EXPOSED AND BURIED GUANOSINE RESIDUES IN $tRNA_1^{Val}$ FROM YEAST

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1. Introduction

Application of the method of chemical modification to studies of the macrostructure of biopolymers is based on the assumption that potentially reactive "exposed" residues are modified quantitatively, while "buried" residues remain unchanged after the reaction proceeds to completion under conditions providing stability of the macrostructure. Change of the macrostructure in the course of modification sometimes leads to exposure of regions that are buried in the native conformation; an example is the reaction of tRNA with water-soluble carbodiimide [1].

It seems that more detailed and correct information on the macrostructure of biopolymers could be obtained by measuring the initial rates of modification of their constituent monomer residues. As a measure of these rates one can take the relative extents of modification of monomer residues after reaction of polymer with a given reagent under conditions in which no monomer residue is modified to an extent greater than 10–15%.

The present paper is concerned with results of such a modification study performed with $tRNA_1^{Val}$, and a water-soluble alkylating reagent synthesized recently in this laboratory [2, 3], 2′,3′-*O*-[4-(*N*-2-chloroethyl-*N*-methylamino)benzylidene]-uridine-5′-methylphosphate (MepURCl). Alkylating reagents of this type modify principally guanosine residues in tRNA (cf. [4]).

Measurements of the initial relative rates of modification of guanosines in tRNA with 2-chloroethylamines allows one to determine whether the essential guanosine residue is "buried" or "exposed" irrespective of its participation in the formation of the secondary structure of the tRNA.

2. Materials and methods

The microspectrophotometer, a modified model described earlier [5], with a 1 μl flow cell allowing the continuous registration of the optical density of effluents at several wavelengths in the course of chromatography, microcollector, micropump and other equipment for micro-column chromatography were elaborated in our institute. Details of the methods will be published elsewhere. The synthesis and characteristics of MepURCl are described in [2]. tRNA was isolated from baker's yeast by a combination of 3 chromatographic procedures [6–8]. The tRNA obtained incorporated 1800–1900 pmoles of valine per 1 A_{260} unit (measured in 0.1 M Tris-HCl, pH 7.5, 0.02 M $MgSO_4$) with rat liver valine tRNA ligase. Pyrimidyl-ribonuclease, covalently bound to carboxymethylcellulose, was prepared according to [9].

Three preparations of modified $tRNA_1^{Val}$ were obtained. The first two preparations, with extent of modification 1.5 and 8 moles of modified residues per 100 moles of nucleotides, were obtained under conditions of "tertiary structure lability" ($tRNA_1^{Val}$, 1 mg/ml, 8 mM MepURCl, 40 mM Tris-HNO_3, pH 7.6, 40°, 25 and 120 min, respectively). The third preparation, with extent of modification 1.5%, was obtained under conditions of "tertiary structure stability" (the same concentration of tRNA and MepURCl, the same buffer plus 2 mM $Mg(NO_3)_2$, 20°, 50 hr).

The reaction mixture (15 μl) was applied to a Sephadex G-25 (Superfine) column (0.8 × 200 mm) equilibrated with 0.1 M sodium acetate, pH 4.0. 30 μl of the same buffer were passed through the column to ensure separation of modified tRNA from excess reagent. The elution was stopped at this moment, the

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column was heated for 40 min at 40° to hydrolyse the acetal bond of modified guanosine residues [10], then the elution was continued to isolate modified tRNA from the MepU split off. The procedure afforded modified tRNA with 7-alkylguanosine residues absorbing at 350 nm due to the presence of benzaldehyde moieties (extinction coefficient of 24×10^3 $mole^{-1} \times cm^{-1}$) [11] and thus the extent of modification was determined from the A_{350}/A_{260} ratio of the polymer peak determined by means of microspectrophotometry. This modified tRNA was desalted by gel-filtration on Sephadex G-25 in 0.01 M Tris-acetate, pH 7.5, and applied to a column of pyrimidyl-ribonuclease bound to CM-cellulose. After 12 hr digestion the column was washed with the same buffer and the effluent applied to a DEAE-cellulose column for chromatographic analysis. The latter was performed as described in the legends to the figures; in the course of the chromatographic runs, the absorbancy of the effluents was continuously recorded at 6 wavelengths: 250, 260, 270, 280, 290 and 350 nm.

