The nucleotide sequence of a major glutamine tRNA from rat liver

Julia A. Yang, Lee W. Tai, Paul F. Agris⁺, Charles W. Gehrke[§] and Ting-Wa Wong

Department of Pathology, The University of Chicago, Chicago, IL 60637, and ⁺Division of Biological Sciences and [§]Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO 65211, USA

Received 25 January 1983; Revised 25 February 1983; Accepted 28 February 1983

ABSTRACT

A major glutamine tRNA from rat liver was purified. Post-labeling techniques showed its nucleotide sequence to be: pG-G-U-U-C-C-A-U-m¹G-G-U-G-ψ-A-A-D-Gm-G-D-D-A-G-C-A-C-U-C-U-G-G-A-Cm-U-C-U-G-A-A-ψ-C-C-A-G-C-G-A-U-m⁵C-m⁵C-G-A-G-ψ-ψ-C-A-m¹A-A-U-C-U-C-G-G-U-G-G-A-A-C-C-U-C-C-AOH.

INTRODUCTION

Several recent reports have indicated that tRNAs from neoplastic tissues may be hypomodified compared to their counterparts from normal tissues (1-6). Hence any specificity of function which modified nucleotides may confer on normal tRNAs are potentially altered in tumor tRNAs. As a part of a study aimed at comparing the primary and tertiary structures of tRNAs from normal rat liver with those from Morris hepatomas, we purified a major glutamine tRNA from rat liver and determined its nucleotide sequence.

MATERIALS AND METHODS

Purification of tRNA^{Gln}. Crude tRNA obtained from the liver of inbred Buffalo rats (7) was fractionated on a BD-cellulose column (8). The first major glutamine accepting peak (hereafter designated as tRNA₁^{Gln}) was further chromatographed on Sepharose 4B (9), followed by repeated polyacrylamide gel electrophoresis (10). During successive stages of purification, the glutamine accepting activity of the tRNA 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 (7). The final gel-purified tRNA₁^{Gln} had a glutamine accepting activity of 1250 pmoles/A₂₆₀ unit and was sufficiently pure for sequence determination.

Sequence Determination. The purified tRNA₁^{Gln} was sequenced by the post-labeling procedures described by Silberklang et al. (11).

Modified Nucleotides. Modified nucleotides present at the 5'-ends of oligonucleotides

from RNase T1 or RNase A fingerprints were analyzed by complete nuclease P1 digestion and thin-layer chromatography (11). An overall identification of the modified nucleotides was also performed by labeling an RNase T2 hydrolysate of the tRNA with ^{32}P to produce $[5'\text{-}^{32}\text{P}]\text{pNp}'\text{s}$; treatment with nuclease P1 yielded the corresponding $[5'\text{-}^{32}\text{P}]\text{pN}'\text{s}$, which were analyzed by thin-layer chromatography (11). Finally, non-radioactive nucleosides 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 (12).

RESULTS

Sequence Analysis. The oligonucleotide fragments obtained from complete RNase T1 or RNase A digests of the purified tRNA₁^{Gln} were labeled at their 5'-ends with ^{32}P , separated by fingerprinting (Figures 1 and 2), and sequenced by partial digestion with snake venom phosphodiesterase or nuclease P1, followed by two-dimensional homochromatography (11). Overlapping of the fragments was achieved by partial digestion of 5'- ^{32}P -labeled intact tRNA or large tRNA fragments with nuclease P1, or by sequencing gel (13). The deduced nucleotide sequence is shown in Figure 3.

Analysis of Modified Nucleotides. 5'- ^{32}P -labeling of a hydrolysate of the tRNA followed by two-dimensional thin-layer chromatography (11) revealed the presence of m¹G, ψ , D, Gm, Cm, m⁵C, and m¹A. While this procedure is useful in identifying the modified nucleotides in a tRNA, it does not yield 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. Hence a quantitative HPLC analysis of the non-radioactive nucleosides derived from the tRNA was also carried out (12). The results indicated the presence of 1 mole of m¹G, 5 moles of ψ , 1 mole of Gm, 1 mole of Cm, 1.5 moles of m⁵C, and 0.8 mole of m¹A. The number of moles of D could not be determined by HPLC, since dihydrouridine is non-aromatic and has no absorbance at 254 or 280 nm, the two wavelengths at which the nucleosides are normally analyzed.

The assignment of m¹G to position 9, ψ to positions 13 and 53, Gm to position 17, and D to position 19 presented no difficulty, since these modified nucleotides were found at the 5'-ends of RNase T1 or RNase A fragments (Figure 3). The uridine at position 16 was present as a mixture of D (60%) and U (40%), as indicated by the relative molar yields of pA-A-D and pA-A-U in the RNase A fingerprint (Figure 2), as well as the relative molar yields of p ψ -A-A-D-Gm-G and p ψ -A-A-U-Gm-G in the RNase T1 fingerprint (Figure 1). The assignment of D to position 20 and ψ to positions 39 and 54 was based on the characteristic resistance of these nucleotides to partial snake venom phosphodiesterase digestion and the abnormally long mobility shifts on two-dimensional homochromatography

Figure 1.

Fingerprint of 5'-³²P-labeled oligonucleotides from a complete RNase T1 digest of rat liver tRNA₁^{Gln}. The unlabeled spots represent contaminants from the [γ-³²P]ATP used in the labeling procedure. B denotes the position of the xylene cyanol blue dye.

