Minireview

Elongation factors from the Guerin epithelioma and rat liver cells

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Translation of the codons in mRNA requires the presence of protein factors, GTP, a sulfhydryl compound and various cations. The two protein factors required for polypeptide chain elongation with rat liver preparations have been designated [1, 2] as aminoacyl transferase I (elongation factor 1; EF-1) and aminoacyl transferase II (elongation factor 2; EF-2); they are analogous to factors designated T and G, respectively, from *E. coli* [3, 4] and have been recognized in many other cells and organisms [5 - 7]. Studies with highly purified translational factors and with cell-free systems that translate a variety of mRNAs provide insight into the exact roles of translational factors, the regulatory mechanisms that affect protein synthesis at the level of these factors, and into essential features of mRNAs that affect their interaction with the translational machinery.

In elongation process, EF-1 is responsible for the aminoacyl-tRNA binding to the ribosomes, while EF-2 catalyses the translocation of peptidyl-tRNA from site A to site P on the ribosome. Peptidyl transferase is an enzyme integrally bound with ribosomes, it participates in the transfer of peptidyl-tRNA from P site to A site and catalyses peptide bound formation. It has been recently suggested [8] that the 23 S ribosomal RNA participates in the peptidyl transferase function. Our studies were performed on the elongation factors (EF-1 and EF-2) isolated from Guerin epithelioma and rat liver cells. Guerin epithelioma is one of the most malignant experimental tumours, and is used in tests for new chemotherapeutics.

ELONGATION FACTOR 1 (EF-1)

The main function of elongation factor 1 (EF-1) is to bind aminoacyl-tRNAs to the acceptor site A on the ribosome. Binding of the aminoacyl-tRNAs (other than the initiator Met-tRNA), in eukaryotic chain elongation, requires the intermediary formation of a ternary complex between the aminoacyl-tRNA, GTP, and the binding factor EF-1.

The first information on the multiple forms of EF-1 in eukaryotic cells was supplied by Shneir & Moldave [9]. The heterogeneity of eukaryotic EF-1 was also described by Collins *et al.* [10] and others [11 - 16]. On the basis of these informations it seems that at least two multiple forms play a specific role in polypeptide chain biosynthesis. Two complementary factors, named EF-1α and EF-1β, were isolated from pig liver by the Japanese group of Dr. Kaziro [17]. EF-1α is the subunit that participates in formation of the ternary complex [18 - 22], *via* formation of a binary complex with GTP [20 - 25]. It binds GTP and the resulting binary complex reacts then with aminoacyl-tRNAs to form a ternary complex; this complex binds to and delivers the aminoacyl-tRNA to the ribosomal particle [16, 26 - 28], GTP is hydrolysed [16, 21, 29], EF-1α•GDP is formed [16], and the factor is released from the ribosome [28]. The other subunit EF-1β, catalyzes the GDP/GTP exchange on EF-1α [15, 30 - 33]. Thus, a model can be proposed according to which EF-1β reacts

with the EF-1α•GDP complex, displacing GDP and forming an EF-1α•EF-1β binary complex; then GTP displaces EF-1β resulting in formation of an EF-1α•GTP complex which can bind another aminoacyl-tRNA.

Isolation of the elongation factors from rat liver and Guerin epithelioma was previously described [34 - 37]. In turn, the EF-1 preparation from Guerin epithelioma was separated into three heterogeneous fractions, EF-1A, EF-1B and EF-1C, while EF-1 isolated from rat liver cells was separated into two fractions only, EF-1A and EF-1B(C) [36]. EF-1 from rabbit reticulocytes dissociates into three subunit forms, too [38]. The cross-experiments showed that EF-1A from the tumour cells was stimulated by EF-1B and EF-1C, but EF-1B and EF-1C were not mutually stimulated [36].

