Isolation and properties of the subunit form EF-1C of elongation factor 1 from Guerin epithelioma cells

Cezary Marcinkiewicz and Władysław Gałasiński

Department of General and Organic Chemistry, Institute of Chemistry, Medical Academy, 15–230 Białystok 8, Poland

EF-1C is a component of the aggregate EF-1B, consisting of the subunit forms EF-1A·EF-1C; it was isolated by dissociation of this aggregate in the presence of GTP. The subunit form EF-1C stimulates binding of aminoacyl-tRNA to ribosomes, catalysed by EF-1A, similarly as EF-1βγ which stimulates the activity of EF-1 in other eukaryotic cells.

EF-1C in the presence of 6 M urea was separated into two polypeptides. Polypeptide of molecular mass 32000 Da is responsible for regeneration of the EF-1A·GTP active complex. Thermal sensitivity of EF-1A was much higher than that of EF-1B, thus a protective role of EF-1C in the EF-1A·EF-1C complex is suggested.

The elongation factor EF-1, isolated from various eukaryotic cells, was resolved into two subunit forms EF-1α and EF-1βγ [1 - 4]. EF-1α is the main subunit which, in the complex with GTP, is responsible for binding of aminoacyl-tRNA to the acceptor site (A) on the ribosome. The subunit form EF-1βγ stimulates EF-1α activity by changing the inactive complex EF-1α·GDP into the active complex

EF-1α·GTP [5] in a three-step process:
- 1) EF-1α·GDP + EF-1βγ → EF-1α·GDP·EF-1βγ
- 2) EF-1α·GDP·EF-1βγ → EF-1α·EF-1βγ + GDP
- 3) EF-1α·EF-1βγ + GTP → EF-1α·GTP+ EF-1βγ

Another factor EF-1δ, which can catalyse the same process of exchange of GDP to GTP in the EF-1α·GDP complex has been also described [6, 7].

In the Guerin epithelioma cells the factor responsible for the nucleotide exchange was also found but its purification by the universally known methods proved unsatisfactory. The procedure of isolation of EF-1C from the EF-1A·EF-1C complex presented in this paper is based on dissociation of the subunit forms EF-1A·EF-1C in the presence of GTP by a modification of the method of Crecht *et al.* [2].

MATERIALS AND METHODS

The following steps of the procedure applied were performed as described in earlier papers: preparation of the ribosomes, [^{14}C]aminoacyl-tRNA and elongation factors [8], synthesis of [^{14}C]polyphenylalanine [9], binding of [^{14}C]phenylalanyl-tRNA to ribosomes [10], isolation of EF-1B [11].

Isolation of homogeneous EF-1C. Dissociation of EF-1B in the presence of GTP [2] was applied to isolate EF-1C. The EF-1B preparation [11], containing approx. 30 mg of protein, was dialysed for 6 h against 0.03 M Tris/HCl buffer, pH 8.0, containing 20% glycerol, 10 mM 2-mercaptoethanol and 100 mM KCl. Then GTP was added to the final concentration of 0.2 M and incubation was carried out for 30 min at 37°C. The incubation mixture was dialysed overnight against 0.03 M Tris/HCl, pH 8.0, con-

taining 20% glycerol, 10 mM MgCl₂, 50 mM KCl, 10 mM 2-mercaptoethanol and 0.05 M GTP. The dialysate was chromatographed on a DEAE-Sephadex A-50 column (10 × 1 cm) equilibrated with the same buffer. Elution was carried out with the use of the buffered solution containing increasing concentrations of KCl. Fractions of 2 ml were collected at the flow rate

of 30 ml/h; EF-1C was eluted with 0.35 M KCl (Fig. 1).

Isolation of polypeptides (32000 Da and 51000 Da) of the EF-1C preparation [4]. To the EF-1C fraction, obtained after GTP-dependent dissociation of EF-1B, urea was added to 6 M concentration and the mixture dialysed overnight against 50 mM Tris/HCl buffer, pH
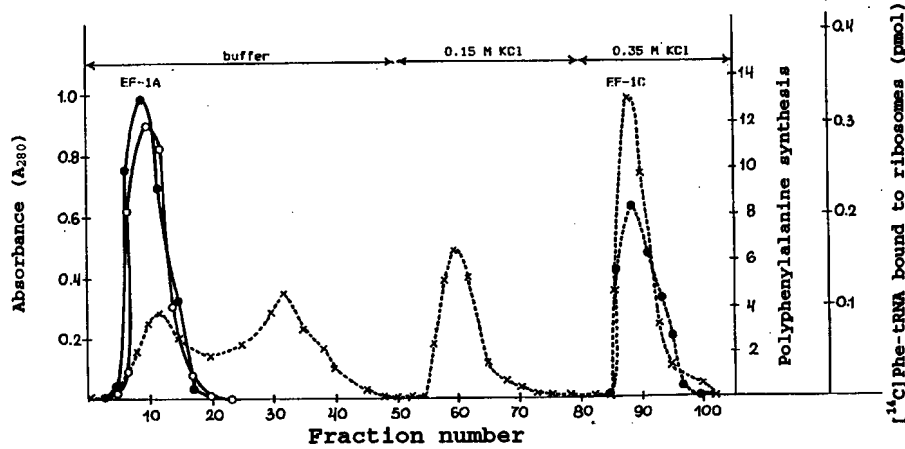

