REMOVAL OF Y-37 FROM $tRNA^{phe}_{yeast}$ ALTERS OLIGOMER BINDING TO TWO LOOPS

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Received December 4,1972

SUMMARY

The association constants of complementary oligomers were used to monitor changes in the structure of $tRNA^{phe}_{yeast}$ as a consequence of excision of a single base Y in the anticodon loop and of clipping the molecule at the point of excision. Significant changes were found not only in the binding constants of oligomers complementary to the anticodon loop but also in the K of an oligomer complementary to the dihydro U loop. The results suggest that either a single base change in a tRNA may alter structure elsewhere in the molecule or that the acid treatment necessary to remove Y irreversibly alters the structure of $tRNA^{phe}_{yeast}$.

INTRODUCTION

Tri and tetraribonucleotides have recently been shown to bind to a variety of available complementary sequences of a tRNA molecule [1-3]. This observation should be useful in helping to determine whether a change of a single base in a tRNA sequence only exerts a local effect on the RNA structure or causes a larger rearrangement of the conformation of the macromolecule. This distinction is especially important if the single base change has been shown to alter the activity of the tRNA. One such case occurs in $tRNA^{phe}_{yeast}$. This tRNA has a fluorescent base "Y" [4] immediately adjacent to the anticodon which can easily be removed at pH 2.9. $tRNA^{phe}_{yeast}$ missing this nucleoside has altered activity in both the aminoacylation and the ribosome binding assays [5,6]. The tRNA chain missing the Y base can also be clipped at the same position in the anticodon loop resulting in two half molecules held together by the hydrogen bonded helices [7]. This communication reports preliminary experiments which indicate

that removal of the Y base and clipping of the anticodon loop not only significantly alters association constants of oligomers complementary to the anticodon loop, but also changes the association constant of an oligomer to the dihydro-U loop. Thus, either the removal of a single base in the anticodon loop alters the structure of a region of the tRNA some distance from it or else the acid treatment of $tRNA^{phe}_{yeast}$ leading to the modification causes irreversible conformational changes. Since acid treatment of $tRNA^{tyr}_{coli}$ does not lead to changes in the association constant of an oligomer to the dihydro U loop, the former interpretation is favored.

MATERIALS AND METHODS

$tRNA^{phe}_{yeast}$ was a gift from Dr. L. Kirkegaard. Its acceptor activity was 1900 pmoles/A_{260} and this figure was used for the calculation of concentrations. $tRNA^{phe}_{yeast}$ missing Y-37 and clipped at that position were both prepared as described by Thiebe and Zachau [5,7,8]. ^3H tri and tetraribonucleotides were synthesized as described by Uhlenbeck, Baller and Doty [1].

The association constants for oligonucleotides binding to tRNA were measured at 0° by equilibrium dialysis in 50 µl chambers in 1 M NaCl, 10 mM MgCl, 10 mM phosphate pH 7 as described by Uhlenbeck [2]. The $tRNA^{phe}_{yeast}$ concentrations were in the range of 30 to 50 µM and greatly exceeded the oligomer concentrations. Under these conditions the association constant K for an oligomer binding to a single site on a tRNA molecule is given by (R - 1)/[tRNA], where R is the ratio of counts on the two sides of the dialysis chamber. A non zero value for K does not necessarily mean that all the residues in the oligomer are binding to the tRNA. The criteria used for determining whether a tetramer is binding completely have been described previously [2].

RESULTS AND DISCUSSION

Since complementary oligomers do not bind to the TψCG loop or the helical regions in two other tRNAs [2], it will be assumed in this preliminary work that those regions will also be unavailable in $tRNA^{phe}_{yeast}$. Four possible regions for complementary oligomer binding remain (see Figure 1): (1) positions 18-21 in the dihydro U (D) loop (since D is not planar, it cannot form a stacked RNA double helix); (2) positions 32-36 in the anticodon loop (the structure of Y probably prohibits base pairing [4]); (3) positions 44-48 in the variable region; and (4) the 3' terminal residues 73-76. In Table 1 the association constants of oligomers complementary to these regions indicate

TABLE I

Oligomer Binding to $tRNA^{phe}_{yeast}$ in 1 M NaCl, 10 mM $MgCl_2$, pH 7

Tetramers	K	ΣK_{TRI}*	Controls	Site
UCCC	43,200	2,200	CCCC	18 - 21
UCAG	13,800	1,000	UCGA, AGUC, CAGU	32 - 35
UUCA	13,700	1,000	AUCA	33 - 36
GACC	14,200	3,800	CCAA	45 - 48
UGGU**	154,000	138,700	--	73 - 76

*ΣK_{TRI} is the sum of the K's of the adjacent constituent trimers in the tetramer. Controls are oligomers of similar sequence which do not bind.

**It is not clear from these data that UGGU is binding to residues 73-76 if one applies the criteria given in reference 7.

that all four of these regions are probably available. This is consistent with oligomer binding patterns to other tRNAs [2] and nearly consistent with trimer binding experiments to $tRNA^{phe}_{yeast}$ [3].

The major purpose of this study was to examine the changes in the oligomer binding pattern as a function of the removal of Y-37 and chain scission at that point. The binding constants of one trimer and four tetramers to the intact and modified $tRNA^{phe}$ samples are given in Table 2. The results clearly show that two of the four regions are effected by the modifications.

