CODON–ANTICODON INTERACTION IN *ESCHERICHIA COLI* $tRNA^{Phe}$

Chemical modification study

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1. Introduction

The possibility of conformational change of tRNA upon codon binding has been widely discussed [1–6]. Experimental attempts to register this conformational change gave contradictory results [2–6].

We have investigated alkylation of tRNAs with chloroethylamine derivatives. It was found that reactivities of guanosines in tRNAs are strongly dependent on their interactions in the tertiary structure of the molecules [7]. The proposed opening of the base pairs between D-loop and T-loop of tRNAs upon codon binding should influence the reactivities of guanosines in these parts of the tRNA structure.

In an attempt to find out whether there is a conformational change in *E. coli* $tRNA^{Phe}$ upon codon complexation we have made a chemical modification study of the interaction of this tRNA with $(pU)_4$. Using this sensitive approach we found no changes in reactivities of guanosines in $tRNA^{Phe}$ in the presence of oligouridylate.

2. Materials and methods

$tRNA^{Phe}$ (*E. coli*) was from Boehringer, Mannheim. 2′,3′-*O*-[4-(*N*-2-chloroethyl-*N*-methylamino)-benzylidene]-uridine-5′-methylphosphate was synthesized as in [8].

$(pU)_4$ and $(pC)_4$ was prepared by Dr V. Ryte in this laboratory, as in [9]. Methods of alkylation of tRNA and analysis of the modified $tRNA^{Phe}$ (*E. coli*) were as in [7,10]. Equilibrium dialysis was done as in [9].

3. Results and discussion

We have investigated alkylation of $tRNA^{Phe}$ (*E. coli*) with the 2-chloroethylamine derivative 2′,3′-*O*-[4-(*N*-2-chloroethyl-*N*-methylamino)-benzylidene]-uridine-5′-methylphosphate [7,10]. The reagent modifies mainly guanosine in tRNA [11]. In order to get information about the native structure of tRNA we modified the polymer to a small extent (1–1.5 mol reagent/mol tRNA) and determined the relative rate constants of alkylation of single guanosines in the tRNA [7]. It was found that guanosines in $tRNA^{Phe}$ differ significantly in their reactivities due to their interactions and positions in the tRNA macrostructure. Guanosines G_{18} and G_{19} are reactive since they are exposed in the tRNA macrostructure [12]. Two guanosines in the D-stem, G_{10} and G_{24} are also reactive. Guanosines G_{22} and G_{57} have low reactivity, apparently due to the participation of their N7 atoms in tertiary hydrogen bond formation [12]. G_{15} was found to have low reactivity in various tRNAs investigated [13] probably due to steric hindrance. All differences in guanosine reactivities are determined by micro-environment of these guanosines in the tertiary structure of the tRNA since these differences disappear when the tRNA macrostructure is destroyed [7].

Obviously the gross conformational change of the $tRNA^{Phe}$, such as disruption of the interaction between T- and D-loops should influence the reactivities of the guanosine residues mentioned. Therefore we tried to detect a conformational change of $tRNA^{Phe}$ (*E. coli*) upon codon complexation by measuring the reactivities of guanosines of this tRNA

in the presence of oligouridylic acid.

