THE PRIMARY STRUCTURE OF TWO MAMMALIAN tRNAs^{Phe}: IDENTITY OF CALF LIVER AND RABBIT LIVER tRNAs^{Phe}

G. KEITH, J. P. EBEL and G. DIRHEIMER

Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, Rue René Descartes, Strasbourg, France

Received 31 August 1974

1. Introduction

During 1973 several authors [1,2] found that three initiator tRNAs$_f^{Met}$ originating from different mammals have the same primary structure. This led us to check whether this is also the case with non-initiator tRNAs, and whether this is a general feature for mammalian tRNAs. Having previously determined the nucleotide sequence of rabbit liver tRNA^{Phe} [3], we therefore looked on the structure of another mammalian liver tRNA^{Phe}, this of calf liver. As several minor features in the rabbit liver tRNA^{Phe} were still to be determined, we first established three not yet known dinucleotide sequences in this tRNA and tried to gain additional information about its Y base. Then we compared the oligonucleotides obtained by exhaustive digestion of calf liver tRNA^{Phe}, with either T_1 or pancreatic ribonucleases, to the corresponding oligonucleotides from rabbit liver tRNA^{Phe} digests. No difference in the nucleotide sequence of the two tRNAs was found.

2. Material and methods

Phenylalanine specific aminoacylation tests were performed in order to check the purity, using a crude aminoacyl tRNA synthetase extract from yeast under conditions previously described [4]. Phenylalanine specific tRNA from calf liver was purchased from Boehringer–Mannheim; it was at least 85% pure.

The structural investigations for the determination of the primary structure and the conditions for complete hydrolyses with either T_1 or pancreatic ribonucleases were as previously described [5]. The conditions for chemical recurrent degradations used to confirm the unsolved dinucleotide sequences in rabbit liver tRNA^{Phe} are in press [6].

For the determination of the Y base we used the acid excision conditions (incubation during 5 hr at 37°C in 0.1 M potassium phosphate adjusted to pH 2.9 with phosphoric acid) and the chromatographic system (silica gel TLC developped using the upper phase of an ethylacetate–1-propanol–water (4:1:2) mixture) described by Blobstein et al. [7].

3. Results

Table 1 shows the sequences or tentative sequences of the oligonucleotides obtained after exhaustive digestion with either T_1 ribonuclease or pancreatic ribonuclease of calf liver tRNA^{Phe} compared to the oligonucleotides from rabbit liver tRNA^{Phe}.

In a previous paper where the nucleotide sequence of rabbit liver tRNA^{Phe} was reported [3], the underlined U–C and C–U sequences in the following oligonucleotides C–U–C–m^{1}A–Gp, A–Cm–U–Gm–A–A–Y–A–ψ–C–U–A–A–A–Gp and m^{7}G–U–C–m^{5}C–C–U–Gp had not been completely established. Using a chemical recurrent degradation method [6] involving periodate oxidation, β-elimination and dephosphorylation, we determined the right sequences of the unknown pyrimidic dinucleotides, which are those indicated above.

The 'Y' base of rabbit liver tRNA^{Phe} had not yet been characterized. Using the acid excision conditions and the thin-layer chromatographic system of

 North-Holland Publishing Company – Amsterdam

Table 1
Comparison of T_1 and pancreatic ribonuclease digests of $tRNA^{Phe}$ from calf and rabbit liver

Rabbit liver $tRNA^{Phe}$		Calf liver $tRNA^{Phe}$	
Gp	6	Gp	6
G–m_2^2Gp**	0.8	C–m_2^2Gp**	0.8
A–Gp	2	A–Gp	2
C–A–C–C–A	1	C–A–C–C–A	1
hU–hU–Gp	1	hU–hU–Gp	1
pGp	1	pGp	1
C–C–Gp	1	C–C–Gp	1
C–U–C–m^1A–Gp	1	(C,U,C)–m^1A–Gp	1
T–ψ–C–Gp	0.7	T–ψ–C–Gp	0.5
U–ψ–C–Gp	0.3	U–ψ–C–Gp	0.5
ψ–ψ–A–Gp**	0.8	ψ–ψ–A–Gp**	0.8
m^1A–U–C–C–C–Gp	1	m^1A–U–C–C–C–Gp	1
U–U–U–C–Gp	1	(U,U,U,C)–Gp	1
C–m_2^2G–ψ–ψ–A–Gp**	0.20	C–m_2^2G–ψ–ψ–A–Gp**	0.2
m^7G–U–C–m^5C–C–U–Gp	0.85	m^7–G–hU–(C,m^5C,C,U)–Gp	1
m^7G–hU–C–m^5C–C–U–Gp	0.15	–	–
A–A–A–U–A–m^2Gp	1	A–A–A–U–A–m^2Gp	1
A–Cm–U–Gm–A–A–Y*–A–ψ–C–U–A–A–A–Gp	1	A–Cm–U–Gm–A–A–Y*–A–ψ–(C,U)–A–A–A–Gp	1
Adenosine	1	Adenosine	1
Cp	11	Cp	11
m^5Cp	1	m^5Cp	1
ψp	2	ψp	2
Up	5	Up	5
hUp	1	hUp	1
A–Cp	1	A–Cp	1
m_2^2G–ψp	1	m_2^2G–ψp	1
m^1A–G–hUp	1	m^1A–G–hUp	1
G–m^1A–Up	1	G–m^1A–Up	1
G–G–Tp	0.7	G–G–Tp	0.5
G–G–Up	0.3	G–G–Up	0.5
G–G–Cp	1	G–G–Cp	1
A–m^2G–Cp	1	A–m^2G–Cp	1
pG–Cp	1	pG–Cp	1
G–G–G–Up	1	G–G–G–Up	1
Gm–A–A–Y*–A–ψp	1	Gm–A–A–Y*–A–ψp	1
G–A–A–A–Up	1	G–A–A–A–Up	1
A–A–A–G–m^7G–Up	0.85	–	–
A–A–A–G–m^7G–hUp	0.15	A–A–A–G–m^7G–hUp	1
A–G–A–Cm–Up	1	A–G–A–Cm–Up	1
G–G–G–A–G–A–G–Cp	1	(G,G,G,A,G,A)G–Cp	1