Fig. 1. Isolation of EF-1A and EF-1C on DEAE-Sephadex A-50 column after previous dissociation of EF-1B in the presence of GTP.

Activity of EF-1A in polyphenylalanine synthesis (●) and binding to ribosomes (○). Activity of EF-1C in stimulation of EF-1A in polyphenylalanine synthesis (●—●). Polyphenylalanine synthesis was carried out in the presence of poly(U) using 80 µl of each fraction and 5 µg of EF-1A
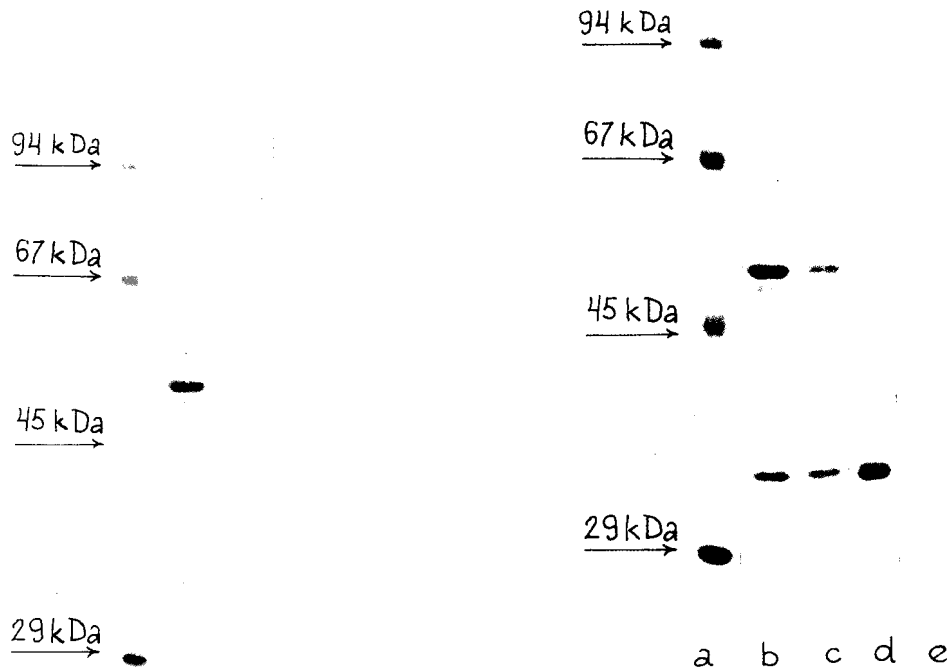

Fig. 2. Electrophoresis on SDS polyacrylamide gel [12] of the EF-1B preparation and EF-1A obtained after GTP-dependent dissociation of EF-1B.

a) Markers: phosphorylase b (M_r 94000), BSA (M_r 67000), egg albumin (M_r 45000), carbonic anhydrase (M_r 29000); b) EF-1B (15 µg of protein); c) EF-1A (10 µg of protein)

Fig. 3. Electrophoretic separation of the polypeptides of EF-1C on SDS-PAGE [12].

a) Markers (see Fig. 2); b) EF-1B (15 µg of protein); c) EF-1C (10 µg of protein); d) polypeptide of M_r 32000 (7 µg of protein); e) polypeptyde of M_r 51000 (8 µg of protein). For further explanation see the text
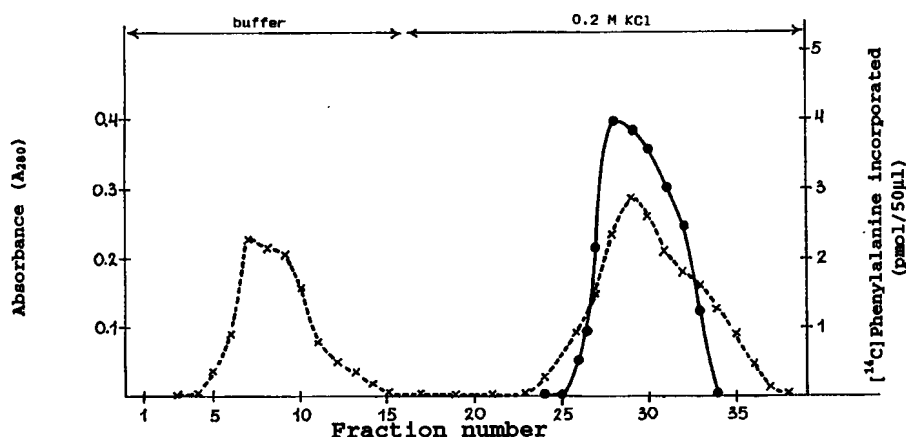
Fig. 4. The separation pattern of EF-1C polypeptides on DE-52 cellulose in the presence of 6 M urea.
(●) Stimulation assay of EF-1A activity in polyphenylalanine synthesis, by separated polypeptides of EF-1C;
(x) absorbance

