THE NUCLEOTIDE SEQUENCE OF A SERINE tRNA FROM *ESCHERICHIA COLI*

H. ISHIKURA
Laboratory of Biochemistry, Institute for Hard Tissue Research, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

Y. YAMADA and S. NISHIMURA
Biology Division, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan

Received 21 May 1971

1. Introduction

The structures of serine tRNA's from yeast [1] and rat liver [2] have been determined. We report here the nucleotide sequence of $tRNA_1^{Ser}$ from *E. coli*. The structure was compared with those of $tRNA^{Ser}$'s from yeast and rat liver with some discussion on the recognition site of seryl-tRNA synthetase.

2. Materials and methods

E. coli $tRNA_1^{Ser}$ was purified with a combined use of two column chromatographic systems, DEAE-Sephadex A-50 and benzoylated DEAE-cellulose, as already reported [3].

The products of complete digestion with RNase T_1 and pancreatic RNase were separated on DEAE-Sephadex columns. The sequences were determined by further enzymatic digestion with the complementary enzyme, snake venom phosphodiesterase, *E. coli* polynucleotide phosphorylase, *Bacillus subtilis* RNase, 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 isolating the products of limited digestion with RNase T_1. Partial digestion of $tRNA_1^{Ser}$ and isolation of the products were carried out as described previously [4]. The products were analyzed, after extensive hydrolysis with RNase T_1 or pancreatic RNase, by two-dimensional thin-layer chromatography [5] and by DEAE-Sephadex A-25 column chromatography monitored with a highly sensitive automatic ultraviolet recorder [6]. In some cases, Sanger's fingerprinting techniques [7] were applied for analysis of the oligonucleotide fragments. Since our samples were not radioactive, spots on DEAE-cellulose paper were detected by ultraviolet absorption. For this purpose, usually an RNase digest of 3 to 5 $A_{260\,nm}$ units of the fragment was applied to the origin of a cellulose acetate strip. Some oligonucleotides necessary for overlapping were obtained by digestion of $tRNA_1^{Ser}$ with RNase U_2 after it was treated with kethoxal for the modification of G residues [8]. Details of these procedures will be published separately.

3. Results and discussion

Fig. 1 shows the nucleotide sequence of $tRNA_1^{Ser}$ and the oligonucleotides used for overlapping. In this sequence, we have insufficient confirmatory evidence to establish the position of 4-thiouridine without making use of analogies with other tRNA's on account of its degradation during the course of analysis.

The structure arranged in a cloverleaf form is shown in fig. 2. It is composed of 88 nucleotides, having a large extra-loop. The unknown minor nucleoside (N) located in the anticodon loop is probably an *O*-methylated nucleoside, since the bond between N and U in NpUp obtained in complete pancreatic RNase digest was resistant to alkali. The other minor nucleosides detected were dihydrouridine, ribothymidine, pseudo-

