGGAGCAGU. It will be quite reasonable to choose the former, since the former sequence fully fits to the common characteristics to all tRNAs, whereas the latter does not. The total sequence has a chain length of 77 nucleotides, starting with pCG and ending in $CAACCA_{OH}$, the same as *E. coli* initiator tRNA [4]. In fig.2, the sequence was arranged in a clover-leaf form. The clover-leaf model of *E. coli* $tRNA_f^{Met}$ [4] is also shown for comparison, where the nucleotide sequences or residues different from *B. subtilis* initiator tRNA are enclosed in brackets.

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                10        20        30        40        50        60        70     77
(a)  pCGCGGGGUGGAGCAGUUCGGDAGCUCGUCGGGCUCAUAACCCGAAGGUCGCAGGTψCAAAUCCUGCCCCCGCAACCA OH
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(b)                      GGDAG                                AGGTψCAAAUCCUG
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     pCGCGGGGUGGAGCAGUUCGGDAGCUCGUCGGGCp   AACCCGAAGGUCGCAGGTψCAAAUCCUGCCCCCGCp
(c)          UGGAGCAGUUCGGDAGCUCGUCGGGCUCAUAACCCGAAGGUCGCAGGTψCAAAUCCUGp
                         (G)DAGCUCGUCGGGCUCAUAACCCGAAGGUCGCAGGTψCAAAUCCUGCCCCCGCAACCA OH
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Fig.1. Nucleotide sequences of *B. subtilis* $tRNA_f^{Met}$ (a) and oligonucleotides used for overlapping. (b) The sequences established by combining the data obtained from complete digestion with RNase T_1 and pancreatic RNase. (c) The oligonucleotides obtained by partial digestion with RNase T_1 or pancreatic RNase. The parenthesized G denotes uncertainty of the presence.

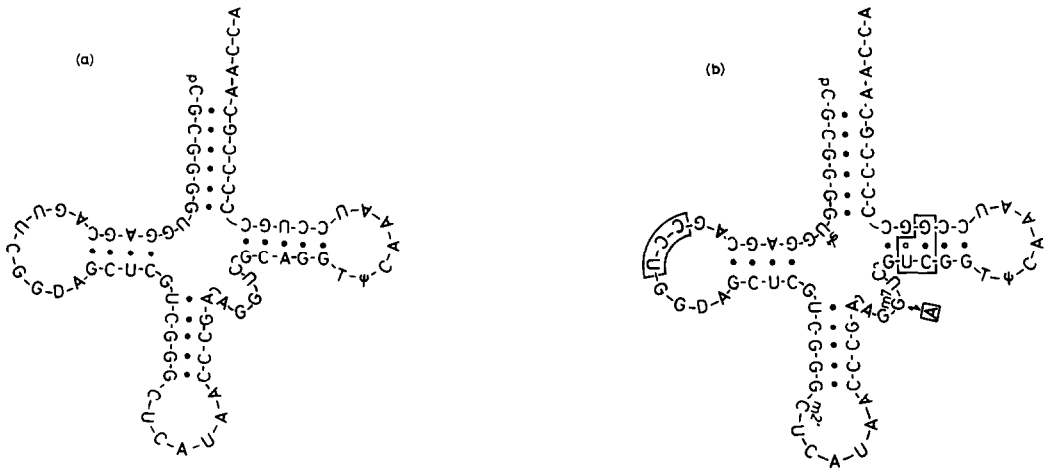

Fig.2. (a) Clover-leaf model of *B. subtilis* $tRNA_f^{Met}$. (b) Clover-leaf model of *E. coli* $tRNA_f^{Met}$ [4]. The arrow points to the substitution found in the minor species of *E. coli* $tRNA_f^{Met}$ [4]. The nucleotide residues or sequences different from *B. subtilis* $tRNA_f^{Met}$ are enclosed with brackets. The differences in the state of modifications were disregarded in comparison.

