The nucleotide sequence of a glutamate tRNA from rat liver

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ABSTRACT

A glutamate tRNA from rat liver was purified. By means of post-labeling techniques, its nucleotide sequence was shown to be: pU-C-C-C-A-C-A-U-m^1G-G-U-C-ψ-A-G-C-G-G-D-D-A-G-G-A-U-U-C-C-U-G-G-ψ-U-mcm^5s^2U-U-C-A-C-C-C-A-G-G-C-G-G-C-m^5C-m^5C-G-G-G-Tm-ψ-C-G-A-C-U-C-C-C-G-G-U-G-U-G-G-G-A-A-C-C-A_{OH}. The sequence is remarkably similar to that of $tRNA_4^{Glu}$ from Drosophila melanogaster. Only 10 out of 75 nucleotides in the two tRNAs are different.

INTRODUCTION

As a result of the rapid burgeoning in cloning techniques, sequencing of tRNA genes has to some extent supplanted the direct sequencing of tRNAs. Nonetheless, isolation and determination of the primary structure of mature tRNAs are still pivotal in such studies as the enzymatic processing of precursor tRNAs to mature-sized tRNAs, the formation of modified nucleotides, the interaction of tRNAs with aminoacyl-tRNA synthetases, and structure-function correlation involving NMR spectroscopy and x-ray crystallography. A continuous interest in our laboratory has been the biosynthesis of thionucleotides in tRNAs (1,2). In connection with such studies, we undertook to purify a 2-thiouridine-containing $tRNA^{Glu}$ from normal rat liver and to determine its nucleotide sequence.

MATERIALS AND METHODS

Purification of $tRNA^{Glu}$. Unfractionated tRNA was obtained from the liver of inbred Buffalo rats (3). The $tRNA^{Glu}$ in question was then isolated from the crude tRNA by successive chromatography on benzoylated DEAE-cellulose (4) and Sepharose 4B (5), followed by repeated polyacrylamide gel electrophoresis (6). The glutamic acid accepting activity of the purified tRNA (hereafter designated as $tRNA_1^{Glu}$) was assayed with a crude synthetase preparation obtained from homologous rat liver. Procedures for preparing the crude synthetase and for assaying aminoacylation were as outlined by Yang and Novelli (3). The final gel-purified $tRNA_1^{Glu}$ could be charged to 1300 pmoles/A_{260} unit and was found to be sufficiently pure for sequence determination on fingerprinting.

Sequence Analysis. The post-labeling procedures used for fingerprinting and sequencing

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the purified $tRNA_1^{Glu}$ were as described by Silberklang et al. (7).

Modified Nucleotides. Modified nucleotides were characterized in several ways. Those present at the 5'-ends of the oligonucleotides from RNase T1 or RNase A fingerprints were analyzed by complete P1 digestion and thin-layer chromatography (7). An overall analysis of the modified nucleotides was also performed by labeling an RNase T2 hydrolysate of the tRNA with [γ-^{32}P]ATP and polynucleotide kinase; after treatment with nuclease P1, the liberated [5'-^{32}P]mononucleotides were identified by thin-layer chromatography (7). Finally, non-radioactive nucleoside mixtures derived from complete digestion of the tRNA with RNase T2, nuclease P1, and alkaline phosphatase were analyzed and quantitated by high-performance liquid chromatography (HPLC) against known nucleoside markers (8).

RESULTS

Sequence Analysis. The purified $tRNA_1^{Glu}$ was digested completely with RNase T1 or RNase A. The resulting oligonucleotide fragments were labeled at their 5'-ends with ^{32}P, separated by fingerprinting, and sequenced by partial digestion with snake venom phosphodiesterase or nuclease P1 followed by two-dimensional homochromatography (7). Overlapping of the fragments was achieved by partial digestion of 5'-end ^{32}P-labeled intact tRNA or large tRNA fragments with nuclease P1 (7), or by sequencing gel (9). The results of these analyses led to the nucleotide sequence shown in Figure 1.

Modified Nucleotides. Post-labeling of a hydrolysate of the tRNA with ^{32}P followed by two-dimensional thin-layer chromatography (7) revealed the presence of m^1G, ψ, D, m^5C, mcm^5s^2U, and Tm. The last two unusual nucleotides had mobility characteristics similar to those reported previously (10,11). While this procedure is useful in identifying the modified nucleotides in a tRNA, it does not give a reliable estimate of the number of moles of each modified nucleotide present, since polynucleotide kinase may not phosphorylate all modified nucleotides with equal facility. For this reason, a quantitative HPLC analysis of the non-radioactive nucleosides derived from the tRNA was also carried out (8). The results indicated the presence of 1 mole of m^1G, 3 moles of ψ, 2 moles of m^5C, 1 mole of mcm^5s^2U, and 0.5 mole of a modified nucleoside tentatively identified as Tm. No T was detected in the molecule. The number of moles of D could not be determined by HPLC, since dihydrouridine is non-aromatic and exhibits no absorbance at 254 nm or 280 nm, the two wavelengths at which the nucleosides are normally analyzed.

