THE NUCLEOTIDE SEQUENCE OF SHEEP LIVER HISTIDINE-tRNA (ANTICODON Q–U–G)

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Received 13 May 1981

1. Introduction

In foetal lamb liver, an isoacceptor of $tRNA^{His}$ has been shown to share a number of features with the $tRNA^{His}_{GUG}$ from reticulocytes [1]:

(i) Both acylated tRNA cochromatographed on RPC-5 columns;

(ii) Their chromatographic pattern was not modified after CNBr treatment;

(iii) In the presence of purified tRNA-guanine transglycosylase from *Escherichia coli* [2] a radioactive guanine could be inserted into the anticodon loop of both molecules.

However, the actual degree of homology between these tRNAs could only be estimated on the basis of their respective nucleotide sequence. As an approach, we have determined the primary structure of the major isoacceptor of sheep liver $tRNA^{His}$, whose purification was published in [3].

2. Materials and methods

Sheep liver $tRNA^{His}_{GUG}$ was purified with an acceptor activity of 1600 pmol histidine/A_{260} unit [3]. [γ-^{32}P]ATP (2000–3000 Ci/mmol) and cytidine 3′,5′-[5′-^{32}P]bisphosphate were from Amersham France. T_4 polynucleotide kinase, T_4 RNA ligase and nuclease P_1 were obtained from P-L Biochemicals; calf intestine phosphatase was from Boehringer/Mannheim, T_1 and T_2 ribonucleases from Sankyo and ribonuclease A from Worthington. Limited hydrolysis of tRNA, polyacrylamide gel electrophoresis of the ^{32}P-labelled oligonucleotides and identification of the terminal 3′,5′-nucleoside diphosphates followed the procedures in [4,5]. Separation of the oligonucleotides

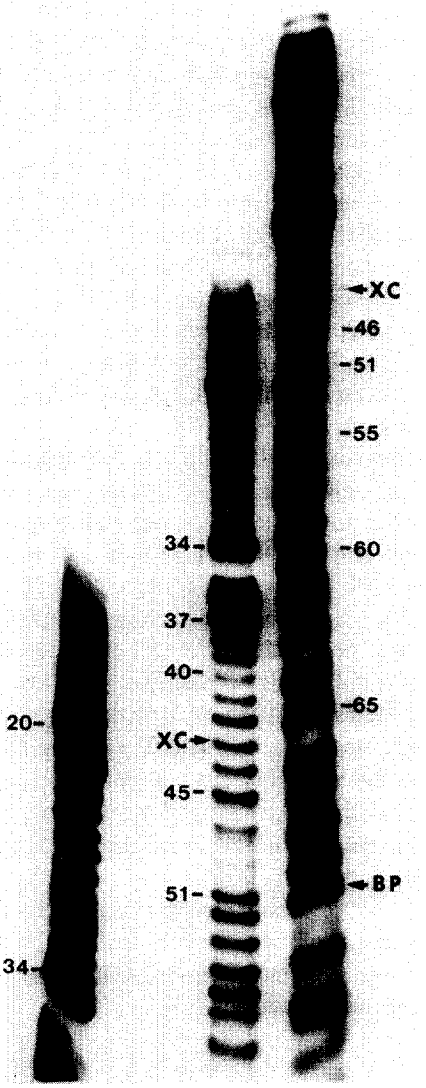

Fig.1. Electrophoretic pattern of a partial digest (bidistilled water, 4 min, 80°C) of $tRNA^{His}$. Three identical samples of the post-labelled oligonucleotides were fractionated on a 15% polyacrylamide gel (1 × 130 × 400 mm) (1000 V). Numbering of the fragments was from the 5′-end to the 3′-end. XC is xylene cyanol F F, BP is bromophenol blue.

* Part of the thesis to be presented by M. B.

after total digestion of tRNA with T_1 RNase was done as in [6,7]. Sequencing of the oligonucleotides and 5′-end labelling of tRNA was performed according to [8]. 3′-End labelling was done as in [9]. The analysis of 2 large fragments isolated from a partial pancreatic digest provided additional results. Further information was obtained by the sequence gel method [10]. Numbering of the residues in the tRNA was according to [11].

3. Results

The sequence of sheep liver $tRNA^{His}_{QUG}$ was derived from the following results.

