INTERCHANGEABILITY OF ELONGATION FACTOR-Tu AND ELONGATION FACTOR-1 IN AMINOACYL-tRNA BINDING TO 70 S AND 80 S RIBOSOMES

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1. Introduction

Recent observations made in this laboratory [1] have indicated that peptide chain elongation factor 1 (EF-1) from ascites tumor cells functions differently from its bacterial counterpart elongation factor-Tu (EF-Tu). While the latter enzyme forms a ternary complex with aminoacyl-tRNA and GTP and leaves the ribosomal receptor site after correct positioning of its substrate and after GTP hydrolysis, EF-1 functions in situ; after being bound to the post initiation complex it does not leave the ribosome until termination of peptide chain elongation. In view of these apparent differences it appeared interesting to study the action of the two factors with heterologous ribosomes.

2. Material and methods

The reagents used in this study came from the same sources as stated previously [2]. The preparation of *Escherichia coli* elongation factors followed instructions given by Arai et al. [3]. Labeling of EF-Tu by reductive alkylation was performed as described by Kleinert and Richter [4]. *E. coli* ribosomes were prepared and washed four times with 0.5 M NH_4Cl as described previously [5]. Washed ribosomes and aggregated and monomeric forms of EF-1 in the labeled and unlabeled form from ascites tumor cells were prepared as published elsewhere [2]. Similarly, the charging of crude, unfractionated *E. coli* tRNA with [^{14}C]phenylalanine (1160 dpm/pmol) followed published procedures [6]. The proportions of tRNA remaining uncharged varied in each preparation. Therefore, the amount of deacylated tRNA introduced into the assays with a given amount of [^{14}C]Phe-tRNA was mentioned in each series of experiments. The enzymatic binding of Phe-tRNA to ribosomes was stopped by the addition of ice cold dilution buffer (30 mM Tris–HCl, pH 7.5, at 20°C, 6 mM magnesium acetate and concentrations of NH_4Cl or KCl as indicated in the legends) followed by filtration through Sartorius nitrocellulose membrane filters. Polymerized phenylalanine was precipitated by 5% trichloroacetic acid and heated at 90°C for 15 min. After cooling at 0°C for 10 min, the reaction mixtures were filtered through Sartorius nitrocellulose filters and washed with 80% (v/v) ethanol in water. The filters were dried, introduced into plastic vials containing 5 ml butyl-PBD (Ciba) and counted in a Packard liquid scintillation counter, model 3385. Gel electrophoresis of the purified elongation factors followed published procedures [2].

The capacity of ribosomes to bind labeled elongation factors was measured by Bio-Gel A 1.5 m chromatography on 1 × 27 cm columns which were developed with 10 mM Tris–HCl (pH 7.5 at 20°C), 15 mM NH_4Cl, 15 mM KCl, 10 mM magnesium acetate and 10 mM 2-mercaptoethanol as described [1].

3. Results

3.1. *Purity of elongation factors prepared from* E. coli *D 10 and Krebs II ascites tumor cells*

The elongation factor preparations used in this

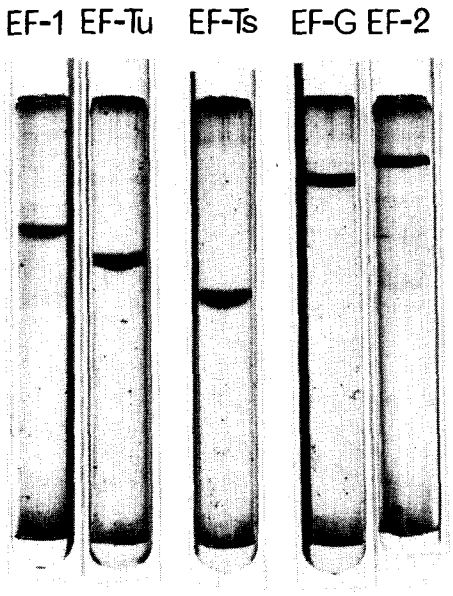

Fig.1. Polyacrylamide gel electrophoresis of elongation factors purified from *E. coli* or ascites tumor cells. 7% (w/v) acrylamide gels containing 0.1% SDS, at pH 7.2, were run as indicated with either 1.6 μg EF-1_L, 1.8 μg EF-Tu, 2.9 μg EF-Ts, 2.8 μg EF-G or with 2.3 μg EF-2 for 1.5 h at 15 mA/tube. The gels were fixed and stained for 1.5 h with 0.25% (w/v) Coomassie Brilliant Blue in 45% (v/v) methanol and 9% (v/v) acetic acid. Destaining was carried out in 7.5% (v/v) acetic acid and 5% (v/v) methanol in water.

