On the mechanism of tRNA methylase-tRNA recognition

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ABSTRACT

In order to further elucidate the mechanism of tRNA methylase-tRNA interaction the methylation of some individual tRNAs separately and by pairs was performed. In conditions of tRNA excess the methylation rates of positionally analogous nucleotides in tRNA molecules are not summed up when two substrates are simultaneously present in the reaction mixture. The inhibitory action of yeast tRNA^{Ser}, possessing m⁵C in position 29, on the methylation of C₂₉ in other individual tRNAs was shown. Yeast tRNA^{Ala} which possesses an A residue in position 27 was shown to inhibit the methylation of G₂₇ in *E.coli* tRNA^{Met}.

The data obtained confirm the suggestion that tRNA methylases recognizes the tertiary structure of tRNAs. They show also that the recognition and the proper catalytic action are two autonomous processes and that the former at least in its first stage is rather unspecific.

INTRODUCTION

tRNA methylases (S-Ado-Met: tRNA methyltransferase, EC 2.1.1) constitute a highly specific group of enzymes; an obligatory condition of their action is the native conformation of tRNA molecules. Long fragments of tRNA molecules including halves^{1,2} and three quarters³ can neither serve as substrates for methylases, nor even exert any inhibitory action on the methylation of native molecules⁴. Some authors believe nevertheless that the specificity of tRNA methylases is determined by the nucleotide sequence in the methylation site⁵.

We obtained some information about tRNA methylation mechanism via methylating individual tRNAs separately and in mixture. The methylation of positionally analogous nucleotides in two tRNAs when present separately and together in the reaction mixture in conditions of proportionality in relati-

on to protein concentration and time was investigated. The action of an tRNA with a completely different structure in the methylation site, as well as the action of a tRNA which cannot be methylated at the respective site on the methylation of different tRNAs was determined. The data obtained confirm the concept about the autonomy of the recognition process and the proper catalytic action of enzymes and allow to suggest that the first step of tRNA-methylase interaction is rather unspecific and consists in recognition of some very general features of tRNA tertiary structure pertinent to the whole population of tRNA molecules.

MATERIALS AND METHODS

Purified preparations of yeast tRNA^{Ser} and tRNA^{Phe} were generous gifts of Professor Zachau (Munich, FRG) that of E.coli tRNA^{Met} and tRNA^{Glu} of Professor Kelmers (Oak Ridge Lab., Tennessee). Yeast tRNA^{Val}₁ was isolated from a total backscattered yeast tRNA preparation by Dr. Axelrod⁶.

tRNA-methylases from rat liver and carcinoma were isolated as described^{1,2}. Methylation was carried out in an incubation mixture containing: 0.01 M Tris-HCl; pH 8.2; 0.001 M DTT; 0.001 M EDTA; 0.4 M NH₄CH₃COO; 0.01 mM S-adenosyl-¹⁴CH₃-methionine. The protein content was 1 mg/ml in the case of carcinoma and 2.5 mg/ml in the case of liver enzyme. The amount of each tRNA was two units A₂₆₀ per mg protein except in inhibition experiments in which the amount of tRNA varied. The total volume of the reaction mixture was 0.2-1 ml. Incubation was for 1 h at 37°C.

To determine the amount of total ¹⁴CH₃-incorporation into tRNA aliquots were withdrawn from the reaction mixture and placed on filter discs; after fixation and washing the latter were analyzed in toluene scintillation counter⁷.

To determine the incorporation of the label into single nucleotides, tRNA was isolated after methylation, hydrolyzed with 1 N HCl for 1 h at 100°. The resulting purine bases and pyrimidine nucleotides together with a small amount of authentic methylated nucleic acid compounds were fractionated by

the method of two dimensional TLC on cellulose FND (Filtrak DDR)⁸. The UV containing sites of the thin layers were fixed with a solution of nitrocellulose in a mixture of acetone and alcohol, taken away from the plates and counted in a toluene scintillator⁹. The extent of $^{14}\text{CH}_3$ -incorporation into each minor component was established and confronted with the data on incorporation into total tRNA (filter data) and with recovery of radioactivity from the cellulose plates. This allowed to establish $^{14}\text{CH}_3$ -incorporation into each methylated compound per mg protein.

