EVIDENCE FROM CHEMICAL MODIFICATION FOR AN UNUSUAL TERTIARY STRUCTURE OF THE TψC LOOP IN RABBIT LIVER $tRNA_1^{Val}$

Valentin V. VLASSOV, Anatoly T. PUSYRIOV and Hans J. GROSS+

Institute of Organic Chemistry, Siberian Division of the Academy of Sciences, Novosibirsk, USSR and *+Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, FRG*

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1. Introduction

Mammalian $tRNA_1^{Val}$, in contrast to all other tRNAs, contains two adenosines A_{59} and A_{60} opposite to U_{54} and ψ_{55}. There is evidence that its TψC stem is extended by an additional A_{60}:U_{54} pair, and that the TψC loop (loop IV) is two nucleotides shorter as compared to normal tRNAs [1,2]. One may suggest that this unique feature may influence the tertiary structure of the $tRNA_1^{Val}$.

Alkylating reagents are particularly suitable for investigations of nucleic acid tertiary structure since they react with single-stranded as well as with helical regions of nucleic acids [3]. Earlier we studied the alkylation of *Escherichia coli* $tRNA^{Phe}$ with 2',3'-*O*-[4-(*N*-2-chloroethyl-*N*-methylamino)-benzylidene]-uridine-5'-methylphosphate and found that reactivities of guanosines in this tRNA towards that reagent are strongly influenced by interactions of their N7 atoms with Me^{2+} and by participation of the N7 atoms in hydrogen bonding [4].

We report here results of the alkylation of rabbit liver $tRNA_1^{Val}$. The data presented here suggest the absence of one of the elements of tRNA tertiary structure in this tRNA: a hydrogen bond between N7 of guanosine G_{57} and the 2'-hydroxyl group of ribose 55.

2. Materials and methods

Rabbit liver $tRNA_1^{Val}$ was isolated as in [1]. RNase A was from Calbiochem, T_2 RNase from Sankyo Co., DEAE-cellulose DE 23 SS was from Serva.

MepURCl was synthesized as in [5].

$tRNA_1^{Val}$ was alkylated under following ionic conditions: (A) 0.1 M $NaClO_4$, 5.8 mM $Mg(ClO_4)_2$; (B) 0.1 M $NaClO_4$; (C) no salt added. In each case, reaction mixtures contained $tRNA_1^{Val}$ (3.9×10^{-5} M), MepURCl (5.4×10^{-3} M), EDTA (5×10^{-4} M) and 0.03 M Tris–ClO_4, pH 7.7.

After alkylation, $tRNA_1^{Val}$ was incubated at pH 4.0 to hydrolyze the acetal bond of the reagent, and was digested with RNase A as in [4,6,7]. Ribonuclease A digests of modified $tRNA_1^{Val}$ were analyzed by means of micro-column chromatography [8] (see fig.1 legend). To determine the nucleotide composition, modified oligonucleotides were digested with T_2 RNase after purification by chromatography on DEAE-cellulose, and the mononucleotides obtained were analyzed by chromatography on Aminex A-27 using a 0.14–1.4 M formate gradient [9]. Relative rate constants of alkylation 'K_j' of guanosines in $tRNA_1^{Val}$ were calculated according to [4,7].

3. Results and discussion

Mammalian $tRNA_1^{Val}$ contains two adenosines A_{59} and A_{60} opposite to U_{54} and ψ_{55}, which could form unusual base pairs in addition to the 5 normal pairs. Investigations of differential melting of a 3'-terminal fragment of this tRNA containing the TψC stem, and of the enzymatic U_{54} methylation in $tRNA_1^{Val}$ strongly suggest the presence of a 6 base pair TψC stem and a 5 nucleotide loop IV in this

Abbreviation: MepURCl, 2',3'-*O*-[4-(*N*-2-chloroethyl-*N*-methylamino)-benzylidene]-uridine-5'-methylphosphate

tRNA [1,2]. The functional significance of this structural peculiarity in $tRNA_1^{Val}$ is not clear.

Earlier we applied the guanosine-specific alkylating reagent MepURCl for investigations of the tertiary structure of yeast $tRNA_1^{Val}$ and of *E. coli* $tRNA^{Phe}$ [4,6,7]. Reactivities of guanosines expressed in terms of relative rate constants of alkylation, K_j, may be used for the detection of some elements of tertiary structure of tRNAs: hydrogen bonds, in which N7 atoms of guanosines participate, and interactions of N7 of guanosines with Me^{2+} [4].