8.0, containing 20 mM KCl, 6 M urea and 10 mM 2-mercaptoethanol. The dialysate was chromatographed on DE-52 cellulose column (5 × 1 cm) equilibrated and washed with the same buffer. Fractions of 1.5 ml were collected at the flow rate of 20 ml/h. The fractions not adsorbed on the column and eluted with 0.2 M KCl (these fractions contain EF-1C polypeptides, cf. Fig. 4) were dialysed against the buffer consisting of: 50 mM Tris/HCl, pH 8.0; 10% glycerol; 100 mM KCl and 10 mM 2-mercaptoethanol. The dialysates free of urea were concentrated and then characterized by SDS-PAGE (polyacrylamide gel electrophoresis with SDS [12], Fig. 3). The results were confirmed by separation of EF-1C components by IEF/SDS-PAGE (isoelectrofocusing/SDS-PAGE [13], Fig. 5).

The exchange of GDP to GTP in the EF-1A GDP complex. The exchange of nucleotides was carried out according to [3] with some modifications. The incubation mixture consisted of: 0.04 ml of the cocktail (5 ml of 0.2 M Tris/HCl, pH 7.5; 30 mg DTT; 2 ml 0.125 M Mg(CH3COO)2; 10 mg BSA; 2.5 ml glycerol; 2 mg GDP) and 10 μg EF-1A, and an adequate volume of bidistilled water to make the total volume of 0.1 ml.

The incubation was performed at 37°C for 5 min and then 0.7 ml of the mixture consisting of: 1.3 ml 0.2 M Tris/HCl, pH 7.5; 30 mg DTT; 0.6 ml 0.125 M Mg(CH3COO)2; 4.5 ml 0.2 M NH4Cl; 10 mg BSA; 0.95 ml glycerol; 2.6 ml H2O was added. To the obtained mixture, 0.02 ml (0.2 pmol) of [14C]GTP and bidistilled water
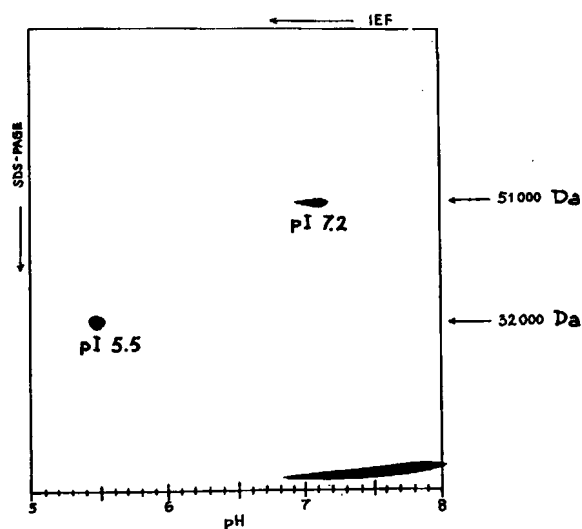

Fig. 5. Two-dimensional resolution of the polypeptides of EF-1C on IEF/SDS-PAGE [13].
I. Dimension: IEF isoelectrofocusing in polyacrylamide gel with the use of ampholine of pH 5 - 8; II. dimension: polyacrylamide gel electrophoresis in the presence of 0.1% SDS [12]. pI, isoelectric point

