FUNCTION OF Y IN CODON-ANTICODON INTERACTION OF $tRNA^{Phe}$

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Summary: Molar association constants of binding oligonucleotides to the anticodon loops of (yeast) $tRNA^{Phe}$, (yeast) $tRNA^{Phe}_{HCl}$ and (E.coli) $tRNA^{Met}_{F}$ have been determined by equilibrium dialysis. From the temperature dependence of the molar association constants, ΔF, ΔH and ΔS of oligomer-anticodon loop interaction have been determined. The data indicate that the free energy change of codon-anticodon interaction is highly influenced by the presence of a modified purine ($tRNA^{Phe}$), of an unmodified purine ($tRNA^{Met}_{F}$) or its absence ($tRNA^{Phe}_{HCl}$). Excision of the modified purine Y in the anticodon loop of $tRNA^{Phe}$ results in a conformational change of the anticodon loop, which is discussed on the basis of the corresponding changes in ΔF, ΔH and ΔS.

The fidelity of proper codon-anticodon interaction between mRNA and tRNA on the ribosome is one of the key steps in protein biosynthesis. Experiments of binding tRNA fragments to ribosomes in the presence of possible codons emphasize the importance of a definite preformed conformation of the anticodon loop for the recognition process (1). A comparison of the known primary structures of tRNA shows that they have general composition and sequence of the anticodon loop in common (2,3). On the 5'-side of the anticodon, there are two pyrimidines, and on the 3'-side two purines. Furthermore, most of the anticodon loops contain a modified purine adjacent to the 3'-side of the anticodon. Though this modified purine appears to be important for many physico-chemical characteristics of tRNA (4), its biological significance is not known as well as its importance for the integrity of the conformation of the anticodon loop.

Recently, the technique of complementary oligonucleotide binding has been developed to explore the structure of tRNA (5,6,7,8). We used this technique to measure the temperature dependencies of the K values of binding complementary oli-

gonucleotides to the anticodon loops of (yeast) $tRNA^{Phe}$, (yeast) $tRNA^{Phe}_{HCl}$† as well as (E.coli) $tRNA^{Met}_{F}$. Thus, ΔF-, ΔH- and ΔS-values of binding oligonucleotides to different anticodon loops were determined. These parameters reflect differences in the conformation of the anticodon loops due to the presence of a modified purine ($tRNA^{Phe}$), of an unmodified purine ($tRNA^{Met}_{F}$) or its complete absence ($tRNA^{Phe}_{HCl}$).

Experimental

(Yeast) $tRNA^{Phe}$ and (E.coli) $tRNA^{Met}_{F}$ were purchased from the Boehringer-Mannheim Corporation and used without further purification. Both tRNA's had acceptance activities in the order of 80-90%. Tritium labelled tetranucleotides were prepared as described previously (8,10). Association constants were determined by equilibrium dialysis experiments as described (5,7,8,10). Oligomer concentrations were in the range of 0.1-0.3 μM. $tRNA^{Phe}_{HCl}$ was prepared by acid treatment of $tRNA^{Phe}$ as described by Thiebe and Zachau (9).

Enthalpies were calculated from least square fits of plots of the logarithms of the association constants versus the inverse absolute temperature.

Results and Discussion

Oligonucleotide binding studies on the structure of (yeast) $tRNA^{Phe}$ have shown that only sequence UMeGAA, i.e. the anticodon and the 5' adjacent base, is fully available for bihelical complex formation with complementary oligonucleotides (10). Molar association constants of binding oligomers to this sequence of the anticodon loop of $tRNA^{Phe}$ and $tRNA^{Phe}_{HCl}$, are summarized in Table 1. A general criterion for tetramer binding is that K of a tetramer is at least greater than 5 times the sum of its constituent trimers (6,7). In accordance with this criterion, the data would indicate that UpUpCpA and UpUpCpG bind to $tRNA^{Phe}$ as tetramers, but do not bind as tetramers to $tRNA^{Phe}_{HCl}$. UpUpCpU and UpUpCpC, the other two 3' extended codons, are not fully complementary to the anticodon loop of $tRNA^{Phe}$ or of $tRNA^{Phe}_{HCl}$. Hence, the binding constants of these tetramers are considerably lower than those

† According to the nomenclature of Thiebe and Zachau $tRNA^{Phe}$, of which Y has been removed by mild acid treatment, is termed $tRNA^{Phe}_{HCl}$ (9).

Table 1. Molar Association Constants of Oligomers with (yeast) $tRNA^{Phe}$ and (yeast) $tRNA^{Phe}_{HCl}$

	$tRNA^{Phe}$ K (10^3 M^{-1})	$tRNA^{Phe}_{HCl}$ K (10^3 M^{-1})
UpUpC	2.0	1.0
UpCpA	1.8	1.8
UpCpG	3.0	2.0
UpUpCpA	75.0	10.0
UpUpCpG	35.0	8.2
UpUpCpU	19.0	3.8
UpUpCpC	8.5	1.5

K was measured in 1.0 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate buffer (pH 7.0) at -2°C.

of the complementary UpUpCpA and UpUpCpG. Upon removal of Y, the K values of all oligomers are markedly reduced. This indicates that the free energy change of codon-anticodon interaction is highly influenced by the presence of the modified base Y adjacent to the anticodon. An analysis of the temperature dependence of the codon-anticodon interactions should give more details about the nature of the conformational change of the anticodon loop of $tRNA^{Phe}$ upon excision of Y.

