# COMPLEMENTARY TRIMER BINDING TO TRANSFER RNA$^{Phe}$

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## 1. Introduction

Much evidence has been obtained by physicochemical measurements that tRNA has a structure of higher order than a loose combination of helical segments as is proposed by the general cloverleaf structure [1, 2, 3]. But it is still not known what parts or segments of the loops of the cloverleaf are involved in the organization of the higher ordered structure of tRNA and what parts are not, i.e. remain single-stranded. Recently, Doty and coworkers have shown that oligonucleotides of at least 3 units interact with available complementary regions of an RNA molecule to such a degree that the association constant can be readily measured by equilibrium dialysis [4, 5]. We applied this methodology to tRNA$^{Phe}$ in order to explore the availability of the bases within the loops of tRNA$^{Phe}$ complementary trimer binding. The results which are reported here, are compatible with the cloverleaf model as far as the doublestranded stem-regions are concerned. In addition, parts of the hU-loop and the rT-loop are not available for complementary trimer binding, wheras the bases in the extra arm are available for complementary trimer binding.

## 2. Materials and methods

Primer dependent polynucleotide phosphorylase (*Micrococcus luteus*) was purchased from Boehringer, Mannheim, Germany. Dinucleoside monophosphates were purchased from Zellstoff-Fabrik Waldhof, Mannheim, Germany. Tritium labeled nucleoside-5′ diphosphates were supplied by Schwarz Biochemicals Inc., Orangeburg, New York, USA. Typical reaction conditions for the synthesis of a trimer XpZpN were: 0.1–1.0 mM $^3$H-NDP (1–14 Ci/mmole), 6 mM XpZ, 10 mM $MgCl_2$, 0.4 M NaCl, 0.2 M glycine buffer (pH 9.3) and 0.3 mg/ml polynucleotide phosphorylase [6]. When $^3$H-CDP or $^3$H-UDP was used, incubation time was 4 hr at 34°. Otherwise, the reaction mixtures were incubated for 14 hr at 34°. The reaction was terminated by boiling and 0.1 mg/ml alkaline phosphatase was added and reacted for an additional hour to degrade unreacted nucleoside-5′ diphosphate. The products were separated from the reactants by descending paper chromatography as described by Thach [7].

tRNA$^{Phe}$ was purchased from Boehringer, Mannheim, Germany. It was used without further purification. Dialysis was carried out in chambers made from a pair of 1.5 cm 'Plexiglas' acrylic disks with a shallow cylindrical depression cut into each (1.8 × 12 mm) as described by Uhlenbeck et al. [4]. The experiments were carried out in 1.0 M NaCl, 10 mM $MgCl_2$ and 10 mM $Na_2HPO_4$ (pH 7.0) buffer. In the experiments, the tRNA concentration (40 μM) greatly exceeds the trimer concentration (5–50 nM). Therefore, the association constant $K$ of a trimer to a single complementary site on the tRNA molecule can be calculated by the relationship $R = 1 + K[\text{RNA}]$, where $R$ is the ratio of trimer counts in the chamber with the tRNA to the chamber without [8]. The experimental error in the evaluation of the association constants is estimated to be 20% for $K$-values above 1000 l/mole, and to be 50% for $K$-values below 1000 l/mole.

## 3. Results

According to the cloverleaf structure the following sequences of $tRNA^{Phe}$ are in stemregions: 1–7, 66–72 (acceptor stem); 10–13, 22–25 (hU-stem). In table 1 trimers are listed, which are complementary to parts of the stemregion and do not interact with $tRNA^{Phe}$. As can be seen from this table, they span the region of the entire anticodon stem (39–43), of the entire hU-stem (22–25) and of almost the complete acceptor stem (3–5, 7; 68–71). This suggests that these stem-regions are indeed in bihelical conformation and therefore not available for complementary trimer binding. In addition, the trimers listed in table 1 have complementary sequences in loop regions of $tRNA^{Phe}$, which are apparently not available for complementary trimer binding. These sequences are in the hU-loop 17–19 and 19–21, and in the rT-loop 54–56, 55–57, 58–60. In table 2 trimers are listed of which complementary sequences in the loop regions are unique and do not occur elsewhere in the $tRNA^{Phe}$ sequence. In reporting these results it is important to note that association constants of less than about 500 l/mole correspond to *R*-values, in the conditions of our experiments, of less than 1.02. Such values are taken to indicate no significant binding for the trimers used. Trinucleotides containing only A and U have association constants in the range of about 500 l/mole [5]. The wobble codon–anticodon interaction of UUU and $tRNA^{Phe}$ belongs to this limited class. On the other hand, *K*-values of about 1200 l/mole can be expected for trimers forming one GC bond. Trimers with more GC content have *K*-values between 2000 and 5000 l/mole, except those containing a GG sequence, in which cases the constants are near 10,000 l/mole, if the GG at the 3′ end of the trimer, and 20,000 l/mole, if at the 5′ end [5]. Accordingly, the association constants of trimer binding to $tRNA^{Phe}$ listed in table 2 have been classified as positive, intermediate and negative. The resulting differences in *K*-values are not due to differences in composition and sequence of the trimer, but instead arise from differences in the accessibility of the antisequence $tRNA^{Phe}$.

