A STUDY OF tRNA METHYLASE ACTION

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1. Introduction

tRNA methylases constitute a group of enzymes each specific for a certain nucleotide. But any particular methylase can transfer methyl groups usually to only one of the many corresponding nucleotides located in a definite position of the tRNA molecule. It is still unsettled what structural features of tRNA molecules determine the specific and selective action of tRNA methylases.

It was suggested earlier that tRNA methylase action is determined by the chemical nature of the methylatable nucleotide and the primary structure in the region of its location. Recently evidence has been accumulated on the involvement of tRNA conformation in the methylation. Studying the *in vitro* methylation of yeast valine tRNA in heterologous systems [1, 2], we obtained some data to support this point of view. In the present study the results of *E. coli* $tRNA_f^{Met}$ methylation allow us to suggest that the predominant role in tRNA methylase action belongs to the position of the corresponding nucleotide in the tertiary structure of tRNA.

2. Methods

Purified *E. coli* $tRNA_f^{Met}$ was a generous gift from Prof. A.D. Kelmers (Oak Ridge National Laboratory, USA). The preparation of tRNA methylases from rat liver and hepatoma and the methylation procedure were described previously [1, 2]. To identify the modified components $tRNA_f^{Met}$ was digested after methylation with 1 N HCl for 1 hr at 100°; the purine bases and pyrimidine nucleotides were fractionated by two-dimensional TLC* on cellulose according to Björk and Svensson [3]. To locate the minor components formed the methylated $tRNA_f^{Met}$ was digested with pancreatic ribonuclease (EC 2.7.7.16) or guanylic ribonuclease from *Actinomyces* (EC 2.7.7.26) and the digests were fingerprinted on cellulose thin layers in the solvent systems previously used for paper fingerprinting of yeast $tRNA_1^{Val}$ oligonucleotides [4, 5]. The oligonucleotides were then recovered from thin layers and analyzed for radioactivity and absorbance according to [6] and then analyzed by the usual chemical, enzymatic, chromatographic and spectrophotometric methods.

3. Results and discussion

Our enzyme preparation from rat liver and hepatomata incorporated three times more methyl groups into *E. coli* $tRNA_f^{Met}$ as compared with yeast $tRNA_1^{Val}$; this makes up 2.5–3 mole $^{14}CH_3$-groups per 1 mole $tRNA_f^{Met}$. The modified bases were identified as 5-methyl-cytidine and 1-methyl-adenosine, formed also in yeast $tRNA_1^{Val}$ [2], and N^2-methyl-guanosine not found when methylating valine tRNA *in vitro*. The modified nucleotides were found in the ratio $m^5C:m^1A:m^2G = 0.5:1:1$. Proceeding from the oligonucleotide composition of the RNAase digests of

* *Abbreviations*: TLC – thin layer chromatography; m^1A, m^5C, m^2G, m^7G – 1-methyl-adenosine, 5-methyl-cytidine, N^2-methyl-guanosine, 7-methyl-guanosine, respectively.

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E. coli $tRNA_f^{Met}$ known from the communications of Dube et al. [7, 8] and from the regularities in oligonucleotide distribution on fingerprints we easily succeeded in locating the modified nucleotides in the $tRNA_f^{Met}$ molecule. 5-Methyl-cytidine was found in the tetranucleotide m^7G–U–C–G of the guanylo-RNAase digest; this proves unequivocally its position in the minor loop of the $tRNA_f^{Met}$ "clover-leaf" model, the usual site of 5-methyl-cytidine location in different tRNA's [9]. As expected 5-methyl-cytidine was found in the pancreatic RNAase digest as a mononucleotide. 1-Methyl-adenosine was detected in T–Ψ–C–A–A–A–U–C–C–G and in A–A–A–U of the guanylo- and pyrimidylo-RNAase digests, respectively. The location of the label in the triadenylic sequence of the tetranucleotide A–A–A–U was established according to the following scheme:

$$A\text{–}m^1A\text{–}A\text{–}U \xrightarrow{\text{Micrococcal nuclease + phosphatase digestion}} A\text{–}m^1A + A + U \xrightarrow{\text{Alkaline digestion}} Ap + m^1A$$

Thus, 1-methyl-adenosine is formed in $tRNA_f^{Met}$ under heterologous methylation in the same position as 1-methyl-adenosine in tRNA's methylated by homologous enzymes *in vivo*, namely in the nineteenth position from the 3′-end. N^2-Methyl-guanosine was identified in the tetranucleotide C–U–C–G of the guanosyl-RNAase digest and in the dinucleotide G–U of the pyrimidylo-RNAase digest, respectively. This is an unequivocal proof of m^2G location in the unpaired region between the H_2U-arm and the anticodon arm of $tRNA_f^{Met}$. In tRNA's methylated *in vivo* this is the site of N^2-dimethyl-guanosine location [9] and therefore the formation in this position of N^2-methyl-guanosine after heterologous methylation *in vitro* is an exceptional case. However, the transfer of only one methyl group to the G in this position of $tRNA_f^{Met}$ has also been stated by others [10, 11].

