The nucleotide sequence of arginine tRNA^{Arg}_{CCG} from bovine liver

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ABSTRACT

The nucleotide sequence of arginine tRNA^{Arg}_{CCG} from bovine liver was determined to be: pG-A-C-C-C-A-G-U-m¹G-m²G-C-C-U-A-A-D-Gm-G-A-D-A-A-G-G-C-A-Ψ-C-A-G-C-Cm-U-C-C-G-m¹G-A-G-C-U-G-G-G-G-A-D-U-G-Ψ-G-G-G-T-Ψ-C-G-m¹A-G-U-C-C-C-A-U-C-U-G-G-G-U-C-G-C-C-A_{OH}. This arginine tRNA is 76 nucleotides in length with 13 modified bases and has an anticodon of CCG. The sequence of this molecule is substantially different from those of other arginine tRNAs sequenced to date and is the only arginine tRNA sequenced which would be expected to recognize the codon CGG.

INTRODUCTION

As an initial step in the study of mammalian arginine tRNAs and tRNA genes, we have isolated and determined the nucleotide sequence of an arginine tRNA from bovine liver. It is important to determine the primary structures of mature mammalian tRNAs in order to elucidate the mechanisms of post-transcriptional processing and modification of the precursor tRNA transcripts, and also to delineate any sequence differences (such as base substitutions) which may occur between normally expressed tRNA genes and potential tRNA pseudogenes.

MATERIALS AND METHODS

Bovine liver tRNA^{Arg}_{CCG} was isolated by a three-step chromatographic procedure involving a BD-cellulose column run at pH 7.6, followed by two successive RPC-5 columns, the first run at pH 4.5 and the second run at pH 7.6. The materials used and details of these procedures have been previously described (1,2).

RESULTS

The primary structure of arginine tRNA^{Arg}_{CCG} from bovine liver is shown in the cloverleaf conformation in Figure 1a.

Fig. 1. (a) Nucleotide sequence of arginine tRNA^{CCG} from bovine liver. (b) RNA sequence gel of partial digests of the [5'-32P]tRNA (1,7). (c) Mobility-shift analysis of the [3'-32P]tRNA, partially digested with alkali (2). (d) Mobility-shift analysis of a [5'-32P]oligonucleotide from a partial formamide digest. (e) A representative 5'-end analysis (4,5) of oligonucleotides generated by partial formamide hydrolysis, using PEI-cellulose sheets and 0.55M (NH₄)₂SO₄.

The 13 modified nucleotides shown in Figure 1a were identified by the [5'-³²P]nucleoside-monophosphate procedure (3), and also by analyses of the 5'- termini of oligonucleotides generated by partial formamide hydrolysis (3-5).

The nucleotide sequence of residues 34-74 was primarily established by identifying the 5'-termini of the oligonucleotides derived from partial formamide digests (4,5) which had been fractionated on 20% polyacrylamide gels. A mobility shift analysis of [3'-³²P]-tRNA^{Arg} (2,6) confirmed the sequence CCG of nucleotides 65-76 (Fig. 1c.) A mobility-shift of a partial T₁-derived oligonucleotide confirmed the sequence of nucleotides 60-72. Data obtained from an RNA sequence gel of partial digests of [3'-³²P]tRNA, as well as mobility-shift analyses of formamide-derived oligonucleotides, verified the sequence of residues 34-76.

The sequence of nucleotides 1-39 was initially determined by identifying the 5'-termini of an array of formamide-derived oligonucleotides, fractionated on a 12% polyacrylamide gel. Due to the inability of formamide to cleave the GmG and CmU bonds, the identities of nucleotides 18 (G) and 33 (U) could not be identified by this method. However, these nucleotides were identified from analyses of the RNA sequence gels of [5'-³²P]-labeled tRNA (3,7). Furthermore, RNA sequence gels of [5'-³²P]-labeled tRNA confirmed the sequence of nucleotides 7-53. (One such gel is shown in Fig. 1b.) A mobility-shift analysis of [5'-³²P]-labeled tRNA established the sequence of nucleotides 1-9. A mobility-shift analysis of a formamide-derived oligonucleotide confirmed the sequence of nucleotides 14-23.

The sequence of nucleotides 14-54 was determined by identifying the 5'-termini of an array of formamide-derived oligonucleotides fractionated on a 15% polyacrylamide gel. A representative autoradiogram of one of the PEI-cellulose sheets is shown in Fig. 1e. Mobility-shifts of several of these formamide-derived oligonucleotides, as well as of partial T₁-derived oligonucleotides, were valuable in confirming the sequences of residues 27-39, 29-39, 37-45, 43-49, and 50-53. An autoradiogram of a representative mobility-shift (in the anticodon region) is shown in Fig. 1d.

DISCUSSION

There are seven eukaryotic arginine tRNAs or tRNA genes of known sequence (8,9). A comparison of these sequences shows that there are conserved residues in these seven molecules which are not universally present

in all tRNAs. These conserved nucleotides are: G₇, the parent nucleotide sequence GGC at nucleotides 9-11, the sequence GCPu Ψ C (or GCPuUC) at nucleotides 24-28, G₃₀, C₃₂ or Cm₃₂, A₃₈, C₄₀, G₄₂, GADU (or GAUU) at nucleotides 45-48, G₅₂, C₆₂, C₆₆ and G₆₉. Moreover, some of these nucleotides appear to be characteristic of eukaryotic, as compared to prokaryotic, arginine tRNAs. Thus, for example, G₄₂ is present in all seven eukaryotic arginine tRNAs or tRNA genes, but is not found in any of the prokaryotic or mitochondrial arginine tRNAs of known sequence (8). One feature of the bovine liver tRNA^{Arg}_{CCG} which is different from other eukaryotic arginine tRNAs is the A₂₉·U₄₁ base-pair in the anticodon stem, which is U₂₉·A₄₁ in the other eukaryotic arginine tRNAs. The bovine liver tRNA^{Arg}_{CCG} would be expected to recognize only the codon CGG, and it most probably would correspond to fraction I of Hatfield *et al.* (10). The overall sequence homologies of bovine liver tRNA^{Arg}_{CCG} to other arginine tRNAs or tRNA genes are: *E. coli* tRNA^{Arg}₂ (53%), *B. subtilis* tRNA^{Arg} (66%), yeast tRNA^{Arg}₂ (64%), Morris hepatoma mitochondrial tRNA^{Arg}_{UCG} (33%), mouse leukemia tRNA^{Arg}₁ (74%), and *Drosophila* tRNA^{Arg}₂ gene (70%).

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