Long-range conformational transition in yeast tRNA^{Phe}, induced by the Y-base removal and detected by chloroacetaldehyde modification

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ABSTRACT

Chemical modification was used to study the conformational changes occurring in yeast tRNA^{Phe} after the Y-base excision. The chemical probe was the adenine- and cytosine-specific reagent chloroacetaldehyde. Comparison of the modification patterns in tRNA^{Phe} and tRNA^{Phe}_{-Y} shows that seven bases, adenines 35, 36 and 38 in the anticodon loop and adenines 73, 76 and cytosines 74, 75 in the 3'-terminus were modified in both tRNAs with a quantitative difference in the modification level of the anticodon loop bases. The most interesting, however, is the qualitative difference consisting in modification of cytosine-60 in the TYC loop of tRNA^{Phe}_{-Y}. Some aspects of the mechanism of this long-distance conformational transition are briefly discussed.

INTRODUCTION

A variety of methods sensitive to many different features of tRNA structure has been used to study the structural effect of the Y-base removal from yeast tRNA^{Phe}. The results of biochemical assays i.e. aminoacylation^{1,2} and ribosome binding^{1,3,4} in most cases could only be best-explained in terms of a conformational change. Some physical methods of lower potentiality could prove the existence of a conformational change but failed in its closer characterization^{2,5}. However, techniques capable of detecting more subtle, local perturbations turned out to be successful in indicating some specific sites of altered conformation. The complementary oligonucleotide binding studies suggest that besides changes in the anticodon loop⁶⁻⁸, some structural alterations can also take place in D-loop region⁸. The comparative analysis of ¹H NMR spectra of tRNA^{Phe} and tRNA^{Phe}_{-Y} revealed several interesting differences in the high-field⁹ and the low-field region^{5,10,11}. Of the methyl and methylene group resonan-

ces of minor nucleosides, those of m^5C40 from the anticodon stem, and m^5C49 from the variable region showed some changes in chemical shifts. Other detectable differences comprising Gm34, T54, m^2G26 and D16 resonances could be observed on temperature-induced "melting" of the tRNA structure⁹. Earlier NMR studies of hydrogen-bonded protons^{5,10} indicate that all but one of the shifted signals involve base-pairs associated with the anticodon stem. The remaining affected resonance was later attributed to a perturbation of U33¹². More recent studies show that one resonance, that of A31-Y39 base pair from the anticodon stem is lost in the spectrum of the Y-base deficient tRNA^{Phe}, while some other resonances change their positions¹¹. The ^{31}P NMR spectra of tRNA^{Phe} have also been examined before and after the removal of the Y-base and a number of changes was detected among about 17 phosphate resonances resolved from the main signal¹¹. However, only a few of them could be attributed to the individual phosphate residues /P34, P36 and P38/.

A single reported so far result of chemical modification studies¹³ shows that m^7G46 is in tRNA^{Phe}_{-Y} more susceptible to the irreversible degradation by sodium borohydride than in tRNA^{Phe}.

In this report we present the results of chloroacetaldehyde modification.

RESULTS

Modification method. Chloroacetaldehyde, like many other base-modifying reagents shows specificity for single-stranded regions and is sensitive to the strength of stacking interactions^{14,15}. The most advantageous feature of chloroacetaldehyde consists, however, in the fact that it modifies two common tRNA bases in the same manner and with comparable rates^{16,17}, being able to supply quantitative correlation between steric accessibilities and reactivities of modifiable bases. Modification conditions: 1M chloroacetaldehyde, 25°C, 20h, are so selected as to ensure a high, nearly entire modification of these adenine and cytosine residues which are most exposed to a solvent and to the reagent in the tRNA tertiary structure. At the same time the reaction pH /5.5/ which at 20 mM Mg^{+2} should maintain the native tRNA conformation¹⁸ is known to ensure equal modification

rates for adenosine and cytidine¹⁶.

