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Alterations at the 3′-CCA end of *Escherichia coli* and *Thermus thermophilus* $tRNA^{Phe}$ do not abolish their acceptor activity

N.A. Moor[a], M.N. Repkova, V.I. Yamkovoy[b], O.I. Lavrik[a,*]

[a]*Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, Prospekt Lavrentiev 8, 630090, Novosibirsk, Russian Federation*

[b]*Novosibirsk State University, 630090, Novosibirsk, Russian Federation*

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Abstract The 3′-CCA end of $tRNA^{Phe}$ from *Escherichia coli* and *Thermus thermophilus* was changed to AAA, CCC, UUU and UUA by the stepwise degradation procedure of the 3′-CCA end of $tRNA^{Phe}$ followed by the ligation with oligoribotrinucleotides. Substrate activity of $tRNA^{Phe}_{UUA}$ and $tRNA^{Phe}_{CCC}$ in tRNA aminoacylation was shown. $tRNA^{Phe}_{AAA}$ is a bad substrate for *E. coli* and *Th. thermophilus* phenylalanyl-tRNA synthetases. $tRNA^{Phe}_{UUU}$ has no detectable activity in tRNA aminoacylation. Therefore the nature of the 3′-end of $tRNA^{Phe}$ plays an important role in tRNA binding and its substrate efficiency. Nevertheless the CCA sequence at the 3′-end of $tRNA^{Phe}$ does not seem to be an absolute requirement for tRNA aminoacylation.

Key words: tRNA recognition; 3′-CCA end; Mechanism of tRNA aminoacylation

1. Introduction

The role of the 3′-terminal CCA sequence, common to all tRNAs in the interaction of tRNAs with their specific aminoacyl-tRNA synthetases, was investigated earlier [1]. The data confirm that the completeness of the CCA-end is an absolute requirement for aminoacylation of tRNA. tRNAs of different species shortened by one and more 3′-terminal nucleotides were shown to lose their acceptor activity.

As to the identity of nucleotides 76, 75 and 74 and their importance for substrate efficiency of tRNA some limited and contradictory data have been published ([1] and refs. therein). The conservative replacement of cytidines by uridines did not deactivate *E. coli* $tRNA^{Tyr}$ and $tRNA^{Arg}$ but completely abolished aminoacylation activity of *E. coli* $tRNA^{fMet}$. Yeast $tRNA^{Phe}$ with a 3′-CCC end could not be aminoacylated and there was no additional information on the interaction of this tRNA with the enzyme [1].

During the last decade this part of the tRNA molecule has not been a subject of the intensive study. The high-resolution crystal structures of two complexes, the *E. coli* glutaminyl system and the yeast aspartyl system, are now known [2]. A common feature of both complexes is that the acceptor stem end of tRNAs undergoes large conformational changes. Recently natural mutants of $tRNA^{His}$ ($tRNA^{His}_{UCA}$) from *Salmonella typhimurium* and $tRNA^{Val}_1$ ($tRNA^{Val}_{GCA}$, $tRNA^{Val}_{ACA}$) from *E. coli* have been isolated and their functional activity in the translation system has been shown [3]. Therefore tRNAs with mutations at the CCA-end exist in vivo.

In the present work we have investigated in detail the contribution of the 3′-CCA end to the specific interaction of *E. coli* and *Th. thermophilus* $tRNA^{Phe}$s with their homologous phenylalanyl-tRNA synthetases. The mutations were introduced into the 3′-CCA end and kinetic parameters of $tRNA^{Phe}$ mutants (K_m, V_{max} and their ratio) were evaluated in respect to tRNA aminoacylation.

2. Materials and methods

E. coli alkaline phosphatase was purchased from 'Fermentas' (Lithuania). *E. coli* $tRNA^{Phe}$ was from Boehringer Mannheim. DTT was from Fluka, sodium periodate was from Sigma, HEPES, Tris, ATP, DS-Na, acrylamide and *N,N′*-methylenbisacrylamide were from Serva. Other reagents were of analytical grade. L-[^{14}C]Phenylalanine (360 mCi/mmol) was from UVVR, L-phenyl-[2,3-^{3}H]alanine (25,000 mCi/nmol) was from Isotop, St. Petersburg.

$tRNA^{Phe}$ from *Th. thermophilus* was purified as described earlier [4]. FRS from *E. coli* and *Th. thermophilus* were purified [5,6]. Oligoribonucleotides pCpCpA and pUpUpA were synthesized using the H-phosphonate procedure [7] followed by phosphorylation [8]. Oligoribonucleotides $(pA)_3$, $(pC)_3$ and $(pU)_3$ were prepared by hydrolysis of the corresponding polyribonucleotides by a snake venom phosphodiesterase according to the procedure of Mudrakovskaya and Yamkovoy [9]. T4 RNA ligase was purified from *E. coli* B infected by bacteriophage T4 amN82 according to the procedure described earlier [10].

