Glycine and asparagine tRNA sequences from the archaebacterium, *Methanobacterium thermoautotrophicum*

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Two tRNA sequences from *Methanobacterium thermoautotrophium* are reported. Both $tRNA^{Gly}_{GCC}$ and $tRNA^{Asn}_{NUU}$, the first tRNA sequences from methanogens, were determined by partial hydrolyses (both chemical and enzymatic) and analyzed by gel electrophoresis. The two tRNAs contain the unusual T-loop modifications, Cm and m^1I, which are present in other archaebacterial tRNAs. Finally the presence of an unknown modification in the D-loop has been inferred by a large jump in the sequence ladder. These tRNAs are approximately equidistant from eubacterial or eukaryotic tRNAs.

tRNA sequence *Evolution* *Methanogen*

1. INTRODUCTION

The kingdom of archaebacteria is composed of a diverse set of organisms including thermoacidophiles, halophiles and methanogens [1,2]. Curiously, halophiles have enjoyed an extensive popularity even though they represent only a relatively small niche within the much larger grouping of methanogens. In our desire to use tRNA sequences as a probe of evolutionary relationships, we have turned our attention to the methanogens [3,4]. To date no tRNA sequences from methanogens have been reported, although a tRNA gene sequence has been published as part of a study of ribosomal RNA genes [5]. The two tRNA sequences determined here, $tRNA^{Asn}_{NUU}$ and $tRNA^{Gly}_{GCC}$ from *Methanobacterium thermoautotrophicum* are compared with other tRNA structures.

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2. METHODS

2.1. *Purification of the tRNAs*

Cells of *M. thermoautotrophicum* were generously supplied by Dr R.S. Wolfe. Crude tRNA was isolated by phenol extraction of cells and then purified by gel electrophoresis [3]: 20 A_{260} units were applied to a 10% polyacrylamide-7 M urea gel which was subjected to electrophoresis at 600 V for 48 h at 4°C in TBE buffer (50 mM Tris–borate and 5 mM EDTA). The tRNA was resolved into 13 main bands, which were isolated and further purified by electrophoresis in a 20% gel at room temperature (1000 V). Pure tRNA contained in the bands was extracted from the gel in a 0.5 M NH_4 acetate, 1 mM $MgCl_2$, 0.1 mM EDTA and 0.1% SDS solution overnight at room temperature, precipitated twice with ethanol and stored dry at −20°C.

2.2. *Preparation and sequencing of ^{32}P-labelled tRNA*

Labelling of tRNAs was carried out as in [6] and [7]. End-labelled tRNAs were electrophoresed for 15 h at 1500 V in thin (0.08 cm) 15% polyacrylamide–7 M urea gels. Under these conditions one

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main band for each of the different tRNAs (purified as above) was observed on the gel. tRNA sequencing was carried out by enzymatic hydrolysis of the 3′- or 5′-end labelled tRNA with RNases T_1, U_2, A, and Phy-M [7]. A variation of the technique in [8] was also used to identify and position modified nucleotides. In this latter technique, 1 μg tRNA was partially hydrolyzed in 5 μl deionized formamide at 100°C for 3–4 min. The resulting fragments were labelled with 100–200 μCi [γ-^{32}P]-ATP (3000 Ci/mmol) using 5 units polynucleotide kinase and separated on 15 and 20% polyacrylamide-7 M urea gels at room temperature in TBE buffer. The individual bands were excised from the gel and extracted with 300 μl of 300 mM NaCl, 0.1% SDS. To the spin filtrate, 2.5 vols of ethanol were added to precipitate the RNA. Digestion of labelled RNA fragments in 5 μg carrier tRNA was carried out with 0.5 units RNase T_2, 1 unit RNase T_1 and 0.5 μg RNase A in 100 mM Na acetate (pH 4.5) overnight at 37°C. The 5′-labelled nucleoside 3′,5′-diphosphates were identified by thin-layer chromatography on PEI–cellulose sheets developed in 0.55 M ammonium sulfate at 4°C or in 1.75 M ammonium formate (pH 3.5) at room temperature.