There is also an experimental evidence showing that the modification process itself does not induce significant conformational changes in the tRNA structure. The usually accepted criterion i.e. low and justifiable by the tRNA secondary structure level of modified bases attained at the modification plateau, is very well fulfilled by chloroacetaldehyde reaction¹⁷.

Modification sites. The strategy used in these studies for identification of the modification sites involves specific enzymatic conversion of the chloroacetaldehyde-modified tRNA into a set of fragments, their subsequent fractionation by preparative one-dimensional polyacrylamide gel electrophoresis and nucleoside content analysis by HPLC. However, it should be mentioned here that RNase T₁ is used in this procedure solely to generate fragments of chloroacetaldehyde-modified tRNA. This implies that for some fragments containing more than one modifiable adenine or cytosine at the internal positions of the chain, the degree of modification cannot be determined for each individual base but for all modified adenines or cytosines as a total.

The electrophoretic separation of tRNA^{Phe} and tRNA^{Phe}_{-Y} fragments, their assignment and HPLC analysis of these fragments in which modification was found are shown in composite Fig.1. It is seen that T₁ RNase hydrolysates of both chloroacetaldehyde modified tRNAs are fractionated into 11 resolved bands on 20% polyacrylamide gel. Since their separation patterns were identical except for the lowest mobility band, they are shown in Fig.1 in a single scheme.

In order to assign the separated oligonucleotides and identify the modified fragments they were analysed twice. Hydrolysis with snake venom phosphodiesterase and bovine alkaline phosphatase /pH 8.8, 37°C, 24h/ was used for analysis of total nucleoside composition while hydrolysis with ribonuclease P₁ /pH 5.3, 20°C, 1h/ was applied for 5'-terminal nucleoside analysis.

Bands 1, 1'. Analysis of total nucleoside composition of these fragments, fluorescent in UV light, shows in both cases the presence of Y, U, G, Gm, A and εA, while the analysis of their 5'-end nucleosides /data not shown/ indicates exclusively A. Bands 1 and 1' were assigned as ACmUGmAAAYAm⁵CUGp and ACmUGm-

Fig.1. Left : Separation of T₁ fragments of the chloroacetaldehyde-modified tRNA^{Phe} and tRNA^{Phe}_{-Y} On 20% gel, 40 x 20 x 0.1 cm /BB refers to bromophenol blue marker migration/ and their assignment. Right : HPLC analysis of total nucleoside composition of the fragments containing modified adenosine or cytidine /Aminex A-6, 0.175 M ammonium formate pH 4.55, 50°C/.

AA-AYm⁵CUGp, respectively. Nucleoside Y could not be detected under HPLC conditions used, due to a much higher retention volume, while Cm and m⁵C comigrated with εA. The overall modification extent for adenosines present in fragment 1 originating from the anticodon loop and stem of tRNA^{Phe} was determined to be 2.1. This modification is shared between three internal adenosines A35, A36 and A38. The corresponding fragment of tRNA^{Phe}_{-Y} /1'/ shows a higher degree of adenosine modification, 2.4, as calculated from the HPLC data.

Band 2. Analysis of total nucleoside content of the fragment derived from tRNA^{Phe} shows the presence of U, G, A, C and m⁶A /which is the product of m¹A rearrangement under slightly alkaline conditions/. The 5'-end nucleoside analysis showed the absence of any uncharged nucleosides and band 2 was assigned as m¹AUCCACAGp fragment from TYC loop and stem. In the correspon-

Fig.2. Chloroacetaldehyde modification pattern in yeast tRNA^{Phe} and tRNA_{-Y}^{Phe}. The values in brackets refer to the latter tRNA.

ding fragment of tRNA_{-Y}^{Phe} the surprising difference was the presence of ϵ C in the total nucleoside hydrolysate. Only one of the three cytidine residues present in the fragment, C60, occupies the single-stranded region and is the only candidate for this modification. The modification extent was determined to be 0.3. It is worth noting that C60, unaffected by chloroacetaldehyde treatment in native tRNA^{Phe} has never been modified in this tRNA by any other cytosine-specific reagent¹⁹. The very low 1% modification of C60 in yeast tRNA^{Phe} by I₂ is the single reported exception²⁰.