Table 1

The trimers listed are complementary to stem regions of $tRNA^{Phe}$ as well as loop regions and did not bind. Binding was measured in 1.0 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate buffer (pH 7.0) at 0° by equilibrium dialysis. $tRNA^{Phe}$ concentration was 40 μM and trimer concentrations were in the range of 20 nM.

| Trimer | Complementary $tRNA^{Phe}$ sequence | Stem | Complement in loop-region |
|---|---|---|---|
| UCC | GGA (3–5) | acceptor | |
| UAA | UUA (7–9) | stem | |
| GAA | UUC (68–70) | (1–7 | rT-loop (54–56) |
| CGA | UCG (69–71) | 66–72) | rT-loop (55–57) |
| GAU | AΨC (38–40) | anticodon | rT-loop (58–60) |
| CCA | UGG (41–43) | stem | hU-loop (17–19) |
| UCC | GGA (42–44) | (27–31 | hU-loop (19–21) |
| | | 39–43) | |
| CUC | GAG (20–22) | (hU-stem | |
| GCU | AGC (23–25) | 10–13 | hU-loop (20–22) |
| | | 22–25) | |

As can be seen in table 2, the extra arm $AGG^{m^7}UC$ (44–48) of $tRNA^{Phe}$ is accessible to complementary triplet binding. The association constants for binding of the three complementary trimers are all below the expected range and thus termed intermediate. The trimers CCC, ACU, and AAC are complementary to the hU-loop AGhUhUGGGA (14–21). It is seen that the association constant for AAC is 1500 l/mole. This value is in the range expected for trimers containing only one G or C base. ACU, the complementary trimer next to the 5′ side, has an association constant of about 250. This *K*-value is so low as to be ambiguous and is termed to indicate no significant binding. Moving to the 3′ side, the trimer CCC has an association constant of 1000 l/mole. Since a *K*-value above 10,000 l/mole is to be expected for such trimers, the *K*-value for CCC binding is termed intermediate and indicates a very weak interaction.

UCG, which is complementary to a part of the rT-loop (56–58), does not bind to $tRNA^{Phe}$ indicating that this part of the rT-loop is not accesible to complementary trimer binding. Next, we turn to the part $UG^mAA$ (32–35) of the anticodon loop, which includes the anticodon sequence $G^mAA$. The complementary trimer UCA, next to the 5′ side, has an association constant of about 500 l/mole, which is at the detection limit of our method. The interaction of UCA with the anticodon loop is apparently not very significant. Also, we measured the association constants of the codon UUC and the wobble codon UUU. The *K*-value for UUC binding is 2700 l/mole. This is

Table 2
Molar association constants of trimers with $tRNA^{Phe}$. $K$ was measured in 1.0 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate buffer (pH 7.0) at 0° by equilibrium dialysis. $tRNA^{Phe}$ concentration was 40 μM and trimer concentrations were in the range of 20 nM.

| Trimer | Complementary $tRNA^{Phe}$ sequence | $tRNA^{Phe}$ region | $K$ (l/mole) | Classification |
|---|---|---|---|---|
| ACU | AGhU (14–16) | | 250 | – |
| AAC | hUhUG (16–18) | hU-loop | 1500 | + |
| CCC | GGG (18–20) | | 1000 | intermediate |
| UCA | $UG^{m}A$ (33–35) | anticodon loop | 500 | intermediate |
| CCU | $AGG^{m^7}$ (44–46) | | 3000 | intermediate |
| ACC | $GG^{m^7}U$ (45–47) | extra arm | 2000 | intermediate |
| GAC | $G^{m^7}UC$ (46–48) | | 750 | intermediate |
| UCG | $CGA^{m^1}$ (55–57) | rT-loop | 0 | – |

in good agreement with the one recently measured by a fluorescence technique [9]. The 3′ wobble codon UUU binds at 0° about 5 times more weakly than the codon. The association constant for UUU binding is about 600 l/mole. The 5′ wobble codon GUG and the codon AUG for the $tRNA_f^{Met}$ did not show such a difference in binding strength [4]. One explanation for this apparent difference in behaviour might be that in the 3′ wobble codon for $tRNA^{Phe}$ one GC base pair is exchanged by a GU base pair, but in the 5′ wobble codon for $tRNA_f^{Met}$, one AU base pair is exchanged by one GU base pair. However, further experiments are needed in order to obtain a good understanding of these differences.

## 4. Discussion

The data summarized in tables 1 and 2 show that parts of the hU-loop of $tRNA^{Phe}$ are not available to complementary trimer binding. This suggests that they are in shielded areas and involved in the three-dimensional structure of this tRNA. On the other hand, the extra arm, although somewhat restricted for an interaction with complementary trimers, seems to be not involved in additional base pairing, which would be relevant for the three-dimensional structure of $tRNA^{Phe}$ as recently proposed [1]. But further work is still needed for a complete mapping of $tRNA^{Phe}$. This is currently done with appropriate tetranucleotides in our laboratory.

## References

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