Lysine tRNAs from rat liver: lysine tRNA sequences are highly conserved

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ABSTRACT

The two major lysine tRNAs from rat liver, tRNA₂^{Lys} and tRNA₅^{Lys}, were sequenced by rapid gel or chromatogram readout methods. The major tRNA₂^{Lys} differs from a minor form only by a base pair in positions 29 and 41; both tRNAs have an unidentified nucleotide, U**, in the third position of the anticodon. Although highly related, the major tRNA₂^{Lys} and tRNA₅^{Lys} differ in four base pairs and four unpaired nucleotides, including the first position of the anticodons, but have the same base pair in positions 29 and 41. The three tRNAs maintain a m²G-U pair in the acceptor stem. Detection of this m²G is in contrast to other reports of lysine tRNAs. Sequences of lysine tRNAs are strongly conserved in higher eukaryotes.

INTRODUCTION

For several years, our laboratories have been involved in studying mammalian isoaccepting lysine tRNAs. A number of isoacceptors have been observed depending on the source of cells or their growth state (1,2). In slowly growing or differentiated tissues, tRNA₂^{Lys} and tRNA₅^{Lys} are the major isoacceptors, whereas the amount of tRNA₄^{Lys} varies with the growth state of cells and correlates with the doubling time of cells in culture (3-5). Changes in the relative amounts of lysine isoaccepting tRNAs also result from stress or availability of growth factors (6-8). As a part of our continuing interest in the possible role of tRNA₄^{Lys} in cell division (9) and the alterations in chromatographic profiles of lysine isoacceptors, some of which are due to differences in modification, we determined the nucleotide sequences of the two major lysine tRNAs from rat liver and a minor species closely related to tRNA₂^{Lys}. These sequences confirm the preservation of a very high degree of homology among nonorganellar eukaryotic lysine tRNAs.

MATERIALS AND METHODS

Rat liver tRNA was chromatographed on RPC-5 and benzoylated DEAE-cellulose in a scheme similar to that of Wittig *et al.* (10) to provide purified tRNA₂^{Lys} and

Fig. 1 Direct readout of the sequence of nucleotides 29-58 of tRNA^{Lys}₂ from PEI-cellulose chromatograms. Unlabeled major nucleotides were included in each lane.

tRNA^{Lys}₅. Further purification was done in 10% polyacrylamide gels with 7 M urea.

Sequencing involved limited chemical, enzymatic, or random hydrolysis methods. For the enzymatic method, tRNA was labeled on the 3' terminus with [5'-³²P]pCp by T₄ RNA ligase (11) and treated with nucleases (12,13) by a modified method including RNase Phy M (U- and A-specific) and the C-specific RNase Cl₃ (14). We used the chemical sequencing method of Peattie (11) with a modified reaction for cytosine (15).

Most of the sequence data were obtained by using limited formamide hydrolysis (16) to produce sequencing ladders in thin polyacrylamide gels (17) as we reported for the sequencing of tRNA^{Lys}₆ from mouse cells (6). Individual bands were cut from sequencing ladders and fragments eluted. The labeled 5'-nucleotide of each fragment was released with RNase T₂ and chromatographed on PEI-cellulose in 0.55 M (NH₄)₂SO₄ (17). Some minor nucleotides were treated with nuclease P₁ and chromatographed on cellulose thin layers according to Silberklang *et al.* (18) (system A; 1st dimension, i-butyric acid/water/NH₄OH, 132/66/2; 2nd dimension, 0.1 M sodium phosphate, pH 6.8/(NH₄)₂SO₄/n-propanol, 100/60/2, v/w/v) or Nishimura (19) (system B: 1st dimension, as in system A; 2nd dimension, i-propanol/HCl/water, 70/15/15).

RESULTS

tRNA^{Lys}₂ separates into two bands upon electrophoresis in a polyacrylamide gel. The minor component, tRNA^{Lys}_{2m}, is about 35% of the total. Sequencing of

Fig. 2 Nucleotides 36 and 37 of $tRNA_2^{Lys}$ after chromatography on cellulose. Samples of the preparations used for Fig. 3 were treated with nuclease P_1 and chromatographed. (A) and (D): system B; (B), (C), and (E): system A. Dotted regions are standards detected by UV light.

$tRNA_2^{Lys}$ by the enzymatic method identified the major nucleotides for positions 14 through 74. Confirmation of these assignments, completion of major nucleotide assignments, and identification of minor nucleotides was done by

Fig. 3 The nucleotide from position 54 of $tRNA_2^{Lys}$ after chromatography on cellulose. A sample of the preparation of the dinucleotide from positions 54-55 used for Fig. 3 was treated with nuclease P_1 and chromatographed in system A.