Excision of Y greatly decreases the value of K of both tetramers complementary to the anticodon loop. Although in the case of the codon tetramer UUCA one could argue that Y stabilizes the neighboring AU base pair between the oligomer and the tRNA, a longer range stabilization would have to be invoked to explain the change in UCAG binding. A more likely explanation for the simultaneous decrease of the two binding constants is that the excision of Y disrupts the rigid conformation of the anticodon loop and the more flexible loop cannot bind oligomers as well. Some support for this view is given by the fact

TABLE 2

Oligomer Binding Constants to $tRNA^{phe}_{yeast}$ in 1 M NaCl, 10 mM $MgCl_2$, pH 7 as Position 37 is Altered

Oligomer	Y^+ tRNA	Y^- tRNA	Clipped tRNA
UCAG	13,800	3,200	44,300
UUCA	13,700	1,700	1,600
UCCC	43,200	5,100	8,000
GACC	14,300	14,800	14,400
GGU	117,100		106,900

that the K of UUCA is nearly the same binding to the Y^- and the clipped anticodon loop. Thus, the average conformation of the anticodon in the Y^- t^{RNA} is quite similar to that of single stranded RNA, the presumed conformation of the clipped molecule. The high value of K for UCAG to the clipped anticodon loop is presumably due to the contribution of an additional stacking interaction as the anticodon helix is extended by four bases (Figure 2).

The binding of UCCC to the D loop of $tRNA^{phe}_{yeast}$ decreases at least five fold when the Y base is removed. Thus, either the acid treatment needed to remove Y irreversibly alters the conformation of the D loop region or the Y base is necessary for the correct conformation of the D loop. It is not easy to distinguish between these possibilities. Treating $tRNA^{tyr}_{coli}$ with acid in a similar way was not found to change the K of GCUC to the D loop. K for GCUC was 24,600 for the acid treated $tRNA^{tyr}_{coli}$ and 25,000 for the untreated. One can argue that the D loop sequence is not the same in $tRNA^{tyr}_{coli}$ so that a difference is not seen. Experiments in progress will attempt to show interaction between the two loops by competition between oligomers.

Theibe and Zachau [5] showed that the aminoacylation reaction of Y^- $tRNA^{phe}_{yeast}$ was different from intact $tRNA^{phe}_{yeast}$. From spectroscopic evidence

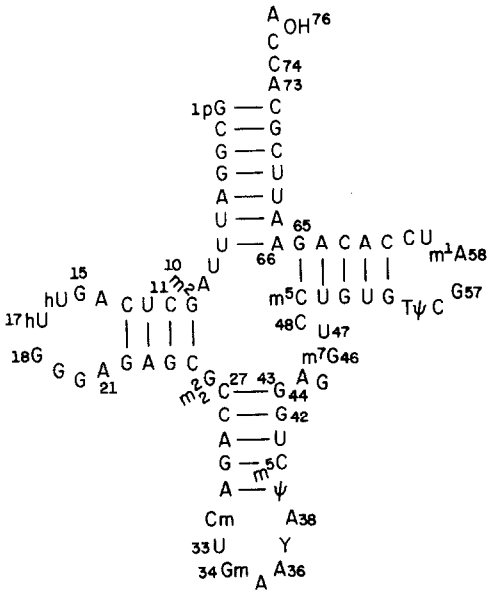

Fig. 1.

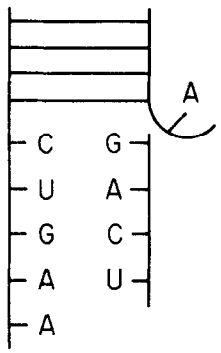

Fig. 2.

Figure 1. The structure of $tRNA^{phe}_{yeast}$ [12]. The abbreviations used are: hU, 5,6-dihydrouridine; Cm^5, 5-methylcytidine; Cm, 2'-O-methylcytidine; Gm, 2'-O-methylguanosine; Gm^2, N^2-methyl-guanosine; Gm^2_2, N^2-dimethylguanosine; Gm^7, 7-methylguanosine; Am', 1-methyladenosine.

Figure 2. A likely structure for the complex of UCAG to the clipped anticodon loop of $tRNA^{phe}_{yeast}$.

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they suggested that the difference was due to a conformational change in the molecule rather than a local difference in the anticodon loop. The present experiments showing just such a conformational change in the area of residues 18-21 are consistent with this interpretation. Furthermore, chemical modification [10] and enzymatic digestion [11] experiments both suggest that altering residues G18-G20 changes the affinity for $tRNA^{phe}_{yeast}$ to its synthetase.

The method of oligomer binding to tRNA was useful for examining the effect of a single base change on the structure of tRNA. Application of the method to chemically or biochemically modified tRNAs should also lead to valuable structural information. Work is also in progress on examining the separated half molecules of $tRNA^{phe}_{yeast}$ in order to learn more about the folding of the macromolecule.

ACKNOWLEDGMENTS

This work was supported by a grant from the U. S. Public Health Service (GM 19059-01). O. C. Uhlenbeck holds a Career Development Award (GM 70,498) from the U. S. Public Health Service.

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