The nucleotide sequence of a glutamate tRNA from rat liver

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ABSTRACT


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^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^Glu could be charged to 1300 pmoles/μg protein unit and was found to be sufficiently pure for sequence determination on fingerprinting.

Sequence Analysis. The post-labeling procedures used for fingerprinting and sequencing...
the purified tRNA$^{\text{Glu}}$ 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 PI 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 [Y-$^{32}$P]ATP and polynucleotide kinase; after treatment with nuclease PI, 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 PI, and alkaline phosphatase were analyzed and quantitated by high-performance liquid chromatography (HPLC) against known nucleoside markers (8).

**RESULTS**

**Sequence Analysis.** The purified tRNA$^{\text{Glu}}$ 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 PI 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 PI (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 $m^1$G, $\psi$, D, $m^5$C, $mcm^5$s$^2$U, 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 $m^1$G, 3 moles of $\psi$, 2 moles of $m^5$C, 1 mole of $mcm^5$s$^2$U, 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 $m^1$G to position 9, D to position 19, $\psi$ to position 32, $mcm^5$s$^2$U 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 $\psi$ 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 $m^5$C's could not be unequivocally
Cloverleaf model of rat liver tRNA\textsubscript{Glu}

\textbf{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\textsubscript{Glu} contains a 2-thiouridine derivative in the first position of the anticodon. Its nucleotide sequence is remarkably similar to that of tRNA\textsubscript{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\textsubscript{Glu} (13) and 23 out of the 75 nucleotides in S. cerevisiae tRNA\textsubscript{Glu} (14) are different.

The divergence from the glutamate tRNAs of bacteria is even more striking; the tRNA\textsubscript{Glu} from E. coli (15) is 76 nucleotides long, of which 31 are different from the rat liver tRNA\textsubscript{Glu}, including an additional nucleotide in the D loop; finally, the tRNA\textsubscript{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\textsubscript{Glu} reported here has the anticodon SUC, where S was identified as mcm\textsuperscript{5}s\textsuperscript{2}U 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^{Glu} reported here.

A peculiar feature of the rat liver tRNA^{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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