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THE PHOSPHORYLATION OF ELONGATION FACTOR EF-1 ISOLATED FROM GUERIN EPITHELIOMA

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The function of EF-1 in various eucaryotic systems has been extensively studied. Subunit forms EF-1α and EF-1βγ were isolated from pig liver [1], from yeast [2, 3], calf brain [4], wheat seeds [5] and rabbit reticulocytes [6]. The binding of aminoacyl-tRNA to ribosomes is catalysed by EF-1 in the presence of GTP, while EF-1βγ stimulates a replacement of GDP for GTP in the complex of EF-1α·GDP [1].

From Guerin epithelioma cells two subunits forms of EF-1 were isolated: EF-1A (functionally analogous to EF-1α) and EF-1B, which is an aggregate of EF-1A and EF-1C [7]. Functionally, EF-1C corresponds to EF-1β (βγ). Recently it was found that EF-1α from rabbit reticulocytes was phosphorylated [8]. EF-1H from wheat seeds was found to show autophosphorylation properties [9]. A serine of EF-1β in EF-1βγ from *Artemia salina* was phosphorylated [10]. It was also shown that casein kinase II is responsible for EF-1β phosphorylation [11].

The subject of this work is the phosphorylation of EF-1 subunit forms isolated from Guerin epithelioma cells.

All the chemicals used were of the reagent grade.

The cell-free system from rat liver or from Guerin epithelioma cells, ribosomes, [^{14}C]phenylalanyl-tRNA and elongation factors EF-1 and EF-2 were prepared according to the methods previously described [12 - 13]. The

subunit forms EF-1A and EF-1B were obtained and polyphenylalanine synthesis and [^{14}C]phenylalanyl-tRNA binding to ribosomes were performed according to [7].

The isolation of EF-1B′. The crude EF-1 preparation, obtained after ammonium sulfate saturation of the eluate from hydroxyapatite [13] was chromatographed (200 mg of protein) on CM-Sephadex C-50 column (35 × 2 cm) equilibrated with the buffer: 0.05M Tris/HCl, pH 8.0, 25% glycerol, 10 mM 2-mercaptoethanol. The column was washed with the same buffer. The flow rate was maintained at 30 ml per hour and 5 ml fractions were collected. The proteins with EF-1 activity were not adsorbed on the CM -Sephadex C-50 column. The active fractions were concentrated at 4°C by vacuum dialysis against the buffer and the preparation (approximately 60 mg of protein) was placed on the top of DEAE-Sephadex A-50 column (25 × 1.2 cm) equilibrated with the same buffer. Elution was performed with 0.4 M KCl in the same buffer at the flow rate 30 ml/h and fraction volume of 5 ml. The active peak fractions were rechromatographed on the CM -Sephadex column (15 × 1.2 cm) equilibrated with the same buffer, but at pH reduced to 6.5. The elution was carried out with a linear KCl gradient (0.0 M/50 ml to 0.4 M/50 ml) in the same buffer, pH 6.5. Fractions of 3 ml were collected at the elution rate of 30 ml/h. The active fractions were concentrated at 4°C by vacuum dialysis against the above buffer, pH 8.0, and used for EF-1B′ characterization.

Phosphorylation of the EF-1 subunit forms. The standard reaction mixture contained in the volume of 0.2 ml: 50 mM Tris/HCl, pH 7.5; 50 mM KCl; 6 mM $MgCl_2$; 5 mM DTT (dithiothreitol); 13.5 nM [γ-^{32}P]ATP and appropriate amounts (depending on the experiment) of EF-1A, EF-1B or EF-1B′ fractions.

Incubation was carried out at 37°C for 30 min and was stopped by adding 0.25 ml of 10% trichloroacetic acid and heating in a boiling water bath for 15 min. The protein precipitates were filtered through glass fibre filters GF/A, than washed three times with 15 ml of 5% trichloroacetic acid and dried. The radioactivities of the protein precipitates were counted in the PPO-POPOP-toluene scintillation liquid on Isocap 300, Nuclear Chicago counter.