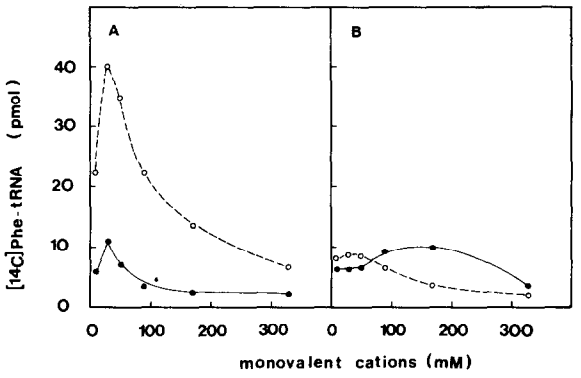

Fig.2. Enzymatic activities of EF-Tu and EF-1_L on *E. coli* or ascites ribosomes dependent on monovalent cations. 0.1 ml reaction mixture containing 2% (v/v) glycerol, 23 mM Tris–HCl (pH 7.5 at 20°C), 6 mM magnesium acetate, 5 mM dithiothreitol, 5 mM phosphocreatine, 20 μg creatine phosphokinase, 57 pmol [^{14}C]Phe-tRNA, 361 pmol deacylated, unfractionated *E. coli* tRNAs, 20 μg poly(U), and 0.2 mM GTP. Where indicated 100 pmol EF-1_L (closed circles) or 40 pmol EF-Tu and 40 pmol EF-Ts (open circles) were introduced. (A) 48 pmol *E. coli* ribosomes, (B) 60 pmol ascites ribosomes. Monovalent cations (equimolar mixture of NH_4Cl and KCl) as indicated. After a 30 min incubation period at 37°C the mixtures were filtered through nitrocellulose filters and counted for radioactivity. Blanks obtained in the absence of factors were subtracted from the experimental values.

study yielded discrete single bands after electrophoresis in sodium dodecyl sulphate acrylamide gels (fig.1).

3.2. *Optimal ionic conditions for the binding of the aminoacyl-tRNA to bacterial and ascites ribosomes stimulated by EF-Tu or EF-1_L*

Ribosomes are very sensitive to the ratio of divalent to monovalent cation concentrations. Therefore, we fixed the magnesium concentration at 6 mM and varied the monovalent cation concentration during EF-Tu and EF-1_L stimulated binding of Phe-tRNA to poly(uridylic) acid programmed *E. coli* and ascites ribosomes. As shown in fig.2 (panel A) there is a sharp optimum at 30 mM monovalent cations for Phe-tRNA binding to *E. coli* ribosomes by both factors. In the analogous experiment carried out with ascites ribosomes the NH_4^+/K^+ optimal concentrations were less sharp (fig.2, panel B). They were found to be 30 mM for EF-Tu and 175 mM for the homologous EF-1_L. Maximal Phe-tRNA binding due to EF-1_L was equal with both types of ribosomes. In contrast, EF-Tu when tested at the optimal concentrations of monovalent cations functioned much better with *E. coli* ribosomes than with ascites ribosomes. However, the extent of Phe-tRNA binding to ascites cell ribosomes catalyzed by EF-Tu was almost equivalent to the magnitude of binding mediated by the homologous factor.

3.3. *Phe-tRNA binding activity of EF-Tu and EF-1_L on* E. coli *and ascites ribosomes over a wide range of factor concentrations*

It is shown in fig.3 (panels A and B) that EF-1_L was equally active with both types of ribosomes over a wide range of concentrations. At concentrations below 70 pmol, EF-Tu was more active on ascites ribosomes than the homologous enzyme; at higher concentrations, however, the activity of EF-Tu leveled