Our preliminary results [36, 37] allowed to conclude that the EF-1A, isolated from Guerin epithelioma cells, corresponds to the light form of EF-1 from other animal tissues. EF-1B was suggested to be an unidentified aggregate. It is commonly known that EF-1 in the tissues appears mainly in aggregates [13], the dimensions of which differ with the kind of the tissue. Fraction EF-1C was thermostabile and stimulated the activity of the free EF-1A added to the elongation system [37].

Next, the components of the EF-1B aggregate were identified [39]. The subunit EF-1A was

not adsorbed on DEAE-Sephedex A-50 column and it was obtained in a free form (Fig. 1 B, b). The homogeneous EF-1A was also obtained after dissociation of the EF-1B aggregate in the presence of GTP [40]. The EF-1A subunit form is functionally similar to EF-1α (eukaryotes) and EF-Tu (prokaryotes).

EF-1B was eluted from DEAE-Sephadex column as the main peak (Fig. 1A) and it was markedly more active in polyphenylalanine synthesis than fraction EF-1A. It was shown that EF-1B consist of at least three polypeptides (Fig. 1B, c.); one of them is EF-1A, while the other two bands may correspond to EF-1βγ. EF-1B seems to be an aggregate similar to EF-1H [39], which is known to be a complex of EF-1α with EF-1β(βγ) [41 - 43].

From the fraction not adsorbed on CM-Sephadex C-50, at pH 8.0, a preparation designated EF-1B' was obtained. It was active in polyphenylalanine synthesis and stimulated this process when added to the EF-1A containing incubation mixture [44].

Autophosphorylation of aggregates EF-1B and EF-1B' from Guerin epithelioma

EF-1B and EF-1B' are aggregates containing both subunit forms: EF-1A and EF-1C, and other polypeptides (Fig. 2, lanes a, c).
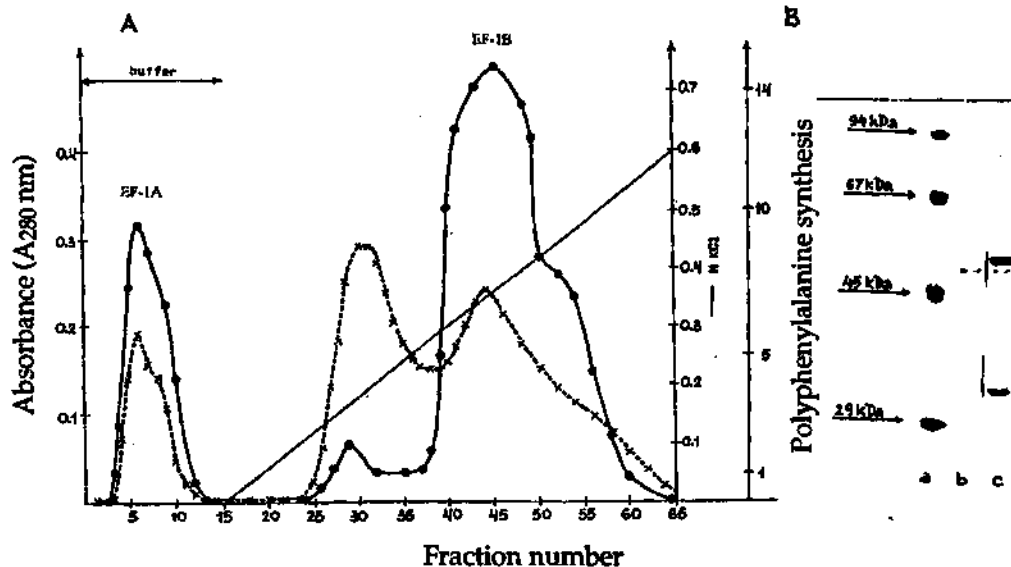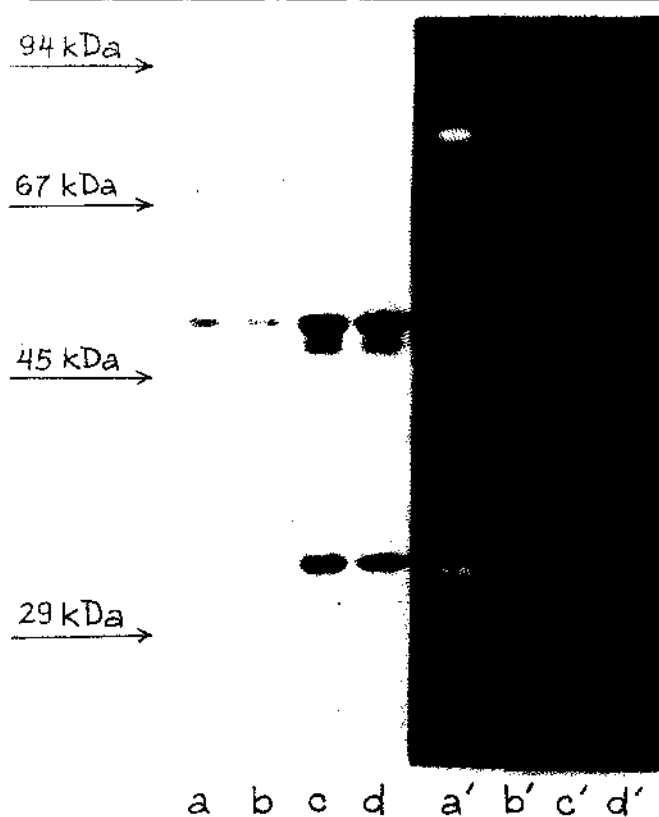

