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Rabbit translation elongation factor 1α stimulates the activity of homologous aminoacyl-tRNA synthetase

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Abstract **Functional and structural sequestration of aminoacyl-tRNA has been recently found in eukaryotic cells and the aminoacyl-tRNA channeling has been suggested [B.S. Negrutskii et al., Proc. Natl. Acad. Sci. 91 (1994) 964–968], but molecular details and mechanism of the process remained unclear. In this paper we have verified a possible interaction between rabbit aminoacyl-tRNA synthetase and homologous translation elongation factor 1α (EF-1α), the proteins which may play a role of sequential components involved into the transfer of the aminoacyl-tRNA along the protein synthetic metabolic chain. The stimulation of the phenylalanyl-tRNA synthetase activity by EF-1α is found. The effect is shown to be specific towards the origin of tRNA and elongation factor molecules. The data obtained favor the direct transfer mechanism of the aminoacyl-tRNA channeling process during eukaryotic protein synthesis.**

Key words: Aminoacyl-tRNA synthetase; Elongation factor; Protein–protein interaction; Channeling; Rabbit liver

1. Introduction

High efficiency of the protein biosynthesis occurring in a relatively large volume of eukaryotic cells suggests some sort of compartmentalization of the translation apparatus. However, apart from well-documented association of different translation components with each other and with cellular structures [1–6], little is known about its functional importance for the polypeptide synthesis.

One of the mechanisms to realize potential advantages of the compartmentalization might be channeling, or direct transfer of aminoacyl-tRNA from aminoacyl-tRNA synthetase to elongation factor, and to ribosome without dissociation into the surrounding medium [1,7–10]. Indeed, functional and structural sequestration of endogenous aminoacyl-tRNA has recently been found in eukaryotic cells and aminoacyl-tRNA channeling in the course of protein synthesis has been suggested [8,11,12]. Molecular details and mechanism of the process remained unknown. It has been hypothesized that aminoacyl-tRNA synthetase and EF-1α may form a complex with subsequent direct transfer of aminoacyl-tRNA from aminoacyl-tRNA synthetase to EF-1α [8].

In this paper we verify a possible interaction between aminoacyl-tRNA synthetase and EF-1α by studying the effect of the latter on the activity of PheRS. A noticeable stimulation of rabbit liver PheRS catalytic activity is observed in the presence of homologous EF-1α. The effect is shown to depend on the origin of tRNA and elongation factor molecules. The data obtained favor the direct interaction between the enzyme and EF-1α.

2. Experimental

^{14}C-labeled phenylalanine and [^{3}H]GDP were purchased from Amersham. Total yeast tRNA, bovine serum albumin, poly(U), ATP and GTP were from Sigma. Sephacryl S-400, SP-sepharose, Q-sepharose were purchased from Pharmacia. Phosphocellulose and DEAE-cellulose were from Whatman. Rabbit liver tRNA was prepared as described [13]. Bacterial elongation factor Tu was a gift from Dr. I. Rublevskaya (this laboratory). All other chemicals were reagent grade.

PheRS was isolated as described earlier [14]. Briefly, homogenate of 6 rabbit livers in 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 6 mM DTT, 2 mM PMSF, 10% glycerol was centrifuged at 10000×*g*. Solution of polyethylene glycol 6000 was added dropwise (final concentration 3%) to postmitochondrial supernatant to precipitate polyribosome fraction. The precipitate was dissolved in 50 mM Tris-HCl, pH 7.5 containing 5 mM $MgCl_2$, 35 mM KCl, 10% glycerol, 1 mM DTT, 0.1 mM EDTA. 4 M KCl solution was added to obtain 0.4 M final concentration of the salt. Polyribosomes were pelleted at 105000×*g* for 2 h. Supernatant was dialyzed overnight (50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10% glycerol, 1 mM DTT) and applied to the DEAE-cellulose column equilibrated with the same buffer. Proteins were eluted by linear (0.035–0.5 M) gradient of KCl concentration in the same buffer. Activity of PheRS in [^{14}C]phenylalanyl-tRNA formation was determined, the most active fractions were combined, diluted five-fold with 50 mM K-phosphate buffer, pH 6.8 and applied to phosphocellulose column equilibrated with the same buffer. The elution was carried out by 0.25–0.5 M gradient of K-phosphate buffer, pH 6.8. The purest fractions were combined, dialyzed overnight in 50 mM HEPES, pH 7.5 containing 20% glycerol and stored in liquid nitrogen. SDS-polyacrylamide gel electrophoresis showed 90% purity of the enzyme.