Each experiment was fulfilled with enzymes from two sources, namely from rat carcinoma and liver, in conditions in which $^{14}\text{CH}_3$ -incorporation was linear in relation to time and protein amount.

RESULTS AND DISCUSSION

To study the mechanism of tRNA methylase-tRNA interaction on three types of experiments were carried out.

First, the methylation of positionally analogous nucleotides was studied by the "mixed substrate" method. In conditions of substrate excess and linearity of $^{14}\text{CH}_3$ incorporation in the time course the velocity of m^5C formation in position 29 (counting from the 3'-end) of yeast tRNA₁^{Val}, tRNA^{Phe} and E.coli tRNA_f^{Met}, and m^1A formation in position 19 of yeast tRNA₁^{Val}, tRNA^{Phe} and tRNA^{Ser} and E.coli tRNA_f^{Met} and tRNA^{Glu} was studied in the case the tRNAs were in the reaction mixtures separately and combined in pairs. Fig.1 shows some examples. One can see that when two tRNAs are put in one reaction mixture the velocities of positionally analogous nucleotide methylation makes in no case the sum of individual tRNA methylation. This was observed for all tRNA pairs which were checked, as well as for both minor components studied (m^1A and m^5C). The data obtained allow to suggest competitive relationship between the mixed tRNAs in relation to the enzyme, and this in its turn indicates that methyl group incorporation into analogous positions of different tRNAs is catalyzed by one and the same enzyme. This conclusion is not unexpected since genetic evidence in favour of this suggestion

Fig.1. Methylation rates of C_{29} (A) and A_{19} (B) in individual tRNAs incubated²⁹apart and¹⁹combined in pairs. The reaction mixture contained 5 A_{260} units of each individual tRNA and 2.5 mg liver protein in 1 ml.

exists; Björk and Isaksson¹⁰ reported the isolation of E.coli mutants with no m^5U in their tRNAs, and Kjellin-Stöby¹¹ reported the isolation of yeast mutants with tRNAs completely devoid of m^2G . The most plausible explanation of these data is that one and the same enzyme methylates positionally analogous nucleotides in all tRNAs of a cell or tissue, and that

these mutants are deprived of the enzyme which methylates the respective nucleotides in all tRNAs of E.coli or yeast.

In experiments of the second type (Fig.2) the action of

Fig.2. Inhibition of C_{29} methylation in yeast $tRNA^{Val}_1$ by yeast $tRNA^{Ser}$. Lineweaver-Burk plot with $tRNA^{Val}_1$ as the variable substrate. Incorporation of $^{14}CH_3$ in pmol/mg protein per hour was determined in a reaction mixture containing 1 mg carcinoma protein in 1 ml. 1 - without $tRNA^{Ser}$; 2 and 3 - 0.2 and 0.5 A_{260} units $tRNA^{Ser}$ in 1 ml, respectively.

yeast $tRNA^{Ser}$ on the methylation of C_{29} in yeast $tRNA^{Val}_1$ and $tRNA^{Phe}$ was studied. The survey of $tRNA^{Ser}$ structure (Fig.2) shows that this tRNA is already methylated in position 29, and it has in contrast to $tRNA^{Val}_1$ and $tRNA^{Phe}$ a rather long extra arm. The data presented in Fig.2 show that nevertheless $tRNA^{Ser}$ exerts a pronounced inhibitory action on the formation of m^5C in $tRNA^{Val}_1$ and that this inhibitory action is of a competitive character. We can conclude that the methylase which converts C_{29} into m^5C_{29} in $tRNA^{Val}_1$ interacts with $tRNA^{Ser}$ too, although the latter is already methylated at position 29 and strongly differs from $tRNA^{Val}_1$ in its structure at the methylation site. $tRNA^{Ser}$ was shown to exert an analogous inhibitory action on the methylation of C_{29} in $tRNA^{Phe}$.