Limited hydrolysis of tRNA in bidistilled water (4 min, 80°C) followed by electrophoretic separation of the 5′-end labelled oligonucleotides (e.g., fig.1) permitted to establish the sequence of 3 long fragments:

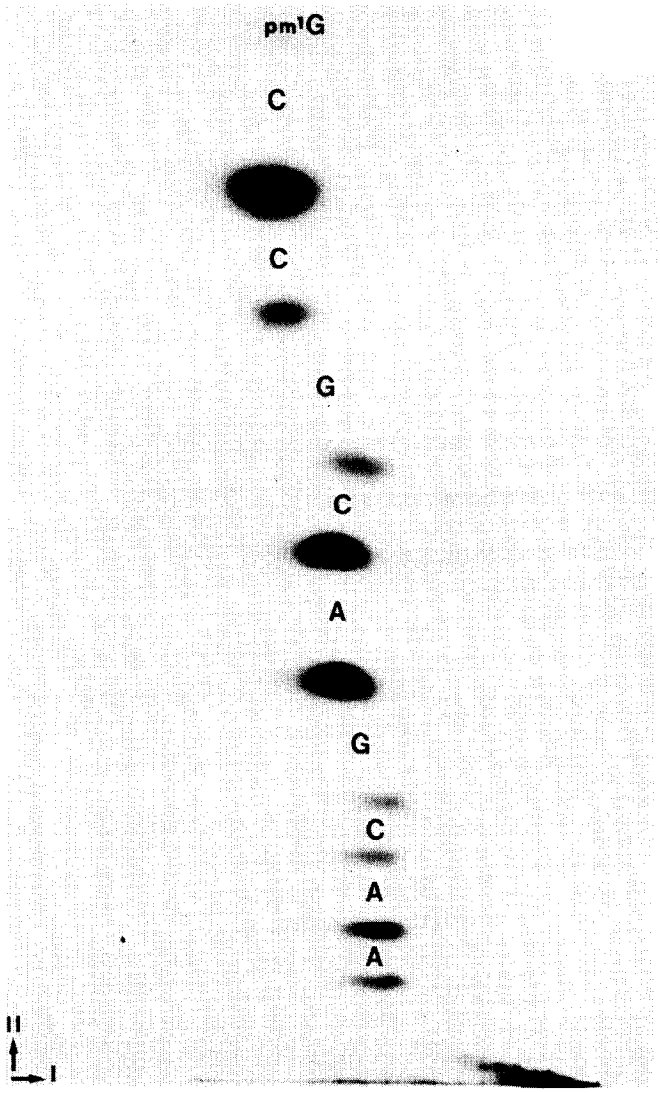

Fig.2. Analysis of a partial nuclease P_1 digest of the oligonucleotide 37 (fig.1): (I) first-dimension, electrophoresis on cellogel (pH 3.5); (II) second dimension, homochromatography in 50 mM KOH-strength 'homomix'.

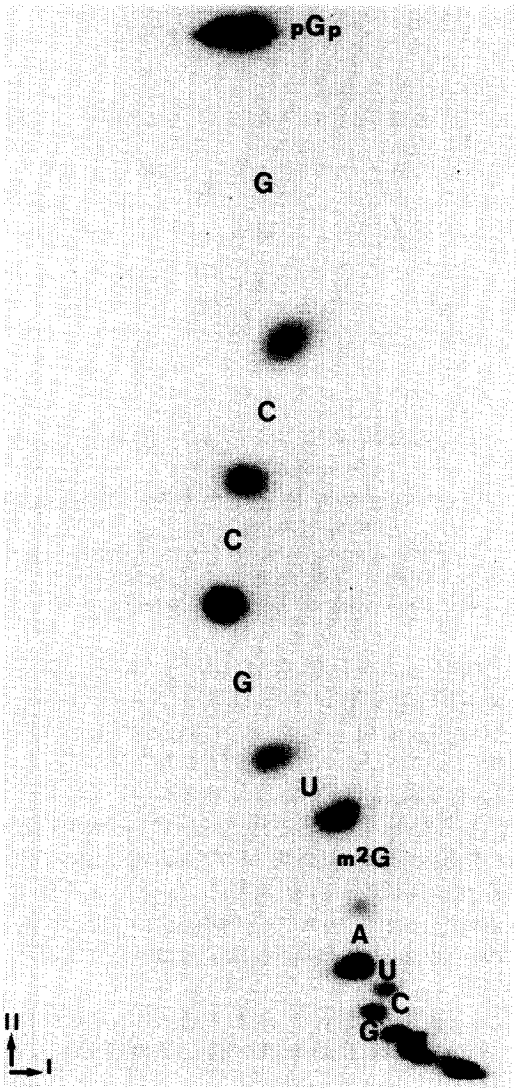

Fig.3. Mobility-shift analysis of a partial P_1 digest of 5′-^{32}-labelled $tRNA^{His}$: (I) first-dimension, electrophoresis on cellogel (pH 3.5); (II) second dimension, homochromatography in 25 mM KOH-strength 'homomix'.