(11), as well as the frequent occurrence of D and ψ at these locations in other tRNA molecules. The placement of Cm at position 32 was based on the RNase A fragment pG-G-A-Cm-U (Figure 2) and the sequencing gel. The fragments pA-U-m⁵C-m⁵C-G and pA-U-m⁵C-C-G were both present in the RNase T1 fingerprint, in roughly equimolar amounts (Figure 1). This finding was consistent with the HPLC estimate of 1.5 moles of m⁵C in the molecule. The assignment of m¹A to position 57 was based on its characteristic reverse mobility shift on two-dimensional homochromatography and the normalization of its behavior after conversion to m⁶A (11). Finally, the HPLC analysis indicated that there may be 5 moles of ψ in the molecule. Only 4 ψ's could be accounted for unequivocally;

Figure 2.

Fingerprint of 5'-³²P-labeled oligonucleotides from a complete RNase A digest of rat liver tRNA₁^{Gln}. The unlabeled spots represent contaminants from the [γ-³²P]ATP used in the labeling procedure. B denotes the position of the xylene cyanol blue dye.

we were unable to locate the fifth, due to the labeling procedure used to analyze the nucleotide sequence.

DISCUSSION

This is the first eukaryotic glutamine tRNA sequenced directly. The previously reported glutamine tRNAs are either from bacteria or phages (14); of these, only one from *E. coli* with the anticodon CUG (15) and one from *H. volcanii* with the anticodon C^{*}-U-G (16) are comparable to the rat liver tRNA₁^{Gln}. Of the two bacterial glutamine tRNAs, the one from *H. volcanii* is structurally more similar to the rat liver glutamine tRNA.

Figure 3.

Cloverleaf model of rat liver tRNA^{Gln}.
The nucleotides are numbered consecutively.

The *H. volcanii* tRNA^{Gln} is 76 nucleotides long and differs from the rat liver tRNA^{Gln} in 29 nucleotides, including an additional nucleotide in the D loop; there is considerable structural homology between the two tRNAs in the acceptor stem, the TΨC loop, and the variable loop.

The tRNA^{Gln} reported here is the major glutamine tRNA in the rat liver. It appears to have the same sequence as the tRNA^{Gln} gene located recently in a human DNA segment which also contains the genes for a tRNA^{Lys} and a tRNA^{Leu} (17). Our finding suggests that this human tRNA^{Gln} gene not only may be functional, but may actually be the locus for specifying the synthesis of a major species of tRNA^{Gln}.

ACKNOWLEDGMENTS

This study was supported by NIH grants CA 28053 (to T.-W.W.) and GM 27662 (to C.W.G.).

REFERENCES

1. Wong, T.-W., Harris, M.A. and Morris, H.P. (1975) Biochem. Biophys. Res. Commun. 65, 1137-1145.

2. Katze, J.R. (1978) *Nucleic Acids Res.* 5, 2513-2524.
3. Okada, N., Shindo-Okada, N., Sato, S., Itoh, Y.H., Oda, K. and Nishimura, S. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4247-4251.
4. Roe, B.A., Stankiewicz, A.F., Rizi, H.L., Weisz, C., DiLauro, M.N., Pike, D., Chen, C.Y. and Chen, E.Y. (1979) *Nucleic Acids Res.* 6, 673-688.
5. Raba, M., Limburg, K., Burghagen, M., Katze, J.R., Simsek, M., Heckman, J.E. RajBhandary, U.L. and Gross, H.J. (1979) *Eur. J. Biochem.* 97, 305-318.
6. Kuchino, Y., Kasai, H., Yamaizumi, Z., Nishimura, S. and Borek, E. (1979) *Biochim. Biophys. Acta* 565, 215-218.
7. Yang, W.-K. and Novelli, G.D. (1971) *Methods Enzymol.* 20, 44-55.
8. Gillam, I.C. and Tener, G.M. (1971) *Methods Enzymol.* 20, 55-70.
9. Holmes, W.M., Hurd, R.E., Reid, B.R., Rimerman, R.A. and Hatfield, G.W. (1975) *Proc. Natl. Acad. Sci. USA* 72, 1068-1071.
10. Ikemura, T. and Dahlberg, J.E. (1973) *J. Biol. Chem.* 248, 5024-5032.
11. Silberklang, M., Gillum, A.M. and RajBhandary, U.L. (1979) *Methods Enzymol.* 59, 58-109.
12. Gehrke, C.W., Kuo, K.C., McCune, R.A., Gerhardt, K.O. and Agris, P.F. (1982) *J. Chromatogr.* 230, 297-308.
13. Simoncsits, A., Brownlee, G.G., Brown, R.S., Rubin, J.R. and Guilley, H. (1977) *Nature* 269, 833-836.
14. Sprinzl, M. and Gauss, D.H. (1982) *Nucleic Acids Res.* 10, r1-r55.
15. Yaniv, M. and Folk, W.R. (1975) *J. Biol. Chem.* 250, 3243-3253.
16. Gupta, R. (1981) Ph.D. Thesis, University of Illinois (Urbana), cited in reference 14.
17. Roy, K.L., Cooke, H. and Buckland, R. (1982) *Nucleic Acids Res.* 10, 7313-7322.