Fig. 4 pm^2G from positions 6 and 10 of tRNA_{Lys} after treatment of pm^2Gp with nuclease P_1 and chromatography in system A.

the method using limited formamide hydrolysis. The chromatographic display of nucleotides 29-58, as pNp's, is shown in Fig. 1. The anticodon was expected to be CUU, but the third position is an unstable, unidentified nucleotide, U**. Bidimensional chromatography of this nucleotide from position 36 (Fig. 2) shows pU and a second component, pU**, just below pU in system B (Fig. 2A)

Fig. 5 Nucleotides in positions 34 and 37 of tRNA_{Lys} . (A) PEI-cellulose chromatogram of the nucleotides 34-39. (B) Nucleotide 34 from (A) after treatment with nuclease P_1 (system A). The arrow indicates the faint spot corresponding to $\text{mcm}^3\text{s}^2\text{U}$. (C) Nucleotide 37 from (A) after treatment with nuclease P_1 (system A).

Fig. 6 Cloverleaf form of the primary structures of tRNA₂^{Lys} and tRNA₅^{Lys}. The arrow indicates the only identified differences between tRNA₂^{Lys} and tRNA_{2m}^{Lys}.

and pU and a component with the same mobility as pψ in system A (Fig. 2B). Confirmation of nucleotide 37 as p⁶A is shown in Fig. 2C. Formamide hydrolysis does not cleave the phosphodiester bond between pTm and its 3' neighbor; thus, pψ is not positively identified except by analogy with the structure of rabbit liver tRNA₂^{Lys} (20). Nuclease P₁ cleaves the linkage to yield a nucleotide with the expected mobility of pTm (Fig. 3). Both positions 6 and 10 are m²G as shown by bidimensional chromatography (Fig. 4).

Positions 21-73 for tRNA_{2m}^{Lys} were determined by the method using limited formamide hydrolysis. This tRNA appears to have the same sequence as tRNA₂^{Lys} except for a G-C pair in positions 29 and 41. Positions 6 and 10 are m²G as in the major species.

The order of major nucleotides in positions 5-76 of tRNA₅^{Lys} was determined by the chemical method and confirmation and completion of the sequence was done by the formamide hydrolysis method. The anticodon is mcm⁵s²UUU. The modified nucleotide in the first position of the anticodon is identified (Fig. 5A and 5B) by analogy with rabbit liver tRNA₅^{Lys} (20) and comparison with mobilities of nucleotides from *Drosophila* tRNA₅^{Lys} (21). Position 37 was expected to be ms²t⁶A (20), and this was confirmed by its chromatographic mobility (22) (Fig. 5C). The sequences of the tRNAs are compared in Fig. 6.

DISCUSSION

These sequences for tRNA₂^{Lys}, tRNA_{2m}^{Lys}, and tRNA₅^{Lys} from rat liver are in agreement with sequences of analogous lysine tRNAs from rabbit liver (21) with two exceptions. We find m²G in position 6 in all three isoacceptors instead of an unmodified G. Also, there is an unstable, unidentified nucleotide comprising all or part of the third position of the anticodon in tRNA₂^{Lys} and tRNA_{2m}^{Lys}. Rat liver tRNA₂^{Lys} differs from that of *Drosophila* (23) only in the number of modifications of nucleotides. Rat liver and *Drosophila* (22) tRNA₅^{Lys} differ in the number of modified nucleotides and in four nucleotides in the anticodon stem.

The sequence for tRNA₅^{Lys} agrees with gene sequences for rat liver (24) and human (25) tRNA₅^{Lys}. It differs in five positions from a mouse lysine tRNA gene (26) closely resembling a tRNA₅^{Lys} sequence. Of several mouse lysine tRNAs sequenced by us (6,27), none has the sequence reported for the mouse lysine tRNA gene.

The available data clearly show a very high degree of homology of lysine tRNAs from higher eukaryotes. It is especially interesting that one G-U pair in the acceptor stem is retained among the different organisms as well as within the different lysine tRNA genes in the same organism and that two G-U pairs are retained in tRNA₂^{Lys}.

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Abbreviations used: PEI-cellulose, polyethyleneimine-impregnated cellulose; t⁶A, N-[9-(β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine; m²t⁶A, the 2-methylthio derivative of t⁶A; mcm⁵s²U, 5-methylcarboxymethyl-2-thiouridine.

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