3. RESULTS

3.1. *Primary sequence*

The primary sequence was elucidated using rapid gel sequencing techniques. In most cases, fragments of the full molecule were essential to the final assignment of sequence. The variable loop region is particularly recalcitrant to structure determination, yet this region is readily available using a labelled fragment. Autoradiograms of typical sequencing gels are shown in figs 1,2. Fig.1 is the enzymatic degradation of the 5′-end labelled $tRNA^{Gly}_{GCC}$ whereas fig.2 is the enzymatic sequencing gel of a 5′-end labelled fragment of $tRNA^{Gly}$ covering A_9–G_{19}. This D-loop region was particularly difficult to determine because of an unknown modified nucleotide at position 15. Our preliminary sequence of this region did not take into account the inordinate jump in the sequence ladder, indicating that the adjacent phosphodiester bond is not cleaved during formamide hydrolysis. Our feeling now is that this position is occupied by a modified G because of its slight susceptibility to

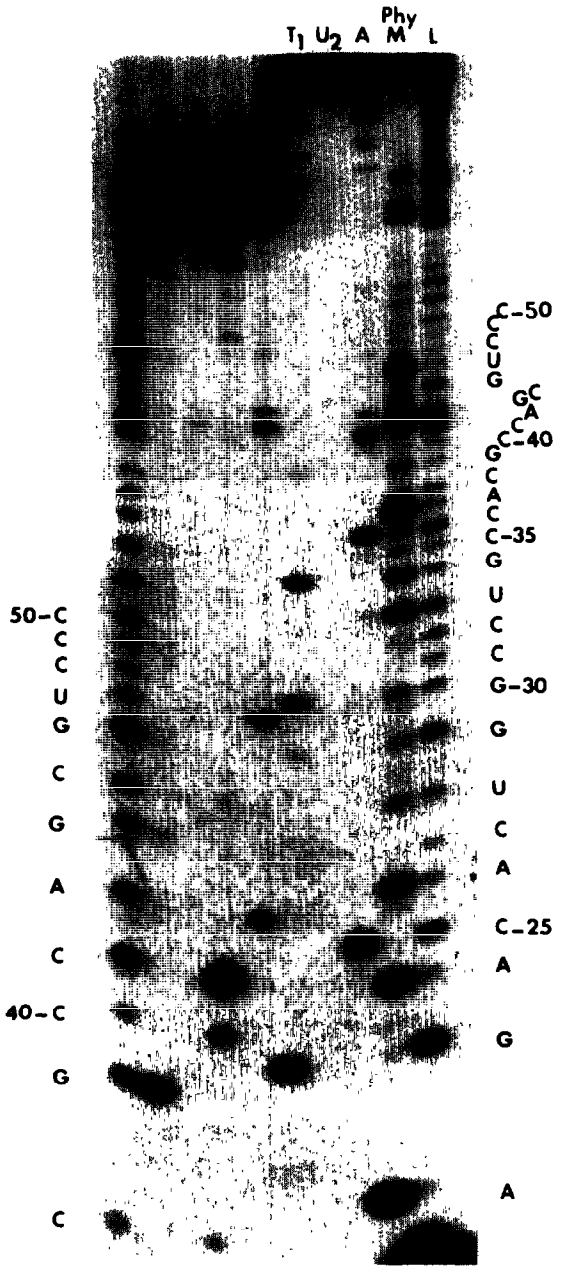

Fig.1. Enzymatic degradation of the 5′-end labelled $tRNA^{Gly}$. L is formamide ladder. T_1, U_2, A and PhyM represent the various enzymatic digestion tracks.

T_1 RNase and is perhaps the same modification which is present in some other archaebacterial tRNAs [9].

Fig.3,4 show the primary structure of $tRNA^{Gly}_{GCC}$ and $tRNA^{Asn}_{NUU}$ as deduced from the above results.

