EXPOSED CYTOSINE RESIDUES IN THE $tRNA_1^{Val}$ FROM YEAST

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1. Introduction

In order to build a detailed 3-dimensional model of tRNA structure, it is necessary to know the location of the exposed base residues which do not interact in non-covalent bonds supporting the spacial structure of the tRNA particle. Chemical modifications which do not destroy the macromolecular structure are obviously of great use in this respect.

Location of exposed adenosine [1] and guanosine [2] residues in $tRNA^{Phe}$ (yeast) has been determined recently by means of monoperphtalic acid and kethoxal treatment, respectively.

It has been shown in this laboratory [3] that in the $tRNA_1^{Val}$ (yeast) only 4–5 cytosine (C) residues are accessible to *O*-methylhydroxylamine action.

The aim of this work is to determine the position of these exposed C's in the known [4] primary structure of the $tRNA_1^{Val}$.

2. Materials and methods

$tRNA_1^{Val}$ (yeast) was isolated by a two-step procedure including ion-exchange chromatography on the DEAE-Sephadex [5] followed by reversed-phase chromatography [6]. According to acceptor activity the purity of the preparation was not less than 85%.

Guanylo-RNAase (EC 2.7.7.26) and exonuclease A5 (EC 3.1.4.1) from Actinomyces were kindly provided by Dr. R.I.Tatarskaya.

^{14}C-*O*-Methylhydroxylamine was synthesized from $^{14}CH_3I$ [7].

Other experimental details can be found in the previous publication [3] and in the legends to figures.

3. Results

The distribution of the oligonucleotides after guanylo-RNAase hydrolysis of the modified $tRNA_1^{Val}$ on a two-dimentional thin-layer chromatogram (TLC) is shown in fig. 1. The position of the radioactive spots is also indicated. Of 12 C-containing oligonucleotides only 3 (R1, R2 and R3) were labelled with $^{14}CH_3ONH_2$ demonstrating the high selectivity of the modification reaction.

The position of the spot R1 on the chromatogram is close to that of oligonucleotide AAAUACC of the non-modified tRNA hydrolysate. Spot R2 occupies a position close to that of the oligonucleotide ACACGp. Thus it was reasonable to assume that R1 and R2 are derived from these two tRNA fragments. To prove this statement directly an additional analysis of the radioactive spots was performed.

Spot R1 was eluted from the paper chromatogram and subjected to pancreatic RNAase hydrolysis. After separation of the digestion products by one-dimensional TLC three spots were found (fig. 2). After autoradiography two of them appeared to be labelled and one additional radioactive spot was found which was not observed in UV light. The composition of these spots was identified after their position on the chromatogram, spectra and radioactivity. It was established that R1 included AAAUp, modified cytisine (C*), modified cytidylic acid (Cp*) and ACp*, containing modified C. This oligonucleotide composition is compatible only with the 3'-end of tRNA molecule lacking terminal adenosine. From the radioactivity of the ACp it follows that C_{75} was modified. The radioactivity of cytidine shows that the terminal C_{76} was exposed in molecules where terminal A was absent. The origin of radioactive cytidylic acid is not