The aim of the third set of experiments was to see the

action on the formation of a methylated nucleotide in a tRNA of a second tRNA, which has in analogous position another nucleotide and therefore cannot be a substrate for the corresponding methylase. For this purpose we examined the action of $\text{tRNA}_{\text{Val}}^{\text{Val}}$ on the incorporation of methyl groups into G_{27} (counting from the 5'-end) of $\text{tRNA}_{\text{f}}^{\text{Met}}$; $\text{tRNA}_{\text{Val}}^{\text{Val}}$ has in position 27 not a G but an A (Fig.3) and cannot be there-

Fig.3. Inhibition of G_{27} methylation in *E. coli* $\text{tRNA}_{\text{f}}^{\text{Met}}$ by yeast $\text{tRNA}_{\text{Val}}^{\text{Val}}$. Lineweaver-Burk plot with $\text{tRNA}_{\text{f}}^{\text{Met}}$ as the variable substrate. 1 - without $\text{tRNA}_{\text{Val}}^{\text{Val}}$; 2 $\pm 5 \times 10^{-5}$ units $\text{tRNA}_{\text{Val}}^{\text{Val}}$ in 1 ml.

fore methylated by a G-methylase. The data presented in Fig. 3 show that $\text{tRNA}_{\text{Val}}^{\text{Val}}$ inhibits the formation of m^2G in position 27 of $\text{tRNA}_{\text{f}}^{\text{Met}}$ and that this inhibition is of a competitive character. The affinity constant of the methylase for $\text{tRNA}_{\text{Val}}^{\text{Val}}$ is only 10-15 times lower than that for $\text{tRNA}_{\text{f}}^{\text{Met}}$.

These data demonstrate the autonomy of the initial enzyme-substrate interaction, which precedes the proper catalytic action and show that the first stage of methylase-tRNA recognition is most probably nonspecific. It seems that methylases recognize some very general features of tRNA three-dimensional structure, pertinent to the whole population of tRNA molecules.

It is interesting to consider the data obtained in the light of the modern concept of tRNA spatial organization

developed by Roberts et al.¹² as well as by Kim et al.¹³ on the basis of X-ray analysis of yeast tRNA^{Phe} crystals. There are arguments which allow to suggest that this model is valid for other tRNAs as well and what is most important one can take it for granted that the conformation of tRNA in solution is the same as in crystals^{14,15}.

Fig.4. A diagrammatic representation of yeast tRNA^{Phe} tertiary structure¹⁴. The dashed line represents a schematic surface of a synthetase enzyme, interacting with the tRNA molecule.

On Fig.4 the model proposed by Rich for the three dimensional structure of tRNA^{Phe} yeast is shown¹⁴. The left site of this L-shaped structure, as Rich emphasizes, is the most constant one, as it is composed of the most constant in respect to nucleotide number and base pairing parts of the molecule, namely, the acceptor stem, the paired part of the D-arm and a part of the anticodon arm. The most variable parts of the molecule - the D-loop and the extra arm - are localized on the right site of the molecule; as Rich supposes the varying parts of tRNA molecules can exert minimal action on the general conformation of tRNA when one suggests that they can loop out from the molecule.

Considering the recognition of tRNA by aminoacyl tRNA-synthetases Rich points out that its first step is rather non-specific and consists in the interaction of all synthetases with that part of the molecule which is constant for

all tRNAs (Fig.4). The correctness of aminoacylation is provided by the following more specific contacts between the aminoacids of the polypeptide chain of the enzyme and the bases of tRNA. One can assume that the recognition of tRNA by other proteins proceeds on the same structural basis. We think that the data presented allow to suggest the same situation in the case of methylases.

First, we take it for granted, that the key role in the interaction of tRNA with methylases belongs to the spatial organization of the molecules. Further on evidence was obtained indicating the low degree of specificity of the first step of tRNA-methylase interaction; this is indicated by the enzyme-substrate complex formation even in those cases in which the catalytic action cannot be realized. The inhibitory action of yeast tRNA^{Ser} possessing a rather long extra arm, on the methylation of C₂₉ in tRNA^{Val}₁ and tRNA^{Phe} with only 5 nucleotides in the extra arm is in accord with the suggestion that this part of the molecule does probably not participate in the recognition process.

We conclude that the first step of tRNA methylase-tRNA interaction is a nonspecific process and consists in the recognition of some very general features of tRNA spatial organization pertinent to all tRNA molecules.

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