Now we applied this approach for an investigation of the structure of mammalian $tRNA_1^{Val}$. This tRNA was alkylated with MepURCl under conditions stabilizing the tertiary structure, under conditions where tRNA should have a cloverleaf structure [10], and under conditions of a partially unfolded cloverleaf (conditions A,B,C, respectively). The tRNA was alkylated in presence of 1–2 mol reagent/mol tRNA in order to obtain information about the initial rates of modification of guanosines, thus characterizing their reactivities in tRNA molecules unchanged by the modification [4,7]. Alkylated $tRNA_1^{Val}$ was digested with RNase A and the oligonucleotides obtained were analyzed by micro-column chromatography. Since alkylated oligonucleotides contain dialkylaminobenzaldehyde moieties absorbing at 350 nm, it is easy to locate them with a multi-wavelength microspectrophotometer in the course of chromatographic runs.

Figure 1 shows results of chromatographic analyses of ribonuclease A digests of rabbit liver $tRNA_1^{Val}$. It is evident that the chromatographic system resolves the majority of the oligonucleotides. Figure 2 shows the results of chromatographic analyses of oligonucleotides of ribonuclease A digests of $tRNA_1^{Val}$ alkylated under conditions stabilizing the tertiary structure of tRNA. Relative rate constants for the alkylation of guanosines in $tRNA_1^{Val}$ under various conditions are summarized in table 1. For comparison, table 1 also contains data

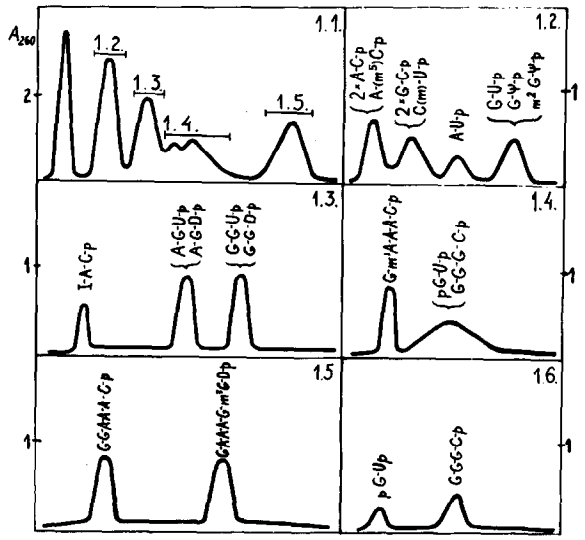

Fig.1. Chromatographic analysis of the RNase A digest of rabbit liver $tRNA_1^{Val}$. All chromatographies were performed on columns with DEAE cellulose (30 µl) in 7 M urea, using linear NaCl gradients; the total elution volume was 0.6 ml. (1.1) Fractionation of a RNase A digest of $tRNA_1^{Val}$; 0.01 M Tris–Cl^-, pH 8.2, NaCl 0.00–0.24 M. (1.2–1.6) Rechromatographies: (1.2) Dinucleotides, pH 3.7 (pH was adjusted with HCOOH), NaCl 0.00–0.10 M; (1.3) Trinucleotides, pH 3.7, NaCl 0.00–0.10 M; (1.4) Fraction 1.4 from (1.1) pH 3.7, NaCl 0.00–0.20 M; (1.5) Fraction 1.5 from (1.1) pH 3.0, NaCl 0.00–0.12 M; (1.6) Fraction containing pG–Up and G–G–G–Cp (not shown in (1.1)), 0.005 M CH_3COONa, pH 4.9, NaCl 0.00–0.20 M.

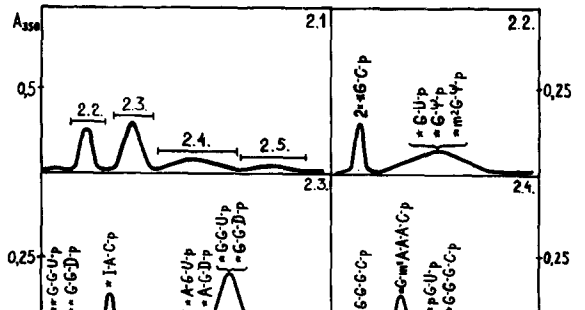

Fig.2. Chromatographic analysis of RNase A digests of $tRNA_1^{Val}$ alkylated under conditions stabilizing the tertiary structure of tRNA (conditions A). Chromatographies were performed as in fig.1 legend. One or two asterisks in diagrams (2.2–2.6) indicate moderate or high reactivities, respectively, of guanosines (inosine); for details see table 1.