The assignment of m^1G to position 9, D to position 19, ψ to position 32, mcm^5s^2U to position 34, and Tm to position 53 presented no problem, since these modified nucleotides were found at the 5'-ends of RNase T1 or RNase A fragments (Figure 1). The assignment of ψ to positions 13 and 54, and D to position 20 was based on their characteristic block to snake venom phosphodiesterase cleavage and the abnormally long mobility shifts on two-dimensional homochromatography. Only the two m^5C's could not be unequivocally

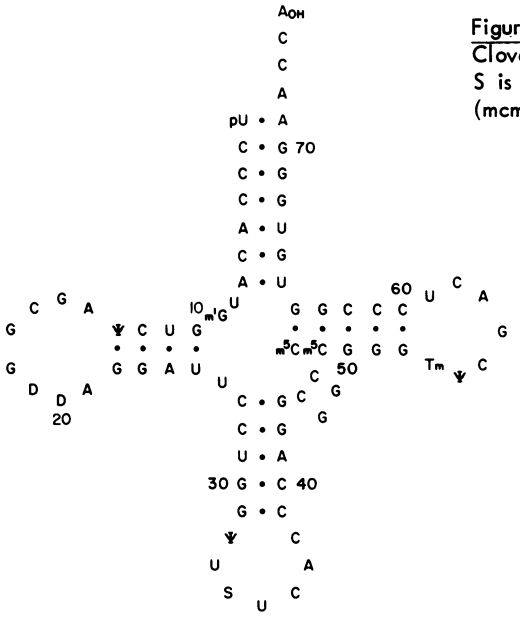

Figure 1.
Cloverleaf model of rat liver $tRNA_1^{Glu}$.
S is 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U). Tm is 2'-*O*-methylribothymidine.

localized. Their assignment to positions 48 and 49 was based on the frequent presence of m^5C at these locations in other tRNA molecules, and the considerable resistance of the third and second nucleotides in the fragment pC-C*-C*-G to snake venom phosphodiesterase digestion, a behavior not shared by the unmodified pC-C-C-G.

DISCUSSION

This is the first mammalian glutamate tRNA sequenced directly. In common with the other glutamate tRNAs from Drosophila, yeasts, and bacteria reported previously (12-16), this rat liver $tRNA_1^{Glu}$ contains a 2-thiouridine derivative in the first position of the anticodon. Its nucleotide sequence is remarkably similar to that of $tRNA_4^{Glu}$ from Drosophila melanogaster (12); both tRNAs are 75 nucleotides long, and only 10 out of 75 nucleotides in the two tRNAs are completely different. The homology to the glutamate tRNAs from yeasts is considerably less; 21 out of the 75 nucleotides in S. pombe $tRNA_1^{Glu}$ (13) and 23 out of the 75 nucleotides in S. cerevisiae $tRNA_3^{Glu}$ (14) are different. The divergence from the glutamate tRNAs of bacteria is even more striking; the $tRNA_2^{Glu}$ from E. coli (15) is 76 nucleotides long, of which 31 are different from the rat liver $tRNA_1^{Glu}$, including an additional nucleotide in the D loop; finally, the $tRNA^{Glu}$ from H. volcanii (16) is 78 nucleotides long and differs in 34 nucleotides, including three extra nucleotides in the D loop.

The rat liver $tRNA_1^{Glu}$ reported here has the anticodon SUC, where S was identified as mcm^5s^2U by HPLC analysis. Like all glutamate tRNAs with a 2-thiouridine derivative in

the first position of the anticodon, it preferentially recognizes the codon GAA; the ability to pair with the codon GAG through wobbling is lost (12,14,17).

A $tRNA^{Glu}$ gene from rat liver has been cloned and sequenced recently by Sekiya et al. (18). The secondary structure of the $tRNA^{Glu}$ deduced from the DNA sequence indicated that its anticodon is CUC, which recognizes the codon GAG. It is not surprising, therefore, that its nucleotide sequence is noticeably different from the $tRNA_1^{Glu}$ reported here.

A peculiar feature of the rat liver $tRNA_1^{Glu}$ is the presence of Tm in place of T in the TψC loop (Figure 1). This modified nucleotide, which contains modifications in both the base and the sugar residues, is distinctly unusual. Of more than 200 tRNA sequences compiled thus far (16), only rabbit liver lysine tRNAs have been shown to contain Tm in the same position (10). Its functional significance remains to be elucidated.

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