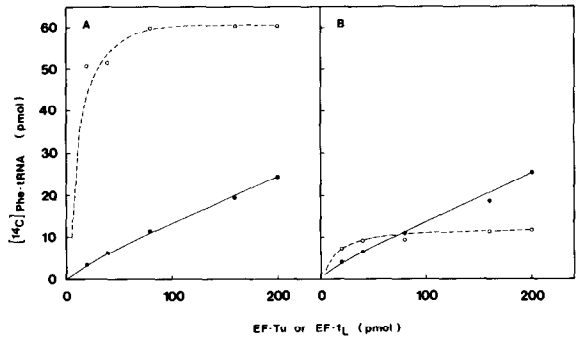

Fig.3. Comparison of [^{14}C]Phe-tRNA binding activity of EF-Tu and EF-1_L with *E. coli* and ascites ribosomes at various factor concentrations. Binding assays contained in 0.1 ml vol: 6% (v/v) glycerol, 30 mM Tris–HCl (pH 7.5 at 20°C), 6 mM magnesium acetate, 5 mM phosphocreatine, 20 μg creatine phosphokinase, 0.2 mM GTP, 6 mM dithiothreitol, 40 μg poly(U), 120 pmol [^{14}C]Phe-tRNA together with 7 nmol deacylated, unfractionated *E. coli* tRNAs and (only (A)) 22 mM NH_4Cl, 27 mM KCl, 128 pmol washed *E. coli* ribosomes or (B) 27 mM NH_4Cl, 152 mM KCl, 120 pmol washed ascites tumor ribosomes and the indicated amounts of EF-Tu (open circles) or EF-1_L (closed circles). After incubation for 40 min at 37°C the tubes were chilled and filtered through nitrocellulose filters, which were dried and counted. Retention of [^{14}C]Phe-tRNA on filters in the absence of factors or ribosomes were subtracted from experimental values. EF-Tu, open circles; EF-1_L, closed circles.

off while there was a further concentration-dependent increase in the activity of EF-1_L (fig.3, panel B). With 128 pmol of *E. coli* ribosomes, EF-Tu activity reached a plateau at 60 pmol.

As observed in the previous experiment, this activity was significantly higher than the stimulation of Phe-tRNA binding to ascites ribosomes.

3.4. *Interchangeability of binding enzymes in peptide chain elongation*

In table 1, three groups of experimental results are presented which illustrate the effect of the complementary elongation factors EF-G and EF-2 on the function of EF-Tu/EF-Ts or EF-1_L and EF-1_H on ribosomes from both sources.

The first group of results demonstrates the function of the bacterial EF-Tu in the binding reactions and its cooperation with EF-G in the bacterial, and with EF-2 in the ascites ribosomal system.

In the second set of experiments [5–8], the monomeric form of ascites EF-1 (EF-1_L) was again found to be equally active on *E. coli* and on ascites ribosomes in the binding reaction. But in contrast to EF-Tu, EF-1_L cooperates only with EF-2 on ascites ribosomes to synthesize poly(phenylalanine). The addition of EF-G to EF-1_L- and poly(U)-programmed *E. coli* ribosomes does not only fail to induce the synthesis of poly(phenylalanine) but inhibits the binding reaction.

In the last group of experiments [9–12] an EF-1 preparation from ascites cells which contained predominantly the aggregated form (EF-1_H) was found to be equivalent to EF-1_L when tested with poly(U)-charged ascites ribosomes in the absence or presence of EF-2. In contrast, its activity in mediating Phe-tRNA binding to *E. coli* ribosomes was poor. When complemented with EF-G, it was absolutely inactive.

3.5. *Confirmation of the different modes of action of [^{3}H]EF-Tu and [^{3}H]EF-1_L*

The availability of tritiated EF-Tu and EF-1_L permitted a more direct assessment of the interaction of the two factors with both types of ribosomes (table 2). The first four experiments in this table show that firm binding of [^{3}H]EF-Tu to poly(U)-charged 70 S ribosomes occurs only in the presence of Phe-tRNA and a non-cleavable GTP analogue. Under comparable conditions, only very little of [^{3}H]EF-Tu became attached to ascites ribosomes and GTP cleavage did not appear to effect the removal of the factor from 80 S ribosomes as completely as from *E. coli* ribosomes (expts 5,6). Interestingly, [^{3}H]EF-1 was bound to *E. coli* ribosomes to the same extent as [^{3}H]EF-Tu under optimal conditions (exp. 4); the presence of aminoacyl-tRNA, however, was no prerequisite for the binding of this factor and GTP cleavage did not clear the *E. coli* ribosome from bound [^{3}H]EF-1 (expts 7–9). These findings indicate that EF-1_L behaves on *E. coli* ribosomes in the same way as on 80 S ribosomes [1].

