RELATIVE REACTIVITIES OF SOME GUANOSINE RESIDUES IN THE "HALVES" OF $tRNA_f^{Val}$ (YEAST) AND IN THEIR COMPLEX

V.V. VLASOV, N.I. GRINEVA, D.G. KNORRE and R.M. PAVLOVA

Institute of Organic Chemistry, Siberian Division of the Academy of Sciences, Novosibirsk, USSR

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1. Introduction

Recently we have proposed the method of investigation of the macrostructure of tRNA based on the determination of reactivities of individual nucleosides in tRNA by measuring their relative initial rates of modification [1]. These initial rates reflect the availability of individual nucleosides for reagent in native tRNA with the macrostructure undisturbed by the modification. We have applied this approach to the study of the macrostructure of yeast $tRNA_f^{Val}$ with water-soluble guanosine-specific (cf [2]) alkylating reagent, 2′,3′-*O*-[4-(*N*-2-chloroethyl-*N*-methylamino)-benzylidene]-uridine-5′-methylphosphate (MepURCl) [3, 4].

The present paper is concerned with results of such a modification study performed with "halves" of $tRNA_f^{Val}$ (fragment pG_1-Ip_{35} = 5′-H and fragment $A_{36}-A_{77}$ = 3′-H) and with their complex.

2. Materials and methods

The equipment for micro-column chromatography was elaborated in our institute. Details of the methods will be published [5]. Fragments of $tRNA_f^{Val}$ were kindly provided by Dr. A.D. Mirzabekov, Institute of Molecular Biology of the Academy of Sciences of the USSR, Moscow, and by N.I. Komarova of our Institute. The complex of the fragments incorporated 840 pmoles of valine per 1 A_{260} unit of the 3′-fragment (optical density at 260 nm A_{260} was measured in 0.1 M Tris-HCl, pH 7.5, 0.02 M $MgSO_4$) with rat liver valine: tRNA ligase. Polynucleotides were modified with MepURCl at 20°, in 0.04 M Tris-H_2SO_4, containing 2×10^{-3} M $MgSO_4$, polynucleotide 2 mg/ml, MepURCl 8 mM (conditions for stability of the macrostructure of tRNA). To obtain complex of the fragments, equimolar amounts of the 5′-H and 3′-H were dissolved in 40 mM Tris-H_2SO_4, pH 7.6, plus 2 mM $MgSO_4$. The solution was kept for 15 min at 20°, and then the reagent dissolved in the same buffer was added. Modified polymers were isolated from the reaction mixture and acetal bonds of modified guanosine residues were hydrolysed at pH 4 as described earlier [1]. The procedure afforded modified polymers with 7-alkylguanosine residues absorbing at 350 nm due to the presence of benzaldehyde moieties (extinction coefficient of 24×10^3). The extent of modification was determined from the A_{350}/A_{260} ratio of the polymer peak determined by microspectrophotometry.

5′-H and 3′-H were modified respectively to the extent of 0.7 and 0.9 moles of the reagent per mole of fragment. The complex of the halves was modified to the extent of 0.9 moles of the reagent per mole of the complex. Modified polymers were desalted and digested with pyrimidyl-ribonuclease, as described in [1]. The digests were applied to DEAE-cellulose columns for chromatographic analysis, as described in [1]. The digests were chromatographed at pH 8 in 7 M urea. Fractions of oligonucleotides were rechromatographed on DEAE cellulose at pH 3.7 in 7 M urea to separate modified oligonucleotides from unmodified ones [1]. Modified oligonucleotides absorb at 350 nm due to the presence of benzaldehyde moieties, and their relative amounts were determined from the A_{350} in their peaks. In the course of the

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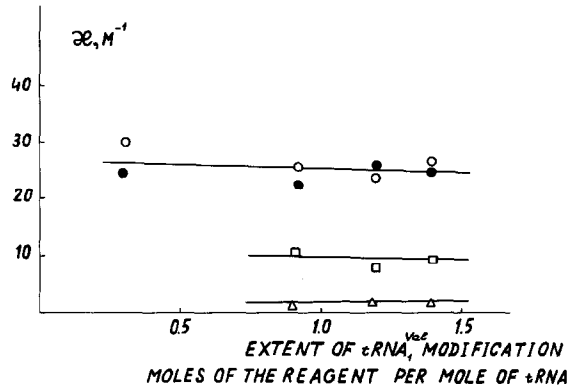

Fig. 1. Relative rate constants of modification of some guanosine and inosine residues in $tRNA_1^{Val}$. ○, Inosine (oligonucleotide I–A–Cp); ●, guanosine (m^1G–G–Up, G–G–Dp); □, guanosine (G–G–Cp); △, guanosine (A–G–Tp, A–G–Dp).

chromatographic runs, the absorbancy of the effluents was continuously recorded at 8 wavelengths: 250, 260, 270, 280, 290, 330, 350 and 380 nm.