Table 1
Compilation of sequences determined by different methods

	Aminoacyl stem				D Stem	D Loop			D Stem		Anticodon stem		Anticodon loop	
	1			5	10	15		20		25		30		35
I			G U m^2G A	U C	G U A Ψ	A G U G		D A	G U A C		U C U G C G		Ψ U Q U G m^1G C	
II						A G U G G		D D A	G U A					
III	pG G C C G U m^2G A			U C	G U									
V								D D A	G					

	Anticodon stem		Extra arm	T Ψ Stem	T Ψ Loop			T Ψ Stem		Aminoacyl stem			
	40		45	50	55		60		65		70		75
I	C G C A G		C A A	C G G	U Ψ C G m^1A A U			C C G A G		U C A C G G			
II		G	C A A C	C U C G G	U U						C G G C	A C C A_{OH}	
IV											C G G C	A C C A_{OH}	
V											m^5C	A C C A_{OH}	

I: Bidistilled water hydrolysis, post-labelling of the fragments and chromatographic identification of the 5′-terminal nucleotides
II: Limited nuclease P_1 digestion of oligonucleotides eluted from the gel and sequencing by two-dimensional homochromatography
III: Mobility-shift analysis of partial digests of 5′-end labelled $tRNA^{His}$ with nuclease P_1
IV: Mobility-shift analysis of partial digests of 3′-end labelled $tRNA^{His}$ with nuclease P_1
V: Sequencing of the oligonucleotides present in a total T_1 digest

G–U–m^2G–A–U–C–G–U–A–Ψ–A–G–U–G, D–A–G–U–A–C–U–C–U–G–C–G–Ψ–U–Q–U–G–m^1G–C–C–G–C–A–G–C–A–A, C–G–G–U–Ψ–C–G–m^1A–A–U–C–C–G–A–G–U–C–A–C–G–G, extending from nucleotides 4–18, 20:1–46 and 51–71, respectively. These results were confirmed by mobility-shift analysis of oligonucleotides eluted from gels of different electrophoretic runs. Fractionation of a partial digest of oligonucleotide 37 (fig.1) by two-dimensional homochromatography is shown in fig.2.

The overlap between fragments 20:1–46 and 51–71 could be assessed by limited nuclease P_1 digestion of the oligonucleotide present in band 43. Analysis of the material present in band 14 by the same technique permitted to bridge the gap between fragments 4–18 and 20:1–46. The presence of 2 guanylic residues at positions 18 and 19 was confirmed by sequencing the 5′-end labelled tRNA as in [10]. The existence of 2 adjacent D residues at positions 20 and 20:1 was ascertained by characterization of D–D–A–G in a total T_1 digest.

5′-End labelling of $tRNA^{His}$ followed by mobility-shift analysis of partial enzymatic digests led us to establish the following sequence: pG–G–C–C–G–U–m^2G–A–U–C–G–U (fig.3).

When the $tRNA^{His}$ was labelled at the 3′-end and similarly processed, the sequence C–G–G–C–A–C–C–A_{OH} could be determined. The modification of C72 was demonstrated by the characterization of a m^5C residue at the 5′-end of the oligonucleotide C–A–C–C–A_{OH} isolated from a T_1 total digest.

Table 1 summarizes the results obtained from the various analytical procedures. Fig.4 shows the sheep liver $tRNA^{His}_{QUG}$ in the classical clover-leaf form.

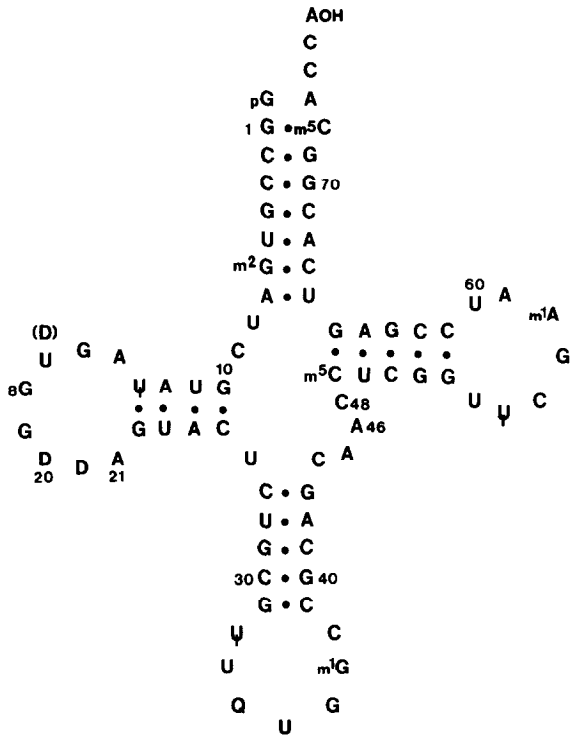

Fig.4. Cloverleaf model of sheep liver $tRNA^{His}_{QUG}$.