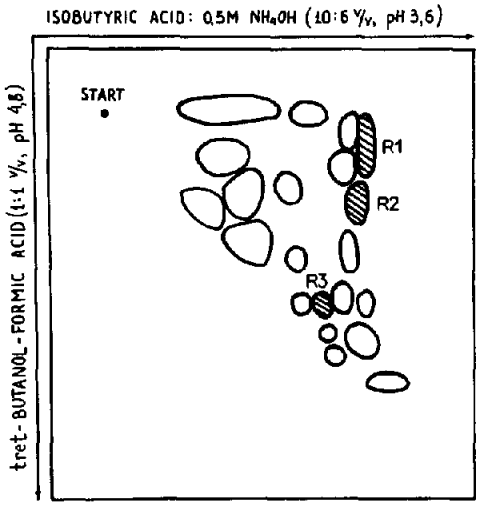

Fig. 1. Two-dimensional thin-layer chromatographic separation of the complete guanylo-RNAase hydrolysate of the $tRNA_1^{Val}$ modified with $^{14}CH_3ONH_2$. *Conditions of modification*: 1–2 mg tRNA/ml; 1.5 M $^{14}CH_3ONH_2$ (S.A. 4.6 mCi/mmole); 1.5 M KCl, pH 5.0; 37°, 3 hr. $^{14}CH_3ONH_2$ was removed after repeated precipitation with ethanol. 3.5 A units were digested at pH 8.0 with 180 units of granylo-RNAase during 24 hr at 37°. 3–5 A units were applied on the cellulose TLC plate (16 × 16 cm). Runs were repeated 2–3 times in both directions. Spots were detected under a mercury lamp and the plate was then contacted with an X-ray film and left for a few days to develop the autoradiogram. The radioactive spots (oblique-lined) were numbered as indicated.

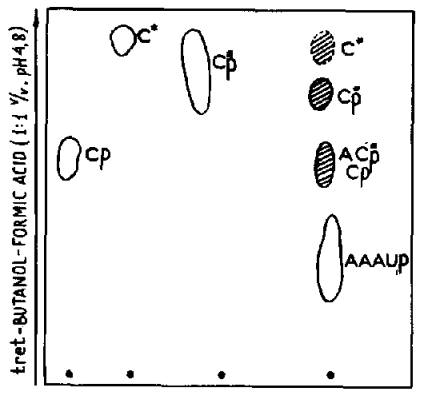

Fig. 2. One-dimensional thin-layer separation of the pancreatic RNAase digest of the spot R1. Spot R1 was cut from paper chromatogram, eluted with water, the material was concentrated and subjected to digestion with P-RNAase (0.3 μg, 37°, 24 hr, pH 7–8). Position of the markers is also shown: C*-4-O-methyl oxime uridine and its C* p-5′-phosphate.

quite clear from this chromatogram since it could be derived both from C_{73} and C_{76} in tRNAase where C is not a terminal nucleoside (in tRNAase with full acceptor end). To distinguish between these two possibilities, $tRNA_1^{Val}$ was oxidized by periodate and treated with amine to remove terminal A and C. After enzymatic hydrolysis of the corresponding R1 spot no radioactive cytidylic acid was found and, hence, C_{73} was resistent to NH_2OCH_3 modification.

Spot R2 eluted from the paper chromatogram and treated with exonuclease A5 and alkaline phosphate. As can be seen from fig. 3 this oligonucleotide is composed of nucleosides G, A, non-modified and modified C's and of a dinucleotide AC. This composition corresponds well to the fragment of the anticodon loop ACACGp. From the radioactivity of the AC, one can conclude that C_{37} (the third letter in the anticodon IAC) was modified. The presence of the labelled C is an indication of the accessibility of C_{39} to NH_2OCH_3 since the rate of hydrolysis of the dinucleotide AC is much less compared with the pentanucleotide.

The position of spot R3 on the chromatogram (fig. 1) probably corresponds to a trinucleotide and according to its mobility may be diHUCGp from the diHU-loop. This suggestion was proved as follows. After pancreatic hydrolysis of the R3 only Gp and modified Cp were revealed on chromatogram under UV light. After $^{14}CH_3ONH_2$ treatment of the non-modified diHUGG this trinucleotide occupies exactly the same position on the TLC plate as R3 after two-dimensional separation.

4. Discussion

The location of the exposed cytidine residues in the primary structure of the $tRNA_1^{Val}$ is shown in fig. 4. These data prove that in conditions when three-dimensional structure was stabilized by high ionic strength and appropriate pH, modification with CH_3ONH_2 could distinghuish between buried and exposed cytosine bases.

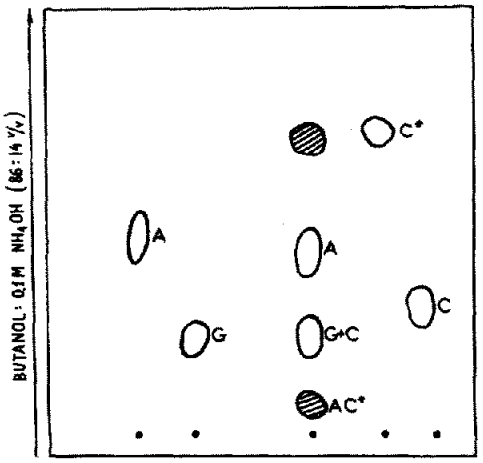

Fig. 3. One-dimensional thin-layer separation of the incomplete exonuclease A5 digest of the spot R2. Spot R2 was cut from paper chromatogram, eluted with water, the solution concentrated, digested with exonuclease A5 (1.3 units) and phosphatase (0.056 units) during 14 hr at 37°. Other details are in the legends to figs. 1 and 2 and the text.