Fig. 1. The separation pattern of EF-1 from Guerin epithelioma cells on DEAE-Sephadex A-50 column (A) and electrophoresis of the active peak fractions EF-1A and EF-1B in 10% polyacrylamide-gel with 0.1% SDS (B) (after [39]).

Designations A: (●), Polyphenylalanine synthesis, [14C]phenylalanine incorporated (pmoles per 50 μl of each fraction); (x), absorbance. Designations B: a, markers; b, EF-1A; c, EF-1B.

94 kDa →

67 kDa →

45 kDa →

29 kDa →

a   b   c   d   a'   b'   c'   d'

Fig. 2. Polyacrylamide-gel electrophoresis with SDS and autoradiography of the autophosphorylated preparations of EF-1B' and EF-1B (15 μg of protein). Electrophoresis, lanes: a, EF-1B'; b, EF-1B' with heparin (2 μg); c, EF-1B; d, EF-1B with heparin (2 μg). Autoradiograms, lanes: a', EF-1B'; b', EF-1B' with heparin; c', EF-1B; d', EF-1B with heparin.

EF-1B and EF-1B', but not EF-1A, can undergo autophosphorylation [44], (Fig. 3). EF-1B and EF-1B' are aggregates containing protein kinase activities and are phosphorylated without addition of the enzyme. On Fig. 2, lanes a, c, a', c' it can be seen that EF-1B differs from EF-1B' in polypeptide composition. The autoradiographic picture shows that, in EF-1B' two polypeptides (of approx. 32 kDa, corresponding to EF-1β and of approx. 90 kDa) were phosphorylated (Fig. 2, lane a'), while in EF-1B only the polypeptide (of approx. 51 kDa, corresponding to EF-1γ), was phosphorylated (Fig. 2, lanes c', d'). EF-1A and EF-1C individually, are unable to become autophosphorylated and they can not phosphorylate other polypeptides. This is in agreement with the suggestion of Janssen *et al.* [45], that the protein kinase phosphorylating EF-1β is a protein integrally bound to this factor, but is not its functional subunit.

Protein kinase (CK II) was found to be the enzyme responsible for autophosphorylation in the EF-1B' preparation. Heparin, a specific inhibitor of CK II [45, 46], distinctly inhibited autophosphorylation of EF-1B' (Fig. 4, Fig. 2, lane b').