Table 1
Relative rate constants of alkylation of guanosines in rabbit liver $tRNA_1^{Val}$, *E. coli* $tRNA^{Phe}$ and yeast $tRNA_1^{Val}$, K_j (M^{-1})

Nucleoside in rabbit liver $tRNA_1^{Val}$	K_j alkylation conditions			K_j for corresponding guanosines			
	A	B	C	*E. coli* $tRNA^{Phe}$ conditions (A)		Yeast $tRNA_1^{Val}$ (alkylation in 0.04 M Tris–SO_4, 2×10^{-3} M $MgSO_4$)	
G_1	5.2	15	20	8.5		14	
G_{10}	1.9	32	68	44		–	
G_{15}	1.9	19	25	3.8		1.8	
G_{17}, G_{18}	19	39	60	21	($G_{18,19}$)	23	($G_{18,19}$)
G_{30}, G_{39}	6	20	37	2.4	(G_{27-30})	16	
I_{34}	30	30	73	31	(G_{34})	21	(I_{35})
G_{42}, G_{45}	7	24	47	8.3	(G_{44}, X_{47})	–	
G_{52}, G_{53}	4	12	43	18		1.8	(G_{53})
G_{57}	20	16	39	1.7		–	
G_{63}, G_{64}, G_{65}	3.9	15	20	3.2	($G_{5,6}, G_{63,65}$)	–	
G_{67}, G_{68}	4.9	12	26	1.6	(G_{69-71})	–	

For the oligonucleotides containing several guanosines, average constants were calculated. Oligonucleotides G–G–Up and G–G–Dp, A–G–Up and A–G–Dp were not separated by the chromatographies used. Their amounts were determined from the Up content according to the nucleotide composition analysis of mixtures of these oligonucleotides

for corresponding guanosines in yeast $tRNA^{Val}$ and *E. coli* $tRNA^{Phe}$. Under conditions stabilizing the tertiary structure of tRNA, inosine I_{34} and guanosines G_{17}, G_{18} and G_{57} are most reactive in $tRNA_1^{Val}$. The least reactive are guanosines G_{10} and G_{15}. Other guanosines showed intermediate reactivities. When alkylation was performed under conditions destabilizing tRNA tertiary structure, reactivities of all guanosines in $tRNA_1^{Val}$ were nearly of the same value, in accordance with results obtained earlier with *E. coli* $tRNA^{Phe}$ [4]. High reactivity of inosine in the anticodon of $tRNA_1^{Val}$ and of guanosines in its D loop are reasonable as they are exposed in the tRNA tertiary structure [11], in accordance with results of other chemical modification studies. Reactivities of guanosines in double-stranded regions of $tRNA_1^{Val}$ are of the same order of magnitude as those of guanosines in double-stranded regions in other tRNAs. The values for yeast $tRNA^{Val}$ are somewhat higher as it was alkylated at lower salt concentrations. Differences in reactivities of guanosines in helical regions of tRNAs may be due to differences in stacking interactions; the lowest values are characteristic for polypurine sequences. As in other tRNAs, guanosine G_{15} in $tRNA_1^{Val}$ is characterized by low reactivity. Probably, this guanosine participates in interaction with Me^{2+} in all tRNAs [4,12]. Reactivities of guanosines G_{10} are different in the tRNAs studied, obviously due to differences in the interactions of their D stems with variable loops. Thus the interaction between G_{10} and G_{45} found in yeast $tRNA^{Phe}$ [11] may occur in rabbit liver $tRNA_1^{Val}$, but not in *E. coli* $tRNA^{Phe}$.

G_{57} in loop IV of $tRNA_1^{Val}$ is one of the most reactive guanosines in contrast to guanosine G_{57} in *E. coli* $tRNA^{Phe}$ [4]. N7 of guanosine G_{57} in yeast $tRNA^{Phe}$ and obviously in *E. coli* $tRNA^{Phe}$ participate in the formation of hydrogen bonds with the 2′-hydroxyl group of ribose 55 [11]. This is one of the important interactions stabilizing the uridine turn (U-turn) in the TψC loop of usual tRNAs and has been proposed to be a universal element of tRNA TψC and anticodon loops [13]. It is yet unknown whether RNAs other than tRNAs also contain U turns [13]. It may therefore be mentioned here that an infectious circular RNA molecule, the viroid of the

potato spindle tuber disease [14], contains UCG and UCA sequences, respectively, in both loops at the ends of its native structure, similar to the situation in the TψC loops of tRNA.

The results discussed here suggest the absence of this interaction in rabbit liver $tRNA_1^{Val}$. Therefore, the extention of the TψC stem and shortening of the TψC loop in this tRNA result in the elimination of the interaction characteristic for tertiary structures of other tRNAs. This may as well affect the interaction between TψC loop and D loop in this tRNA; however, with this method we did not find any other peculiarities in the organisation of the tertiary structure of this tRNA. On the other hand it seems possible that the unusual loop IV structure of rabbit liver $tRNA_1^{Val}$ may be related to its codon response [1], which is in contrast to the wobble hypothesis [15–17]. Interestingly, the corresponding *Drosophila* $tRNA_4^{Val}$ which seems to have a conventional TψC loop with a pyrimidine opposite to T_{54}, shows a less abnormal codon response [18].

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