

NUCLEOTIDE SEQUENCE OF tRNA₃^{Ser} FROM *ESCHERICHIA COLI*

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1. Introduction

The nucleotide sequence of tRNA₁^{Ser}, one of the two tRNA^{Ser}s purified from *E. coli* with the use of several column chromatographic systems [1], was reported previously [2]. The present communication reports the nucleotide sequence of the other one, tRNA₃^{Ser}. Two tRNA^{Ser}s are different in coding properties: tRNA₁^{Ser} recognizes codons UCA, UCG and less effectively UCU, whereas tRNA₃^{Ser} recognizes codons AGU and AGC [1]. The primary sequence of tRNA₃^{Ser} consists of 93 nucleotide residues including six minor nucleotides. A threonine-containing carbamoyl derivative of adenosine is located at the position adjacent to the 3'-end of the anticodon sequence G-C-U. The sequence can be arranged in a clover-leaf structure with extra-loop region larger than that of tRNA₁^{Ser}. Some discussion about the recognition sites

for seryl-tRNA synthetase is also given on the basis of structural comparison between these two isoaccepting tRNA's.

2. Materials and methods

E. coli tRNA₃^{Ser} was purified with a combined use of three column chromatographic systems, DEAE-Sephadex A-50, reversed-phase and benzoylated DEAE-cellulose, as already reported [1].

The products of complete digestion with RNAase T₁ and bovine pancreatic RNAase were separated on DEAE-Sephadex A-25 columns. The sequences were determined by further enzymatic digestion with complementary enzyme, snake venom phosphodiesterase, *E. coli* polynucleotide phosphorylase, *Bacillus subtilis* RNAase, RNAase U₂, RNAase T₂ and/or *E. coli* alkaline phosphatase, followed by column, paper and/or thin-layer chromatography.

Overlapping sequences were constructed by isolating the products of limited digestion with RNAase T₁ or pancreatic RNAase. Partial digestion of tRNA₃^{Ser} with pancreatic RNAase and isolation of the products were carried out according to Kimura et al. [3]. In the case of partial digestion with RNAase T₁, 10 µg of the enzyme was used per 250 A₂₆₀ units of tRNA₃^{Ser}. tRNA₃^{Ser} was partially digested with RNAase T₁ at

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Abbreviations:

s⁴U, 4-thiouridine; s²C, 2-thiocytidine; t⁶A, *N*-[9-(β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine; m²'G, 2'-O-methylguanosine; ms²iA, 2-methylthio-*N*⁶-(Δ²-isopentenyl)adenosine; V, uridin-5-oxyacetic acid; D, 5,6-dihydrouridine; N, unidentified nucleoside. A₂₆₀ unit, an amount of material which has an absorbance of 1.0 at 260 nm when dissolved in 1 ml of water and measured with a 1 cm light path.

37° for 10 min with or without 0.02 M magnesium acetate. The other conditions were the same as those used for limited digestion with pancreatic RNAase. The products were analyzed, after extensive hydrolysis with RNAase T₁ or pancreatic RNAase, with the use of two dimensional fingerprinting technique developed for analysis of ³²P-labeled RNA by Sanger et al. [4]. The first electrophoresis on cellulose acetate strip was carried out in 7 M urea (pH 3.5, adjusted by acetic acid). In both first and second runs, n-hexane was used as cooling solvent instead of Versol. Since our samples were not radioactive, spots on DEAE-cellulose paper were detected by ultraviolet absorption. Some oligonucleotides necessary for overlapping were obtained by digestion of tRNA₃^{Ser} with RNAase U₂, after it was treated with kethoxal for the modification of guanosine residues [5]. Details of these procedures will be published separately.

3. Results and discussion

Fig. 1 shows the total sequence of tRNA₃^{Ser} and the sequences of oligonucleotides used for overlapping. The detection of 2-thiocytidine and *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine in tRNA₃^{Ser} and their identification were already reported [1]. The structure arranged in a cloverleaf form is shown in fig. 2a. It is composed of 93 nucleotide residues, five more than tRNA₁^{Ser}. It results in a longer stem in extraloop region. As for minor nucleosides, a dihydrouridine residue is located in dihydrouridine loop, a ribothymidine and a pseudouridine in

G-T-ψ-C loop, a 4-thiouridine at the eighth position from 5'-terminal, and a 2-thiocytidine and a threonine-containing adenosine derivative in anticodon loop. The anticodon sequence G-C-U properly fits to the coding properties of tRNA₃^{Ser} (AGU and AGC). We have previously reported the separation of tRNA₃^{Ser} into two fractions (3-a and 3-b). Analysis of oligonucleotides produced by complete digestion with RNAase T₁ of these two fractions revealed that the difference lies in the state of modification of an adenosine derivative [1]. In this respect, the structure reported here is that of tRNA_{3-a}^{Ser}. The adenosine derivative occupies the position adjacent to 3'-end of the anticodon sequence G-C-U. This finding confirms the specific role of this threonine-containing carbamoyl derivative of adenosine in the codon-anticodon interaction in the tRNA's recognizing codons starting with A [6, 8, 9]. In tRNA_{3-b}^{Ser}, this adenosine derivative seems to be replaced by another adenosine derivative of similar structure. 2-Thiocytidine is located in anticodon loop at the position adjacent to the uridine residue which precedes the anticodon. The presence of 2-thiocytidine at the same position was reported in *E. coli* tRNA₁^{Arg} [10].