The temperature dependence of association of UpUpCpA, UpUpCpG, UpUpCpU and UpUpCpC with $tRNA^{Phe}$ (50 µM) in 1.0 M NaCl, 10 mM $MgCl_2$ and 10 mM $Na_2H\ PO_4$ buffer, pH 7.0, is shown in Fig.1, which depicts the fraction of free oligomers as function of the temperature. Plotting the logarithms of the molar association constants, which were calculated from the data of Fig.1 against the inverse absolute temperatures, yielded a straight line. This justified an analysis based on the van't

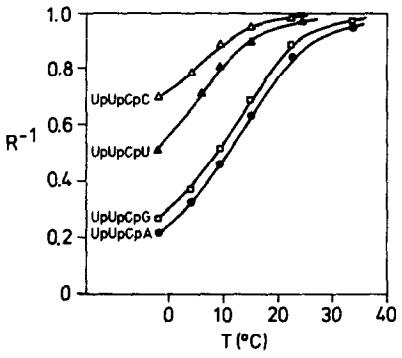

Fig. 1.

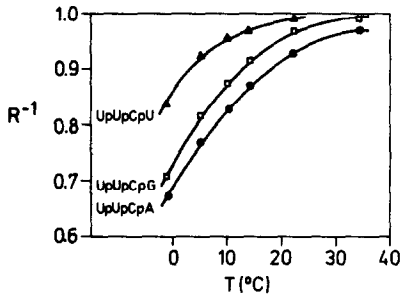

Fig. 2.

Fig. 1. Relation between R^{-1}, the fraction of free oligomers, and temperature for binding UpUpCpA, UpUpCpG, UpUpCpU, and UpUpCpC to (yeast) $tRNA^{Phe}$ at 50 µM concentration in 1.0 M NaCl, 10 mM $MgCl_2$ and 10 mM Na_2HPo_4, pH 7.0.

Fig. 2. Relation between R^{-1}, the fraction of free oligomers, and temperature for binding UpUpCpA, UpUpCpG, and UpUpCpU to (yeast) $tRNA^{Phe}_{HCl}$ at 50 µM concentration in 1.0 M NaCl, 10 mM $MgCl_2$ and 10 mM Na_2HPo_4 pH 7.0.

Hoff equation in order to calculate the enthalpies of oligomer – tRNA interaction. Accordingly, the free energy of binding (ΔF) is given by the changes of enthalpy (ΔH) and entropy (ΔS) upon binding:

$$\Delta F = -RT \ln K = \Delta H - T\Delta S \qquad \text{eq. 1.}$$

Thus, average values of these thermodynamic quantities for the temperature range which we have studied, have been determined. The corresponding values for oligomer binding to $tRNA^{Phe}$ at 10°C are given in Table 2.

A ΔH-value of -7.4 to -8.2 kcal/mole per base pair has been obtained by calorimetric studies of the melting of PolyA : PolyU double strands (11). The major contribution to the enthalpy of helix formation in aqueous solution is presumably the stacking of bases. Thus, the formation of the first base pair in the helix would have a negligible enthalpy, because it is devoid of stacking interaction (12). Since (n+1) base pairs would correspond to n "stacking units", enthalpies of short helix formation are better calculated per "stacking unit" instead per base pair. Therefore, the ΔH-values of about -20 kcal/mole for UpUpCpA- and UpUpCpG - $tRNA^{Phe}$

Table 2. Thermodynamic Quantities for Binding of Tetranucleotides to the Anticodon Loop of (yeast) $tRNA^{Phe}$ and (yeast) $tRNA^{Phe}_{HCl}$ at 10°C

Complex	ΔH (kcal/mole)	ΔF (kcal/mole)	TΔS (kcal/mole)
$tRNA^{Phe}$: UpUpCpA	-(19.5 ± 1.5)	-(5.6 ± 0.1)	-(13.9 ± 1.6)
$tRNA^{Phe}$: UpUpCpG	-(20.5 ± 1.5)	-(5.5 ± 0.1)	-(15.0 ± 1.5)
$tRNA^{Phe}$: UpUpCpU	-(16.5 ± 2.0)	-(4.8 ± 0.2)	-(11.7 ± 2.2)
$tRNA^{Phe}$: UpUpCpC	-(16.0 ± 2.0)	-(4.4 ± 0.2)	-(11.6 ± 2.2)
$tRNA^{Phe}_{HCl}$: UpUpCpA	-(14.0 ± 2.0)	-(4.7 ± 0.2)	-(9.3 ± 2.2)
$tRNA^{Phe}_{HCl}$: UpUpCpG	-(15.0 ± 2.0)	-(4.5 ± 0.2)	-(10.5 ± 2.2)
$tRNA^{Phe}_{HCl}$: UpUpCpU	-(15.4 ± 3.0)	-(3.9 ± 0.3)	-(11.5 ± 3.3)