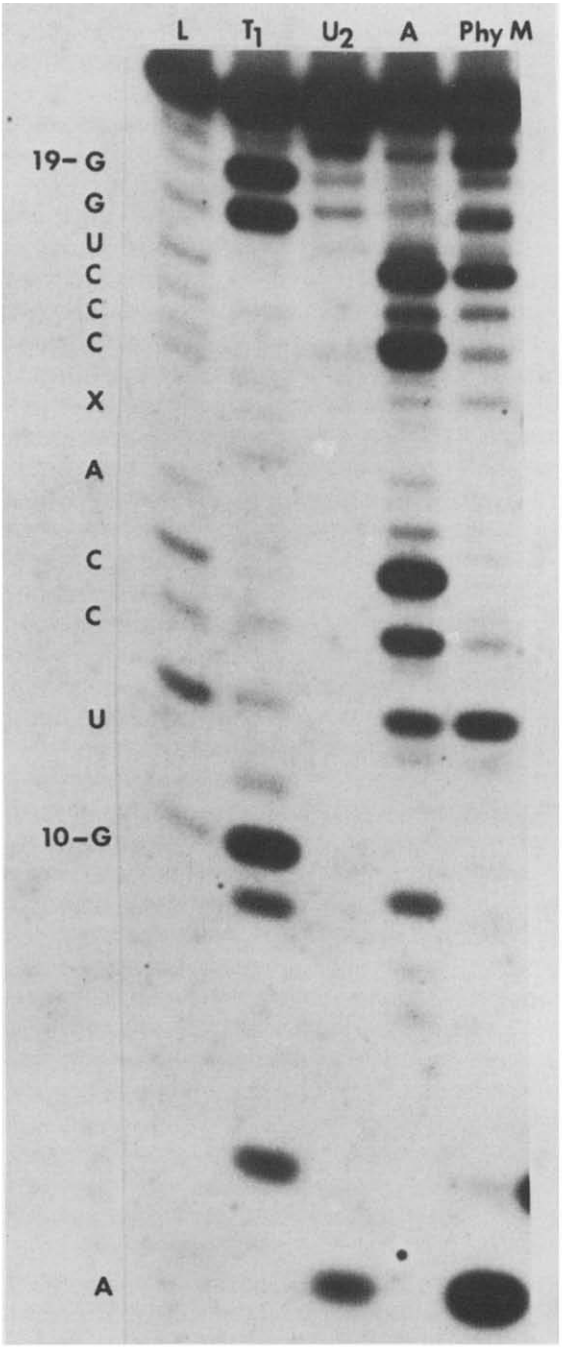

Fig.2. Enzymatic degradation of the 5′-end labelled fragment of $tRNA^{Gly}$ covering the sequence A-9–G-19.

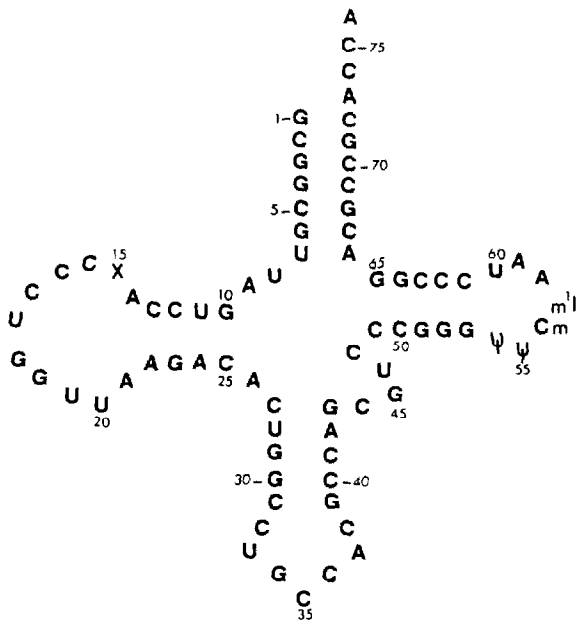

Fig.3. Cloverleaf folding of $tRNA^{Gly}$. Numbering is according to the Cold Spring Harbor convention.

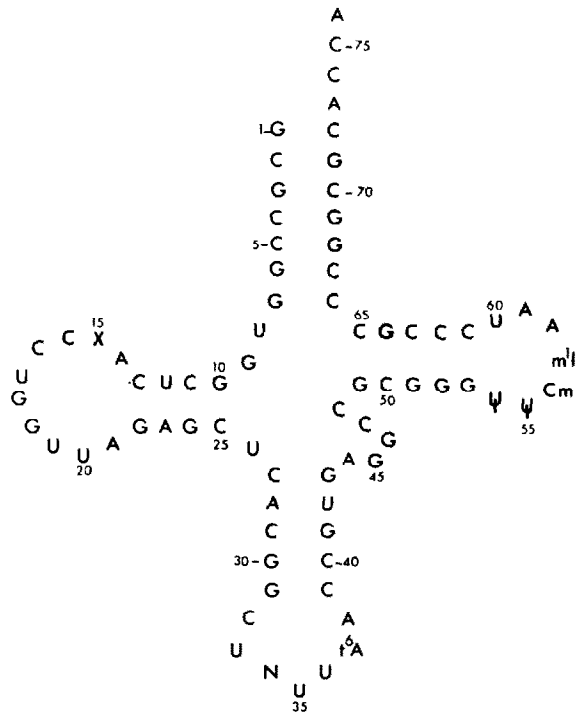

Fig.4. Cloverleaf folding of $tRNA^{Asn}$. Numbering is according to the Cold Spring Harbor convention.