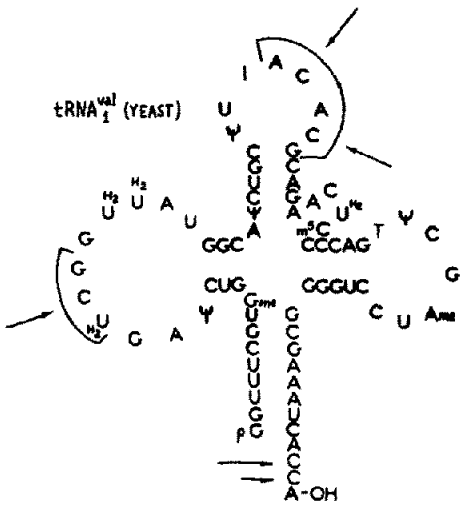

Fig. 4. Sites of modification of yeast $tRNA_1^{Val}$ by CH_3ONH_2. The primary sequence was taken from Bayev et al. [4]. The arrows indicate the modified C's. The brackets indicate the modified oligonucleotes from the guanylo-RNAase digest.

The number of the exposed C's is small (5 out of 21) and coincides with the value determined previously from kinetic data [3]. Three regions containing exposed C's were revealed, namely, anticodon loop, 3′ACC-end and the diHU-loop. It was shown earlier that the anticodon is able to form intramolecular hydrogen bonds with the codon in the absence of ribosomes [8–10], i.e. it is exposed. This statement is accepted in all models suggested for tRNA structure.

The accessibility of the C_{39} in the anticodon loop is in agreement with the exposure of the A_{38} in analogous position in the $tRNA^{Phe}$ [11] to monoperphtalic acid treatment. The exposed state of the ACC-end is in accordance with its high RNAase sensitivity [12, 13]. The exposed character of the C_{17} in the diHU-loop is relevant to the same nature of G_{20} in the $tRNA^{Phe}$ [2] and also to a preferential enzymatic attack of phosphodiester bonds between G_{19} and G_{20} and between G_{20} and G_{21} in $tRNA^{fMet}$ (*E. coli*) [14]. Thus at least part of the diHU-loop is exposed. The same three exposed regions were found by methylation of $tRNA^{Phe}$ [15].

The important point shown here is a total resistance of the two C's located in the common TψC-loop. It was shown earlier that in some tRNAs ψ [16], G [1] and mA [17] are protected against modification. Taking into account that both C_{60} at the 3′-end of the looped region and ψ_{55} near the 5′-end are resistant, it is reasonable to assume that the TψC-loop is buried as a whole. This seems to be a general feature in the structure of all tRNAs.

The extra arm containing C_{46} and 5-MeC_{48} is resistant suggesting that at least part of it is not exposed to the modifying agent. This is not surprising since the non-standard length of the extra arm suggests that it is located on the surface of the tRNA macromolecule.

The pG-C_{73} base pair at the edge of the helical "stem" segment and in the neighbourhood of a non-Watson-Crick type G-U base pair is nevertheless strong enough to prevent the chemical modification of the C_{73}.

These data are in agreement with some features of tRNA models proposed but support none of them fully. For example, in Cramer's model [1] for $tRNA^{Phe}$ CC from the ACC-end is paired whereas this is not the case for $tRNA_1^{Val}$ as shown here. In Levitt's model [18] the first and last bases of the anticodon loop

interact with each other; this suggestion is hardly compatible with the reactivity of the C_{39} in the $tRNA^{Val}$.

A detailed knowledge of all exposed bases in one tRNA molecule is a necessary prerequisite for constructing any definite three-dimensional model of the tRNA.

Acknowledgements

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