The cloverleaf structure of tRNA₁^{Ser} [2] is shown for comparison in fig. 2b where the nucleotide residues common to tRNA₃^{Ser} are enclosed with brackets. Three major regions common in both *E. coli* tRNA₃^{Ser}'s are (i) the stem part of dihydrouridine loop and its neighboring region, (ii) 5'- and 3'-terminal region and (iii) G-T-ψ-C loop. Since the common sequence in G-T-ψ-C loop is also found in *E. coli* tRNA's specific to the other amino acids [11-17],

	10	20	30	40	50	60	70	80	90	93
(a)	pGGUGAGGs ⁴ UGGCCGAGAGGCCDGAAGGCGCUCCCs ² CUGCt ⁶ AAGGGAGUAUGCGGUCAAAAGCUGCAUCCGGGTψCGAAUCCCCGCCUCACCGCA _{OH}									
(b)	PyGAGGs ⁴ UGp PyGCUt ⁶ AAGGGAGUp GUCAAAAGCp PyGGGGTψCGp									
(c)	pGGUGAGGs ⁴ UGGCCGAGAGGCCDGAAGGCGCUCCCs ² CUGp CUt ⁶ AAGGGAGUAUGCGGUCAAAAGCUGCAUCCGGGTψCGAAUCCCCGCCUCACCGp									
	pGGUGAGGs ⁴ UGGCCGAGAGGCCDGAAGGCP AGUAUGCGGUCAAAAGCUGCAUCCGp									
	pGGUGAGGs ⁴ UGGCCGp UAUGCGGUCAAAAGCUGCAUCCGp									
(d)	CGCUCCCs ² CUGCt ⁶ AAp UGCGGUCAp UCCGGGTψCGAp									

Fig. 1. Nucleotide sequences of *E. coli* tRNA₃^{Ser} (a) and oligonucleotides used for overlapping. (b) The sequences established by combining the data obtained from complete digestion with RNAase T₁ and pancreatic RNAase. (c) The oligonucleotides obtained by partial digestion with RNAase T₁ or pancreatic RNAase. (d) The oligonucleotides obtained by RNAase U₂ digestion of kethoxal-treated tRNA₃^{Ser}

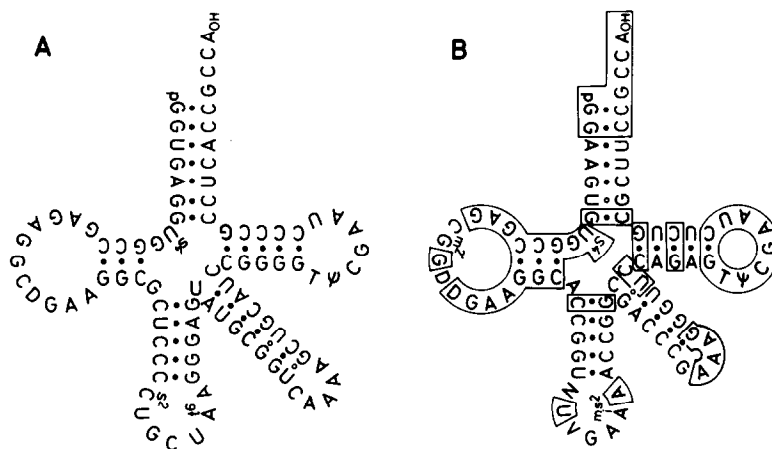


Fig. 2. (a) Cloverleaf model of *E. coli* tRNA₃^{Ser}. (b) Cloverleaf model of *E. coli* tRNA₁^{Ser} [2]. The nucleotide residue or sequences common to tRNA₃^{Ser} are enclosed with brackets. Base paired regions are indicated by closed circles (G—C and A—U) and open circles (G—U).

it can be excluded from a specific sequence for recognition of seryl-tRNA synthetase. The common structures seen in dihydrouridine stem region and CCA-terminal region are possible candidates for enzyme recognition sites. The participation of these regions to the enzyme recognition has been proposed [18, 19]. Each region by itself may indeed contribute in some degree to the construction of recognition sites. It will be more reasonable to consider that the specific structure which seryl-tRNA synthetase does recognize is determined by cooperation of several regions or sequences. In this sense, these two regions can be said to give the necessary conditions for enzyme recognition. On tRNA₃^{Ser} serine can be charged, though slightly less efficiently, by the aid of yeast or rat liver seryl-tRNA synthetase [1]. The large size of extra region is a remarkable characteristic of tRNA^{Ser} including yeast and rat liver tRNA^{Ser}'s [20, 21], although the sizes and sequences are different in all tRNA^{Ser}'s. It is certain that this large extra region plays a significant role in the formation of an overall structure specific to tRNA^{Ser}. Therefore, the large size of extra region can be added as a necessary condition for the enzyme recognition. The anticodon loop is probably not an important recognition site, since it is so different in the two *E. coli* tRNA^{Ser}'s. For the complete elucidation of enzyme recognition site of tRNA^{Ser}, we must await the structural deter-

mination of other isoaccepting tRNA^{Ser}'s and other lines of studies on tRNA^{Ser}.

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