Volume 238, number 1, 211–213 FEB 06349 September 1988

$tRNA^{Phe}$ deprived of 3′-terminal adenosyl residue does not stimulate adenosine aminoacylation catalyzed by phenylalanyl-tRNA synthetase from *Escherichia coli*

A.S. Boutorin, V.A. Gordienko, O.I. Lavrik and N.A. Moor

Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Novosibirsk 630090, USSR

Received 11 July 1988

Phenylalanyl-tRNA synthetase from *Escherichia coli* does not catalyze the [^{14}C]phenylalanyl residue transfer from phenylalanyl-adenylate to adenosine either in the presence or absence of homologous $tRNA^{Phe}$ and $tRNA^{Phe}_{-A}$. When the reaction mixture contained dithiothreitol, radioactive substance was detected having a mobility on HPLC column close to that of aminoacyladenosine. The amount of this product depended on the concentration of dithiothreitol in the mixture. Phenylalanyl residue was suggested to undergo transfer from aminoacyladenylate to dithiothreitol molecule.

Phenylalanyl-tRNA synthetase; Aminoacyladenosine; Aminoacylation; Dithiothreitol; tRNA

1. INTRODUCTION

Remy and his group [1,2] showed that phenylalanyl-tRNA synthetase from yeast catalyzed transfer of the aminoacyl residue from phenylalanyladenylate to Ado or CCA in case $tRNA^{Phe}$ deprived of one or several 3′-terminal nucleotides was present in the reaction mixture. $tRNA^{Phe}_{-A}$ was proposed to induce the formation of 'catalytically active' conformation of the enzyme active site, providing its binding with the substrates and subsequent aminoacylation of Ado.

These results opened an attractive possibility to evaluate the contribution of various tRNA molecule regions in the conformational adaptation of the aminoacyl-tRNA synthetases active sites. We tried to apply this test to the studies of aminoacylation mechanisms in the *Escherichia coli* system. However, using microcolumn HPLC we have demonstrated that in the presence of $tRNA^{Phe}_{-A}$ PRS from *E. coli* does not catalyze aminoacylation of the adenosine added.

At the same time, we observed the formation of [^{14}C]phenylalanine-labelled products with chromatographic mobility close to that of phenylalanyl-adenosine. The amount of these products in the reaction mixture was proportional to the concentration of DTT.

Correspondence address: A.S. Boutorin, Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Lavrentiev prospekt 8, Novosibirsk 630090, USSR

Abbreviations: Ado, adenosine; DTT, dithiothreitol; $tRNA^{Phe}$, phenylalanyl-specific tRNA; $tRNA^{Phe}_{-A}$, $tRNA^{Phe}$ deprived of 3′-terminal adenosyl residue; PRS, phenylalanyl-tRNA synthetase

2. MATERIALS AND METHODS

Homogeneous PRS from *E. coli* MRE-600 was isolated and its homogeneity and functional activity were tested as described [3]. PRS from yeast was kindly provided by Dr P. Remy; $tRNA^{Phe}$, by Dr B. Lorber. $tRNA^{Phe}$ from *E. coli* was purchased from Boehringer Mannheim; Nucleosil C18 was from Machery Nagel; [^{14}C]phenylalanine was from UVVVR; ATP was from Reanal (analyzed for homogeneity by microcolumn HPLC); Ado and AMP were from Reanal; puromycin was from Serva; DTT was from Fluka. Phenylalaninyladenylate was synthesized as described [4]. The removal of the 3′-adenosyl residue was performed as described [5].

Hydrolysis of [^{14}C]phenylalanyl-tRNA with the pancreatic ribonuclease (from Sigma) was carried out at 37°C for 2 h. The

