BINDING OF COMPLEMENTARY OLIGONUCLEOTIDES TO AMINOACYLATED $tRNA^{Phe}$ FROM YEAST

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SUMMARY

The effect of the aminoacylation on the structure of $tRNA^{Phe}$ from yeast has been studied by equilibrium dialysis experiments. The association constants of oligomers were determined which were complementary to the dihydrouridine-, the anticodon-, the TΨC loop; to the extra-arm and to the 3'-terminus of $tRNA^{Phe}$-A_{73}, $tRNA^{Phe}$-A-C-C-3'NH_2A and Phe-$tRNA^{Phe}$-A-C-C-3'-NH_2A in which the phenylalanine is bound by a stable amide bond. The results show that removal of the 3'-terminus or aminoacylation of $tRNA^{Phe}$ from yeast does not cause a gross conformational change of the molecule. However, the aminoacylation renders the 3'-terminus inaccessible to binding complementary oligonucleotides. Based on this finding, it is proposed that the α-amino group of Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A folds back to the 5'-terminal phosphate to form a salt bridge.

Abbreviations: $tRNA^{Phe}$-A-C-C-A = $tRNA^{Phe}$ = phenylalanine specific tRNA from yeast, $tRNA^{Phe}$-A_{73} = $tRNA^{Phe}$-A-C-C-A with missing 3'-C-C-A end, $tRNA^{Phe}$-A-C-C-3'NH_2A = $tRNA^{Phe}$ in which the 3'-terminal adenosine is replaced by 3'-deoxy-3'-aminoadenosine. Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A = phenylalanylated $tRNA^{Phe}$-A-C-C-3'NH_2A.

INTRODUCTION

Substantial progress in the study of the structure of tRNA has been achieved by the recent X-ray diffraction studies on uncharged, crystalline tRNA (1,2). However, it was suggested that the tRNA may change its

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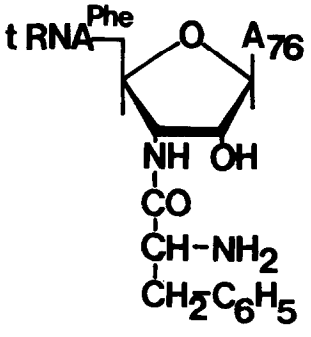
structure during its functional cycle in protein biosynthesis (3). The understanding of the conformation of aminoacyl-tRNA in solution is therefore a prerequisite to the elucidation of such a functional mobile tRNA structure. Attempts to identify conformational changes upon aminoacylation of tRNA by spectroscopic methods gave, however, conflicting results (4-6). Serious limitation of such investigations is the lability of the ester bond by which the amino acid is bound to the 3'-terminal adenosine of the tRNA.

It was shown in several laboratories that the binding of oligonucleotides to single-stranded regions of RNA molecules is an effective method for studying the secondary and tertiary structure of tRNA in solution (7). Previously we have explored the ability of non-aminoacylated $tRNA^{Phe}$ from yeast for interaction with complementary oligonucleotides by equilibrium dialysis (8,9). Now we have studied by this technique the structure of aminoacylated Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A from yeast, on which the aminoacyl residue is bound by a stable amide bond to the 3'-position of the terminal adenosine (10) (Fig. 1).



Legend to Fig. 1:

Structure of the 3'-end of Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A.

MATERIAL AND METHODS

Micrococcus luteus polynucleotide phosphorylase (E.C.2.7.7.8) was isolated and rendered primer dependent according to Gassen et al. (11). Dinucleoside monophosphates were purchased from Zellstoff-Fabrik Waldhof, Mannheim, Germany, and from Boehringer, Mannheim, Germany. Tritium labeled nucleotide-5'diphosphates were supplied by Schwarz Biochemicals Inc.,Orangeburg, New York, U.S.A.. Tritium labeled oligonucleotides (1-14 Ci/mmole) were synthesized as previously described (8). $tRNA^{Phe}$-A_{73} from yeast was prepared from $tRNA^{Phe}$-A-C-C-A_{76} by limited degradation with snake venom phosphodiesterase (E.C. 3.1.4.1) and analysed as described previously (12). Its composition was 74% $tRNA^{Phe}$-A_{73}, 25% $tRNA^{Phe}$-A-C_{74} and less than 1% of $tRNA^{Phe}$-A-C-C_{75}. After incorporation of the missing 3'-nucleotides (12) this tRNA accepted 1440 pmoles phenylalanine/A_{260}-unit. $tRNA^{Phe}$-A-C-C-3'NH_2A from yeast was prepared by incorporation of 3'-deoxy-3'-aminoadenosine-5'-phosphate into $tRNA^{Phe}$-A-C-C_{75} by tRNA-nucleotidyl transferase from yeast (E.C. 2.7.7.2.1) using a described procedure (13). It accepted 1520 pmoles Phe/A_{260}-unit tRNA. Preparative aminoacylation, leading to Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A was performed with purified phenylalanyl-tRNA synthetase (E.C. 6.1.1.20) from yeast according to (14). Final purification of this aminoacylated tRNA was performed by chromatography on a RPC-5 column (15). As could be shown by determination of the 3'-end nucleoside (12), this preparation of Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A did not contain any native $tRNA^{Phe}$-A-C-C-A or not aminoacylated $tRNA^{Phe}$-A-C-C-3'NH_2A.

