PRIMARY SEQUENCE OF GLUTAMIC ACID tRNA II FROM *ESCHERICHIA COLI*

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1. Introduction

We have previously reported that *E. coli* $tRNA_2^{Glu}$ contained 5-methylaminomethyl-2-thiouridine [1] and 2-methyladenosine [1,2] as minor constituents. Preliminary sequential study of this tRNA strongly suggested that 5-methylaminomethyl-2-thiouridine and 2-methyladenosine are located in the first position of the anticodon, and in the next to the 3'-hydroxyl end of the anticodon [1], respectively. The specific function of the 2-thiouridine derivative in the decoding process in protein synthesis has been previously discussed [1]. In addition, it was shown that selective modification of the 2-thiouridine derivative in *E. coli* $tRNA^{Glu}$ with cyanogen bromide caused loss of the glutamic acid acceptor ability, suggesting that the first letter of the anticodon, or the anticodon region of the $tRNA^{Glu}$, might be important for the recognition of glutamyl-tRNA synthetase [3]. For further understanding of the involvement of the 2-thiouridine derivative in biological functions of tRNA, we have tried to obtain a total primary sequence of *E. coli* $tRNA_2{}^{Glu}$. This communication deals with the nucleotide sequence of *E. coli* $tRNA_2^{Glu}$. *E. coli* $tRNA_2^{Glu}$ consists of 76 nucleotide residues with relatively few minor nucleosides, and the sequence can be arranged as clover-leaf structure as in the case of other tRNA's.

Abbreviations:
m^2 A: 2-methyladenosine
S: 5-methylaminomethyl-2-thiouridine
OD unit: an amount of material which has an absorbance of 1.0 at 260 mμ when dissolved in 1 ml of water and measured with a 1 cm light path

2. Materials and methods

2.1. *Isolation of E. coli* $tRNA_2^{Glu}$

Unfractionated *E. coli* tRNA obtained from cells of *E. coli* B harvested at late log phase was first fractionated by DEAE-Sephadex A-50 column chromatography as described previously [4]. Glutamic acid tRNA was separated into 2 fractions by this procedure [5]. The major species, designated as $tRNA_2^{Glu}$ which was eluted later from the column, was further fractionated by benzoylated DEAE-cellulose column chromatography, then reverse phase partition column chromatography as previously described [5]. In later work, the $tRNA_2^{Glu}$ fraction obtained by DEAE-Sephadex A-50 column chromatography at pH 7.5 was purified by successive application of DEAE-Sephadex A-50 column chromatography at pH 4.0 [6]. The tRNA thus isolated was found to accept 1.66 nmoles of ^{14}C-glutamic acid per OD unit of tRNA, and estimated to be more than 95% pure judged from its chromatographic profiles of RNase T_1 and pancreatic RNase digests. Details of this purification procedure will be published elsewhere.

2.2. *Isolation and characterization of oligonucleotides derived from RNase T_1 and pancreatic RNase digests*

The general methods used for extensive hydrolysis of tRNA with RNase T_1 or pancreatic RNase, subsequent isolation of the oligonucleotides by DEAE-Sephadex columns, sequence determination of the oligonucleotides by the use of RNase T_1, T_2, RNase U_2,

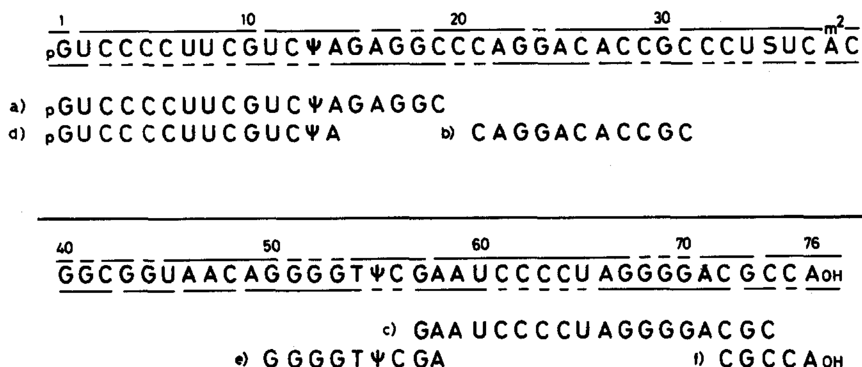

Fig. 1. The nucleotide sequence of *E. coli* $tRNA_2^{Glu}$ and the large oligonucleotides used for overlapping. Fragments a, b and c were obtained by limited digestion with pancreatic RNase; fragments d, e and f were obtained by RNase U_2 digestion of kethoxal-treated $tRNA_2^{Glu}$. The 2 lines over or under the sequence show fragments from complete RNase T_1 and pancreatic RNase digestion.