Activity of PheRS was determined in 50 μl reaction mixture containing 80 mM Tris-HCl, pH 7.8, 100 mM KCl, 10 mM $MgCl_2$, 3 mM ATP, 130 μg total rabbit liver tRNA, 60 μM [^{14}C]phenylalanine and different amounts of PheRS. The mixture was incubated 3 min, unless otherwise indicated, at 35°C and the reaction was stopped with ice-cold 10% TCA. Precipitates were collected on GF/C filters (Whatman). Radioactivity of dried filters was counted using toluene-based scintillation fluid (counting efficiency 80%).

EF-1α was isolated using combination of gel-filtration and ion-exchange chromatographies. Rabbit liver was homogenized in 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 15% glycerol, 2 mM 2-mercaptoethanol, 1 mM PMSF. After 40 min centrifugation at 10000×*g* postmitochondrial supernatant (25 ml) was loaded on Sephacryl S-400 column (2.6×95 cm) equilibrated with 25 mM KH_2PO_4, pH 7.5, 1 mM $MgCl_2$, 15% glycerol, 2 mM 2-mercaptoethanol, 0.1 mM PMSF. [^{3}H]GDP binding activity of EF-1α was tested in all fractions according to [15]. The most active fractions were combined and loaded on Q-sepharose column (2.8×16 cm) equilibrated with the same buffer. Flow-through protein fraction was applied to SP-sepharose column (0.8×3.5 cm) and washed with the same buffer. Elution

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Abbreviations: EF-1α, translation elongation factor 1α; PheRS, phenylalanyl-tRNA synthetase (EC 6.1.1.20); TCA, trichloroacetic acid; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride

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was carried out by 50–500 mM KCl gradient in the same buffer. Purity of EF-1α preparation was about 95% as judged from the SDS-polyacrylamide gel electrophoresis. The factor was highly active in the [^{3}H]GDP-binding assay, the stimulation of [^{14}C]phenylalanyl-tRNA binding to poly(U)-programmed 80S ribosomes and the stimulation of poly[^{14}C]phenylalanine synthesis on poly(U)-programmed 80S ribosomes. The activity of EF-1α preparation in [^{14}C]phenylalanyl-tRNA synthesis was negligible.

To analyze EF-1α-nucleotide complex by HPLC 300 pmoles of EF-1α were loaded on Bakerbond Wide-Pore PEI(WAX), 5 μM column (4.6×250 mm), Baker Research Products. After washing with 0.02 M NaH_2PO_4, pH 2.8 the gradient of 0.02 M NaH_2PO_4, pH 2.8–0.05 M NaH_2PO_4, pH 2.8, 0.6 M $(NH_4)_2SO_4$ was applied and optical density of the eluate was measured at 254 nm wavelength.

3. Results

Since EF-1α may exist in the cell as either GDP- or GTP-bound form, the nature of nucleotide bound was studied using HPLC analysis. The chromatographic behavior of a nucleotide bound to EF-1α was found identical to that of GDP. No other nucleotides including GTP were detected in the EF-1α preparation. Thus, the EF-1α*GDP form was used in further experiments.

Table 1 shows the effect of EF-1α on the catalytic activity of PheRS. About two-fold increase of k_{cat} is revealed in the presence of the factor while the maximum level of tRNA phenylalanylation does not change (Fig. 1). GDP alone in equimolar concentration has no effect on PheRS activity (data not shown). The concentration-dependent effect of EF-1α on PheRS activity is shown in Fig. 2 and the sigmoidal shape of the curve is observed.

To check whether the stimulation depends on the origin of elongation factor the effect of EF-Tu on PheRS activity was tested (Fig. 2). EF-Tu revealed no detectable stimulatory effect in spite of its similar function in protein synthesis. Since stimulation of PheRS activity could be caused by non-specific stabilization rather than specific EF-1α-PheRS interaction the effect of bovine serum albumin on the initial rate of [^{14}C]phenylalanyl-tRNA formation was studied. No effect was found in the presence of non-specific protein (Fig. 2).