4. Discussion

The primary structure of *Drosophila melanogaster* $tRNA^{His}_{GUG}$ was determined in [12]. This tRNA was the first cytoplasmic $tRNA^{His}$ from eucaryotic origin to be sequenced. An extensive homology (84%) exists between this molecule and sheep liver $tRNA^{His}_{QUG}$. In view of the quasi-identity observed between *Drosophila* and mammalian $tRNA^{Phe}$ [13], this result was not unexpected. Two essential features are common to insect and mammalian $tRNA^{His}$:

(i) The uridine in position 54 is not modified into ribothymidine;

(ii) The left strand of the acceptor stem is 8 nucleotides long, as found also in other procaryotic [14] and eucaryotic [15] $tRNA^{His}$, but in both cytoplasmic $tRNAs^{His}$ from eucaryotic origin, the 5′-terminal residue is unpaired.

Only 5 basepairs are found to differ between sheep liver and *Drosophila* $tRNA^{His}$, namely 12–23, 28–42, 29–41, 50–64 and 51–63. In addition, cytidine 26 and uridine 44 in *Drosophila* tRNA are changed in sheep liver tRNA for uridine and cytidine, respectively. These differences have been confirmed by various analytical procedures.

Table 2
Comparison of eucaryotic $tRNA^{His}$ sequences

Source	Anti-codon	Aminoacyl stem 1	5	D Stem 10	D Loop 15	20	D Stem 25	Anticodon stem 30	Anticodon loop 35
Sheep liver	QUG	pG G C C G U m^2G A		U C G U A Ψ	A G U G G D D A		G U A C	U C U G C G	Ψ U Q U G m^1G C
Drosophila	GUG		G	C	D		G C	C A	G
Yeast mt	GUG	U G A A	U	A U	U C	A D	A A A	Ψ A C G C	G Ψ

Source	Anti-codon	Anticodon stem 40	Extra arm 45	T Ψ stem 50	T Ψ loop 55	60	T Ψ stem 65	Aminoacyl stem 70	75
Sheep liver	QUG	C G C A G	C A A C	m^5C U C G G	U Ψ C G m^1A A U		C C G A G	U C A C G G m^5C	A C C A_{OH}
Drosophila	GUG	U G	U	C m^5C A			U G		
Yeast mt	GUG	G C G U U	A U	C G A	T A U		U C	A U U C A	C C

The sequence of sheep liver $tRNA^{His}_{QUG}$ is presented at the top. In the other tRNAs, only those nucleotides are shown which are different from the corresponding nucleotides in sheep liver $tRNA^{His}_{QUG}$

The modified nucleosides m^2G, Q, m^1G, m^1A and D have been unambiguously identified as well as the 3 pseudouridine residues. m^2G is located at position 6 whereas an unmethylated guanosine appears at the same place in *Drosophila* $tRNA^{His}$. The same absence of methylation of G6 was observed in another tRNA from insects, when silkworm and human $tRNA^{Gly}_{GCC}$ were compared [16]. The uridine residue at position 16 was only partially modified into dihydrouridine.

The presence of m^5C residues in sheep liver $tRNA^{His}$ could be assumed from its nucleotide composition [3]. One of these modified nucleosides was located at position 72. The other one(s) should be present in the part of the molecule whose sequence was deduced from mobility-shift analyses of partial digests. C48 and C49 are potential candidates for a possible methylation. Chromatographic identification of the labelled nucleotides eluted from the gel fragment extending from A46–C51 clearly revealed the presence of m^5C. Additional data, obtained by sequencing a large oligonucleotide purified from a partial pancreatic digest, supported the methylation of C49.

As reported in [17], a 7-methylguanosine residue does exist in human liver $tRNA^{His}$. This modified nucleoside was not found in *Drosophila* $tRNA^{His}$ Although our results were indicative of the presence of m^7G in sheep liver $tRNA^{His}_{QUG}$ [3], we failed to detect it during our sequencing work.

Since the structural identity between $tRNA^{His}_{GUG}$ and $tRNA^{His}_{QUG}$ from *Drosophila* seems to be established [12], the differences between *Drosophila* $tRNA^{His}_{GUG}$ and sheep liver $tRNA^{His}_{QUG}$, reported here, should be accounted for by the different origin of these molecules. Their sequences are shown on table 2 comparatively to the sequence of yeast mitochondrial $tRNA^{His}_{GUG}$.

Acknowledgements

We thank Dr G. Keith for valuable advice and suggestions and Professor H. Clauser for critically reading the manuscript. This work was supported by a special grant from the Institut National de la Recherche Agronomique.

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