COMPLEX FORMATION BETWEEN TRANSFER RNA'S WITH COMPLEMENTARY ANTICODONS

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SUMMARY

$tRNA^{phe}$ (anticodon GAA) and $tRNA^{glu}$ (anticodon presumably UUC) have been found to form a complex with an association constant of about $5\times10^{5}M^{-1}$ at 0°C. This binding is much stronger than the binding of trinucleotide UUC to $tRNA^{phe}$ but has a weaker temperature dependence. This suggests that the anticodon regions of tRNA have similar and complementary structures, such as Watson and Crick helices.

INTRODUCTION

It has recently been shown that oligonucleotides bind to portions of the anticodon loop of tRNA with binding constants which are of the order of $10^{3}M^{-1}$ for complementary trinucleotides and of the order of $10^{4}M^{-1}$ for complementary tetranucleotides.[1,2,3,4] In these systems the oligomers generally have little secondary structure before binding while the anticodon loop can be assumed to have a well defined conformation. It has been pointed out[2] that the binding between codon and the tRNA anticodon could be expected to be much stronger if the structure of codon were complementary to that of the anticodon, and that this is envisioned to occur during protein synthesis. If the codon and anticodon conformations under these circumstances are short sections of Watson and Crick helices a pair of tRNA molecules, one of which carries an anticodon which serves as the codon of the other, could be expected to bind strongly to each other.

The present paper describes the successful observation of such an anticodon-anticodon complex involving yeast $tRNA^{phe}$ (anticodon GAA) and E.coli $tRNA^{glu}$ (anticodon presumably UUC, but see discussion section). Evidence for this comes from the quenching of the fluorescence of the Y base adjacent to the anticodon of $tRNA^{phe}$ upon the formation of the complex.

EXPERIMENTAL

Yeast $tRNA^{phe}$ was purchased from the Boehringer Mannheim Corporation and used without further purification except for passing it through Sephadex G-25 to remove the salt. It had an acceptance activity of about 1200 μμ moles of phenylalanine per A_{260}. $tRNA^{glu}$, $tRNA^{val}$ and $tRNA^{arg}$ from E.coli K-12MO were kindly donated by the Biological Macromolecular Separations Technology Project of the Oak Ridge National Laboratory.[5] All samples were dissolved in 0.05 M cacodylate buffer at pH 6.7 and contained 10^{-2} $MgCl_2$ unless stated otherwise. All t-RNA concentrations were calculated by using a molar extinction coefficient of 6.3×10^5 at the 260 nm peak.

All fluorescence spectra were obtained by means of a variable temperature recording fluorimeter which has been described previously.[6] The excitation light was 313 nm and its intensity was monitored by deflecting a small portion to a photomultiplier by means of a 45° quartz plate and all fluorescence intensities were corrected for variations in the intensity of the excitation light. The excitation and emission monochromators had slits corresponding to 6 nm and the sample tubes (inside diameter 1.5 mm) were carefully matched. In preparing a set of samples the concentration of $tRNA^{phe}$ was kept constant and the concentration of the other tRNA was varied.

The fraction of light absorbed by a sample whose absorbance at a particular wavelength is A is given by $f_a(A) = 10^{-A}(10^A-1)$. At the excitation wavelength a sample containing only $tRNA^{phe}$ has an absorbance A_{phe}, while a sample containing the same concentration of $tRNA^{phe}$, as well as other, non-fluorescent tRNA has a somewhat higher absorbance A_{tot}. In such mixed samples a fraction A_{phe}/A_{tot} of the light absorbed was absorbed in $tRNA^{phe}$. In order to correct for the light absorbed by components other than $tRNA^{phe}$ in mixed samples the observed fluorescence intensities were multiplied by correction factor $(A_{tot}/A_{phe})[f_a(A_{phe})/f_a(A_{tot})]$. The values for A_{phe} and A_{tot} (between .09 and .14) were determined spectrophotometrically using the same quartz sample tubes as were used in the fluorescence experiments and the correction factors obtained in this way were between unity and 1.08.