Relative rate constants κ_j of modification of the individual guanosine residues G_j in tRNA (j is the number of the guanosine residue in the tRNA primary structure) were calculated similar to [6] from the equation:

$$\kappa_j = \frac{K_G^j}{\sum_i K_i [N_i]} = \frac{[\text{MepUR-G}_j]}{\{[\text{MepURCl}]_0 \cdot (1 - e^{-K_0 t}) - [\text{MepUR-tRNA}]\}} \cdot \frac{1}{[G_j]}$$

where K_0 is the rate constant of ionisation of the MepURCl ($K_0 = 6.3 \times 10^{-6}$ sec^{-1} at 20° [7]). K_G^j is the rate constant for the reaction of G_j with $MepUR^+$ (active intermediate ethyleneimmonium cation formed from the MepURCl). K_i are the rate constants for bye-reactions of $MepUR^+$ with low-molecular nucleophiles. Concentrations of low-molecular nucleophiles $[N_i]$ were the same in all the experiments. $[\text{MepUR-G}_j]$ is the concentration of the modified guanosine residues G_j. This value can be obtained from the concentration of the reagent, attached to the polymer [MepUR-tRNA] and from the ratio $[\text{MepUR-G}_j]/[\text{MepUR-tRNA}]$ determined by chromatographic analysis. $[\text{MepURCl}]_0$ is the initial concentration of the reagent. $[G_j]$ is equal to molar concentration of the tRNA.

3. Results

In the preceding communication [1] we reported the results of determinations of the relative amounts of modified oligonucleotides in pyrimidyl-ribonuclease digests of $tRNA_1^{Val}$ modified with MepURCl to the extent of 1.2 moles of the reagent per mole of tRNA. Now we have determined relative rate constants of modification of some individual guanosine residues in $tRNA_1^{Val}$, in the halves of $tRNA_1^{Val}$ and in their complex. These values were calculated from the data of chromatographic analysis of pyrimidyl-ribonuclease digests of modified polymers (see Materials and methods). Relative rate constants of modification of some guanosine residues in $tRNA_1^{Val}$ under conditions of stability of the tertiary structure of tRNA are listed in table 1. These values are not dependent on the degree of modification of the $tRNA_1^{Val}$ in the initial step of reaction (fig. 1) in accord with the idea that these values reflect the reactivities of guanosine residues in the native structure of the polymer.

Guanosine residues in the halves of $tRNA_1^{Val}$ react with MepURCl faster than guanosine residues in intact tRNA do under conditions of stability of the macrostructure of tRNA, with rates close to that of modification of guanosine residues in $tRNA_1^{Val}$ under conditions of lability of the tertiary structure of tRNA. Relative rates of modification of G_{54} in 3′-H and of guanosine residues of the oligonucleotide G–G–Cp in 5′-H are decreased as compared with the exposed in $tRNA_1^{Val}$ guanosine residues. In 5′-H, G_{15} (A–G–Dp), practically unreactive in $tRNA_1^{Val}$, reacts with the same rate as guanosine residues of the exposed in $tRNA_1^{Val}$ oligonucleotides m^1G–G–Up and G–G–Dp. G_{15}, G_{54} and G–G–Cp have decreased reactivities in the complex of the halves of $tRNA_1^{Val}$ as well as in intact $tRNA_1^{Val}$ molecule.

Table 1

Relative rate constants κ_j of modification of some guanosine residues in $tRNA_1^{Val}$, in halves of $tRNA_1^{val}$ and in their complex with MepURCl (20°, 0.04 M Tris-H_2SO_4, pH 7.6, containing 2×10^{-3} M $MgSO_4$).

Nucleoside number j	(Oligonucleotide)	$\kappa(M^{-1})$ 5′-H	3′-H	Complex	$tRNA_1^{Val}$
31,40	(G–Cp)				16
35	(I–A–Cp)				21
24,25	(G–G–Cp)	22*		13	9.2
54	(A–G–Tp)		17	10	1.8
15	(A–G–Dp)	80			
9,10	(m^1G–G–Up)	78		36	23
18,19	(G–G–Dp)				
1,2	(pG–G–Up)				14

*All guanosine residues in $tRNA_1^{Val}$ are equal reactive under conditions of lability of the tertiary structure of tRNA (40°, 0.04 M Tris-HNO_3, pH 7.6, absence of Mg^{2+}) [1]. κ in this case is equal to about 70 M^{-1} for guanosine and about 20 for inosine.

4. Discussion

The ionisation of the reagent is the rate determining step in reactions with MepURCl [7]. Therefore, relative rates of modification of polymers may be obtained only, but for the comparison of the reactivities of individual guanosine residues in polymers, the absolute values are not necessary.

It was shown, that in solution, halves of $tRNA_1^{Val}$ form a complex with physical and biological properties close to that of intact $tRNA_1^{Val}$ [8, 9]. Halves of the $tRNA_1^{Val}$ can form a complex without special renaturation under conditions of stability of the macrostructure of tRNA. Therefore, it can be proposed that there are no major reorganisations of the macrostructure of halves during complex formation and that macrostructure of halves may reflect the essential features of the macrostructure of $tRNA_1^{Val}$. Results obtained show, that halves of $tRNA_1^{Val}$ contain spatially ordered sections under conditions of stability of the macrostructure of tRNA. Decreases of reactivity of the guanosine residues of the oligonucleotides G–G–Cp and A–G–Tp in halves of $tRNA_1^{Val}$ can not be explained by the fact that these residues are located in the helical regions of the structure. It was shown that guanosine residues of oligonucleotides G–Cp and pG–G–Up located in the helical regions of the structure of $tRNA_1^{Val}$ are relatively highly reactive (table 1).

The decreased reactivity of the G_{15} (A–G–Dp) in the complex of the halves of $tRNA_1^{Val}$ and in intact $tRNA_1^{Val}$ and high reactivity of this residue in 5′-H show, that the 3′-H takes part in protection of the section of the D-loop in $tRNA_1^{Val}$, where G_{15} is located.

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