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Nucleotide sequence of valine tRNA$_{mo^5UAC}$ from Bacillus subtilis

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ABSTRACT

A valine tRNA was purified from *Bacillus subtilis* W168 by a combined use of several column chromatographic systems. The nucleotide sequence was determined to be pG-G-A-G-G-A-U-U-A-G-C-U-C-A-G-C-D-G-G-G-A-G-A-G-C-A-U-C-U-G-C-C-U-mo^{5}U-A-C-m^{6}A-A-G-C-A-G-A-G-G-m^{7}G-U-C-G-G-C-G-G-T-Ψ-C-G-A-G-C-C-G-U-C-A-U-C-C-U-C-C-A-C-C-A$_{OH}$ with the main use of non-labeled tRNA and with the subsidiary use of [^{32}P]-post-labeled sample. This tRNA contains 5-methoxyuridine (mo^{5}U) at the wobble position of the anticodon. A binding experiment of valyl-tRNA to ribosomes revealed that mo^{5}U is recognized by A and G, and fairly well by U.

INTRODUCTION

We have isolated a large amount of *B. subtilis* tRNA and studied their structures using non-labeled tRNA with the intention of preventing from missing or mistaking modified nucleotides. As a result, we found that *B. subtilis* tRNA contains some interesting minor components, i.e. 5-methoxyuridine (mo^{5}U)[1], 5-carboxymethylaminomethyluridine[2] and 5-carboxymethylaminomethyl-2-thiouridine[3], in the anticodon sequences. The main valine tRNA from *B. subtilis* as well as tRNA^{Ala} and tRNA^{Thr} contains mo^{5}U instead of uridin-5-oxyacetic acid (cmo^{5}U) as in *E. coli* tRNAs[4-6]. Here we report the purification, complete nucleotide sequence and coding properties of the tRNA^{Val} containing mo^{5}U.

MATERIALS AND METHODS

Most of the materials, enzymes and methods used for the purification and sequence analysis of tRNA$^{Val}_1$ have been described[7,8].

RESULTS

Purification of tRNA$^{Val}_1$: Valine accepting activity was separated into two peaks on the DEAE-Sephadex A-50 column chromatography of bulk tRNA (1-2g) from *B. subtilis* W168. The first eluting main peak of tRNA^{Val} (referred to as tRNA$^{Val}_1$) was further purified by a combined use of sepharose 4B, BD-cellulose

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and RPC-5 column chromatographic systems. The purity of $tRNA^{Val}_1$ reached more than 90% judged by chromatographic profile and complete digestion analysis with RNases.

Sequence analysis: The products of complete digestion with RNase T_1 and RNase A of cold $tRNA^{Val}_1$ were separated by two-dimensional fingerprinting technique or column chromatographies on DEAE-Sephadex A-25 at pH 7.5 and 2.7 as reported[1]. Nucleotide composition and nucleotide sequence were determined by enzymatic digestion with RNase T_1, RNase A, RNase T_2, nuclease P_1, RNase U_2 and/or silkworm nuclease followed by cellulose thin-layer and/or paper chromatography[7]. Overlapping procedure was performed by analysis of partial digest of cold $tRNA^{Val}_1$ with nuclease S1 followed by fingerprinting analysis and one- or two-dimensional polyacrylamide gel sequencing technique of 5'- or 3'-[^{32}P]-end-labeled $tRNA^{Val}_1$ (Fig.1). The deduced nucleotide sequence is shown in Fig.2.

Codon recognition of [^{14}C]-valyl-$tRNA^{Val}_1$: *B. subtilis* $tRNA^{Val}_1$ was aminoacylated with an *E. coli* aminoacyl-tRNA synthetase preparation and purified by gel filtration on Sephadex G-75 followed by acetylated DBAE-cellulose chromato-

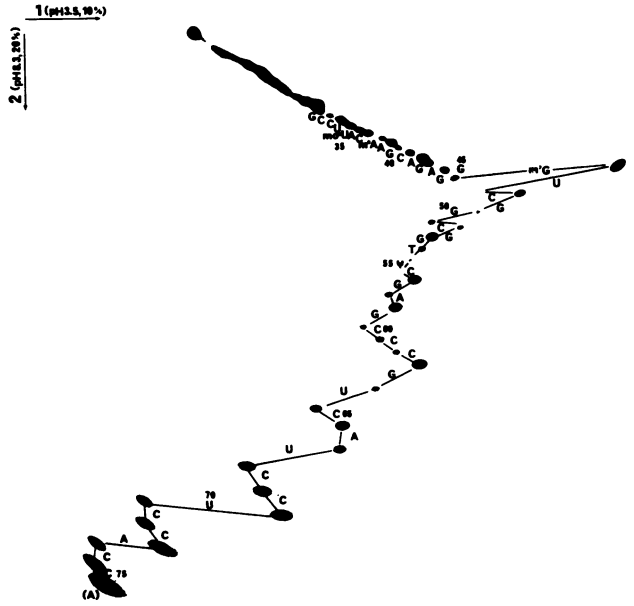

Fig.1 Two-dimensional gel electrophoresis of partial alkaline hydrolysate of [^{32}P]-3'-end-labeled $tRNA^{Val}_1$.
1st dimension: 10% polyacrylamide gel electrophoresis at pH 3.5
2nd dimension: 20% polyacrylamide gel electrophoresis at pH 8.3