RESULTS

The base Y whose structure has recently been determined[7] is on the 3' side of the anticodon of $tRNA^{phe}$ and absorbs to the red of all other bases in the molecule and is (with exception of 7 Me guanine whose quantum yield is an order of magnitude lower) the only base to fluoresce in aqueous solution at room temperature. As a result it can be excited to the exclusion of the remaining bases, energy transfer to other bases is negligible, and, since its fluorescence yield and spectral characteristics are very sensitive to its micro-environment, it represents a powerful tool for the study of the conformation and binding properties of the anticodon loop.[1,2]

While binding of UUC to $tRNA^{phe}$ produces a small blue shift (~5 nm) in the fluorescence spectrum of Y, which is accompanied by a 30 percent decrease in yield,[2] the addition of the "complementary" transfer RNA to $tRNA^{phe}$,

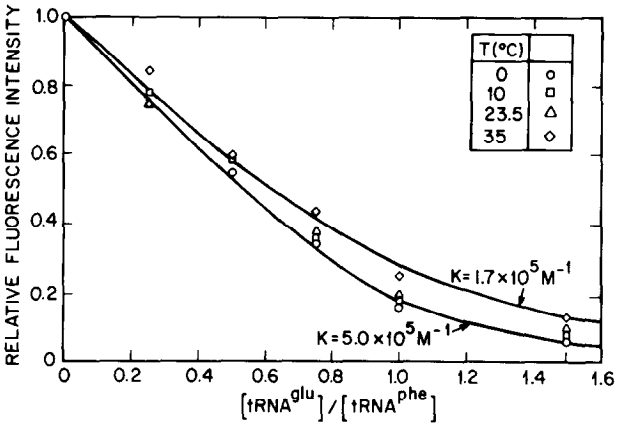

Fig. 1 The relative fluorescence yield of $tRNA^{phe}$ in the presence of various concentrations of $tRNA^{glu}$ and different temperatures. The concentration of $tRNA^{phe}$ was kept constant at 0.51×10^{-4} M and the observed fluorescence yields were corrected for exciting light absorbed in $tRNA^{glu}$ (see experimental section). The theoretical binding curves which provide the best fit to the experimental points are shown for two temperatures.

$tRNA^{glu}$, resulted in almost complete quenching of the Y fluorescence. Figure 1 shows the result of titrating $tRNA^{phe}$ with $tRNA^{glu}$ and measuring the fluorescence intensities as a function of the molar ratios. The association constant for forming the tRNA complex is

$$K = \frac{p:g}{p_f g_f}$$

where p:g, p_f and g_f are the concentrations of the $tRNA^{phe}$:$tRNA^{glu}$ complex and the concentrations of the unbound $tRNA^{phe}$ and $tRNA^{glu}$, respectively. If the Y fluorescence of $tRNA^{phe}$ is quenched completely in the presence of $tRNA^{glu}$ the fraction of $tRNA^{phe}$ complexed with $tRNA^{glu}$, f, is given by F/F_o, where F is the observed fluorescence intensity and F_o is the fluorescence of $tRNA^{phe}$ in the absence of $tRNA^{glu}$.

The experimental points shown in Fig. 1 were fitted by a least square fitting program to theoretical binding curves

$$f = \frac{1}{2}\{y - [y^2 - 4g/p]^{1/2}\}$$

where

$$y = (g/p) + (Kp)^{-1} + 1 .$$

The association constants obtained in this way are given below

T(°C)	0	10	23.5	35
$K(10^5 M^{-1})$	5.0	3.7	2.9	1.7

The experimental errors are estimated to be 30 percent.

Evidence for the specificity of the interaction between the anticodons of $tRNA^{phe}$ and $tRNA^{glu}$ (see Fig. 2) comes from the fact that in the presence of equimolar concentrations of $tRNA^{val}$ (anticodon ?AC) or $tRNA^{arg}$ (anticodon UCU or CCU) the $tRNA^{phe}$ fluorescence was quenched by only about 10 percent. In contrast to this an equimolar concentration of $tRNA^{glu}$ quenched $tRNA^{phe}$ by 80 percent.

