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The nucleotide sequence of glycine tRNA from Mycoplasma mycoides sp. capri

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ABSTRACT

Using in vitro labelling techniques, a $tRNA^{Gly}$ from M. mycoides sp. capri PG3 has been shown to have the sequence : pGCAGGUGs^{4}UAGUUUAAUGGCAGAACUUC AGCCUUCCm^{6}AAGCUGAUUGUGAGGGUΨCGAUUCCCUUCACCUGCUCCA$_{OH}$. The anticodon is UCC and no other $tRNA^{Gly}$ has been detected in the crude tRNA isolated from this organism. As is the case with some mitochondrial tRNAs, where the genome size of the organelle is small, it is possible that this tRNA is used to read all four glycine codons GGN.

INTRODUCTION

The Mycoplasmas are the smallest self-replicating prokaryotes known,have a genome size of only 0.5×10^9 daltons and the DNA of most species has a high A + T content (60-75%)[1]. Although self-replicating, the species are normally parasitic, presumably as a result of theirsmall genome size. This is probably reflected further in the small number of modified nucleosides which occur in mycoplasma tRNA. The sequences of only two tRNAs[2,3] from Mycoplasma species have so far been published and we here report the sequence of a $tRNA^{Gly}$ from Mycoplasma mycoides sp. capri PG3.

MATERIALS AND METHODS

Sources of tRNA, enzymes, radioactive materials, chemicals and most techniques were all as described previously.[3] The $tRNA^{Gly}$ was purified to homogeneity on both BD-Cellulose and DEAE-Sephadex and either hydroxylapatite or RPC-5 columns. Sequence data was obtained (1) by analysis of the products obtained by complete T_1-RNase and pancreatic RNase digestion of the tRNA followed by 5'-end labelling and (2) by using the modification described by Tanaka et al.[4] of the method originally reported by Stanley and Vassilenko.[5]

RESULTS

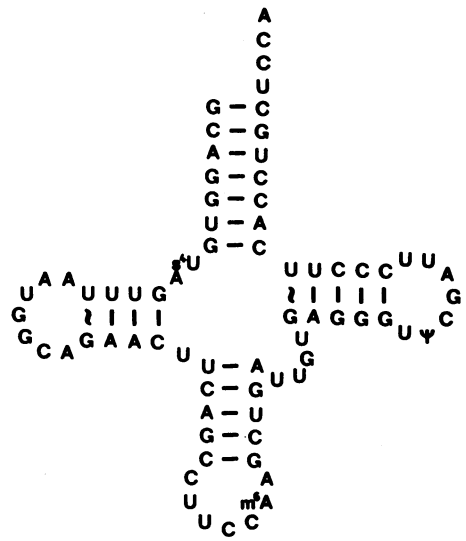
1. Nucleotide Composition of M. mycoides sp. capri $tRNA^{Gly}$. Two-dimensional thin layer chromatography of the $[^{32}P]N_p$ spots resulting from a T_2-RNase digest of the tRNA followed by incubation with polynucleotide kinase in the presence of γ-$[^{32}P]$ATP, showed that besides the four major nucleoside diphosphates, only pψp and pm^6Ap were present. The ultraviolet absorption spectrum of the tRNA had a 338/260 nm ratio of 0.027 with a distinct maximum at 338 nm, which corresponds to one mole of s^4U per tRNA molecule.

2. Total Sequence of M. mycoides sp. capri $tRNA^{Gly}$. The oligonucleotides obtained from complete T_1-RNase and pancreatic RNase digests had their sequences determined in the usual way.[3] No particular problems were encountered but the labelling of some oligonucleotides was far from quantitative. All attempts to 5'-label this tRNA failed. Thus, methods based on P_1 – nuclease digestion or gel sequencing methods which require intact 5'-labelled tRNA could not be used. We have no explanation for this failure, particularly as a contaminating tRNA comprising <5% of the total could be specifically and apparently quantitatively 5'-labelled under the conditions used. The method of Stanley and Vassilenko[5] as made practicable by Tanaka et al[4] was thus used to obtain the necessary overlaps. Nucleotides 9 - 67 could be identified unequivocally following DEAE-electrophoresis of the $[^{32}P]N_p$ spots and this together with the T_1-RNase and pancreatic RNase data enabled the sequence shown in the figure to be deduced.

DISCUSSION

Considerable homology is shown between this tRNA and E. coli $tRNA_1^{Gly}$ (78%) and E. coli $tRNA_2^{Gly}$ (77%).[6] In fact, more often than not, when a difference is observed in sequence between the mycoplasma $tRNA^{Gly}$ and E. coli $tRNA_1^{Gly}$, the sequence is there identical to E. coli $tRNA_2^{Gly}$ (10/16 residues). However, Mycoplasma are thought to be related to Gram-positive organisms[3,7] but unfortunately, no $tRNA^{Gly}$ sequence from such an organism is yet available.

Mycoplasma tRNAs are in general undermodified and this tRNA contains only 3 modified nucleosides : s^4U-8, ψ-55 and m^6A-37. The presence of the latter is of particular interest as none of the E. coli $tRNA_{1-3}^{Gly}$ have modified nucleosides at this position.[6] We have previously argued,[3] and we are to see in further discussion here, that the small genome size of the mycoplasmas plays a large part in reducing the number of "unnecessary" gene products and it is thus likely that this modification of A-37 serves



M. mycoides sp. capri tRNA^{Gly}

an essential purpose.

We have only ever detected one tRNA^{Gly} species in this mycoplasma even though crude tRNA has been subjected to fractionation and assay on columns of four different materials (see Materials and Methods). It has recently been suggested that in mitrochondria,[8] where, as in mycoplasma, the size of the genome is small and thus there may be expected to be pressure to reduce the number of non-essential gene products, it is possible to read all codons with a total of 24 tRNAs. This can be done by one tRNA recognizing all four codons in a family (GGN for glycine) by having an unmodified U in the 5'-position of the anticodon of the appropriate tRNA (position 34). tRNA^{Gly} from M. mycoides sp.capri is the only tRNA involved in protein synthesis other than those from mitochondria which has so far been found to have a U-34. Thus it is tempting to speculate that under conditions where the genome size is limited, the mitochondria and the mycoplasma have overcome the problem in a similar way by reducing the number of tRNAs required.

We are quite convinced that position 34 in tRNA^{Gly} is in fact U. Firstly, no other modified nucleoside has been detected on the base analysis other than ψ and m^6A. Secondly, following the sequencing by the method of Tanaka et al.,[4] the nucleoside diphosphate liberated from the 5'-end of oligonucleotide number 34 co-migrated with a pUp marker in the normal solvent system upon two-dimensional thin layer chromatography. Thirdly, partial snake venom digestion of the T_1-RNase fragment [^{32}P]-

CCUUCCm^{6}AAG (31-39) gives only one series of oligonucleotides upon two-dimensional electrophoresis and homochromatography, corresponding to the sequence shown. Also no block to snake venom digestion is seen in the time points of the digest.

Of course it is possible that in the original tRNA, the U is modified by a substituent which is labile and which is removed completely during purification of the tRNA or during the acidic conditions required to identify the relevant nucleoside diphosphate or 5'-nucleotide produced upon enzymatic digestion. This still does not explain however why only one tRNA^{Gly} species appears to be present in this tRNA and these two facts taken together suggest that M. mycoides sp. capri can read all four glycine codons GGN with one tRNA^{Gly} species whose anticodon is UCC.

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