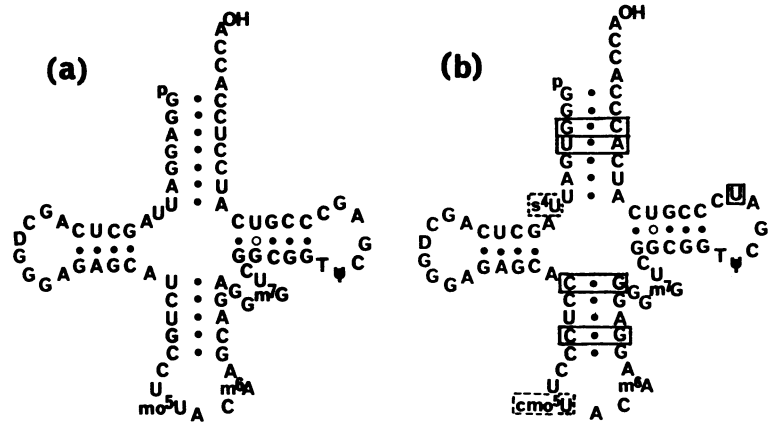

Fig.2 Clover-leaf structures of $tRNA^{Val}$'s from *B. subtilis* and *E. coli*.
(a) Structure of $tRNA_1^{Val}$ from *B. subtilis*.
(b) Structure of $tRNA_1^{Val}$ from *E. coli*. Nucleotides different to those of $tRNA_1^{Val}$ from *B. subtilis* are shown in boxes. Differences in the state of modification are enclosed with broken line.

graphy. Aminoacyl-tRNA binding to *E. coli* ribosomes was assayed by the procedure as described[9]. The binding of valyl-$tRNA_1^{Val}$ to ribosomes was stimulated by the triplet codons GpUpA and GpUpG, and less efficiently by GpUpU. Recognition by GpUpU was 30-50 per cent efficiency of the others (data not shown).

DISCUSSION

B. subtilis $tRNA^{Val}$ can be aminoacylated by *E. coli* aminoacyl-tRNA synthetase. Comparison of the sequence of *B. subtilis* $tRNA_1^{Val}$ with those of *E. coli* $tRNA_1^{Val}$ (anticodon -cmo^5U-A-C-) shows that only nine nucleotides are different and two different base modifications occur at U_8 and U_{34} (Fig.2). The wobbling nucleoside cmo^5U in *E. coli* $tRNA_1^{Val}$ was replaced by mo^5U in *B. subtilis* $tRNA_1^{Val}$. The pairing properties of cmo^5U in $tRNA_1^{Val}$ and $tRNA_1^{Ser}$ from *E. coli* were previously examined[10,11]. These results indicate that cmo^5U can be recognized by U in the third letter of codons at about 20 per cent of efficiency when compaired with A and G. A similar pattern of specificity for codon recognition was observed with mo^5U. Both modified nucleosides show the structural similarity, that is, 5-substitution of uracil ring by O-methyl derivatives. This structure was not observed in other uridine derivatives in the wobble position of two codon letter tRNAs so far investigated[12].

The other valine tRNAs from *E. coli* in which the first letter of the anticodon is occupied by G differ from *B. subtilis* $tRNA_1^{Val}$ in about 30 nucleotides. A difference between *B. subtilis* and eucaryote cells (yeast, rabbit liver, mouse myeloma and human placenta) enlarges to 33-39 nucleotides even if post-transcriptional modifications of nucleotides are disregarded in comparison[12].

Total primary sequence analysis of *B. subtilis* $tRNA_1^{Val}$ was carried out by the convensional methods with the main use of non-labeled samples. The separation of mo^5U nucleotide from U nucleotide is difficult in usual paper or thin-layer chromatography and electrophoresis. We have succeeded in the separation between nucleoside form of mo^5U and U by the thin-layer chromatography on an aluminium roll cellulose sheet in the Rogg's system[7,13]. These two components can be also separated in chromatography when they have different numbers of phosphate from each other and a distinction between them is established by measurement of UV absorption spectra[1]. Sequencing techniques using radioactive tracers possess many advantageous characteristics such as rapid analysis with infinitestimal amount, but base identification should be carefully executed especially in tRNA studies.

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