The kinase, present in EF-1B has not been classified. It was only shown that the histone H2A can serve as a substrate of the enzyme

responsible for autophosphorylation of EF-1B (Fig. 5).

Polyphenylalanine synthesis was decreased when autophosphorylated EF-1B' was used [44]. Such an effect was not observed in the presence of autophosphorylated EF-1B, even if one component of EF-1B was phosphorylated.

In the process of autophosphorylation of EF-1B', its subunit form EF-1C was probably modi-
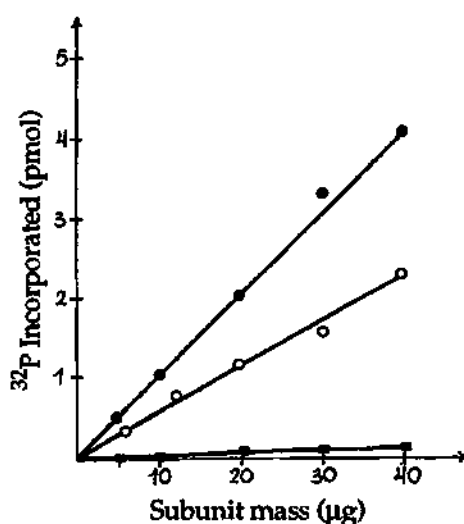

Fig. 3. Autophosphorylation of the subunit forms of EF-1 from Guerin epithelioma (after [44]). (O), EF-1B; (●), EF-1B'; (■), EF-1A. Autophosphorylation was carried out according to the phosphorylation procedure but without addition of protein kinase.
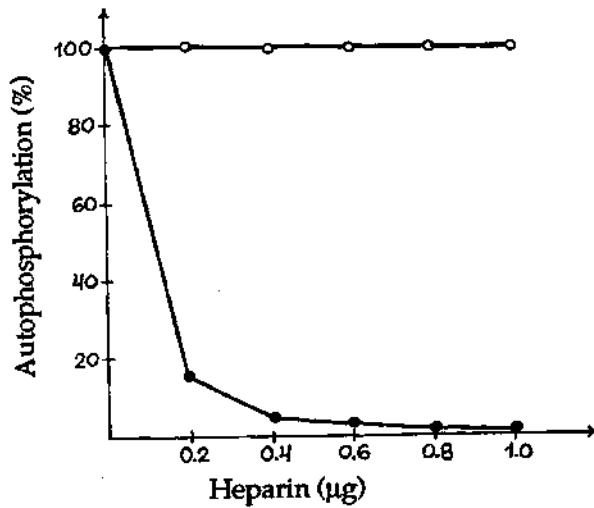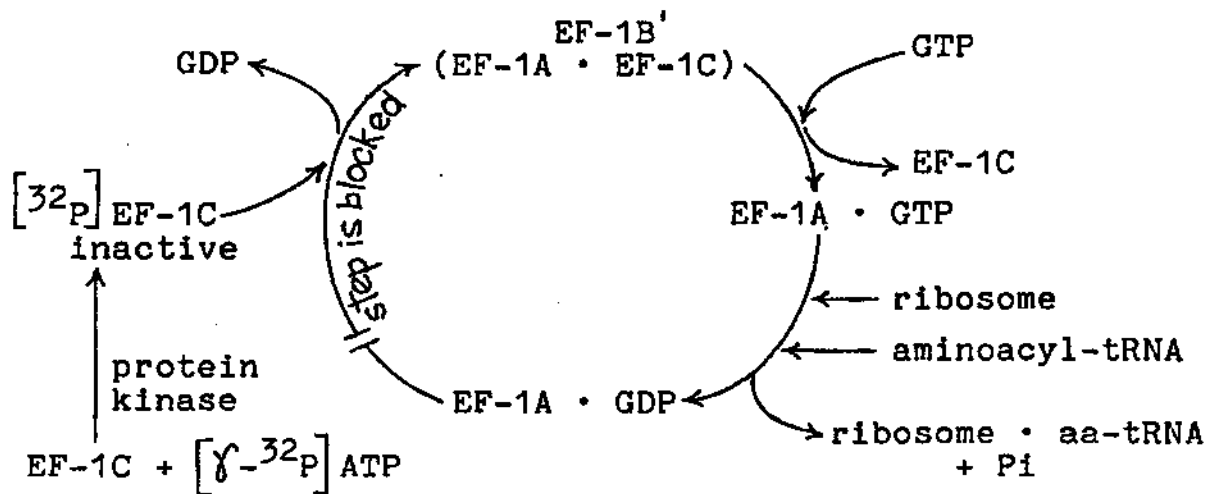
Fig. 4. The effect of heparin on autophosphorylation process of EF-1B (O) and EF-1B' (●).