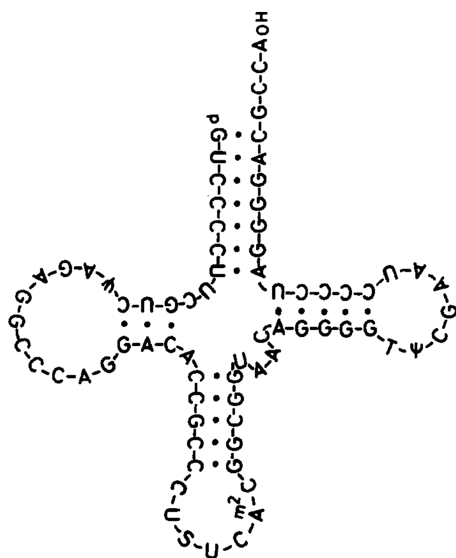

Fig. 2. Clover-leaf model of *E. coli* $tRNA_2^{Glu}$.

pancreatic RNase, *B. subtilis* RNase, polynucleotide phosphorylase, snake venom phosphodiesterase, silkworm nuclease and/or periodate oxidation, were essentially the same as those described previously [7].

2.3. *Isolation of large fragments used for overlapping*

Limited digestion of *E. coli* $tRNA_2^{Glu}$ was performed in the presence of 0.02 M magnesium chloride as described previously [8,9]. Isolation of the large fragments by DEAE-cellulose column chromotography, followed by DEAE-Sephadex A-25 column chromatography and the procedure used for their characterization has been also previously described [8,9]. Oligonucleotides ending with A which were necessary for overlapping were obtained by digestion of *E. coli* $tRNA_2^{Glu}$ with RNase U_2 after it was treated with kethoxal for the modification of G residue as described previously [10,11].

3. Results and discussion

Fig. 1 shows primary sequence of *E. coli* $tRNA_2^{Glu}$ and the large fragments used for overlapping. By combining the data on the sequences of oligonucleotides derived from complete RNase T_1 and pancreatic RNase digests with the sequences of the large fragments as shown in fig. 1, the sequence up to C_{19} from the 5′-terminus and the sequence up to G_{43} from the 3′-terminus have been unambiguously determined. Between these 2 sequents, the 3 following sequences were theoretically still considered as possible :

1) –CCAGGACACCGCCCUSUCm^2ACGGC–,
2) –CCAGGACACCGCGGCCCUSUCm^2 AC–, and
3) –GGCCCAGGACACCGCCCUSUCm^2AC–.

However, it is virtually certain that the unique sequence –CCCUSUCm^2ACG– derived from the RNase T_1 digest includes the anticodon loop, in which the sequence SUC is placed as the anticodon.

This conclusion was obtained by consideration of the general features of anticodon region structure of tRNA, as we have previously discussed [1]. By taking into account this fact, the sequences (2) and (3) can be excluded. Therefore, we conclude that the correct primary sequence of $tRNA_2^{Glu}$ is as indicated in fig. 1. The most difficult task for obtaining the sequence of *E. coli* $tRNA_2^{Glu}$ is characterization of the oligonucleotides A-G-G-G-G-Tp and A-G-G-G-G-A-Cp, derived from the pancreatic RNase digest. These 2 oligonucleotides were eluted together as a broad peak from a column of DEAE-Sephadex A-25. They were extremely resistant to attack by RNase T_1, RNase T_2, silkworm nuclease and snake venom phosphodiesterase, suggesting that they form an aggregate. Isolation of each oligonucleotide was partly achieved by fractionation of the pancreatic RNase digest with DEAE-Sephadex A-25 column chromatography carried out at 65°. In addition, A-G-G-G-G-A-Cp and G-G-G-G-Tp have been separately sequenced by using oligonucleotides obtained from the large fragments c and e, respectively.

The structure arranged in a clover-leaf form is shown in fig. 2. It contains relatively few minor components: 2 moles of pseudouridine and 1 mole each of ribothymidine, 5-methylaminomethyl-2-thiouridine and 2-methyladenosine. 7-Methylguanosine, dihydrouridine and 4-thiouridine, which were found in most *E. coli* tRNA's, were not present in *E. coli* $tRNA_2^{Glu}$. *E. coli* $tRNA_2^{Glu}$ has an unusual structure consisting of 4 unpaired nucleotides between the anticodon-stem and the TψC-stem*. *E. Coli* tRNA's so far sequenced contain either a pentanucleotide sequence with 7-methylguanosine, or a longer stem in that position (see review by Zachau [12] and also [11, 13–16] for sequences of other *E. coli* tRNA's). A sequence of 4 unpaired nucleotides between the 2 stems has only previously been found in yeast $tRNA^{Ala}$ [17] and $tRNA^{Asp}$ [18]. It should also be mentioned that the $tRNA_2^{Glu}$ can form tertiary structures either of the type proposed by Levitt [19] with a base pair between G_{15} and C_{48}, or by Cramer et al. [20] with base pairs between A_{22}–G_{23} and ψC of GTψC. Isolation of other large fragments to obtain final overlapping, and sequence determination of a minor species of *E. coli* glutamic acid tRNA, i.e. $tRNA_1^{Glu}$, are currently being undertaken.

* It is also conceivable that U_{45} forms a base pair with A_{27}, resulting in 6 base pairs in the anticodon-stem, leaving 3 unpaired nucleotides between the anticodon-stem and the TψC-stem.

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