3. Results

Fig. 1 shows the results of the chromatography at pH 8 and rechromatography at pH 3.7 of the pyrimidyl-ribonuclease digests of $tRNA_1^{Val}$ (A) and of $tRNA_1^{Val}$ modified 8% with MepURCl (B). Control experiments showed that the modification of tRNA does not inhibit pyrimidyl-ribonuclease action. The pK_a of the NH-group of the alkylguanosine is approx. 7 [12]. Thus the modification does not change appreciably the charges on oligonucleotides at pH 8, and makes them one unit less at pH 3.7 due to the positive charge on alkylguanosine residues. Positions,

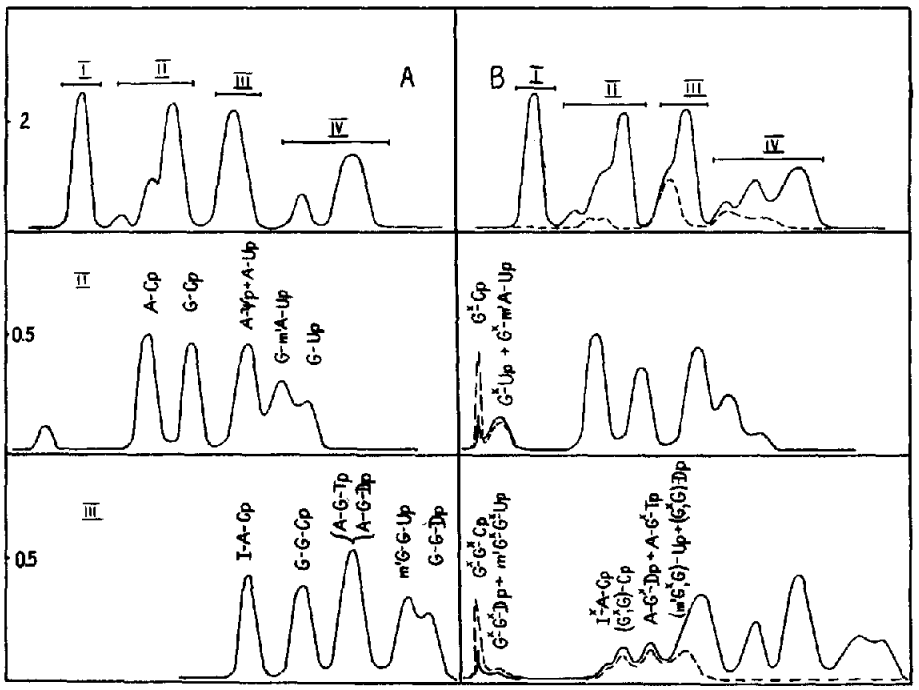

Fig. 1. Chromatography of pyrimidyl-ribonuclease digests of (A) (yeast) $tRNA_1^{Val}$ and (B) (yeast) $tRNA_1^{Val}$ modified 8% with MepURCl. About 0.4 A_{260} units of digests were applied to DEAE-cellulose column (0.6 × 100 mM) and eluted with a linear gradient of NaCl concentration (from 0.00–0.24 M) in 0.01 M Tris-Cl^-, pH 8, 7 M urea (top figures). II and III, rechromatography of fractions II (dinucleotides) and III (trinucleotides), respectively. Fractions from the first chromatography were 5 times diluted with 7 M urea, applied to the same DEAE-cellulose column and eluted with a linear gradient of NaCl concentration (from 0.00–0.08 M) in 7 M urea adjusted to pH 3.7 with HCOOH. ——: Absorbance at 260 nm; - - - -: absorbance at 350 nm.