Fig. 5. Phosphorylation of histone H2A by the preparations of EF-1B' (O) and EF-1B (●).

fied and inactivated. [^{32}P]EF-1C was unable to form the EF-1A•EF-1C intermediary complex and formation of EF-1A•GTP active complex was stopped (Scheme 1).

and by two dimensional SDS-PAGE/IEF electrophoresis [40]. The 32 kDa peptide may be a homologue of EF-1β, while the second one of 51 kDa could correspond to EF-1γ of other eu-

Scheme 1. The inhibitory effect of EF-1B' autophosphorylation on formation of the active EF-1A•GTP complex (after [44]).

Purification and characteristics of the EF-1C subunit form

EF-1C was shown to be the factor responsible for the GDP/GTP exchange in the EF-1A•GDP complex. We tried to isolate EF-1C from the EF-1B preparation, consisting of the subunit forms EF-1A•EF-1C. This complex was dissociated in the presence of GTP [41] with some modifications [40]. EF-1C was resolved into two polypeptides after reversible denaturation in 6 M urea according to [47, 48]. The results were confirmed by SDS-gel electrophoresis
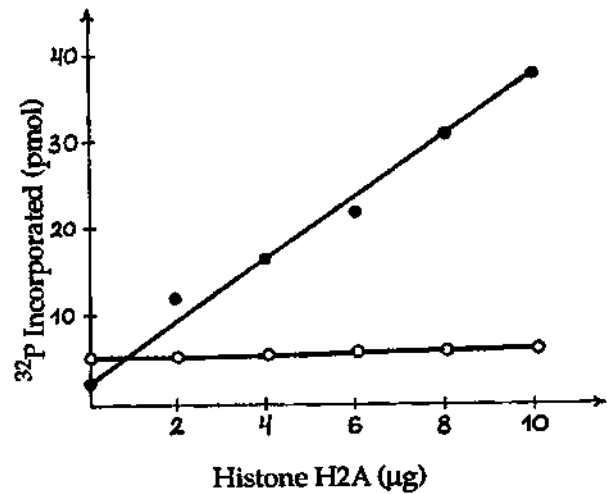
karyotic cells.

In Guerin epithelioma cells EF-1C is present probably in aggregate with EF-1A. High tendency to aggregation of the subunits EF-1A and EF-1C of the elongation factor 1 from Guerin epithelioma suggests some analogies with the early developmental period of the lower eukaryotic organisms [49, 50], in which EF-1 exists as a high molecular complex EF-1H. EF-1H, during the growth season, is transformed to the light form EF-1L (EF-1α). However, EF-1H has low activity and it represents some kind of

storage form of EF-1L, which can be activated only when separated from other proteins. On the other hand, EF-1 isolated from Guerin epithelioma was highly active in all aggregate forms.

EF-1C from rat liver did not show any tendency to aggregate with EF-1A, so it can be isolated without the GTP-dependent dissociation step (not shown). EF-1C in these cells can exist in the free form or it can be aggregated with other proteins, which is in agreement with the reports from other laboratories [32, 41, 48, 51, 52].

