COMPLEMENTARY TRIMER BINDING TO TRANSFER-RNA$_1^{Val}$

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1. Introduction

Oligonucleotides of three 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 [1–4]. We applied such oligonucleotide binding studies to *E. coli* tRNA$_1^{Val}$ in order to explore the availability of sequences of tRNA$_1^{Val}$ to complementary trimer binding. The results which are reported here are compatible with the cloverleaf model as far as the double stranded regions are concerned. Furthermore, the data indicate 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.

2. Material and methods

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. Trimers XpZpN were synthesized according to Thach as described earlier [4, 5]. *E. coli* tRNA$_1^{Val}$ was purchased from Boehringer, Mannheim, Germany. It was isolated as described by Nishimura [6] and has been assayed to have more than 90% valine accepting activity. Dialysis was carried out in 1.0 M NaCl, 10 mM $MgCl_2$ and 10 mM Na_2HPO_4 (pH 7.0) buffer as described [2–4]. In the experiments, the tRNA concentration (50 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 $R = 1 + K$ [RNA], where R is the ratio of trimer counts in the chamber with the tRNA to the chamber without [7]. 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 and discussion

According to the cloverleaf structure the following sequences of tRNA$_1^{Val}$ are in stem regions: 1–7, 66–72 (acceptor stem); 10–13, 22–25 (hU stem); 27–31, 39–43 (anticodon stem); 49–53, 61–65 (rT-stem). In table 1 trimers are listed, which are complementary to parts of the stem regions and do not interact with tRNA$_1^{Val}$. As can be seen from this table, they span the region of the entire anticodon stem (39–43) and of parts of the hU-stem (22–24), of the acceptor stem (2–7) and of the rT-stem (62–65). This suggests that these stem regions are indeed in bihelical conformation and therefore not available for complementary trimer binding.

In table 2 trimers are listed of which complementary sequences occur in the loop regions of tRNA$_1^{Val}$ In reporting these results it is important to note that association constants of less than about 400 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. Trimers containing only A and U have association constants in the range of about 500 l/mole [2,3]. K-values of 1000–2000 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

Table 1
The trimers listed are complementary to stem regions of *E. coli.* $tRNA_1^{Val}$ as well as loop regions and did not bind.

Trimer	Complementary $tRNA_1^{Val}$ sequence	Stem	Complement in loop-region
ACC	GGU (2–4)	acceptor stem	extra-arm (45–47)
CAC	GUG (3–5)	1–7	
AUC	GAU (5–7)	66–72	
CUC	GAG (22–24)	hU-stem	
		10–13	
		22–25	
UCC	GGA(39–41)	anticodon stem	hU-loop (19–21)
CUC	GAG (40–42)	27–31	
CCU	AGG (41–43)	39–43	
		rT-stem	
ACG	CGU (62–64)	49–53	
GAC	GUC (63–65)	61–65	extra-arm (46–48)

Binding was measured in 1.0 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate buffer (pH 7.0) at -2° by equilibrium dialysis. $tRNA_1^{Val}$ concentration was 50 μM and trimer concentrations were in the range of 20 nM.

cases the constants are near 10,000 l/mole, if GG is at the 3′ end of the trimer, and 20,000 l/mole, if at the 5′ end [2]. Accordingly, the association constants of trimer binding to $tRNA_1^{Val}$ listed in table 2 have been classified as positive, intermediate and negative. Based on the data in table 1, it has been assumed that binding of trimers, which are complementary to a stem and a loop region, takes place at the loop region.

As can be seen from table 2, the 5′ end of the hU-loop (14–17) is fully available to complex formation with complementary trimers. The remaining part of the hU-loop (18–21) is not fully available to trimer binding. CCC, having a relatively low K value of 2200 l/mole, could bind as well to part of the extra arm (44–46). CAG is also complementary to the anticodon loop (32–34) and will probably bind there. Oligonucleotide binding studies to *E. coli* $tRNA_F^{Met}$, *E. coli* $tRNA^{Tyr}$ [3], and *yeast* $tRNA^{Phe}$ [8] have shown, that these tRNA's have an asymmetric anticodon loop structure. Always, oligomers extended to the 5′ side of the anticodon bind much more strongly than oligomers extended to the 3′ side. This general pattern is also observed with $tRNA_1^{Val}$. The four possible codons for valine – GUU, GUC, GUG, GUA – bind well to the anticodon (34–36). In addition, trimers, which are complementary to the sequences 32–35 and 35–37, show also some interaction with the anticodon loop, whereas sequence 36–38 is not available to trimer binding.