Dephosphorylation of EF-1 subunit forms was carried out according to [10]. Electrophoresis was performed on 1% SDS-10 % polyacrylamide gel plates (11 × 14 × 0.1 cm) according to Laemmli [16]. For destaining of the

gels Coomassie Brilliant Blue R 250 was applied. Phosphorylated peptides were identified autographically on Roentgen XM-1 films.

The crude EF-1 preparation, obtained after ammonium sulfate precipitation from the hydroxyapatite eluate [13] was separated into active fractions EF-1A, EF-1B and EF-1B′. It was shown that EF-1A is a single polypeptide (M_r 53 000) while EF-1B and EF-1B′ are aggregates containing both subunit forms EF-1A and EF-1C (M_r 36 000), and additionally other polypeptides (Plate 1A, c). As it was previously shown [7], EF-1A and EF-1B can bind [^{14}C]Phe-tRNA to ribosomes and they participate in [^{14}C]polyphenylalanine synthesis. EF-1B′ preparation was found to have the same properties (not shown). EF-1B and EF-1B′ can undergo autophosphorylation, while EF-1A can not (Fig. 1). Visible differences in the

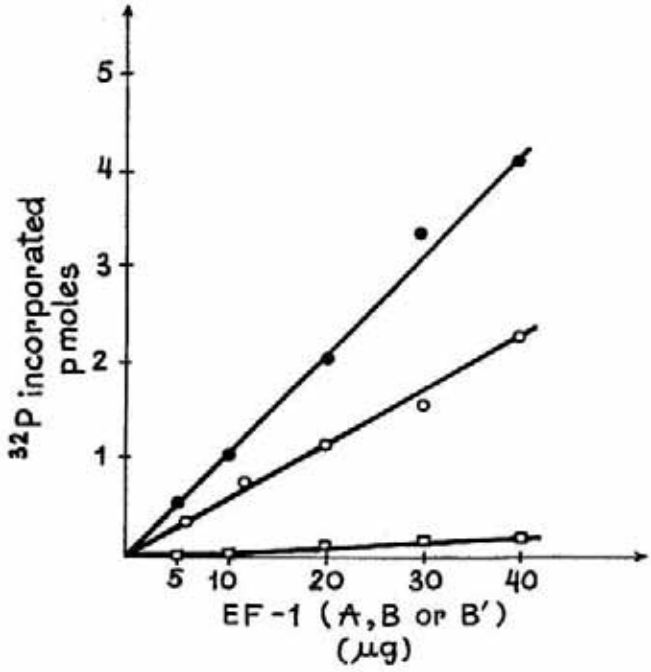


Fig. 1. Autophosphorylation of the subunit forms of EF-1 from Guerin epithelioma. ○, EF-1B; ●, EF-1B′; □, EF-1A

polypeptide composition of EF-1B and EF-1B′, were found on electrophoresis (Plate 1 a, c) and can be also seen on the autoradiograms. In the fraction of EF-1B, the polypeptide of 53 000 was phosphorylated (Plate 1B, c), while on the autoradiogram of EF-1B′ (Plate 1B, a) two bands of phosphorylated proteins are visible. One of them, with M_r of about 36 000,

