

COMPLEMENTARY OLIGONUCLEOTIDE BINDING TO YEAST tRNA^{Phe}_{HCl}

O. PONGS

Max-Planck-Institut für Molekulare Genetik, 1 Berlin-Dahlem, Ihnestrasse 63-73, Germany

Received 30 October 1972

1. Introduction

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 [4]. The biological significance of this modified purine as well as its importance for the integrity of the conformation of the anticodon loop or of the entire tRNA is not known.

Recently, the technique of complementary oligonucleotide binding has been developed to explore the structure of tRNA [5-8]. We used this technique in order to explore structural changes induced by an excision of the base Y [9], which is adjacent to the anticodon of yeast tRNA^{Phe}. The results presented in this communication show that excision of Y does not influence the overall tRNA^{Phe} structure. However, it markedly decreases the stability of codon-anticodon interaction.

2. Experimental

Yeast tRNA^{Phe} was purchased from the Boehringer-Mannheim Corporation and used without further purification. Acceptance activity was in the order of

80%. Tritium labelled oligonucleotides were prepared as described [8, 10]. Association constants were determined by equilibrium dialysis experiments [5, 7, 8, 10]. Oligomer concentrations were in the range of 0.1-0.3 μ M. tRNA^{Phe}_{HCl} was prepared by acid treatment of tRNA^{Phe} as described by Thiebe and Zachau [9][†]. Association constants were classified as previously discussed [7, 10].

3. 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, are fully available for bihelical complex formation with complementary oligonucleotides [10]. All other parts of tRNA^{Phe} were partially or not available to complementary oligonucleotide binding. Table 1 summarizes binding data of a representative number of oligonucleotides, which are complementary to tRNA^{Phe} except to the anticodon loop sequence. The data show that the overall structure of tRNA^{Phe} is not altered upon excision of Y. Similar values of *K* have been obtained for binding oligonucleotides to tRNA^{Phe} and to tRNA^{Phe}_{HCl}. This indicates that the D-loop, the extra arm, and the rT-loop of tRNA^{Phe} are not affected by the modification of the anticodon loop. The same observation holds for the 3'-terminus. UpGpG and GpGpU have similar values of *K*, whether they are bound to tRNA^{Phe} or to tRNA^{Phe}_{HCl}.

Molar association constants of binding oligomers to the anticodon loops of tRNA^{Phe} and tRNA^{Phe}_{HCl} are

[†] 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}.

Table 1
Molar association constants of trimers and tetramers with tRNA^{Phe} and tRNA^{Phe}_{HCl}

Oligomer	tRNA Antisequence	K (l/mole)	
		tRNA ^{Phe}	tRNA ^{Phe} _{HCl}
UpCpC	GpGpA (19-21)	0	0
CpCpC	GpGpG (18-20)	2000	1400
CpCpA	GpGpD (17-19) ^{D-loop}	0	0
CpCpCpA	GpGpGpD (17-21)	500	400
GpApC	GpUpC (46-48)	0	0
ApCpC	GpGpU (45-47)	1500	1400
CpCpU	ApGpG (44-46) ^{extra-arm}	3800	3000
ApCpCpU	ApGpGpU (44-47)	2500	2200
GpApU	ApUpC (58-60)	400	0
CpGpA	ψpCpG (55-57)	0	0
GpApA	TrψpC (54-56)	1000	800
ApApC	GpTpψ (53-55) ^{rT-loop}	1500	1200
GpApApC	GpTpψpC (53-56)	3400	3000
ApUpCpG	CpGpApU (56-59)	0	0
UpGpG	CpCpA (74-76) ^{3'-terminus}	4500	5000
GpGpU	ApCpC (73-75)	30000	28000

K was measured in 1.0 M NaCl, 10 mM MgCl₂, 10 mM sodium phosphate buffer (pH 7.0) at -2° by equilibrium dialysis. tRNA concentration was 40-60 μM and oligomer concentrations were in the range of 20 nM.

Table 2

Molar association constants of trimers and tetramers, which are complementary to the anticodon loops of tRNA^{Phe} and of tRNA^{Phe}_{HCl}

Oligomer	tRNA Antisequence	K (l/mole)	
		tRNA ^{Phe}	tRNA ^{Phe} _{HCl}
CpApG	CpUpG (32-84)	750	3000
UpCpA	UpGpA (33-35)	1800	1800
UpCpG	UpGpA (33-35)	3000	2000
UpUpC	GpApA (34-36)	2000	1000
GpUpU	ApApY (35-37)	1700	0
UpCpApG	CpUpGpU (32-35)	2000	4000
UpUpCpA	UpGpApA (33-36)	75000	10000
UpUpCpG	UpGpApA (33-36)	35000	8200
UpUpCpU	UpGpApA (33-36)	19000	3800
UpGpUpU	ApApYpA (35-38)	2000	0

K was measured in 1.0 M NaCl, 10 mM MgCl₂, 10 mM sodium phosphate buffer (pH 7.0) at -2° by equilibrium dialysis. tRNA concentration was 40-60 μM and oligomer concentrations were in the range of 20 nM.