Band 3. Analysis of total nucleoside composition of this fragment, highly fluorescent in UV light, shows C, ϵ C, A and ϵ A. The only oligonucleotide generated by RNase T₁ which contains no guanosine is 3'-terminal fragment CACCA. This assignment is supported by the exclusive presence of C at the 5'-end of this fragment derived from both modified tRNA molecules. Total modification extent for the two cytidines C74 and C75 was found to be 1.7 in tRNA^{Phe} and 1.8 in tRNA_{-Y}^{Phe}, while for the two adenosines A73 and A75 it was 1.5 and 1.6 in tRNA^{Phe} and tRNA_{-Y}^{Phe}, respectively. It should be noted, that despite extensive modification of four 3'-neighbours, C72 remains unaffected by chloroacetaldehyde.

The summary of chemical modification data is shown in Fig.2.

DISCUSSION

The most striking fact which emerges from our studies is the accessibility of C60 to modification with chloroacetaldehyde in tRNA^{Phe}_{-Y}. This cytosine residue located some 50 Å away from the Y-base is the last unit of the TΨC loop, being at the same time the terminal base of the vertical stacking system present in yeast tRNA^{Phe} crystal structure²¹. The C60-U59 stacking interaction is considered to be a weak one and modification of C60 suggests that this base no longer remains in tRNA^{Phe}_{-Y} in the stacking array.

The higher modification extent of adenine residues in the anticodon loop of tRNA^{Phe}_{-Y} /most probably connected with the increased accessibility of A38 to chloroacetaldehyde/ shows that the gap created by the Y-base removal is not completely closed by the stacking interaction between A36 and A38.

It is also interesting to note that our data and the results of ¹H NMR studies on tRNA^{Phe} and tRNA^{Phe}_{-Y} conformations¹¹ can be explained by the loss of some Mg⁺² ions from their strong binding sites as a result of the Y-base removal. In yeast tRNA^{Phe} crystal structure, the Mg⁺² ion located in the anticodon loop has one direct coordination to phosphate-37 and several indirect coordinations, via water molecules to various base residues, Y-base among them²². The removal of this Mg⁺² ion could explain the alteration of the A31-Ψ39 base pair in tRNA^{Phe}_{-Y}¹¹ since both A31 and Ψ39 belong to its coordination sphere. The lack of A31-Ψ39 resonance also in yeast tRNA^{Phe} spectrum recorded in the absence of Mg⁺² ions²³ strongly supports the above explanation.

The other Mg⁺² ion located in the "corner region" of yeast tRNA^{Phe} crystal structure is bound directly to oxygen from phosphate-19 and via water molecules, among others, to C60-N3²². Its absence in tRNA^{Phe}_{-Y} would be in agreement with the result of our chemical modification studies showing the susceptibility of C60 to chloroacetaldehyde modification. In favour of this interpretation is the result of another chemical modification experiment²⁴, showing that C60 is the first cytosine residue to become available to methoxyamine probe on "melting" the yeast tRNA^{Phe} tertiary structure in the absence of magnesium ions.

MATERIALS AND METHODS

Materials. A highly purified yeast tRNA^{Phe} of specific phenylalanine acceptance 1600 pmoles/A₂₆₀ was a generous gift of Professor F. Cramer. Aqueous chloroacetaldehyde was prepared according to Yasnitzki and Dolberg procedure²⁵. Ribonuclease T₁ and bacterial alkaline phosphatase were obtained from Worthington, snake venom phosphodiesterase was purchased from Merck and ribonuclease P₁ from Boehringer. Acrylamide and N,N'-methylene bisacrylamide were from Merck and Biomol, respectively. Aminex A-6 cation-exchange resin was a product of Bio-Rad Laboratories. The Y-base deficient yeast tRNA^{Phe} was prepared according to the procedure described by Thiebe and Zachau¹. Its chromatographic and aminoacylation properties were identical with those described in literature. Moreover, tRNA^{Phe}_{-Y} similarly to tRNA^{Phe} gave a single band on 12.5% polyacrylamide gel and no changes were detected by HPLC in the nucleoside composition of its T₁ fragments. After Biogel P-2 filtration both tRNA^{Phe} and tRNA^{Phe}_{-Y} samples were lyophilized and dissolved in modification buffer immediately before modification reaction.