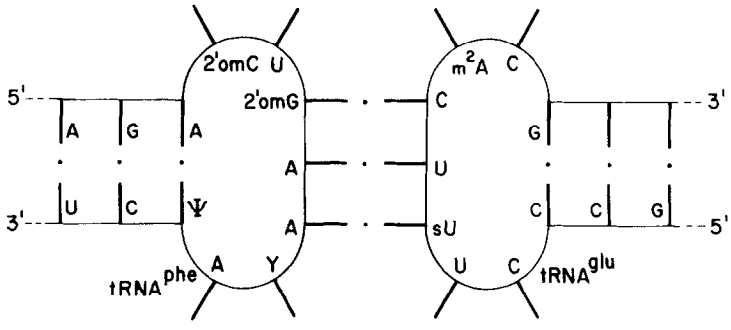

Fig. 2 A schematic representation of the anticodon-anticodon complex formed between $tRNA^{phe}$ and of $tRNA^{glu}$. The primary sequence for the anticodon loop of $tRNA^{glu}$ is the one proposed by Ohashi et al.[7] for E.coli $tRNA_2^{glu}$.

Table I shows the quenching of $tRNA^{phe}$ fluorescence in the presence of an equimolar concentration of $tRNA^{glu}$ as a function of the Mg^{2+} concentration. While there is an increase in binding strength at high Mg concentrations a complex is formed even when $[Mg^{2+}]$ is low. $[tRNA^{phe}]$ was 0.5×10^{-4} M throughout.

Table I

$\frac{[tRNA^{glu}]}{[tRNA^{phe}]}$	$[Mg^{2+}]$	Relative Fluorescence Yield (23°C)
1.0	10^{-4}	0.25
1.0	10^{-3}	0.25
1.0	10^{-2}	0.20
1.0	10^{-1}	0.14
0	10^{-2}	1.00

DISCUSSION

The primary sequence of $tRNA^{glu}$ (E.coli) is not known. Since the codon is GAA or GAG the anticodon may be presumed to be UUC or CUC. Since C---A hydrogen bonding does not occur and almost complete binding between $tRNA^{phe}$ and $tRNA^{glu}$ was observed, one may conclude that UUC is the only anticodon of the $tRNA^{glu}$ (E.coli) used in these experiments.[5] It is interesting that Yoshida et al.[8] recently identified the anticodon of $tRNA_3^{glu}$ (yeast) as SUC, where S is a 2-thiouridine derivative and that Ohashi et al.[9] also reported an odd base (2-thio,5-methylamino-methyl uridine or sU) in the first position of the anticodon of $tRNA_2^{glu}$ of E.coli. It is therefore likely that in the $tRNA^{glu}$ used in these experiments the first U of the anticodon is also replaced by a uridine derivative for which hydrogen bonding to A is possible.

