THE STRUCTURE OF THE CCA END OF tRNA, AMINOACYL-tRNA AND AMINOACYL-tRNA IN THE TERNARY COMPLEX

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1. Introduction

The 3′-terminal CCA end of tRNA is an essential part of the molecule for its biological function since it participates directly in the following reactions during protein biosynthesis: aminoacylation of tRNA, interaction of aminoacyl tRNA with elongation factor Tu (EF-Tu), binding of aminoacyl-tRNA to the ribosomal A-site, binding of peptidyl-tRNA to the ribosomal P-site and during the stringent response where uncharged tRNA is bound to the ribosomal A-site [1]. In order to characterize the structure of the 3′-end of tRNA during some of the above-mentioned functional states we have analyzed the accessibility of this part of the molecule by complementary oligonucleotide binding. These results were obtained from using 6 different tRNAs. The analysis included uncharged tRNAs, charged tRNAs and aminoacylated tRNAs involved in the ternary complex with elongation factor Tu and GTP. The results show that the 3′-terminal adenine becomes least accessible towards oligonucleotide interaction after aminoacylation. Addition of EF-Tu re-exposes this adenine and at the same time reduces the accessibility of the fourth base from the 3′-end for oligonucleotide interaction.

2. Materials and methods

2.1. *tRNA, aminoacyl-tRNA and elongation factor Tu*

Crude and partly purified tRNA from *Escherichia coli* were obtained from The Microbiological Research Establishment, Porton Down, England. $tRNA_m^{Met}$ and and $tRNA_3^{Ser}$ were obtained from Plenum Science, NJ, $tRNA^{Arg}$, $tRNA^{Cys}$ and $tRNA^{Phe}$ were purified from crude tRNA by BD-cellulose–Sepharose 4B and RPC5 column chromatography [2], $tRNA^{Val}$, was purified by BD-cellulose and Sephadex A-50 column chromatography [3] from partly purified tRNA. Elongation factor Tu : GDP was the generous gift from Dr D. L. Miller, Roche Institute of Molecular Biology, NJ. Crude synthetase was prepared as in [2].

For aminoacylation tRNA (20–80 μM) was incubated at 37°C for 20 min with 10-times excess of the corresponding ^{14}C-labelled amino-acid (spec. act. 1 mCi/mmol) in the following charging mixture: 50 mM Hepes (pH 7.5), 10 mM KCl, 10 mM $MgCl_2$, 2 mM ATP, 0.4 mM CTP, 6 mM β-mercaptoethanol and the amount of crude synthetase required to give maximal charging within 20 min for this specific tRNA. The reaction was stopped by making it 0.2 M sodium acetate (pH 4.5) and the mixture was extracted with an equal volume of water-saturated phenol. The aqueous phase was applied to a Sephadex G-25 column (0.6 × 20 cm) and eluted with 5 mM sodium acetate (pH 4.5). The [^{14}C]aminoacyl-tRNA eluting in the exclusion volume was collected and concentrated by ethanol precipitation (−20°C, overnight).

The extent of charging was for $tRNA^{Arg}$ 1210 pmol/A_{260}, $tRNA^{Cys}$ 1100 pmol/A_{260}, $tRNA_m^{Met}$ 1210 pmol/A_{260}, $tRNA^{Phe}$ 1300 pmol/A_{260}, $tRNA_3^{Ser}$ 1180 pmol/A_{260} and $tRNA^{Val}$ 1350 pmol/A_{260}.

The ternary complex EF-Tu:GTP:aminoacyl-tRNA was typically prepared as follows [4]: 7.5 nmol EF-Tu:GTP was incubated at 37°C with 180 nmol GTP, 1.4 μmol phosphoenolpyruvate and 20 μg pyruvate kinase in 190 μl of buffer A: 10 mM Hepes (pH 7.0), 50 mM KCl, 50 mM NH_4Cl and 5 mM $MgCl_2$, after 15 min 5 nmol aminoacyl-tRNA was

added and the incubation continued for 2 min, the mixture was cooled on ice and appropriate aliquots were used for equilibrium dialysis.

2.2. *Oligonucleotide synthesis and equilibrium dialysis*

The synthesis of trinucleoside diphosphates from nucleoside diphosphate (^{3}H, 5–20 Ci/mol) and dinucleoside monophosphates with primer-dependent polynucleotide phosphorylase (*Micrococcus luteus*; EC 2.7.7.8) as well as their characterization was done [5].

The equilibrium dialysis experiments were done as in [5,6] with the following alterations:

(i) Membrane spectrapor 2 (mol. wt cutoff 12 000–14 000; Spectrum Medical Indust., Los Angeles);
(ii) Buffer, 10 mM Hepes (pH 7.0), 50 mM KCl, 50 mM NH_4Cl, 5 mM $MgCl_2$;
(iii) Time and temperature: 24 h at 0°C.

One compartment of the dialysis chamber was filled with 40 μl sample (tRNA, aa-tRNA, aa-tRNA:EF-Tu:GTP; 20–40 μM) and the other with 40 μl oligonucleotide solution (0.2–2 nM).

To determine the association constant (K_a) for the trinucleoside diphosphates (triplets) 30 μl of each compartment were withdrawn and applied to a glass fibre filter (Schleicher and Schüll, Einbeck) dried and counted in toluene 10.4%/PPO/0.02% POPOP scintillation cocktail for 20 min in a Beckman LS7000 Liquid Scintillation Counter. For the determination of the association constant the following equation was used: $(R-1)/[S]$, where R is the ratio of oligonucleotide in the chamber with the macromolecule to that without [7]. $[S]$ corresponds to molar concentration of the sample (macromolecule) to which no triplet has bound. The maximum possible error in determining the K_a is <20% [5]. Each K_a-value represents the average of 4 different equilibrium dialysis experiments. K_a Values <500 l/mol are assumed to indicate non-binding of the oligonucleotide [6,8].