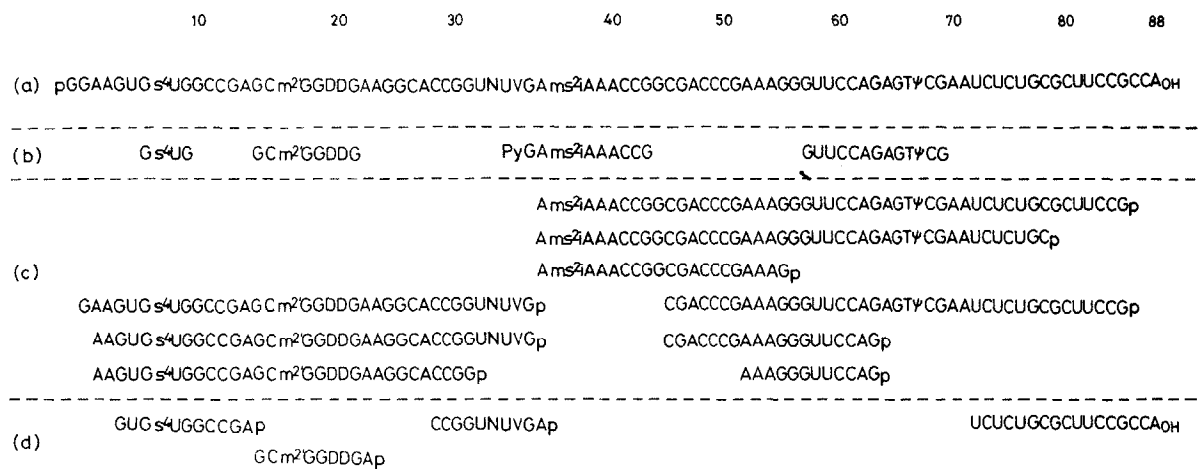

Fig. 1. Nucleotide sequences of *E. coli* $tRNA_1^{Ser}$ (a) and the oligonucleotides used for overlapping. (b) The sequences established by combining the data obtained from complete digestion with RNase T_1 and pancreatic RNase. (c) The oligonucleotides obtained by partial RNase T_1 digestion. (d) The oligonucleotides obtained by RNase U_2 digestion of kethoxal-treated $tRNA_1^{Ser}$.

uridine, 2′-*O*-methylguanosine, uridine-5-oxy acetic acid, and 2-methylthio-N^6-(Δ^2-iso-pentenyl)adenosine. This $tRNA_1^{Ser}$ recognizes codons of UCA and UCG, and less effectively UCU [3]. The presence of uridine-5-oxy acetic acid in the first position of the anticodon as in *E. coli* $tRNA_1^{Val}$ [9, 10] gives a satisfactory explanation for the codon specificity of $tRNA_1^{Ser}$. 2-Methylthio-N^6-(Δ^2-iso-pentenyl)adenosine occupies the position adjacent to the 3′-end of the anticodon. This finding confirms the specific role of this minor nucleotide in the codon-anticodon interaction in the tRNA's recognizing codons starting with U (ref. [11] and unpublished results).

Serine can be charged on *E. coli* $tRNA_1^{Ser}$ by enzyme preparations from yeast and rat liver, though the efficiency is slightly low as compared with enzyme preparation from *E. coli* [3]. A comparison of the nucleotide sequence among serine tRNA's of *E. coli*, yeast and rat liver is shown in fig. 3. *E. coli* $tRNA_1^{Ser}$ contains three additional nucleotides, one in dihydrouridine loop and two in extra-loop region. Of the sequences common to all three tRNA's enclosed with brackets (differences in the state of modification were not taken into account for this comparison), the most striking feature is the resemblance in dihydrouridine region. This region may participate in the recognition of seryl-tRNA synthetase. Five nucleotides from 3′-terminus and 5′-pG are also common. This part is another possible candidate for the recognition of

Abbreviations to the figures: s^4U, 4-thiouridine; $m^2'G$, 2′-*O*-methylguanosine; ms^2iA, 2-methylthio-N^6-(Δ^2-isopentenyl)-adenosine; V, uridine-5-oxy acetic acid; D, 5,6,-dihydrourinine; acC, N^4-acetylcytidine; m_2^2G, N^2-dimethylguanosine; iA, N^6-isopentenyladenosine; m^5C, 5-methylcytidine; m^3C, 3-methylcytidine; $m^2'\psi$, 2′-*O*-methylpeudouridine; $m^2'U$, 2′-*O*-methyluridine; m^1A, 1-methyladenosine, N, unidentified nucleoside; $A_{260\ nm}$ 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.

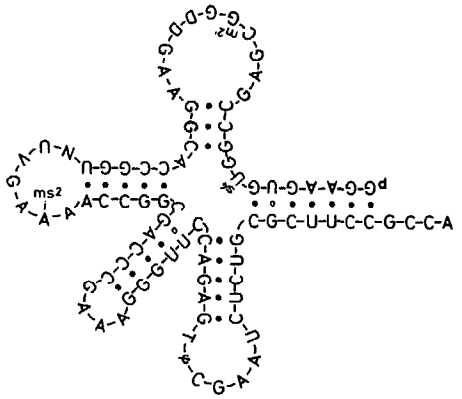

Fig. 2. Cloverleaf model of *E. coli* $tRNA_1^{Ser}$. Base paired regions are indicated by closed circles (G–C and A–U) and open circles (G–U).