The attempt to substitute rabbit liver tRNA for yeast tRNA in the experiments with EF-1α-mediated PheRS stimulation proved to be ineffective. The difference between yeast and rabbit $tRNA^{Phe}$ was studied by comparison of PheRS activity depending on their concentrations (Fig. 3). k_{cat} value of phenylalanyl-tRNA formation was shown to be about the same in the presence of yeast or rabbit tRNA though 3-fold decrease in K_m for rabbit tRNA was observed (Table 1). Addition of EF-1α caused about two-fold increase in k_{cat} and K_m values for rabbit tRNA while having no effect on kinetic parameters for yeast tRNA, suggesting rather specific interactions in the higher eukaryotic enzyme–tRNA–EF-1α system (Table 1).

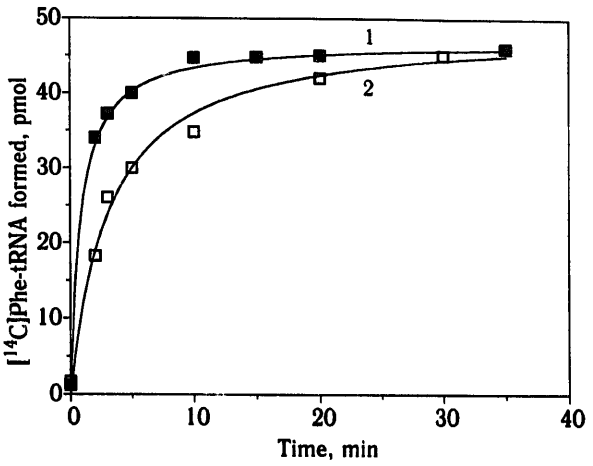

Fig. 1. Time-course of [^{14}C]phenylalanyl-tRNA formation in the presence (1) and absence (2) of EF-1α.

4. Discussion

Recent data strongly suggest channeling of cellular aminoacyl-tRNA in the course of eukaryotic protein synthesis [11,12]. Two mechanisms might be involved in the channeling process: (i) colocalization of translation components in microcompartments which does not require formation of the complex between sequential enzymes, and (ii) direct transfer of aminoacyl-tRNAs from one translational component to another in protein–protein complex formed. Consequently, knowing whether EF-1α can interact with aminoacyl-tRNA synthetase is the principal point for the clarification of aminoacyl-tRNA channeling mechanism in eukaryotic protein synthesis.

Eukaryotic valyl-tRNA synthetase has been shown to form a stable complex with the multi-subunit EF-1 [16], though there have been no such indications for other synthetases so far. Recently the activity of human aspartyl-tRNA synthetase expressed in *E. coli* has been found to be stimulated by incubation in the presence of rabbit EF-1α and GTP in vitro [17]. The question of physical association of the proteins was not addressed experimentally in this work, but transient complex formation was suggested. EF-1α*GTP was shown to influence the rate-limiting step of tRNA aspartylation which was found to be an extremely slow dissociation of aspartyl-tRNA from the synthetase [17]. Since the rate-limiting step of tRNA aminoacylation by eukaryotic PheRS is shown to be a step before phenylalanyl-tRNA dissociation from the enzyme [18,19], the mechanism of EF-1α-induced stimulation appears different for these two enzymes. Moreover, opposite to aspartyl-tRNA synthetase PheRS stimulation by EF-1α does not require GTP. In fact, it is EF-1α*GDP complex which affects

Table 1
Effect of EF-1α on kinetic parameters of tRNA aminoacylation by PheRS

Source of tRNA	Addition of EF-1α	k_{cat} (s^{-1})	K_m (μM)
Yeast	minus	0.15	1.5
Yeast	plus	0.15	1.5
Rabbit	minus	0.22	0.5
Rabbit	plus	0.42	1.2

Kinetic parameters for aminoacylation reaction were calculated at 1.5 min time point. Amount of $tRNA^{Phe}$ in total tRNA preparation is determined to be 3%.