Equilibrium dialysis was carried out under conditions previously reported (8). One compartment of the cell was filled with 40 - 60 µM tRNA and the other one with 10 - 50 nM oligomer, both dissolved in 10 mM $MgCl_2$, 1.0 M NaCl and 10 mM sodium phosphate buffer pH 7.0. The system was allowed to equilibrate at -2°C for 2 - 3 days. Details of the criteria used for classification of oligomer binding are given in ref. 8. The experimental error in the evaluation of the association constants is estimated to be in the order of 20% for K values above 1000 M^{-1}. In most of the conditions of our experiments, association constants of less than about 500 M^{-1} correspond to K values, which are within the detection limit of the equilibrium dialysis method.

RESULTS AND DISCUSSION

Since the ester bond by which the amino acid is coupled to the native aminoacyl-tRNA is not stable at pH 7 during the prolonged times required for

equilibrium dialysis, it was not possible to investigate the structure of Phe-$tRNA^{Phe}$-A-C-C-A by complementary oligonucleotide binding studies. The equilibrium dialysis experiments were therefore performed with 3'-phenylalanylated $tRNA^{Phe}$-A-C-C-3'NH_2A, where the aminoacyl residue is bound to the tRNA by a stable amide bond (13). However, the synthesis of this aminoacyl-tRNA involved several steps, in which the native three-dimensional structure might have become altered. At first, the terminal adenosine is removed by treatment with snake venom phosphodiesterase, then the missing CMP residues as well as the 3'-deoxy-3'-aminoadenosine nucleotide is incorporated by the use of tRNA-nucleotidyl transferase. Thus, in order to be on safe grounds in interpreting any apparent difference in the pattern of oligonucleotide binding upon aminoacylation we had to investigate the binding of complementary oligonucleotides to key intermediates in the Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A synthetic pathway.

The following tRNA species were accordingly investigated: (i) $tRNA^{Phe}$-A_{73}; if the absence of the -C-C-A end does have an effect on the tertiary structure of the tRNA, the equilibrium dialysis data of $tRNA^{Phe}$-A_{73} should differ from those of the native $tRNA^{Phe}$-A-C-C-A. (ii) $tRNA^{Phe}$-A-C-C-3'NH_2A; data obtained with this tRNA served for determination of the structural changes introduced by the modification of the 3'-terminal ribose, on which the 3'-hydroxyl is replaced by, at pH 7, partially protonated amino group. (iii) Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A, which served as an analog of aminoacyl-tRNA.

The binding studies have been carried out with oligomers which according to the clover leaf structure of $tRNA^{Phe}$(16) are complementary to the loop regions, to the extra arm or to the 3'-terminus, respectively. Thus we investigated the accessibility of the sequences 18 - 21, 32 - 36, 40 - 44,

53 - 60 and 73 - 76 of $tRNA^{Phe}$. If there is any detectable change in the structure of tRNA upon aminoacylation these sequences were expected to be most sensitive.

The data in Table 1 show that the association constants, which were obtained by binding complementary oligonucleotides to $tRNA^{Phe}$-A_{73} and to $tRNA^{Phe}$-A-C-C-3'NH_2A, are in good agreement with those, which have been previously obtained for unmodified $tRNA^{Phe}$(8). However, there is a notable difference between the association constants of oligonucleotides, which are complementary to the 3'-terminus. Since $tRNA^{Phe}$-A_{73} lacks the terminal -C-C-A sequence, it is, of course, a trivial observation that binding constants of U-G-G, G-G-U and U-G-G-U to this tRNA are zero. But also the substitution of the terminal A_{76} by 3'-deoxy-3'-aminoadenosine almost completely suppressed binding of U-G-G. Binding of G-G-U, on the other hand, is not affected by this substitution. Consequently the binding constant of U-G-G-U to $tRNA^{Phe}$-A-C-C-3'NH_2A is about 3-fold lower than that to $tRNA^{Phe}$, which was found to be 90 - 120 000 M^{-1} (8). These binding data indicate that the 3'-terminus of $tRNA^{Phe}$-C-C-3'NH_2A has not exactly the same conformation as the 3'-terminus of native $tRNA^{Phe}$.