The extent of deacylation for the aa-tRNAs during the 0°C incubation was calculated from cpm due to the ^{14}C radioactivity of the amino acid in both compartments after the experiment.

3. Results and discussion

Although many investigations have been devoted to the comparison of the structure of aminoacylated and uncharged tRNA, we still have no clear answer to the problem whether the two structures are identical or not. In this study we have measured the binding of trinucleoside diphosphates to the 3′-terminal single stranded sequence of 6 tRNAs (tables 1,2). $GpGpN_1$ binds very strongly to the $-NpCpCpA_{OH}$ sequence (when N and N_1 are complementary) probably because of stacking on top of the aminoacyl stem of the tRNA. This binding is not changed upon aminoacylation whereas the binding of UpGpG to the terminal $-CpCpA_{OH}$ sequence is 4 out of 6 cases is significantly weakened. These observations indicate that aminoacylation of tRNA gives rise to a perturbed structure of the 3′-terminal end in which base pairing to the terminal A is less favoured. Likewise, the interaction between aminoacyl-tRNA and EF-Tu:GTP was studied by oligonucleotide binding to the 3′-terminal sequence of tRNA. Aminoacyl-tRNA but not uncharged or *N*-acetylated aminoacyl tRNA binds strongly to EF-Tu:GTP [9], reflecting the function of EF-Tu which promotes binding of aa-tRNA to the ribosome before peptide bond formation.

There are several lines of evidence that the 3′-terminal region of aa-tRNA is in contact with EF-Tu:GTP, for example the free amino group of the aminoacyl residue is crucial for the interaction [9], EF-Tu:GTP protects the –CCA-end against nuclease digestion [10], and in this study we find that although UpGpG binds somewhat better to the ternary complex than to free aa-tRNA (table 1), the binding of GpGpN to complexed aa-tRNA is considerably lowered (table 2). Thus, either EF-Tu:GTP directly inhibits base pairing to the fourth base from the 3′-end or it causes a structural rearrangement of the 3′-end of aa-tRNA, leaving the fourth base less favoured for base pairing. On the other hand, the –CpCpA-sequence is positioned even better for base pairing when aa-tRNA is bound to EF-Tu:GTP (table 1) in agreement with the observation that attachment of a bulky spin label to the 2-position of the penultimate base of yPhe-tRNA does not prevent ternary complex formation [11]. For all tRNAs tested, a clear protection against deaminoacylation by EF-Tu:GTP was observed (table 1) showing that aa-tRNA was bound to EF-Tu:GTP during the experiment [12]. These results suggest that EF-Tu:GTP is in contact with the amino group of the aminoacyl residue and with the backbone of the single-stranded 3′-end of aa-tRNA. That additional contact points

Table 1
Apparent binding constants of UpGpG to the 3′-terminal CCA-sequence of tRNAs

tRNA species	tRNA	aa-tRNA		EF-Tu:GTP:aa-tRNA	
	K_a (M^{-1})	K_a (M^{-1})	% deacylation	K_a (M^{-1})	% deacylation
$tRNA_1^{Arg}$	3340	1740	19	4020	1
$tRNA^{Cys}$	3440	1360	33	3400	2
$tRNA_m^{Met}$	3390	1650	35	2220	2
$tRNA^{Phe}$	3660	4360	22	3030	1
$tRNA_3^{Ser}$	5350	3590	41	5760	2
$tRNA_1^{Val}$	850	930	3	2710	0

are needed for strong binding is suggested by the observations that the 3′-halfmolecule of Val-$tRNA^{Val}$ as well as the denatured form of yLeu-$tRNA^{Leu}$ do not bind to EF-Tu:GTP [13]. Although it was shown [2] and I. Weygand-Durasević, T. A. K., B. F. C. C., unpublished) in spin-labelling and oligonucleotide binding experiments (not shown) that EF-Tu:GTP induces a structural change of the anticodon loop of aa-tRNA, EF-Tu:GTP does not interact directly with the loop [2,13] and I. Weygand-Durasević, T. A. K., B. F. C. C., unpublished). Thus, it is probable that besides binding the aminoacyl residue and the backbone of the single-stranded 3′-end of aa-tRNA, EF-Tu:GTP interacts with other parts such as double-stranded regions in the core of the tRNA-molecule. Further experiments are needed to clarify this point.

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Table 2
Apparent binding constants of oligonucleotide to the sequence $-NpCpCpA_{OH}$ of tRNAs

tRNA species	Oligonucleotide used	tRNA K_a (M^{-1})	aa-tRNA K_a (M^{-1})	EF-Tu:GTP:aa-tRNA K_a (M^{-1})
$tRNA_1^{Arg}$	GpGpU	215 000	254 000	53 000
$tRNA^{Cys}$	GpGpA	306 000	244 000	196 000
$tRNA_m^{Met}$	GpGpU	60 400	90 000	54 500
$tRNA^{Phe}$	GpGpU	87 400	88 500	36 400
$tRNA_3^{Ser}$	GpGpC	551 800	493 000	142 000
$tRNA_1^{Val}$	GpGpU	196 000	144 000	8600

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