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

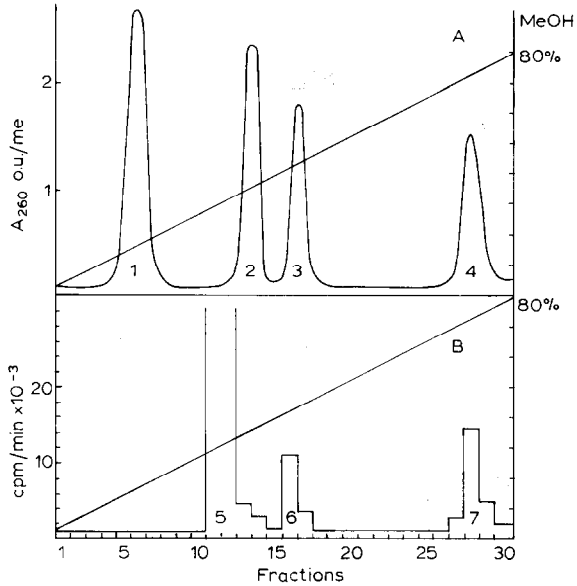

Fig.1. Microcolumn chromatography of the model compounds (A) and reaction mixture (B) on the Nucleosil C-18 column in methanol gradient 4–80% (see section 2). (A) 1, ATP; 2, Ado; 3, phenylalaninyladenylate; 4, puromycin; (B) 5, [^{14}C]phenylalanine; 6, [^{14}C]phenylalanyladenylate; 7, [^{14}C]phenylalanyladenosine (from ribonuclease hydrolysate of [^{14}C]phenylalanyl-tRNA).

enzyme concentration was 100 μg/ml. RNase was removed by the phenol extraction.

Aminoacylation reaction mixtures composition is shown in the tables. Each mixture (50 μl) was incubated for 20 min at 25°C (in the case of *E. coli* enzyme) or at 37°C (in the case of yeast). The reaction was stopped by 5% CH_3COONa, pH 3.5, and the enzyme precipitated by centrifugation. Then the mixture was applied to a Nucleosil C-18 column (2 × 60 mm, 150 μl) preliminarily washed with 0.05 M $LiClO_4$. Chromatography was carried out on the Millichrom chromatograph (Orel, USSR) in methanol gradient from 4 to 80%, the volume of gradient being 1400 μl. 50 μl fractions were applied to 2 × 2 cm Whatman 3 MM paper stripes; the latter were dried and their radioactivity measured on the Minibeta liquid scintillation counter (LKB) in toluene scintillator. The reaction mixture components, products and model compounds were identified both by optical absorbance at 260 nm and by radioactivity.

3. RESULTS AND DISCUSSION

In previous work [1,2], separation of the components from the reaction mixture by paper electrophoresis with subsequent autoradiography was used to test [^{14}C]phenylalanyladenosine. As this method does not seem to be quite suitable for quantitative determination of the products, in the present work microcolumn HPLC was used [6].

Microcolumn chromatographic separation of ATP, adenosine, phenylalaninyladenylate, puromycin and [^{14}C]phenylalanyladenosine is shown in fig.1. A distinct separation of [^{14}C]phenylalanine, [^{14}C]phenylalanyladenosine and [^{14}C]phenylalanyladenylate was evidently achieved.

Puromycin was used as an analogue of aminoacyladenosine in order to mark more exactly its elution point. Our experiments showed that electrophoretic mobilities of both substances in the system described in [1] were identical. Phenylalaninyladenylate served as a marker of the elution point of [^{14}C]phenylalanyladenylate.

Table 1

Radioactivity (cpm) in peak 7 (see fig.1) after chromatographic separation of complete and incomplete aminoacylation mixtures containing PRS from *E. coli* (average values from a series of experiments)

Number of system	Components and concentration (M)						Radioactivity (cpm)
	ATP (10^{-2})	Ado (5×10^{-3})	$tRNA_{-A}$ (8×10^{-6})	tRNA (8×10^{-6})	DTT	PRS (10^{-6})	
1	–	–	–	–	10^{-2}	–	bg
2	–	–	–	–	10^{-2}	+	bg
3	+	–	–	–	–	+	bg
4	+	+	–	–	–	+	bg
5	+	+	+	–	–	+	bg
6	+	+	–	+	–	+	bg
7	+	–	–	–	8×10^{-2}	+	300000

The mixtures contained 6×10^{-5} M [^{14}C]phenylalanine, 2×10^{-2} M $MgCl_2$, 0.1 M Tris-HCl, pH 7.5, 0.03 M KCl, and the components indicated. bg, background, no more than 150 cpm