The results of EF-1C participation in the exchange of GDP to GTP in the EF-1A•GDP complex were presented and it was found that the 32 kDa polypeptide stimulated the GDP/GTP exchange, while the 51 kDa polypeptide was inactive in this process [40].

The hydrophobic character of the latter polypeptide was evidenced (unpublished) and is in agreement with the results described for polypeptide γ by Janssen & Möller [47]. The EF-1B fraction, isolated from Guerin epithelioma [44], was thermostable [40]. Slobin & Möller [53] have observed that the aggregate of EF-1 from *A. salina* was not inactivated at 42°C but, on addition of GTP, dissociated and lost its activity. Our observations could suggest that EF-1C protects EF-1A against thermal inactivation. It would be interesting to know whether polypeptide γ, the function of which is still unknown, is responsible for the thermostability of EF-1C.

It should be noted that, in the EF-1B fraction isolated from rat liver cells, EF-1C was present while EF-1A was absent (to be published). This means that the subunit forms of EF-1 from rat liver show lesser tendency to aggregate with each other and can exist separately in the liver tissue.

ELONGATION FACTOR 2 (EF-2)

For the elongation of ribosome-bound polypeptides, in addition to EF-1 and other components of the elongation system, EF-2 and GTP are required for translocation of mRNA and peptidyl-tRNA from the aminoacyl site (A) to peptidyl site (P), which allows the ribosome to bind new incoming aminoacyl-tRNA at the aminoacyl site [54 - 57].

The pure translocation factor (EF-2) was obtained from rat liver [34]. On the analytical ultracentrifuge the purified protein moved as an essentially homogeneous peak. The $S_{20,\omega}$ value was 4.7 S and molecular mass about 64 kDa. The homogeneity of the purified EF-2 was confirmed by gel-electrophoresis and electrofocusing [34].

Evidence has been presented [58] that the translocation reaction exhibits characteristics consistent with those of the enzyme-catalyzed reaction with respect to the enzyme (EF-2), the substrate (ribosomal peptidyl-tRNA$_a$) and the factor (GTP); a single enzyme catalyzes translocation of several ribosomes, suggesting that the interaction between ribosomes and EF-2 is a reversible process, and that the active EF-2 dissociates from the complex on formation of the product. One of the models for the interaction between EF-2 and ribosomes postulated [58] that the EF-2•ribosome complex would dissociate when the substrate of the reaction, peptidyl-tRNA$_a$, becomes converted to peptidyl-tRNA$_p$; the released EF-2 could then react with the same or another ribosome.

Tanaka *et al.* have shown [59] that EF-2 from pig liver makes with GTP the EF-2•GTP complex which very fast binds to ribosomes. The ternary complex EF-2•GTP•ribosome is transformed during the translocation step into the EF-2•GDP•ribosome complex, which becomes dissociated into ribosome and the binary complex EF-2•GDP. The latter in the reaction with GTP, reproduces the EF-2•GTP complex, releasing GDP.

It is known that EF-2 activity is inhibited by the diphtheria toxin in the presence of NAD^+. The mechanism of this inhibition appears to lead through adenosine diphosphate ribosylation of the EF-2. Ultracentrifugal studies suggest [60] that formation of a ternary complex consisting of the toxin, EF-2 and NAD^+ may be the first step of this inactivation. Further experiments suggested that the complex dissociates when the nicotinamide riboside linkage is cleaved, resulting in formation of the ADP-ribosylated EF-2. This could indicate that the mechanism of inhibition may not involve simply a transfer of ADP-ribose from the toxin to EF-2 [60].