The first base of the anticodon of $tRNA_1^{Val}$ is uridine-5-oxyacetic acid. Apparently, it can pair with all four bases, since all four valine codons bind to this RNA. This degeneracy in pairing is also observed with trimers complementary to the 5′ half of the anticodon loop, where almost all possible combinations interact. The different association constants for codon–anticodon interaction correlate well to the specificity in the presence of ribosomes. GUA and GUG gave the highest K-values in the dialysis experiments as well as the best response in ribosome binding experiments [9]. The extra arm of $tRNA_1^{Val}$ (44–48) is not available to bihelical formation with complementary trimers. CCC shows some interaction, having a low binding constant. It might as well interact with the hU-loop. The TψC-sequence at the 5′ end of the rT-loop is available to complementary binding of GAA, having a K-value of 1200 l/mole. TψC Pu represents the sequence which is common to all tRNA's so far sequenced. Since in the four tRNA's examined by oligonucleotide binding studies [2,3,8], TψC is available to complementary GAA binding, it might indicate a common environment as well as function of this particular tRNA sequence.

The data summarized in tables 1 and 2 show that parts of the hU loop, of the extra arm and of the rT-loop of $tRNA_1^{Val}$ are not available to complementary trimer binding. This suggests that they are in shielded areas and involved in the three-dimensional structure of this RNA. On the other hand, the 5′ end of the hU-loop (14–17) is fully available to complex formation with complementary trimers. These observations are incompatible with the three-dimensional structure of $tRNA_1^{Val}$ as recently proposed [10]. But further work is needed for a complete mapping of $tRNA_1^{Val}$. This is currently done with appropriate tetranucleotides and competition experiments.

Table 2
Molar association constants of trimers with *E. coli* $tRNA_1^{Val}$.

Trimer	Complementary $tRNA_1^{Val}$ sequence	$tRNA_1^{Val}$ region	K(l/mole)	Classification
UCC	GGA (19–21)		0	–
CCC	GGG (18–20)	hU-loop	2200	intermediate
CCA	hUGG (17–19)	(14–21)	800	–
CAG	ChUG (16–18)		900	intermediate
AGC	GChU (15–17)		1300	+
GCU	AGC (14–16)		2500	+
UUG	C^mAA(36–38)		0	–
UGU	AC^mA(35–37)		1000	intermediate
GUU	*VAC (34–36)	anticodon	1400	+
GUC	VAC (34–36)	anticodon	1400	+
GUG	VAC (34–36)	anticodon	2200	+
GUA	VAC	loop	3000	+
UUA	UVA		0	–
UCA	UVA (33–35)	(32–38)	600	intermediate
UGA	UVA (33–35)	(32–38)	400	intermediate
UAA	UVA (33–35)	(32–38)	400	+
UAG	CUV		500	intermediate
CAG	CUV (32–34)		800	intermediate
AAG	CUV (32–34)		800	intermediate
GAG	CUV		1800	intermediate
GAC	GUC (46–48)	extra-arm	0	–
ACC	GGU (45–47)	(44–48)	400	–
CCC	GGG (44–46)		2200	intermediate
GAU	AUC (58–60)		400	–
AUC	GAU (57–59)	rT-loop	600	intermediate
UCG	CGA (56–58)	rT-loop	0	–
CGA	ψCG (55–57)	(53–60)	0	–
GAA	TψC (54–56)		1200	+
AAC	GTψ (53–55)		800	intermediate

K was measured in 1.0 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate buffer (pH 7.0) at −2° by equilibrium dialysis. $tRNA_1^{Val}$ concentration was 50 μM and trimer concentrations were in the range of 20 nM.

* The V in the anticodon arm is uridine-5-oxyacetic acid.

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