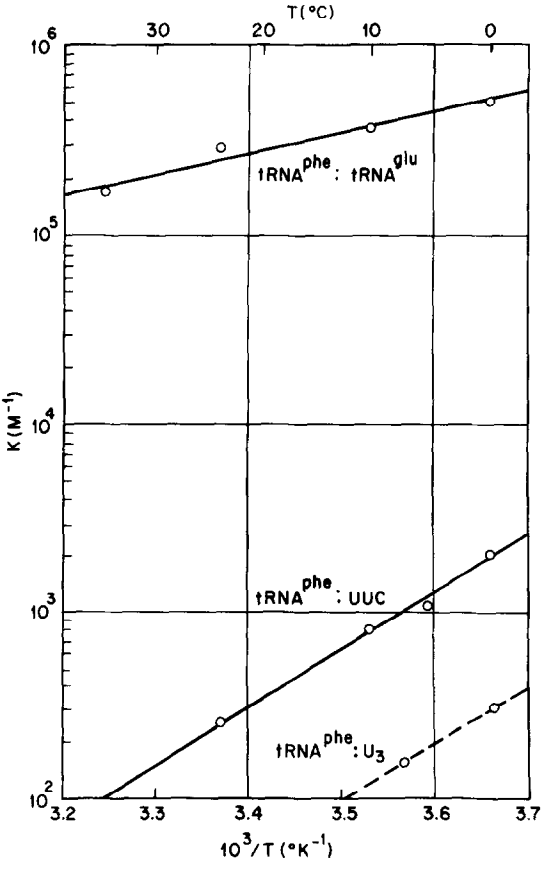

Fig. 3 The association constants of the $tRNA^{phe}$:$tRNA^{glu}$, $tRNA^{phe}$:UUC and $tRNA^{phe}$:UUU complexes as functions of the inverse absolute temperatures.

Figure 3 shows the association constants of various codon-anticodon complexes as functions of the inverse absolute temperature. The most striking result of these experiments is the fact that the binding between complementary tRNA anticodons is about three orders of magnitude stronger than the binding of complementary trinucleotides to tRNA anticodons and that it displays a far smaller temperature dependence.[2] The binding between complementary trinucleotides in aqueous solution is not observable.[10]

In as much as Figure 3 yields good linear relationships one is justified in interpreting these results in terms of the free energy of binding (ΔF) which is given by the changes of enthalpy (ΔH) and entropy (ΔS) upon binding:

$$\Delta F = -RT \ln K = \Delta H - T\Delta S \ . \qquad (1)$$

In this way one obtains the following thermodynamic parameters (in kcal M^{-1} at 10°C) for codon-anticodon binding*

System	ΔF	ΔH	$T\Delta S$
$tRNA^{phe}$:UUC	-3.7	-14.3	-10.6
$tRNA^{phe}$:$tRNA^{glu}$	-7.2	- 5.2	+ 2.0

The errors are between 1 and 2 kcal M^{-1}. It is seen that the free energy of binding is almost doubled when UUC has a suitable conformation imposed on it by the secondary and tertiary structure of $tRNA^{glu}$. This change in ΔF is the result of ΔH decreasing by 9 kcal M^{-1} and $T\Delta S$ increasing by 13 kcal M^{-1}. While a detailed understanding of these changes in enthalpy and entropy cannot be obtained without including the contributions of water of solvation, it is safe to say that the great increase in the entropy term for the $tRNA^{phe}$:$tRNA^{glu}$ system reflects the fact that the two anticodons have well defined complementary conformations. The fact that $T\Delta S$ is positive means that before binding occurred the anticodons were surrounded by well ordered water.

The characteristics of the complex between transfer RNA's with comple-

* The binding of the wobble codon UUU for which data are shown in Fig. 3 is 6 times weaker than that of the codon UUC, but the values of ΔH and $T\Delta S$ (-15 and -12 kcal M^{-1}, respectively) are not very different.[2]

mentary anticodons are indicative of considerable structural integrity of the anticodon and of a "lock and key" interaction similar to that which characterizes many enzyme-substrate interactions. A similar conformational complementarity between tRNA and its codon on the mRNA bound to the ribosome may explain the great fidelity with which codon recognition occurs during protein synthesis. If one accepts this hypothesis it is likely that the codon at the recognition site has the same conformation, independent of the particular codon being read. The fact that tRNA molecules with complementary anticodons form a complex of the kind described above shows therefore that the conformations of anticodons on tRNA are identical and complementary to each other. This suggests that the m-RNA codon at the recognition site of the ribosome and anticodon of the tRNA are Watson and Crick helices. Such a structure for anticodon region has been suggested by Fuller and Hodgson as a result of model building studies.[11]

The results of this investigation show that steric factors are as important in nucleic acid interactions as they are for proteins. They also suggest the use of complementary tRNA binding in the purification of tRNA.

The existence of the $tRNA^{phe}$:$tRNA^{phe}$ complex described here has recently been confirmed by gel electrophoresis experiments.[12]

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