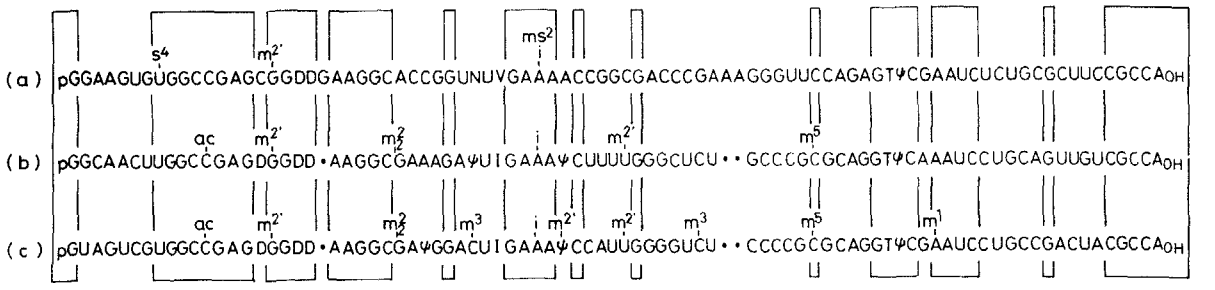

Fig. 3. Comparison of nucleotide sequences in *E. coli* $tRNA_1^{Ser}$ (a), yeast serine tRNA I (b) and rat liver serine tRNA (c). The regions common to all three are enclosed with brackets. The differences in the state of modification are not taken into account for this comparison.

seryl-tRNA synthetase. The sequence in extra-loop region of $tRNA_1^{Ser}$ is completely different from those of the other two. Nevertheless, the large size of extra-loop region present in all three serine tRNA's may contribute to construct a higher-order structure specific to serine tRNA's. In this respect, large extra-loop can also be an important factor for enzyme recognition. We are now carrying on the structural studies of the other *E. coli* serine tRNA's with different codon responses. The elucidation of their nucleotide sequences will give us more detailed information about the enzyme recognition.

Acknowledgements

We are indebted to the Laboratories of Kaken Chemicals for large scale isolation of crude *E. coli* tRNA. Thanks are also due to Drs. G.W. Underwood and P.W. O'Connell of the Upjohn Company for generous gift of kethoxal. This work is partly supported by research grant from the Japanese Ministry of Education.

References

[1] H.G. Zachau, D. Dütting and H. Feldmann, Z. Physiol. Chem. 347 (1966) 212.
[2] M. Staehelin, H. Rogg, B.C. Baguley, T. Ginsburg and W. Wehrli, Nature 219 (1968) 1363.
[3] H. Ishikura, Y. Yamada and S. Nishimura, Biochim. Biophys. Acta 228 (1971) 471.
[4] K. Oda, F. Kimura, F. Harada and S. Nishimura, Biochim. Biophys. Acta 179 (1969) 97.
[5] F. Harada, F. Kimura and S. Nishimura, Biochim. Biophys. Acta 195 (1969) 590.
[6] F. Kimura-Harada, F. Harada and S. Nishimura, Biochemistry (in press).
[7] F. Sanger, G.G. Brownlee and B.G. Barell, J. Mol. Biol. 13 (1965) 373.
[8] M. Litt and V. Hancock, Biochemistry 6 (1967) 1848.
[9] F. Harada, F. Kimura and S. Nishimura, Biochim. Biophys. Acta 182 (1969) 590.
[10] K. Murao, M. Saneyoshi, F. Harada and S. Nishimura, Biochem. Biophys. Res. Commun. 38 (1970) 657.
[11] S. Nishimura, Y. Yamada and H. Ishikura, Biochim. Biophys. Acta 179 (1969) 517.