A STUDY OF tRNA METHYLASES BY THE DISSECTED MOLECULE METHOD

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1. Introduction

It is well known that tRNA is the substrate of many modifying enzymes, primarily the methylases. It is not yet clear why methyl groups are incorporated by these enzymes onto only a few of the many bases in the tRNA molecule. The use of the 'dissected molecule method' [1] now allows us to conclude that the spatial arrangement of tRNA molecule is the main factor which controls the tRNA-methylase recognition.

2. Methods

$tRNA_1^{Val}$ was isolated from baker's yeast as described earlier [2]. Unfractionated aminoacyl-tRNA-synthetase was isolated according to [3]. Half molecules of $tRNA_1^{Val}$ were generous gifts of Dr. A.Mirzabekov.

Methylases were isolated from rat liver and Novikoff hepatoma cells by homogenizing the tissue with 6 volumes of cold 0.32 M sucrose solution with 0.002 M $MgCl_2$ and 0.005 M dithiothreitol (DTT) [4]. The homogenate was filtered through cheese-cloth and centrifuged successively at 23,000 *g* for 20 min and at 105,000 *g* for 60 min. Ammonium sulfate was added to the high-speed supernatant. The protein fraction precipitated between 0 and 50% saturation was collected by centrifugation and dissolved in 0.01 M tris-HCl buffer, pH 8.2, containing 0.002 M EDTA and 0.001 DTT. This solution was used as a source of methylases. Protein was estimated by the method of Lowry et al. [5].

A standard assay mixture in a 0.4 M ammonium acetate buffer pH 9.25 [6] contained 0.65 μmole DTT, 1 μmole EDTA, 2.5 μmoles tris-HCl (pH 8.2), 6.5 nmoles ^{14}C-methyl-*S*-adenosylmethionine (specific activity 58 Ci/mole), 1.2 A_{260} units of the polynucleotide and 1.2 mg of protein in a total volume of 0.77 ml. The incubation was carried out for 6 hr at 37°. tRNA and its halves were precipitated by cetyltrimethylammonium bromide and converted to Na^+ salts as described in [1]. The precipitate was dissolved in water and aliquots were removed for absorbance (A_{260}) and radioactivity assays. Radioactivity was counted in an Intertechnique SL liquid scintillation counter.

3. Results and discussion

Yeast $tRNA_1^{Val}$, its halves and their mixture were methylated with rat liver and hepatoma enzymes, i.e., in a heterologous system. Under the conditions chosen the extent of $tRNA_1^{Val}$ methylation was 1 methyl group per 25–30 molecules of liver tRNA and 1 methyl group per 5–7 molecules of hepatoma tRNA. There was no drop in amino acid accepting activity after the incubation. Preliminary experiments performed on the whole molecule showed that methyl groups are incorporated into several sites of $tRNA_1^{Val}$ which are located in both the 3′- and 5′-halves of the molecule. If the only factor determining the incorporation of methyl groups were a definite nucleotide sequence, there should be an appreciable methylation of the halves and the sum of methylation in the two halves should be approximately equal to the incorporation into the whole molecule. As one can see from the data in table 1, the incorporation into the half molecules is insignificant and in total amounts to only 1/4–1/5 of the incorporation into the whole

Table 1
Methylation of yeast $tRNA_1^{Val}$ and its halves by rat liver and hepatoma enzymes.

Substrate	Methylase from: Liver		Hepatoma	
	cpm/A_{260} unit	%	cpm/A_{260} unit	%
$tRNA_1^{Val}$	7950	100	38100	100
3′-half	325	4.1	2100	5.1
5′-half	2030	25.8	5700	14.9
3′- + 5′-halves	7350	93	30200	82

For conditions see text.

molecule of $tRNA_1^{Val}$. Methyl group incorporation into the premixed halves is rather high, amounting to 82% with hepatoma and to 93% with liver enzymes of the degree of methylation of the native molecule. As was shown by Bayev et al. [1] the two halves of the $tRNA_1^{Val}$ molecule, when mixed, assemble into an aggregate molecule, which has the same physico-chemical properties as the native molecule despite the broken phosphodiester bond in the anticodon. We suggest that the requirement for tRNA methylase activity is not only a certain nucleotide sequence but also the conformation of this sequence within the molecule. When mixed, the two halves aggregate into a molecule so similar to the native one that it can serve as a substrate for the methylases.

The incorporation into the 3′-half is negligible (4–5%). The somewhat higher incorporation into the 5′-half of $tRNA_1^{Val}$ (19–25%), which is structured to a lesser extent that the 3′-half, may be due to some contamination with whole $tRNA_1^{Val}$ molecules, since the latter elutes from the column just behind the 5′-half during fractionation [1].

Some data suggest that the tRNA methylases may be sensitive to the tertiary structure of tRNA. Baguley et al. [6] showed that the sequence A–A–A–Up in the TΨ-loop of yeast $tRNA^{Ser}$ is not methylated by a methylase specific for this sequence. On the other hand 1-adenyl-methylase from mammalian tissues can methylate this sequence, converting it into A–m^1A–A–Up. The authors suggest that in order for this adenine to be methylated, the tRNA structure should be opened. Mammalian methylase could induce this conformational change and so expose the adenine residue.

Kuchino and Nishimura [7] when studying the methylation of individual *E. coli* tRNAs with known primary structures also mentioned the significance of conformation for tRNA methylase action.

Our data support this suggestion in a more direct manner, and also suggest that when synthesized, the tRNA molecule probably acquires its conformation before being subjected to the modifying action of the methylases.

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