The oligonucleotide binding data, which are summarized in Table 1, are not compatible with reported binding data of Cameron and Uhlenbeck (17). These authors report a binding constant of 43,200 M^{-1} for U-C-C-C, which is complementary to the dihydrouridine loop, and a value of 13,700 M^{-1} for U-U-C-A, which is complementary to the anticodon loop. These values differ considerably from ours, which have been determined many times. A comparable K-value for binding U-U-C-A to $tRNA^{Phe}$ was only obtained after excision of the Y-base in

Table 1: Molar association constants K of oligomers which are complementary to $tRNA^{Phe}$ from yeast

Oligomers	Association constants K (M^{-1})+				Complementary regions of $tRNA^{Phe}$
	$tRNA^{Phe}$-A-C-C-A++	$tRNA^{Phe}$-A_{73}	$tRNA^{Phe}$-A-C-C-3'NH_2A	Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A	
					Dihydrouridine loop
U-C-C	0	600	n.d.	n.d.	18 - 20
U-C-C-C	500	1 500	1 500	800	18 - 20
					Anticodon loop
U-C-A-G	2 000	1 800	0	0	32 - 35
U-U-C	2 000	1 800	2 000	500	34 - 36
U-U-C-G	35 000	35 000	36 000	14 000	33 - 36
U-U-C-A	75 000	64 000	63 000	35 000	33 - 36
U-U-U	400				34 - 36
U-U-U-G	n.d.	2 400	2 800	900	33 - 36
U-U-U-A	4 000	4 000	3 500	1 400	33 - 36
					Extra arm
G-A-C-C	3 500	6 500	3 500	3 500	40 - 43
A-C-C-U	2 500	5 000	1 000	2 000	41 - 44
					TΨC loop
A-U-C-G	0	500	1 000	1 600	56 - 60
C-G-A-A	0	500	2 000	2 300	54 - 57
G-A-A-C	3 400	3 000	1 900	1 800	53 - 56
					3'-terminus
U-G-G	4 500	0	500	500	74 - 76
G-G-U	30 000	0	27 400	8 080	73 - 75
U-G-G-U	90 000	0	35 200	19 700	73 - 76

+ K represents the average of six different determinations and was measured in 1.0 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate buffer pH 7.0 at -2°C by equilibrium dialysis. tRNA concentration was 40 - 60 μM and oligomer concentrations were in the range of 20 nM.

++ Data taken from ref. 8.

the anticodon loop (9). But it was not possible to obtain the high K-value for binding U-C-C-C. This might only occur in a denatured form of $tRNA^{Phe}$, especially, if one takes into consideration the information on the structure of $tRNA^{Phe}$, which is now available by X-ray crystal data (1,2).

The major difference in the oligonucleotide binding pattern of Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A compared to non-aminoacylated $tRNA^{Phe}$-A-C-C-3'NH_2A was observed with respect to the 3'-terminal sequence. Binding constants of G-G-U and of U-G-G-U to this aminoacylated tRNA are four to five times lower than to non-aminoacylated tRNA. The 3'-terminus of aminoacyl-$tRNA^{Phe}$ is therefore hardly available for the binding of complementary oligonucleotides.

An attractive explanation for this observation would be that the amino acid of the aminoacylated tRNA folds back to the 5'-terminal phosphate group, forming a salt bridge between the negatively charged phosphate and the positively charged α-amino group (18). This renders the phenylalanylated 3'-terminus as not accessible to bind complementary oligonucleotides. Such a salt bridge could be a recognition point for the elongation factor Tu, since it is known that this factor does not bind aminoacyl-tRNAs from which the 5'-terminal phosphate has been removed (18).

The significance of the observed decrease in the binding constants of oligomers complementary to the anticodon loop, which was repeatedly measured in the case of Phe-$tRNA^{Phe}$-A-C-C-3'NH_2A is not clear. However, it should be pointed out that this observed decrease is significant in so far that it is not due to the variability of binding data. The data for bind-

ing U-U-C-A to $tRNA^{Phe}$ vary between 65,000 M^{-1} and 80,000 M^{-1} as determined by at least twenty independent measurements with ten different $tRNA^{Phe}$ batches over a period of four years. This indicates that the twofold decrease in the K-value of U-U-C-A cannot be reconciled on the basis of the usual variations in determining K-values.

The binding of complementary oligonucleotides to parts of $tRNA^{Phe}$ which are directly involved in the stabilisation of the tertiary structure, such as G19, G20 or T54, Ψ55 residues (1,2) did not increase after aminoacylation. This indicates that the aminoacylation of the $tRNA^{Phe}$ from yeast does not induce a gross conformational change, which could be detected by the complementary oligonucleotide binding technique. Therefore it is suggested that a conformational change of the aminoacyl-tRNA, which is required for its interaction with the ribosomal A-site (19-23) is not induced by aminoacyl-tRNA synthetase but takes place in some of the following steps of the ribosomal binding process.

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