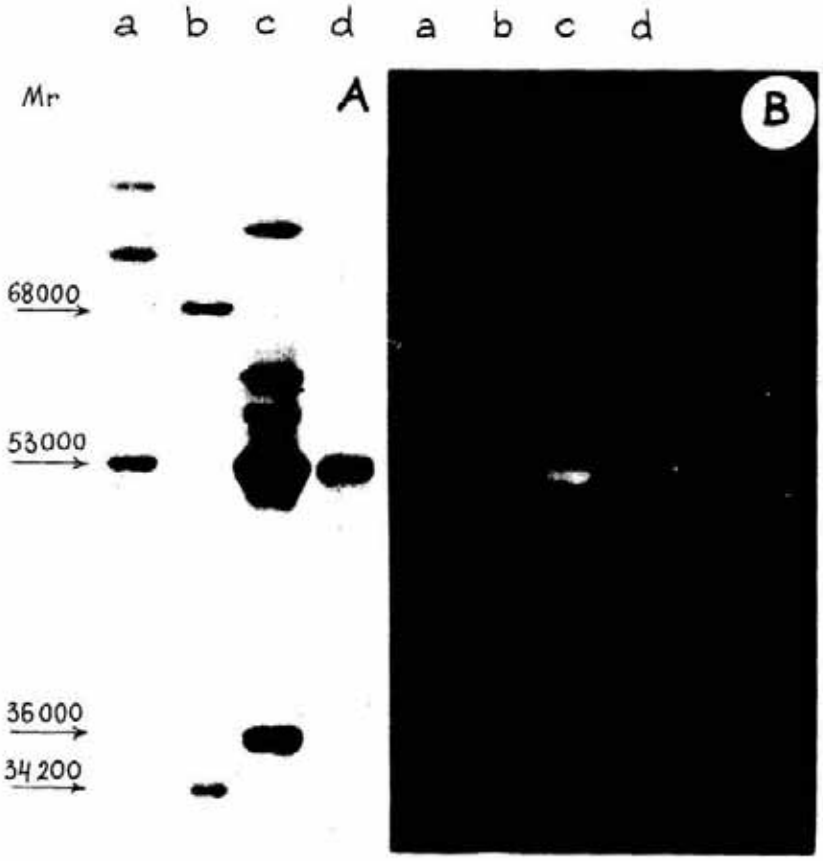


Plate 1. Electrophoresis of the crude EF-1 fraction from Guerin epithelioma on SDS -polyacrylamide. A, electrophoregram; B, Autoradiogram; a, EF-1B′ (10 μg); b, markers: BSA (M_r 68 000), carboxypeptidase (34 000); c, EF-1B (15 μg); d, EF-1A (12 μg)

corresponds to EF-1C, which is responsible for the replacement of GDP for GTP in the EF-1A·GDP complex. Purified EF-1A does not undergo autophosphorylation (Fig. 1) and consequently no bands on the autoradiogram are visible (Plate 1B, d). These results show that fractions EF-1B′ possess kinase activities.

The results illustrated in Fig. 2 show that polyphenylalanine synthesis was decreased by about 25% when EF-1B′, autophosphorylated for 3 - 4 min, was used. This effect was not observed in the case of autophosphory-

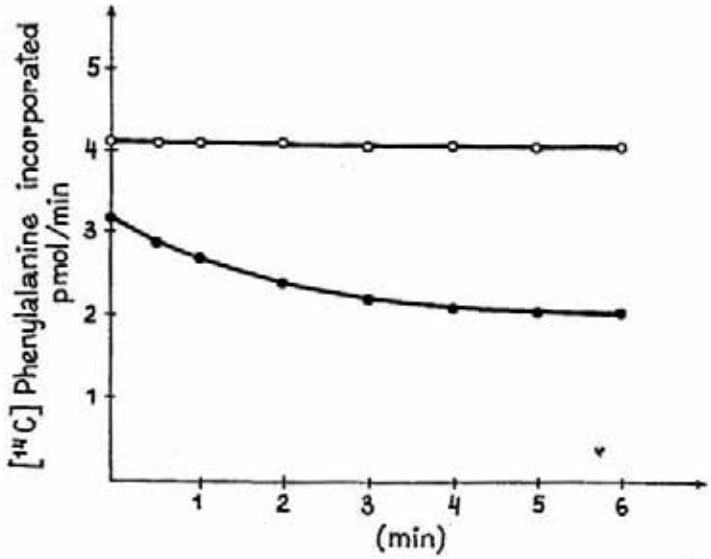
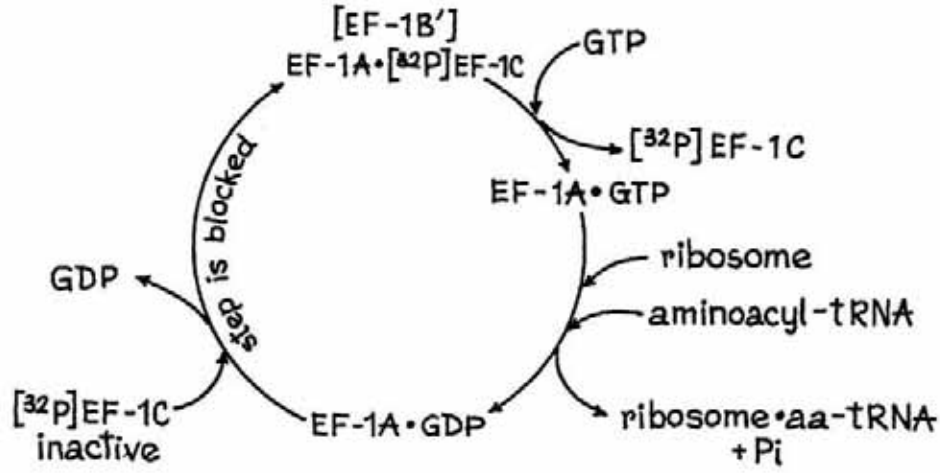