4. Discussion

Although EF-Tu and EF-1_L play analogous roles in the peptide chain elongation process of bacteria and eukaryotic cells, they apparently do so by

Table 1
Functions of prokaryotic and eukaryotic elongation factors on homologous and heterologous ribosomes

Expt.	Additions	[^{14}C]Phe bound or polymerized (pmol)	Trichloroacetic acid insoluble [^{14}C]Phe (pmol)
1	*E. coli* ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-Tu	56	6
2	Ascites ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-Tu	6.3	0.1
3	*E. coli* ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-Tu + EF-Ts + EF-G	85	54
4	Ascites ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-Tu + EF-2	48	45
5	*E. coli* ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_L	25	0.2
6	Ascites ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_L	26	0.5
7	*E. coli* ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_L + EF-G	14	0.2
8	Ascites ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_L + EF-2	67	66
9	*E. coli* ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_H	4.0	0.1
10	Ascites ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_H	19	0.2
11	*E. coli* ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_H + EF-G	0.0	0.1
12	Ascites ribosomes + [^{14}C]Phe-tRNA + poly(U) + EF-1_H + EF-2	62	61

Incubation mixtures contained in 0.1 ml: 6.5% (v/v) glycerol, 30 mM Tris–HCl (pH 7.5 at 20°C), 25 mM NH_4Cl, 6 mM magnesium acetate, 7 mM dithiothreitol, 5 mM phosphocreatine, 20 μg creatine phosphokinase, 0.2 mM GTP, 120 pmol [^{14}C]Phe-tRNA, 7 nmol deacylated, unfractionated *E. coli* tRNAs, 40 μg poly(U). Odd numbered assays contained 128 pmol washed *E. coli* ribosomes. Even numbered tubes had 40 mM sucrose, 140 mM KCl and 120 pmol washed ascites ribosomes. Where indicated, the following amounts of elongation factors were used: 20 pmol EF-Tu, 20 pmol EF-Ts, 18 pmol EF-G, 20 pmol EF-1_L, 20 pmol EF-1_H (based on subunit content) and 8 pmol EF-2. The tubes were incubated at 37°C for 40 min and either filtered through nitrocellulose filters to estimate bound or polymerized phenylalanine or heated at 90°C for 15 min in 5% trichloroacetic acid, as described under Materials and methods. Values obtained in the absence of elongation factors or in their presence but in absence of ribosomes were subtracted

Table 2
Interaction of labeled prokaryotic and eukaryotic binding factors with homologous and heterologous ribosomes

Expt.	Additions	[^{3}H]EF-Tu or [^{3}H]EF-1 eluting with the ribosomal region (pmol)
1	*E. coli* ribosomes + poly(U) + GTP + [^{3}H]EF-Tu + EF-Ts	0.3
2	*E. coli* ribosomes + Phe-tRNA + poly(U) + GTP + [^{3}H]EF-Tu + EF-Ts	0.3
3	*E. coli* ribosomes + poly(U) + GMP-P(NH)P + [^{3}H]EF-Tu + EF-Ts	0.8
4	*E. coli* ribosomes + Phe-tRNA + poly(U) + GMP-P(NH)P + [^{3}H]EF-Tu + EF-Ts	13.3
5	Ascites ribosomes + Phe-tRNA + poly(U) + GTP + [^{3}H]EF-Tu + EF-Ts	1.1
6	Ascites ribosomes + Phe-tRNA + poly(U) + GMP-P(NH)P + [^{3}H]EF-Tu + EF-Ts	2.6
7	*E. coli* ribosomes + poly(U) + GTP + [^{3}H]EF-1	12.3
8	*E. coli* ribosomes + Phe-tRNA + poly(U) + GTP + [^{3}H]EF-1	8.0
9	*E. coli* ribosomes + Phe-tRNA + poly(U) + GMP-P(NH)P + [^{3}H]EF-1	13.2

Reaction vol. (0.1 ml) contained: 170 pmol *E. coli* ribosomes, 40 μg poly(uridylic)acid, 78 pmol [^{3}H]EF-Tu (2810 dpm/pmol, 1 pmol = 45 ng), 20 pmol EF-Ts, 2.5% (v/v) glycerol, 15 mM NH_4Cl, 15 mM KCl, 7 mM magnesium acetate, 6 mM Tris–HCl (pH 7.5 at 20°C), 3 mM 2-mercaptoethanol (expts 1, 3); 200 pmol unlabeled Phe-tRNA with 9.8 nmol unfractionated, deacylated *E. coli* tRNAs were added for expts 2, 4. Experiments 5 and 6 as exp. 2, except for the following changes: 170 pmol ascites ribosomes, 2.5% (v/v) glycerol, 60 mM sucrose, 30 mM NH_4Cl, 6 mM magnesium acetate, 7 mM Tris–HCl (pH 7.5 at 20°C) and 2 mM dithiothreitol. Experiments 7, 8 and 9 as expts 1, 2, 4, respectively, with [^{3}H]EF-Tu and EF-Ts replaced by 340 pmol (80 pmol biologically active) [^{3}H]EF-1 monomers (44 dpm/pmol, 1 pmol = 47 ng), GTP (0.2 mM) and GMP-P(NH)P (0.4 mM) as indicated. Incubations lasted for 30 min at 37°C. Filtrations were carried out on Bio-Gel columns as described under methods. ^{3}H-radioactivity appearing in the ribosomal peak fractions was expressed as pmol EF-Tu or pmol EF-1