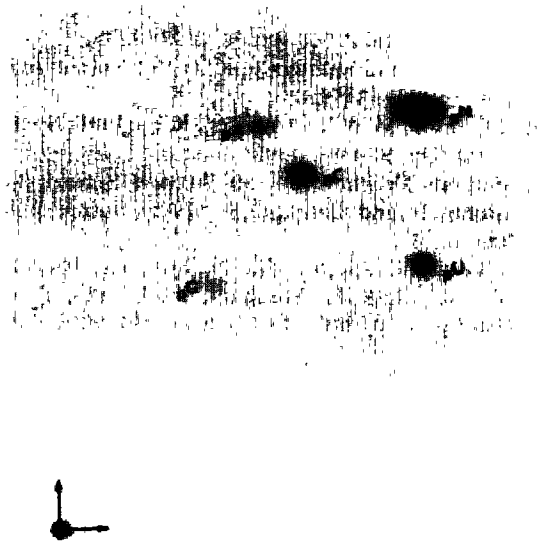

Fig.5. Two-dimensional thin-layer chromatography of position N_{34} of $tRNA^{Asn}$. First dimension is isobutyric acid: 0.5 M NH_4OH, 5:3 (v/v) and second dimension is isopropanol:conc.HCl:H_2O, 20:15:15 (v/v).

3.2. *Modified nucleosides*

Separate chromatographic analyses of the nucleoside diphosphates in 0.55 M ammonium sulfate and 1.75 M ammonium formate as well as the two-dimensional chromatography of the 5′-nucleoside monophosphates [10] indicated the presence of: ψ_{54}, ψ_{55}, Cm_{56} and m^1I_{57} [2] in these tRNAs and the presence of t^6A_{37} in $tRNA^{Asn}_{NUU}$. The dinucleotide triphosphate pCmpm^{1}Ip has the following R_f values: the R_{pAp} in 0.55 M ammonium sulfate is 1.95 and the R_{pUp} in 1.75 M ammonium formate (pH 3.5) is 1.56. N-34 in $tRNA^{Asn}_{NUU}$ is an unknown modification of G that could not be identified with the available standards: its position in a two-dimensional system on cellulose thin-layer plates is seen in fig.5.

4. DISCUSSION

Methanogens along with halobacteria, thermoacidophiles and some thermophiles are classified together in the kingdom of archaebacteria. We report here the first tRNA sequences from the methanogens, although a $tRNA^{Ala}$ gene sequence has been reported [5]. Among present data, only in the case of alanine, are representatives of both halobacteria and methanogens available for comparative analyses. The number of structural differences between these tRNAs (78% identical) could be a result of the phylogenetic divergence, or the fact that the two tRNAs have different anticodons and therefore may not have a recent ancestor [4].

The sequences reported here have many features found in other archaebacterial tRNAs. The *M. thermoautotrophicum* sequences however, exhibit rather long (10–11 bases) D-loops. The consensus sequence NψC$_m$m^{1}IAAU is present in the T-loop, but rather than m$^1\psi$, as in the halobacterial sequences, N is ψ in the methanogen sequences. The high G-C content (Gly and Asn 65%) is characteristically archaebacterial [1], and moreover inherent to thermophiles like *M. thermoautotrophicum* [11]. Finally, these tRNAs, as other archaebacterial tRNAs, have no dihydrouridine which may be because of the instability of this nucleotide under the growth conditions of these organisms. The D-loop is complicated however by an unknown nucleotide which defies hydrolysis in formamide or chemical degradation.

Comparison of the methanogen tRNAs with either eubacterial or eukaryotic tRNAs of the same family shows both methanogen sequences to be too distant to enable any conclusion as to their phylogeny (average number of differences was 31 after conversion of modified nucleotides to parents [4]). In the case of $tRNA^{Asn}$ and particularly $tRNA^{Gly}$, the D-loop is longer than either the eubacterial or the eukaryotic sequence. The length of the variable loop is different only in the case of the eukaryotic glycine tRNA. More precise comparisons will be possible as new sequences become available.

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