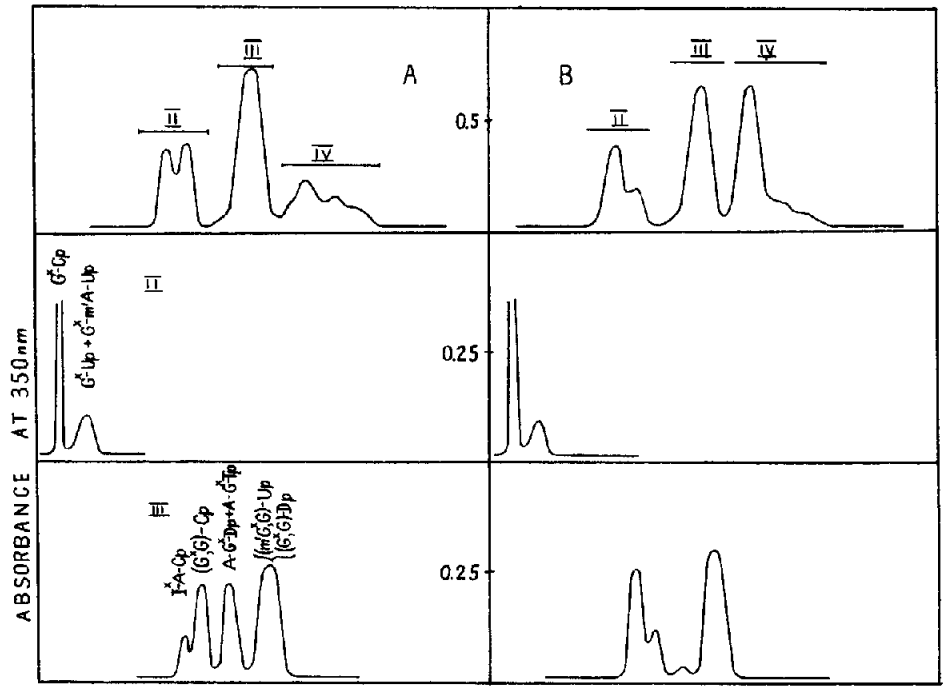

Fig. 2. Chromatography of the pyrimidyl-ribonuclease digests of (yeast) $tRNA_1^{Val}$ modified 1.5% with MepURCl under the conditions of lability of tertiary structure (A) and under the conditions of stability of tertiary structure (B). About 1.2 A_{260} units of digests were applied to DEAE-cellulose column (0.6 × 100 mm) and chromatographed as described in the legend to fig. 1.

spectra and amounts of A_{260} of peaks of oligonucleotides of the $tRNA_1^{Val}$ are in accord with its primary structure. Modified dinucleotides and trinucleotides were identified on the basis of their spectral ratios, which were compared with ones calculated from the summed spectra of constituent 3′-mononucleotides and alkylguanosine [13]. Details of the identification will be published elsewhere. After determination of the positions of the modified di- and trinucleotides in the elution profiles, we chromatographed in the same way digests of the $tRNA_1^{Val}$ modified 1.5% (fig. 2). Modified di- and trinucleotides were identified by their positions, and their relative α values were calculated from A_{350} of the peaks (table 1).