This chromatographic system was used for separation of the reaction mixtures shown in table 1; radioactivity of the fractions collected at the elution point of phenylalanyladenosine was measured. As is seen from table 1, the radioactivity was detected neither in the presence nor in the absence of $tRNA_{-A}$ or native tRNA at the elution point of aminoacyladenosine. It must be noted that according to SDS electrophoresis data and all methods used for activity testing, *E. coli* phenylalanyl-tRNA synthetase was completely homogeneous and entirely active [3]. In control experiments we compared aminoacylation kinetics of $tRNA^{Phe}$ from *E. coli* and yeast in the presence and absence of DTT (data not shown). The kinetic curves were identical, showing that DTT had no effect on the aminoacylation reaction. The same was shown by Murayama et al. [7] for 2-mercaptoethanol in a homologous yeast system. The radioactive product obtained could be neither aminoacyladenosine nor tRNA derivative because of the absence of both the components in the reaction mixture. The same product was detected in the yeast aminoacylation mixture containing ATP and phenylalanyl-tRNA synthetase without tRNA and Ado. The latter effect was observed to be highly dependent on DTT concentration (table 2). The product was stable in the acidic conditions (pH 3.5) and degraded in the alkaline conditions. The transfer of the activated [^{14}C]phenylalanyl residue from aminoacyladenylate to DTT which is present in the reaction mixture seems to be the most probable interpretation of these results. Aminoacylation of nucleophilic reagents other than tRNA was described [7–11].

Thus, the data obtained showed that phenylalanyl-tRNA synthetase from *E. coli* catalyzes aminoacylation of adenosine neither in the presence of $tRNA^{Phe}_{-A}$ nor in the presence of $tRNA^{Phe}$.

Table 2

Formation of radioactive derivative in the incomplete aminoacylation yeast system

DTT concentration (M)	Radioactivity in peak 7 (see fig.1) (cpm)
0	bg
10^{-2}	1300
3×10^{-2}	6500
8×10^{-2}	40000

The reaction mixture contained 6×10^{-5} M [^{14}C]phenylalanine, 2×10^{-2} M $MgCl_2$, 0.1 M Tris-HCl, pH 7.5, 0.03 M KCl, 10^{-2} M ATP, 10^{-6} M PRS, 2.5% sucrose

Acknowledgement: The authors are indebted to Dr S.K. Vassilenko for helpful discussion of the results.

REFERENCES

[1] Renaud, M., Bacha, H., Remy, P. and Ebel, J.-P. (1981) Proc. Natl. Acad. Sci. USA 78, 1606–1608.
[2] Bacha, H., Renaud, M., Lefevre, J.-F. and Remy, P. (1982) Eur. J. Biochem. 127, 87–95.
[3] Ankilova, V.N., Lavrik, O.I. and Khodyreva, S.N. (1984) Prikladn. Biokhim. Mikrobiol. 20, 208–216.
[4] Cassio, D., Lemoine, F., Waller, J.-P., Sandrin, E. and Boissonnas, R.A. (1967) Biochemistry 6, 827–835.
[5] Paulsen, H. and Wintermeyer, W. (1984) Eur. J. Biochem. 138, 117–123.
[6] Baram, G.I., Grachev, M.A., Komarova, N.I., Perelroyzen, M.P., Bolvanov, Yu.A., Kuzmin, S.V., Kargaltsev, V.V. and Kuper, E.A. (1983) J. Chromatogr. 264, 69–90.
[7] MurayAma, A., Raffin, J.P., Remy, P. and Ebel, J.-P. (1975) FEBS Lett. 53, 15–22.
[8] Andrews, D., Trezequet, V., Merle, M., Graves, P.-V., Muench, K.M. and Labouesse, B. (1985) Eur. J. Biochem. 146, 201–209.
[9] Kern, D., Lorber, B., Boulanger, Y. and Giégé, R. (1985) Biochemistry 24, 1321–1322.
[10] Loftfield, R.B. and Eigner, E.A. (1968) Biochemistry 7, 1100–1105.
[11] Nakajima, H., Kitabake, S., Tsurutani, R., Tomioka, I., Yamamoto, K. and Imaheri, K. (1984) Biochim. Biophys. Acta 790, 197–199.