EF-2 can be markedly altered in some organisms, for instance in mutants [61] or under changed conditions [60, 62 - 67]. The isolation,
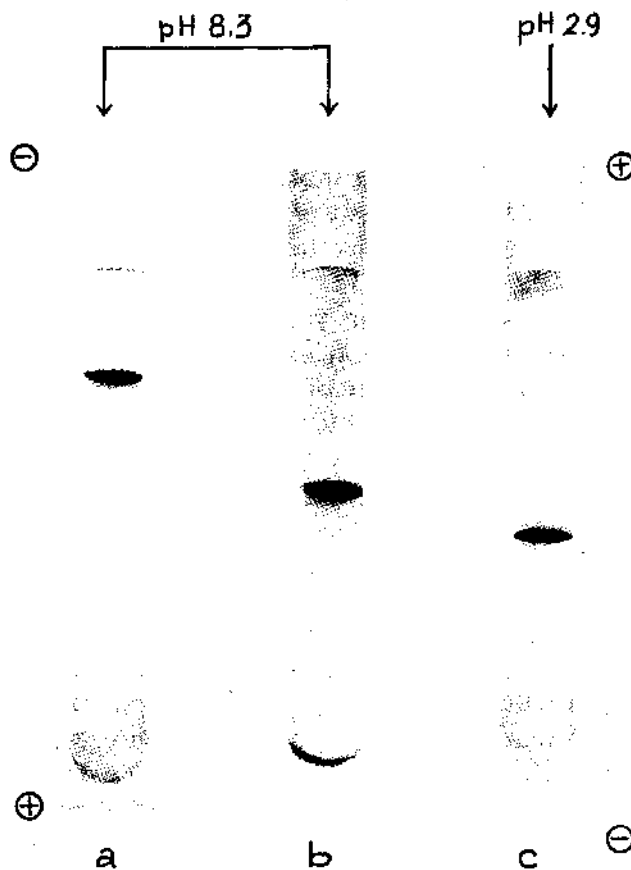
Fig. 6. Polyacrylamide-gel electrophoresis of the purified EF-2 from Guerin epithelioma: a and c, without SDS; b, with SDS.

purification and characterization of EF-2 from Guerin epithelioma cells was carried out and described [68, 69]. The final EF-2 preparation was homogeneous when tested by polyacrylamide-gel electrophoresis (Fig. 6 a, c) and it was found to be a protein consisting of identical polypeptides (Fig. 6 b).

The pI value of Guerin tumour EF-2 was found to be 6.5 [68]. The molecular mass of tumour EF-2, determined by ultracentrifugation in sucrose gradient was about 68 kDa and was very close to that of rat liver EF-2 [34]. No visible differences were found between amino acid compositions of EF-2 from Guerin epithelioma and from rat liver cells [68].

Studies on specificity of the elongation factors have shown that the prokaryotic EF-T can replace EF-1 in animal systems, while EF-G from E. coli was inactive when added instead of animal EF-2 [61]. The lack of tissue specificity has been demonstrated by cross-examinations between systems isolated from rat liver and Guerin epithelioma cells [69]. However, affinity of the Guerin EF-2 to the liver elongation system was lower by half as compared with the homologous tumour or liver systems.

The N-terminal and C-terminal amino acids of EF-2 proteins from normal rat liver and Guerin epithelioma cells were identified [69]. In either EF-2 preparation the only N-terminal amino acid was alanine. In EF-2 isolated from various sources there was also only one N-terminal amino acid [65 - 67, 70, 71]. C-terminal amino acids of EF-2 were determined after hydrazinolysis of EF-2 [69]. The C-terminal amino acid in EF-2 from rat liver was glycine, while in EF-2 from Guerin epithelioma cells it was serine.

N- and C-terminal fragments of EF-2 molecule are important for the functional activity and for the molecule interaction with ribosomes [72]. Structural differences between translocation factors were observed as an effect of mutation [61] or other alterations accompanied by dramatic changes of their activity [71].