Modification procedure. Modification of tRNAs /5-10 A₂₆₀/ with 1M chloroacetaldehyde has been done following the procedure described earlier¹⁷ at 25°C for 20h, with the exception that more concentrated buffer 40 mM CH₃COOK, 20 mM /CH₃COO/₂ Mg of pH 5.5 was used. The reaction was quenched by the removal of chloroacetaldehyde on Biogel P-2 / 1x25cm / column. Then "maturation" of chloroacetaldehyde-modified tRNA was performed /12h incubation in H₂O at 50°C/ in order to transform the remaining reaction intermediates into the stable ethenoderivatives¹⁷. Each tRNA sample after modification and maturation procedures was controlled for its structural integrity by polyacrylamide gel electrophoresis.

Analysis of chloroacetaldehyde-modified tRNAs. Digestions of modified tRNAs with RNase T₁ were performed at pH 5.0 in 7M urea according to²⁶. 20% gel /acrylamide-bisacrylamide 29 : 1/, 40x20x0.1cm, with one 1cm and two 3cm wide slots was used for separation of tRNA T₁ fragments. The gel was kept at a room temperature for at least two days prior to use. During preelectrophoresis /800V, 4°C/ slots were filled with 1xTBE, 7M urea

Table 1. RBB values of T₁ fragments of the chloroacetaldehyde-modified tRNAs /band numbers correspond to those from Fig.1./.

Band No	1	1'	2	3	4	5	6	7	8	9	10
R _{BB}	0.73	0.77	0.87	0.91	0.96	0.99	1.06	1.12	1.20	1.26	1.40

and when current fell to the constant value the urea was washed away from the slots with reservoir buffer /90mM TBE, pH 8.3/ and the tRNA T₁ hydrolysates were loaded into the wider slots while the mixture of markers into the narrow one. Electrophoresis was carried out at 4°C at a constant voltage of 1000 V. The runs were terminated when bromophenol blue marker had migrated 22cm /8-9 h/. Then the separated bands, localised in UV light, were cut out and after further fragmentation extracted for 24h, at room temperature, with 4 ml of distilled water. The co-extracted polyacrylates were removed on small /0.3 x 2cm/ DEAE-cellulose columns with 100 mM TEABC of pH 7.8, and pure oligonucleotides were recovered with 0.5 M TEABC. After evaporation of the volatile buffer each oligonucleotide or oligonucleotide fraction was subjected to both 5'-end and total nucleoside analysis. Digestions of tRNA fragments with snake venom phosphodiesterase and alkaline phosphatase were carried out according to Sen and Ghosh²⁷. Hydrolysis of tRNA fragments with ribonuclease P₁ /2 µg/ was carried out in 0.05 M ammonium acetate of pH 5.3, 0.005 M KH₂PO₄, at 20°C for 0.5-1 h.

HPLC analysis was performed on IChF PAN type 302 apparatus /0.4 x 25 cm column/ equipped with air-thermostated column chamber. The column operated at 100 psi, 50°C in 0.175 M ammonium formate buffer pH 4.55. Separations were monitored at 254 nm, usually in 0.0-0.05 absorbance scale. Nucleosides were quantitated on the basis of their peak areas and UV extinction coefficients, which were determined at pH 4.55 and 254 nm. The error in determination of modification extents was estimated to be not higher than ± 0.1 for each given value.

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