Trace amounts of di- and tri-nucleotides found in the T_1 and pancreatic hydrolysates of calf liver $tRNA^{Phe}$ are not shown. They are due to the fact that this tRNA is only 85% pure and therefore is contaminated by a small amount of other tRNAs. The sequences between brackets from calf liver $tRNA^{Phe}$ might have the same structure as the corresponding oligonucleotides from rabbit liver tRNA but have not yet been confirmed. Their composition is identical. The underlined dinucleotide sequences correspond to the pyrimidic sequences previously published [3].

*Y is peroxy Y.

** C–m_2^2G–ψ–ψ–A–Gp is a T_1 RNase resistant oligonucleotide. Only high amounts of T_1 RNase led to nearly complete breakage (500 units T_1 RNase for 25 A_{260} units of tRNA).

Blomstein et al. [7], we compared it to the yeast $tRNA^{Phe}$ Y base and to the peroxy-Y base found in calf liver $tRNA^{Phe}$. The chromatographic properties of the 'Y' from rabbit liver were identical with those of the peroxy-Y from calf liver. It seems therefore probable that 'Y' from rabbit liver is proxy-Y.

The comparison of the oligonucleotides obtained by exhaustive enzymatic digestion of rabbit and calf liver $tRNA^{Phe}$ showed no difference in the nucleotide sequences (table 1). Only minor differences located at the odd nucleotides were observed.

The ratio T–ψ–C–G/U–ψ–C–G is 1 in calf liver $tRNA^{Phe}$ whereas it is 2 in rabbit liver $tRNA^{Phe}$. This means that the modification of U into T in position 23 from the 3′ end is less important in calf liver $tRNA^{Phe}$ than in rabbit liver $tRNA^{Phe}$. The hU/U ratio in position 30 from the 3′ end is 1/6 in rabbit liver $tRNA^{Phe}$, whereas this position is completely occupied by hU in the calf liver $tRNA^{Phe}$. All other minor nucleotides were found in the same oligonucleotides in both $tRNAs^{Phe}$. Particularly m^1A was found again in two oligonucleotides arising from either T_1 or pancreatic ribonucleases digests: here like in rabbit liver $tRNA^{Phe}$ one of these m^1A is located in position 14 from the 5′ end, a place where up to now this minor nucleoside was only found in rabbit liver $tRNA^{Phe}$.

The fluorescent base adjacent to the 3′ end of the anticodon was previously found in calf liver $tRNA^{Phe}$ to be a mixture of 90% peroxy-Y base and 10% Y base [7]. With the calf liver $tRNA^{Phe}$ we used in this work, we obtained only one spot which had an R_F similar to that described for peroxy-Y. We therefore conclude that this calf liver $tRNA^{Phe}$ contains only peroxy-Y. This result can be explained by the observation of Blomstein et al. [7] who found that beef liver $tRNA^{Phe}$ also contains only peroxy-Y. Our result therefore can be due to the fact that the calf liver $tRNA^{Phe}$ purchased from Boehringer–Mannheim may have been extracted from older calves than the calf liver $tRNA^{Phe}$ prepared by Blomstein et al. [7] where the maturation of Y into peroxy-Y was not completely achieved.

In conclusion, it is interesting to point out that this is the second case where isoaccepting tRNAs from different mammals have the same primary structure. Therefore structural identity is not restricted in mammalian tRNAs to initiator tRNAs, but could be a general feature. To confirm this hypothesis, other comparisons between mammalian tRNAs will be necessary.

Acknowledgements

We wish to thank Mrs C. Fix for excellent technical collaboration. This work was partly supported by grants from the Université Louis Pasteur de Strasbourg, from the Délégation à la Recherche Scientifique et Technique, from the Institut National de la Santé et de la Recherche Médicale (contrat 731006 1) and from the Commissariat à l'Energie atomique.

References

[1] Piper, P. W. and Clark, B. F. C. (1974) Nature 247, 516.
[2] Simsek, M., Raj Bhandary, U. L., Boisnard, M. and Petrissant, G. (1974) Nature 247, 518.
[3] Keith, G., Picaud, F., Weissenbach, J., Ebel, J. P., Petrissant, G. and Dirheimer, G. (1973) FEBS Letters 31, 345.
[4] Keith, G., Gangloff, J. and Dirheimer, G. (1971) Biochimie 53, 661.
[5] Keith, G., Roy, A., Ebel, J. P. and Dirheimer, G. (1972) Biochimie 54, 1405.
[6] Keith, G. and Gilham, P. T., submitted to Biochemistry.
[7] Blomstein, S. H., Grunberger, D., Weinstein, I. B. and Nakanishi, K. (1973) Biochemistry 12, 188.