PheRS activity. Since EF-1α*GDP is not considered to bind aminoacyl-tRNA [20], the stimulation of PheRS activity by the factor occurs most likely through direct protein–protein interaction. The sigmoidal shape of EF-1α concentration-dependent curve, which is typical for allosteric enzyme activation, seems to favor this assumption. The study on detailed mechanism of the stimulation, in particular, direct detection of synthetase*EF-1 complex is in progress.

The question is whether the ability to interact with EF-1α is the property of all aminoacyl-tRNA synthetases. Contrary to higher eukaryotic aspartyl-tRNA synthetase which is known to function as a part of high molecular weight synthetase complex, rabbit PheRS represents the fraction of so-called individual synthetases (4). According to our preliminary results rabbit seryl- and histidyl-tRNA synthetases which are not involved in "core" complex (4) can be activated by EF-1α as well. Altogether these data favor the "all synthetases" model and suggest direct transfer rather than microcompartmentalization mechanism of aminoacyl-tRNA channeling.

On the other hand, it can not be excluded that stimulation of aminoacyl-tRNA synthetases by EF-1α*GDP form may be of regulatory importance controlling the rate of aminoacyl-tRNAs formation and their concentration inside the cell.

A possible interplay of GDP/GTP-bound EF-1α, tRNA, aminoacyl-tRNA and aminoacyl-tRNA synthetases during channeling, involvement of GTP-recycling βγδ subunit complex of EF-1 in the process as well as the entire role of these interactions in the modern scheme of eukaryotic protein synthesis will be the subject of future investigations.

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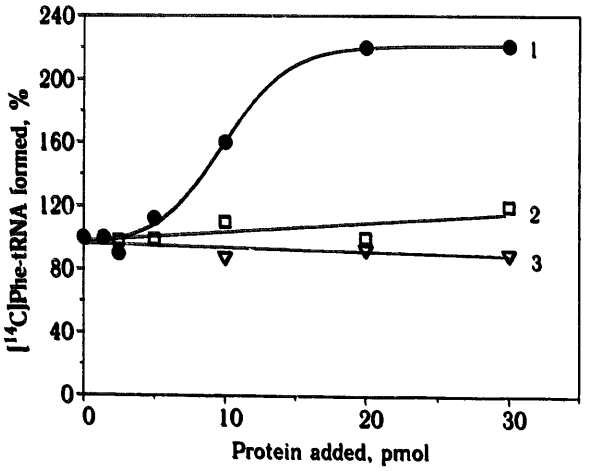

Fig. 2. Effect of EF-1α (1), EF-Tu (2) and bovine serum albumin (3) on the initial rate of [^{14}C]phenylalanyl-tRNA synthesis by 2 pmoles of PheRS. Amount of [^{14}C]phenylalanyl-tRNA (11.4 pmoles) synthesized by PheRS in the absence of EF-1α is considered to be 100%.

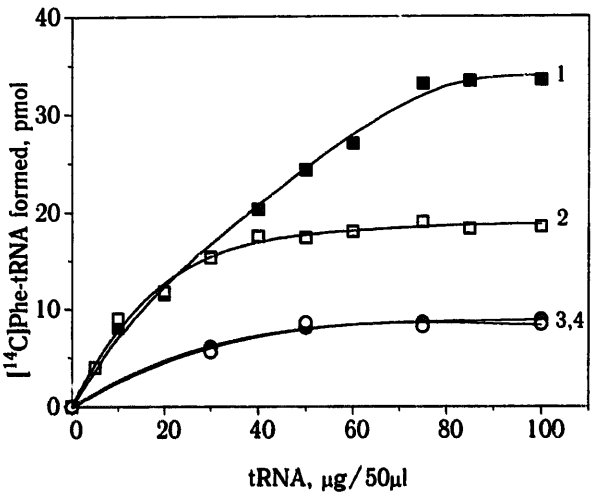

Fig. 3. Effect of rabbit (1,2) and yeast (3,4) tRNAs on the initial rate of [^{14}C]phenylalanyl-tRNA synthesis with (1,3) or without (2,4) EF-1α. 3.6 pmoles of PheRS were incubated with 20 pmoles of EF-1α and indicated amounts of total tRNA in 50 μl aminoacylation mixture.

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