interaction would correspond to a ΔH of -6 to -7 kcal/mole per "stacking unit". This indicates that the UpUpCpA- and UpUpCpG - $tRNA^{Phe}$ "helices" consist of 3 "stacking units" or, respectively, of 4 base pairs, which was already suggested by the high values of K at -2°C (Table 1) (10). It is interesting to note that binding of the "wobble" tetramer UpUpCpG only gives a slightly different ΔF from that of UpUpCpA binding. This difference is mainly reflected in the entropy term. Binding of UpUpCpU and UpUpCpC to $tRNA^{Phe}$ gives ΔH-values of - (16.5 ± 2.0) kcal/mole and - (16.0 ± 2.0) kcal/mole, respectively, which indicates that the UpUpCpU- and UpUpCpC-$tRNA^{Phe}$ "helices" consist of 2 "stacking units", i.e. of 3 base pairs. Indeed, the ΔH-values are similar to a ΔH of - (15 ± 1.5) kcal/mole obtained by binding UpUpC to $tRNA^{Phe}$ (13) (Table 2). The lower stability of UpUpCpU- and UpUpCpC - $tRNA^{Phe}$ "helices" as compared to the UpUpCpA- and UpUpCpG - $tRNA^{Phe}$ "helices" results from a decrease of the number of base pairs. This could be expected since the 3' extended codons UpUpCpU and UpUpCpC cannot base-pair with U at the 5'-side of the anticodon, whereas UpUpCpA and UpUpCpG can.

The observed changes in enthalpy upon UpUpCpN to $tRNA^{Phe}_{HCl}$ are in the range of - (15.0 ± 2.0) kcal/mole, which is similar to that of binding UpUpC (13), UpUpCpC or UpUpCpU to $tRNA^{Phe}$. This suggests the existence of only "two stacking units" or three base pairs, respectively, in all UpUpCpN - $tRNA^{Phe}_{HCl}$ "helices", if it is assumed that the water structures are similar around the anticodon loop of $tRNA^{Phe}$ and of $tRNA^{Phe}_{HCl}$. Apparently, the anticodon loop of $tRNA^{Phe}_{HCl}$ cannot extend the bihelical codon-anticodon complex by one more base pair. This observation may find further support by the entropy data of Table 2. All ΔS-values are in the same range except those for binding UpUpCpA and UpUpCpG to $tRNA^{Phe}$, which bind as tetramers to the anticodon loop.

The anticodon loop of (E.coli) $tRNA^{Met}_{F}$, which does not contain a modified base adjacent to the anticodon, has the sequence (3' to 5') MeCUCAUAA (14). The complementary tetramer ApUpGpA has a molar association constant of 15×10^3 l./mole at -2°C. From the temperature dependence of the molar association constant of ApUpGpA binding, one obtains a ΔH-value of - (19.5 ± 1.5) kcal/mole, which is similar to the reported one (7) and which indicates the formation of four base pairs between ApUpGpA and the anticodon loop of $tRNA^{Met}_{F}$. At 10°C, ΔF equals - (4.6 ± 0.1) kcal/mole and TΔS - (14.9 ± 0.6) kcal/mole. The smaller change in free energy of binding ApUpGpA to $tRNA^{Met}_{F}$ as compared to binding UpUpCpA to $tRNA^{Phe}$ is caused by a different TΔS term and not by a different ΔH. Again, the data demonstrate an important implication of the modified base in the codon-anticodon interaction by its influence on the conformational integrity of the anticodon loop. In accordance with these data, the association constants of all oligomer-anticodon interaction, so far investigated (7,11,15,16), are significantly higher if a modified base is adjacent to the anticodon.

Fuller and Hodgson (17) have proposed a general anticodon loop structure in which the bases on the purine side of the anticodon loop, including the anticodon, form a single stranded stack and the two remaining bases on the pyrimidine side are in an extended conformation. An alternative possibility could be that the pyrimidine side and the anticodon form a single stranded stack and then the

loop bends back at the position of the modified purine. However, neither model is compatible with present oligonucleotide binding data of various tRNA's (5,7,11,15). Neither the 3'- nor the 5'-end of the anticodon loop were available for bihelical complex formation with complementary oligonucleotides. Therefore, an anticodon loop structure has to be envisaged, which can accommodate for bihelical complex formation with tetramers complementary to the anticodon and the 5' adjacent pyrimidine, but which cannot pair with tetramers complementary to other sites of the anticodon loop. The modified purine on the 3'-side of the anticodon loop has to play an important role in stabilizing this particular structure.

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