The stepwise degradation of $tRNA^{Phe}$ from the 3′-end was performed [11]. tRNA was isolated after every cycle by chromatography on DEAE-cellulose [12]. The attachment of trinucleotides: pCpCpA, pUpUpA, $(pU)_3$, $(pC)_3$, $(pA)_3$, to $tRNA^{Phe}$ shortened by the 3′-CCA end was performed with T4 RNA ligase according to the procedure described in [11] with some modifications. The products were separated by electrophoresis on a polyacrylamide gel in the presence of urea. $tRNA^{Phe}$ was isolated from the gel by 0.1% DS-Na in 0.3 M sodium acetate. Acceptor activity of $tRNA^{Phe}$ ligated with CCA was determined to be equal to 1600–1700 pmol/A_{260}. This sample was used as a control for various $tRNA^{Phe}$ mutants. $tRNA^{Phe}$ aminoacylation was carried out as described earlier [13]. K_m and V_{max} values were evaluated by the Enz Fitter program. The standard errors were within 10% of the indicated values.

3. Results and discussion

The essential steps for the preparation of a $tRNA^{Phe}$ modified at the 3′-CCA-end were the removal of three nucleotides and ligation of the shortened $tRNA^{Phe}$ with trinucleotides. *Th. thermophilus* and *E. coli* $tRNA^{Phe}$ were shortened through three cycles, each consisting of the oxidation of the 3′-end ribose by periodate, the cleavage of the oxidized nucleoside in an aniline-

*Corresponding author. Fax: (7) (38) 3235 1665.

Abbreviations: FRS, phenylalanyl-tRNA synthetase (E.C. 6.1.1.20.); $tRNA^{Phe}$, transfer ribonucleic acid specific to phenylalanine; DTT, dithiothreithol; DS-Na, sodium dodecyl-sulfate.

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Table 1
Aminoacylation properties of tRNA^{Phe}s modified at the 3′-CCA-terminus

tRNA	K_m (μM)	V^*_{max} (rel.)	V_{max}/K^*_m (rel.)
	E. coli		
tRNA-CCA	0.60	1.0	1.0
tRNA-UUA	1.3	0.25	0.12
tRNA-CCC	1.6	0.015	0.0056
tRNA-AAA			< 0.0001
tRNA-UUU	not aminoacylable		
	Th. thermophilus		
tRNA-CCA	0.30	1.0	1.0
tRNA-UUA	0.98	0.40	0.12
tRNA-CCC	1.5	0.035	0.0070
tRNA-AAA			< 0.0001
tRNA-UUU	not aminoacylable		

*V_{max} and V_{max}/K_m are normalized to the control tRNA which was restored by ligation of pCCA to tRNA^{Phe}, shortened stepwise by three nucleotides.

catalyzed reaction, and finally, the removal of the 3′-phosphate. tRNA was isolated after every cycle by chromatography on DEAE-cellulose to separate the product of incomplete dephosphorylation. The attachment of trinucleotide (pCpCpA, pUpUpA, $(pU)_3$, $(pC)_3$, $(pA)_3$) to shortened tRNA^{Phe} was performed with T4 RNA ligase. tRNA of the native length was isolated electrophoretically on a denaturating 8% polyacrylamide gel.

K_m, V_{max} values and their ratio obtained for modified tRNA^{Phe} in tRNA aminoacylation catalyzed with *Th. thermophilus* and *E. coli* FRS are presented in Table 1. tRNAs with a conservative change of cytidines 75 and 74 to uridines are substrates for both *E. coli* and *Th. thermophilus* FRS. They exhibit 2- to 3-fold increase in K_m value and 2.5 to 4-fold decrease in V_{max} resulting in one order of magnitude decrease in the V_{max}/K_m ratio as compared with the native tRNA. tRNAs having adenosines instead of cytidines are very poor if any substrates for both enzymes. When the terminal nucleotide in tRNA with the 3′-UUA end was changed to uridine, no detectable tRNA aminoacylation was observed for both *E. coli* and *Th. thermophilus* FRS. When the adenosine 76 was changed on cytidine, $tRNA^{Phe}_{CCC}$ exhibits the substrate activity in both systems. However $tRNA^{Phe}_{CCC}$ demonstrates the significant change in parameters (K_m and V_{max} (Table 1) with predominant influence on the catalytic rate of tRNA aminoacylation.

Earlier we studied the effect of the acceptor end change of *E. coli* tRNA^{Phe} on its interaction with FRS [14]. The removal of the 3′-terminal adenosine decreased the tRNA affinity to the enzyme by one order of magnitude. A further stepwise degradation of tRNA^{Phe} did not result in an additional decrease in its affinity. Therefore the 3′-terminal adenosine provides the crucial contribution to the tRNA binding to the enzyme. The replacement of A76 by 1,N^6-ethenoadenosine abolished the substrate activity and decreased the affinity 4-fold as compared to the native tRNA [14]. Yeast tRNA^{Phe} modified in such a way has been shown to be unchargeable [11]. The data suggest a significant influence on tRNA^{Phe} substrate activity by modification of the 3′-terminal adenosine of tRNA^{Phe}.

Nevertheless $tRNA^{Phe}_{CCC}$ and $tRNA^{Phe}_{UUA}$ exhibit substrate activity in tRNA aminoacylation catalyzed by FRS from *E. coli* or *Thermus thermophilus*. Therefore the 3′-CCA end sequence of tRNA^{Phe} has no unique requirement for tRNA aminoacylation and tRNA^{Phe}s with a modified 3′-end are substrates for phenylalanyl-tRNA synthetases.

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