summarized in table 2. As can be seen, K values of all oligomers, which bind to the anticodon loop, are markedly altered upon removal of Y. GpUpU and UpGpUpU, which bind to the 3'-half of the anticodon loop of tRNA^{Phe} [10], do not bind to tRNA^{Phe}_{HCl}. This was to be expected, since the 3'-half of the anticodon loop of tRNA^{Phe}_{HCl} is missing one base. Binding of CpApG and UpCpApG, which are complementary to the 5'-half of the anticodon loop, bind more strongly to tRNA^{Phe}_{HCl} than to tRNA^{Phe}. Apparently, this half of the anticodon loop can accommodate a helical complex with complementary oligomers more easily, if Y is not present in the anticodon loop. However, the most dramatic effects are seen with oligomers, which are complementary to the anticodon and the 5'-adjacent pyrimidine, i.e. to sequence (3'-5') U-G-A-A. The value of K of binding UpUpCpA to tRNA^{Phe} is approx. one order of magnitude higher than that of binding UpUpCpA to tRNA^{Phe}_{HCl}. Similarly, the values of K of binding other oligomers, which are in part complementary to the anticodon and the 5'-adjacent pyrimidine, are considerably reduced upon removal of Y. This indicates that the modified base Y, which

is 3'-adjacent to the anticodon, has an important influence on the stability of codon-anticodon complexes.

A general criterion for tetramer binding is that K of a tetramer is at least greater than 5 times the sum of the K values of its constituent trimers [6, 7]. In accordance with this criterion, the data of table 2 would indicate that UpUpCpA and UpUpCpG bind to the anticodon loop of tRNA^{Phe} as tetramers. However, these tetramers only bind intermediate to the anticodon loop of tRNA^{Phe}_{HCl} since the values of K of UpUpCpA and UpUpCpG binding are not five times greater than the sum of the K values of the constituent trimers ($K_{\text{UpUpC}} + K_{\text{UpCpA}}$ or K_{UpCpG}). 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 of the complementary UpUpCpA and UpUpCpG.

In summary, the data of table 2 indicate that upon removal of Y the codon-anticodon interaction is considerably weakened. In other words, the presence of the modified purine, adjacent to the anticodon, is important for a stable codon-anticodon interaction. It has been reported that tRNA^{Phe}_{HCl} cannot form a stable complex with mRNA on the ribosome [9]. This observation apparently reflects the weak codon-tRNA^{Phe}_{HCl} interaction reported in table 2, which cannot be enhanced or restored by the ribosome. Three lines of evidence are now available to support the notion that the modified base, which is adjacent to the anticodon, is important for a stable codon-anticodon interaction. 1) Binding constants of tetramers, such as UpUpCpA, which are complementary to the anticodon and the pyrimidine on the 5'-side, are in the range of

80,000 ℓ /mole, if the base on the 3'-side is modified [7, 10, 11], but in the range of 15,000 ℓ /mole, if the base on the 3'-side is not modified as in *E. coli* tRNA^{Met}_F. ii) The codon-anticodon interaction is increased upon modification of the base, which is adjacent to the anticodon, e.g. the yeast tRNA^{Met}_F-ApUpG complex is more stable than the *E. coli* tRNA^{Met}_F-ApUpG complex [12]. iii) The codon-anticodon interaction is decreased upon excision of the modified base, which is adjacent to the anticodon, e.g. the tRNA^{Phe}-UpUpCpA complex is approx. one order of magnitude more stable than the tRNA^{Phe}_{HCl}-UpUpCpA complex.

References

- [1] S.K. Dube, P.S. Rudland, B.F.C. Clark and K.A. Marcker, Cold Spring Harbor Symp. Quant. Biol. 34 (1969) 161.
- [2] H.G. Zachau, Angew. Chem. 81 (1969) 645.
- [3] F. Cramer, Progr. Nucleic Acid Res. Mol. Biol. 11 (1971) 391.
- [4] S. Nishimura, Progr. Nucleic Acid Res. Mol. Biol. 12 (1972) 50.
- [5] O.C. Uhlenbeck, J. Baller and P. Doty, Nature 225 (1970) 508.
- [6] P. Doty and J.B. Lewis, Nature 225 (1970) 570.
- [7] O.C. Uhlenbeck, J. Mol. Biol. 65 (1972) 25.
- [8] O. Pongs, E. Reinwald and K. Stamp, FEBS Letters 16 (1971) 275.
- [9] R. Thube and H.G. Zachau, European J. Biochem. 5 (1968) 546.
- [10] O. Pongs, R. Bald and E. Reinwald, European J. Biochem. (1972) in press.
- [11] P.R. Schimmel, O.C. Uhlenbeck, J.B. Lewis, L.A. Dickson, E.W. Elfred and A.A. Schreier, Biochemistry 11 (1972) 642.
- [12] G. Högenauer, F. Turnowsky and F.M. Unger, Biochem. Biophys. Res. Commun. 46 (1972) 2100.