Fig. 1 represents *E. coli* $tRNA_f^{Met}$ in the "clover-leaf" pattern. The modified nucleotides are marked with an asterisk. Examination of $tRNA_f^{Met}$ primary structure reveals two identical sequences of seven nucleotides each including two trinucleotides UCG and one G residue. In fig. 1 the identical heptanucleotides are boxed. One of them located between the

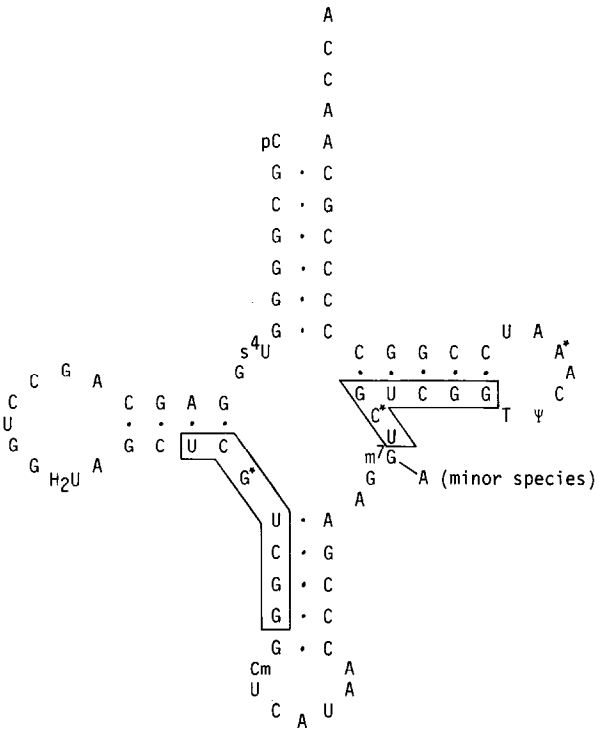

Fig. 1. $tRNA_f^{Met}$ of *E. coli* in the "clover leaf" pattern. Asterisks indicate the nucleotides modified *in vitro* by liver and hepatoma methylases. The two identical sequences are in boxes.

H_2U and the anticodon loop is the substrate for guanosine-tRNA-methylase only. The determination of radioactivity distribution between the nucleotides in the tetranucleotide C–U–C–G showed that 96–97% of the label is located in N^2-methyl-guanosine and only 3–4% in C. Vice versa the same sequence located in the minor loop and in the paired region of the TΨ-arm is methylated only by cytidine-tRNA-methylase; 95–96% of the radioactivity in m^7G–U–C–G was due to 5-methyl-cytidine, whereas the radioactivity of guanosine, corresponding to the one methylated in the first case, was negligible. The two trinucleotides U–C–G located in 3′-position to C–U–C–G and m^7G–U–C–G, respectively, do not contain any label, although they could contain any if the specificities of the methylases were determined by the sequence of three nucleotides. Our data show that of the four identical trinucleotides UCG, one is the substrate for the C-tRNA-methylase, the

other for a G-tRNA-methylase, and the nucleotides of the remaining two are not modified at all. Further we can conclude that the sequence of seven nucleotides is also not a sufficient condition for tRNA-methylase action, since two identical sequences including seven nucleotides located in different parts of the molecule are substrates for different enzymes. This allows us to conclude that at least in this particular case the chemical nature of the methylatable nucleotide and the primary structure of the relevant part of the tRNA polynucleotide chain are not the sole factors governing tRNA-methylase action. We believe that the tertiary structure of the tRNA molecule is also essential for the methylation specificity. The location of methylated nucleotides in sequenced tRNA's, the results obtained by the dissected molecule method [1, 10] and the results of tRNA heterologous methylation *in vitro* support this conclusion.

It is worthy of mention that specificities of tRNA-methylases as to tRNA tertiary structure seem to be quite different from those of other enzymes, for instance, nucleases. Indeed they attack nucleotides in those sites of the tRNA molecule that are protected against nucleases.

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