different modes of action. No information has yet become available whether each of the two mechanisms of action is an inherent property of the respective elongation factor or whether it is also represented in the homologous type of ribosome. Therefore, EF-Tu and EF-1_L were tested for their ability to function with the heterologous ribosome alone or in conjunction with EF-G and EF-2, respectively.

As reported by Krisko et al. [7], EF-Tu can function with 80 S ribosomes both in the Phe-tRNA binding – and after addition of EF-2 – also in peptide chain elongation. In contrast, these authors found EF-1 from reticulocytes unable to substitute for EF-Tu on bacterial ribosomes. These experiments, however, were carried out with an EF-1 preparation which according to present convention would be classified as EF-1_H and which represents aggregates of a single monomer with approx. mol. wt 50 000.

Techniques for the preparation of active EF-1_L monomers in pure form have recently become available [8]. Since the monomer of EF-1 which in ascites tumor cells was shown to have mol. wt 47 000 [9] is much more comparable in size to EF-Tu than the aggregate form of EF-1, the question of EF-1 activity in a prokaryotic ribosomal system could now be re-examined.

When EF-1_L and EF-Tu were compared with respect to their ability to attach Phe-tRNA to *E. coli* ribosomes, several facts emerged:

1. EF-1_L does function in the binding reaction with 70 S ribosomes.

2. Both factors display equal dependencies on the concentration of monovalent cations.

3. EF-Tu is significantly more active than EF-1_L but its higher activity can be attributed at least in part to the incorporation of Phe-tRNA into acid precipitable material due to some residual contamination of the *E. coli* ribosomes with EF-G.

We have previously shown [1] that EF-1_L from ascites cells remains bound to the 80 S ribosome during the whole peptide chain elongation cycle without interfering with the action of EF-2. This is in contrast to the established sequence of events as observed in prokaryotic systems (for review see [10]) according to which EF-Tu has to leave the ribosomal surface in order to render the bound aminoacyl-tRNA accessible to EF-G.

If EF-1_L, in analogy to its behaviour on 80 S ribosomes, would also remain bound to the *E. coli* ribosome after Phe-tRNA attachment and GTP hydrolysis, it should block the subsequent interaction of EF-G with the ribosome. Our finding that no synthesis occurs when EF-G is added to *E. coli* ribosomes to which Phe-tRNA had been attached by EF-1_L, is entirely compatible with this hypothesis. Experiments depicted in table 2, in which labeled EF-Tu and EF-1_L were used, confirmed this conclusion.

Unpublished data from this laboratory have indicated that the aggregated form of EF-1 (EF-1_H) does not dissociate into its monomers in the presence of GTP and aminoacyl-tRNA unless programmed ascites ribosomes are added. The latter, therefore, should be predominantly involved in the disaggregation event. From the data presented in table 1 (expts 9–12), we conclude that the *E. coli* ribosome is unable to dissociate EF-1_H, which is almost inactive with *E. coli* ribosomes, whereas it displays activity similar to EF-1_L in conjunction with ascites ribosomes. These findings explain the inability of reticulocyte EF-1 (aggregated form) to substitute for EF-Tu on *E. coli* ribosomes [7,11].

Artemia salina EF-1 was also found to be inactive on *E. coli* ribosomes but, interestingly, was active on hybrid ribosomes composed of large subunits from *E. coli* and small subunits from *Artemia salina* [11]. It can be speculated from these data that it is the small subunit of eukaryotic ribosomes which is involved in the disaggregation of EF-1_H.

It should not go without notice that EF-G and EF-2 were found to be active only with homologous ribosomes. EF-Ts was not able to stimulate the EF-1_L mediated binding of Phe-tRNA to 70 S or 80 S ribosomes (data not shown).

The work reported in this communication confirms an older study [7] which indicated that EF-Tu is also active on eukaryotic ribosomes and shows for the first time that the monomeric form of the eukaryotic EF-1 promotes the binding of Phe-tRNA to prokaryotic ribosomes. Furthermore, our observations support the concept that EF-Tu and EF-1 function by different mechanisms.

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