The alkylation was performed: (a) under the conditions in [5] – 0.01 M Tris–HCl, (pH 7.5), 0.015 M $MgCl_2$, 37°C, $tRNA^{Phe}$ 0.02 mM, $(pU)_4$ 1 mM; (b) under the conditions where binding of the oligouridylates to $tRNA^{Phe}$ was guaranteed according to [14,15] – 0.01 M Tris–HCl (pH 7.5), 0.03 M $MgCl_2$, 4°C, $tRNA^{Phe}$ 0.02 mM, $(pU)_4$ 5 mM. By equilibrium dialysis experiments we determined the association constant (K_a) describing the interaction between $(pU)_4$ and $tRNA^{Phe}$ to be 2000 M^{-1} under these conditions. This means that at the concentrations used ~90% of the codon binding sites should be occupied. In control experiments $(pC)_4$ was substituted for $(pU)_4$. The modified $tRNA^{Phe}$ was digested with ribonuclease and the oligonucleotides obtained analysed as in [7,10]. It was found that the pattern of differences of reactivities of guanosines in different parts of the $tRNA^{Phe}$ structure was not dependent on the presence of the oligonucleotides within experimental error of K_i determination of 20% (table 1). The results obtained show that the interaction of $tRNA^{Phe}$ with the oligouridylate does not change the microenvironments of guanosines G_{10}, G_{18}, G_{19}, G_{15}, G_{24} and does not destroy the tertiary hydrogen bonds of guanosines G_{22} and G_{57}. This means that the codon–anticodon interaction does not result in gross conformational change of tRNA, which is in accord with the NMR data [6]. The proposed codon-induced rearrangement of tRNA structure was supported by the observation that significant binding of the oligonucleotide CGAA occurs when $tRNA^{Phe}$ is complexed to oligouridylates [2–4]. Some effect of codons on the kinetics of modification of tRNAs by kethoxal was observed, however no definite structural information was obtained in this case [5]. On the contrary an NMR study of interaction of yeast $tRNA^{Phe}$ with the oligonucleotide UUCA suggested that the codon–anticodon interaction does not result in a major conformational change in the $tRNA^{Phe}$ molecule [6]. The experiments with kethoxal [5] are not very convincing since the extent of modification of tRNA in these experiments was very high; up to 8–12 mol reagent/mol tRNA. This figure is much greater than the number of guanosines available for modification with kethoxal in native tRNAs [16]. It is clear that in this case the conformation of tRNA would change in the course of modification due to the appearance of modified guanosines unable to participate in hydrogen bond formation. In this case the pattern of guanosine modification in the tRNA does not reflect their reactivities in the native polymer. It is determined by the conformational states of the tRNA molecules appearing in the course of modification. It should be noted further that the K_a between oligouridylates and $tRNA^{Phe}$ is rather low [14,15] and under conditions used in [5] the complex would hardly exist.

Table 1
Relative reactivities of guanosine residues in $tRNA^{Phe}$ and in $tRNA^{Phe}$ in the presence of oligonucleotides under conditions (b) (see text)

Guanosine G_i	K_i/K_{18}	
	$tRNA^{Phe}$ + $(pC)_4$	$tRNA^{Phe}$ + $(pU)_4$
G_{10}	1.5	1.6
G_{15}, G_{57}	0.2	0.18
G_{18}, G_{19}	1.0	1.0
G_{22}	0.1	0.1
G_{24}	2.3	2.4

Reactivities of guanosines are given as the ratios of their alkylation rates to that of the exposed guanosine G_{18}

NMR experiments and measurements of the initial rates of modification of tRNA bases are the methods providing information about the native structure of tRNA in solution. On the contrary the oligonucleotide binding and chemical modification are, to a considerable extent (as in experiments with kethoxal [5]), methods where the tRNA structure is influenced by the probes. This principal difference in the nature of the experimental approaches may account for the observed contradiction in results. The contradiction could be overcome by assuming that codon binding does not change the conformation of tRNA but rather makes the structure of the molecule more flexible. It could be proposed that one of the conformations of the tRNA is the 'unfolded' one where the interaction of D- and T-loops is disrupted and that the equilibrium between the conformations of tRNA is frozen in free tRNA. If the unfolded conformer is present in minor proportion it could not be detected by NMR or by the method used here. It could be proposed further that the codon–anticodon interaction promotes interconversion between conformers. If the binding of the oligonucleotide CGAA stabilizes the unfolded conformer the addition of CGAA in the presence of the codon will result in a shift of the equilibrium and in accumulation of this conformer as proposed in [6]. Therefore, according

to the model described, the role of the codon–anticodon interaction is the 'unfreezing' of the tRNA structure facilitating interconversion between conformational states. The driving force for the conversion of the tRNA into an unfolded conformer is the interaction with the CGAA sequence of the 5 S RNA.

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