It may be seen that for $tRNA_1^{Val}$ modified under conditions of lability of the tertiary structure, α values (α_L) are close to that calculated for statistical distribution, taking into account the difference in guanosine and inosine reactivities. For $tRNA_1^{Val}$ modified under conditions of stability of the tertiary structure, α values (α_S) differ significantly from calculated values. There is a 3-fold increase of α for inosine residues in the anticodon. Peaks of modified G–G–Dp and m^1G–G–Up are not resolved, but the resulting peak is rather wide thus indicating that both oligonucleotides are modified to a similar extent. α's for G–G–Dp and m^1G–G–Up do not change significantly due to stabilisation of the macrostructure, nor does α for G–Cp. Oligonucleotides with unchanged and enhanced reactivities may be regarded as "exposed" in the tertiary structure. Relative amounts of modified G–G–Cp, A–G–Dp and A–G–Tp are significantly diminished in $tRNA_1^{Val}$ modified under conditions of stability of the tertiary structure, therefore guanosine residues of this oligonucleotide are regarded as "buried". Peaks of G–m^1A–Up and G–Up were not resolved; therefore no conclusions may be drawn concerning these regions without additional fractionation. Fig. 3 shows "exposed" and "buried" nucleosides in the tertiary structure of the $tRNA_1^{Val}$.

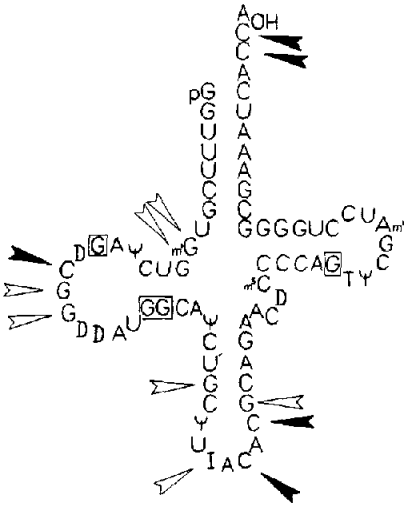

Fig. 3. "Exposed" and "buried" nucleosides in yeast $tRNA_1^{Val}$ (structure given according to [25]). Open arrows indicate cytosines attacked by methoxyamine [13], full arrows indicate guanosines attacked by MepURCl; [G], guanosines not attacked by MepURCl.

4. Discussion

The enhanced reactivity of inosine in the anticodon loop is in accord with all data on chemical modification of tRNAs [14–18]. A 10-fold decrease in relative reactivity of A–G–Tp and A–G–Dp is in agreement with data that the T–Ψ–C loop is buried [15, 16, 19, 20], and with the data that A^{14} in the D loop of $tRNA^{Phe}$(yeast) and $tRNA^{Ser}$(yeast) are not available to oxidation with monoperphthalic acid [22, 23]. Modification of the G–G–Dp suggests that the middle of the D loop is exposed in accord with results on modification of $tRNA_1^{Val}$(yeast) and su^+tRNA^{Tyr} (*E. coli*) with methoxyamine [14, 23], of $tRNA^{Phe}$ with $NaBH_4$ [18], ketoxal [17] and monoperphthalic acid [22]. There are no data in the literature on the region where m^1G–G–Up is located.

According to [14], cytosine residues of G–Cp in the anticodon stem of $tRNA_1^{Val}$ are not attacked by methoxyamine under conditions providing stability of the macrostructure; this indicates that they take part in double-helix formation. Availability of guanosine residues of G–Cp to MepURCl under similar conditions suggests that double-helix formation is insufficient to prevent the alkylation of guanosine. This is supported by the fact that α_L values are close to the calculated values, at 40° tRNA has secondary structure, and is in accord with the data [12] that guanosine in native DNA may be alkylated with 2-chloroethylamines. Therefore the diminished reactivity of guanosine residues of G–G–Cp in the D stem may be regarded as a result of protection by the tertiary structure. As was shown [22, 23], adjacent to this region adenosine in the D loop is not attacked by monoperphthalic acid in $tRNA^{Phe}$(yeast) and $tRNA_2^{Ser}$(yeast).

Modified di- and trinucleotides studied contain approx. 50–60% of the total A_{350} for $tRNA_1^{Val}$ modified under conditions of both lability and stability of the tertiary structure. A more complex mixture of tetra- and pentanucleotides, containing oligoguanilate sequences, is under investigation.

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