Some differences in the polypeptide composition of trypsinolysed homogeneous EF-2 preparations from Guerin epithelioma and rat liver have been also demonstrated [69]. In tumoural EF-2 hydrolysates the lack of the acidic peptide was observed while more of basic peptides were present as compared to those in EF-2 from rat liver.

The active center of EF-2, responsible for the binding reaction with ribosomes, is situated at the C-terminal domain of the molecule [73]. In

our experiments it was found that the rat liver EF-2 lost about 70% of its activity after 0.5 h incubation with carboxypeptidase. In the same conditions, EF-2 from Guerin epithelioma cells, lost only about 30% of its activity. It was also shown that after digestion of the later EF-2 with carboxypeptidase, this elongation factor was almost unable to bind to ribosomes.

The sensitivity of both tumoural and normal EF-2 to N-terminal degradation was lesser than that from the C-terminal. Incubation of EF-2 with aminopeptidase for 0.5 h resulted in about only 10% losses of the activity.

In various animal tissues the presence of an identical substrate of 100 kDa, specific for the calmodulin and Ca^{2+}-dependent protein kinase has been demonstrated [74] and this substrate was identified as EF-2 [75 - 77]. The enzyme that specifically phosphorylated EF-2 was named kinase III [78]. The activity of EF-2 was decreased after phosphorylation and this effect was reversed by dephosphorylation [79]. When the phosphorylation of EF-2 was carried out in the presence of EGTA [ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid] – a kinase inhibitor, EF-2 activity was preserved, but if NaF – a phosphatase inhibitor was added, EF-2 activity was decreased. These results are in agreement with the reports from other laboratories [75, 80 - 83].

Some results that were obtained in our laboratory (unpublished) have shown the possibility of existence of EF-2 in multiple forms. EF-2 was isolated from the postribosomal supernatant of rat liver or Guerin epithelioma cells and then purified consecutively by: filtration on Sephadex G-25, precipitation at 30 - 80% ammonium sulphate saturation, chromatography on DEAE-Sephadex A-50, CM-Sephadex C-50, hydroxylapatite and Ultrogel AcA-44. The active fractions of EF-2 eluted from the Ultrogel AcA-44 column were separated by electrophoresis according to Laemmli [84] into two bands. One of them showed electrophoretic mobility corresponding to molecular mass of approx. 90 kDa, the second of approx. 65 kDa. Two fractions (90 kDa and 65 kDa) with EF-2 activity were obtained also by ultracentrifugation in sucrose gradient. Only the 65 kDa EF-2, was isolated from the active polyribosomes. Thus, it can be concluded that EF-2 exists in at least two forms differing in molecu-lar mass and that EF-2 of 65 kDa may take part directly in the translocation process.

Summarizing, the elongation factors EF-1 and EF-2, isolated from Guerin epithelioma cells, are functionally similar to those from liver of normal rats. Differences were observed in relation to composition of the aggregates of EF-1 subunit forms and their ability to undergo autophosphorylation. In Guerin epithelioma cells, EF-1C exists in the aggregate with EF-1A and can be isolated after dissociation of the aggregate EF-1B in the presence of GTP. In rat liver, the subunit forms EF-1A and EF-1C can exist separately and they can be isolated without the GTP dependent dissociation. Moreover some peculiarities of the EF-2 isolated from Guerin epithelioma were demonstrated. The C-terminal amino acid and peptide map of trypsinolysed tumour EF-2 molecules are different from those of EF-2 from liver cells of normal rats. Furthermore, sensitivity of tumour EF-2 to digestion by carboxypeptidase was lesser than that of EF-2 from rat liver.

We can conclude that our studies on the elongation factors of rat liver and Guerin epithelioma yielded high purified preparations of EF-2 and subunit forms of the heterogeneous factor EF-1. Their structural and functional characterization revealed some peculiarities of the tumoural elongation factors that will be taken advantage of the search specific inhibitors of protein biosynthesis, especially in some tumour cells, as it was suggested in previous papers [85 - 88].

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