Fig. 2. Polyphenylalanine biosynthesis by the autophosphorylated fractions: ○, EF-1B; ●, EF-1B′. For the autophosphorylation, dephosphorylated fractions EF-1B (20 μg) and EF-1B′ (22 μg) were used. For the dephosphorylation see [10].

lated EF-1B, though one of EF-1B components was modified by phosphorylation. Autophosphorylation of EF-1B or EF-1B′ had no effect on [^{14}C]phenylalantyl-tRNA binding with ribosomes (not shown).

From the presented results it can be concluded that EF-1B and EF-1B′ isolated from Guerin epithelioma possess kinase activities. Probably, during EF-1B′ autophosphorylation, its subunit form, EF-1C, was modified and inactivated. Since [^{32}P]EF-1C is unable to form the EF-1A·[^{32}P]EF-1C active complex, making possible the interaction with GTP, the formation of the EF-1A·GTP complex was stopped (Scheme 1).



Scheme 1. Inhibitory effect of EF-1B′ autophosphorylation on the formation of the active EF-1A·GTP complex

From the present results it can be concluded that EF-1B and EF-1B′ isolated from Guerin epithelioma possess kinase activities. Probably, during EF-1B′ autophosphorylation, its subunit form, EF-1C, was modified and inactivated. Since [^{32}P]EF-1C is unable to form the EF-1A·[^{32}P]EF-1C active complex, making possible the interaction with GTP, the formation of the EF-1A·GTP complex was stopped (Scheme 1).

Janssen *et al.* [10] also found that phosphorylation of EF-1β decreased its activity in the nucleotide replacement.

It is a striking observation that phosphorylation of the 53 000 Da polypeptide, which is present in EF-1B fraction, does not influence the elongation process, though its molecular mass is similar to that of EF-1A. It seems possible that an unknown polypeptide was phosphorylated in fraction EF-1B, so that electrophoretic bands of this polypeptide and EF-1A were overlapping. Our assumption may be in agreement with the observation that molecular mass of the third subunit EF-1γ, is similar to/or identical with that of EF-1α [4, 17, 18]. EF-1γ does not affect directly protein biosynthesis and its role is not completely elucidated [2, 19]. We are

interested in the problem whether some analogy between the 53 000 Da protein from Guerin epithelioma and EF-1γ does exist.

Autoelectrographic pictures show (Plate 1) that both subunit forms, EF-1A and EF-1C, are unable to become autophosphorylated or to phosphorylate other polypeptides. This is in agreement with the suggestion of Janssen *et al.* [10] that protein kinase, phosphorylating EF-1β, is a protein integrally bound with the factor complex but is not the functional subunit of EF-1β.

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