4. Discussion

The primary structure of *B. subtilis* $tRNA_f^{Met}$ thus determined bears a considerable degree of resemblance to that of *E. coli* origin. The base difference is seen in six positions when comparison was made with major *E. coli* $tRNA_f^{Met}$ and seven with the minor one [4]. T-ψ-sequence is contained as in *E. coli* initiator tRNA The presence of T-ψ-sequence in the initiator tRNAs of blue-green alga [13], *Streptococcus faecalis* [14] and *Mycoplasma mycoides* var *capri* [15] has been recently reported. It is also found that the initiator tRNA of *Thermus thermophilus* contains one molar amount of 5-methyl-2-thiouridine followed by ψ-C-sequence ([16], and Watanabe, K. and Nishimura, S., personal communication). It is now concluded that G-T-ψ-sequence in loop IV is a common characteristic to all the initiator tRNAs of prokaryotic origin if we disregard different states of modification observed in occasion.

In all the initiator tRNAs of prokaryotic origin, pC-G is detected as 5′-terminal sequence [4,13–15]. Although the nucleoside at the 5th position from 3′-terminus are not always identical [13], 5′-pC seems commonly unpaired [4,13–15]. On the other hand, the terminal regions of all the eukaryotic tRNAs form Watson-Crick type of base-pairs [1–3,17]. The absence of the first base-pair in the terminal region thus appears to be a unique feature common to all the prokaryotic initiator tRNAs. The responsibility of this unpairedness for inability of *E. coli* $tRNA_f^{Met}$ to bind with *E. coli* T_u factor and for the resistance of *E. coli* fMet-$tRNA_f^{Met}$ toward peptidyl tRNA hydrolase has been proposed by Schulman *et al.* [18–20].

The position adjacent to 3′-end of anticodon sequence in *B. subtilis* $tRNA_f^{Met}$ is occupied by unmodified adenosine, like that of *E. coli* [4]. No modified adenosine residue was detected also in *S. faecalis* or mycoplasma $tRNA_f^{Met}$'s [14,15]. In the initiator tRNAs of eukaryotic origin, however, this position is occupied by modified adenosine residues [1–3]. The unmodified state of adenosine residue at this position seems to be another feature common to all the prokaryotic initiator tRNAs. It also might be related to their unique properties.

One of the two different regions between initiator tRNAs of *B. subtilis* and *E. coli* is in the stem part of loop IV [4]. The pattern of base-pairings in this region seems common in those of *B. subtilis* and *S. faecalis* [14]. The nucleotide sequence in loop IV is identical among *E. coli, B. subtilis, S. faecalis* and blue-green alga $tRNA_f^{Met}$'s, but not in mycoplasma $tRNA_f^{Met}$ [15], in disregard of modification at the thymidine site. As for loop I, *E. coli* and *S. faecalis* initiator tRNAs share the identical sequence [4,14],

whereas base replacement is observed at three consecutive positions in the *B. subtilis* $tRNA_f^{Met}$. All of the regions different between $tRNA_f^{Met}$'s of *E. coli* and *B. subtilis* belong to the sites where bases are different among the compared $tRNA^{Met}$'s, both in the case of $tRNA_f^{Met}$'s formylatable by *E. coli* transformylase [2], and in the case of $tRNA^{Met}$'s aminoacylatable by *E. coli* methionyl-tRNA synthetase [3].

Another distinct feature of *B. subtilis* initiator tRNA is a low degree of modification. Only three modified nucleoside residues were detected. Three positions occupied by 4-thiouridine, 2′-*O*-methylcytidine and 7-methylguanosine in the major species of *E. coli* $tRNA_f^{Met}$ [4] receive no modification in *B. subtilis* $tRNA_f^{Met}$. This type of modification pattern is similar to that seen in the initiator tRNA of *S. faecalis* grown in plus-folate medium [14]. Further lower degree of modification is observed in the initiator tRNAs of *S. faecalis* grown in minus-folate medium [14] and of *Mycoplasma* [15].

Chromatographic differences in RPC-5 system between tRNAs from spores and cells in exponential growth of *B. subtilis* were reported with several isoaccepting tRNAs [21,22]. Although no difference was observed chromatographically with $tRNA_f^{Met}$ [21], the modification pattern of $tRNA_f^{Met}$ in spores might be different from that in exponentially growing cells.

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