to make the total volume of 1 ml was added and incubation at 0°C for 3 min was carried out. The incubation was stopped by adding 5 ml of the cooled solution containing: 20 mM Tris/HCl, pH 7.5; 10 mM Mg(CH3COO)2; 0.1 mg/ml BSA; 25% glycerol and the mixture immediately filtered through nitrocellulose filters (Millipore, 0.45 μm). The filters were washed twice with 5 ml of the same buffered solution, but without glycerol added. The washed filters, containing EF-1A [14C]GTP, were dried and their radioactivity was measured in the PPO-
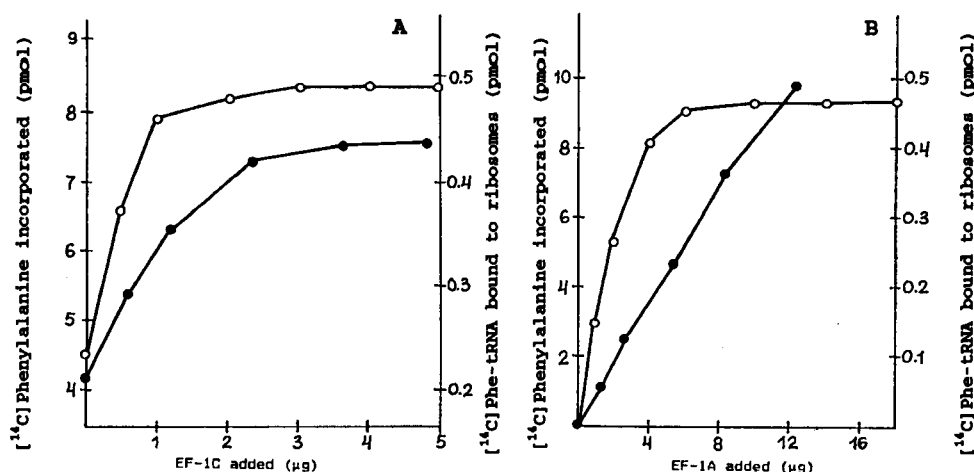
Fig. 6. The effect of EF-1C on EF-1A activity.
A. EF-1A constant (10 μg of protein). B. EF-1C constant (4 μg of protein). (●) Polyphenylalanine synthesis [9]. (O) [^{14}C]Phe-tRNA binding to ribosomes [11]

POPOP-toluene scintillation liquid on the Iso-cap-300 Nuclear Chicago counter.

Chemicals. 2-Mercaptoethanol was from Fluka A.G., DEAE-Sephadex A-50 from Pharmacia Fine Chemicals, DE-52 cellulose from Whatman, DDT (dithiothreitol) from Loba Chemie, Austria, BSA (bovine serum albumin) from Koch Light, GTP (guanosine-5'-triphosphate, Na salt), GDP (guanosine-5'-diphosphate, Na salt), ATP (adenosine-5'-triphosphate, Na salt), poly(U) and protein markers for electrophoresis from Sigma, ampholine from LKB, [^{14}C]phenylalanine (475 mCi/-mmol) and [^{14}C]GTP (575 mCi/mmol) from Amersham. All chemicals were of analytical grade and obtained from commercial sources.

RESULTS AND DISCUSSION

The elongation factors isolated from Guerin epithelioma and not adsorbed to the DEAE-Sephadex A-50 column, were active in polyphenylalanine synthesis and Phe-tRNA binding to ribosomes (Fig. 1, EF-1A). The combined and concentrated fractions (EF-1A) migrated on SDS-PAGE as a single protein band (Fig. 2c).

EF-1C eluted with 0.35 M KCl when applied alone was inactive in polyphenylalanine biosynthesis, but if it was added to the incubation mixture containing EF-1A, it stimulated distinctly the incorporation of [^{14}C]phenylalanine into the polypeptide (Fig. 1).
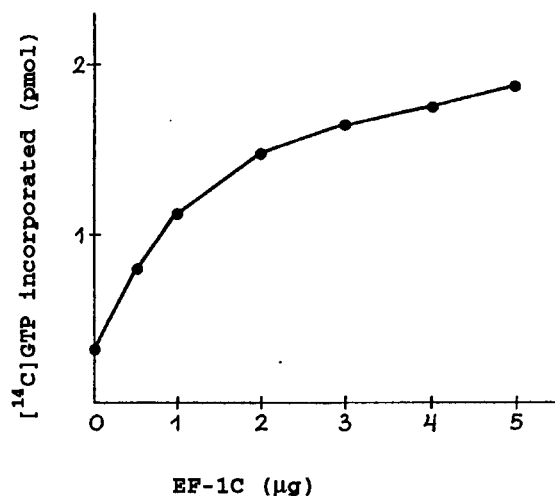

Fig. 7. The graph of GDP/GTP exchange in the EF-1A·GDP complex catalysed by increasing amounts of EF-1C.
The GDP/GTP exchange reaction was carried out according to [3]

We can suggest that EF-1C in Guerin epithelioma cells is present probably in aggregate with EF-1A. It can be supposed that the bulk of EF-1A molecules "running out" from the elongation cycle as the inactive EF-1A·GDP complex is "caught" immediately by EF-1C (at present free) according to the reaction:

EF-1A·GDP + EF-1C → EF-1A·EF-1C + GDP
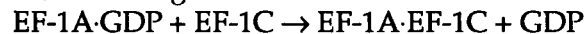
High tendency to aggregation of the subunit forms EF-1A and EF-1C of the elongation factor EF-1 from Guerin epithelioma suggests some analogies with the early developmental period
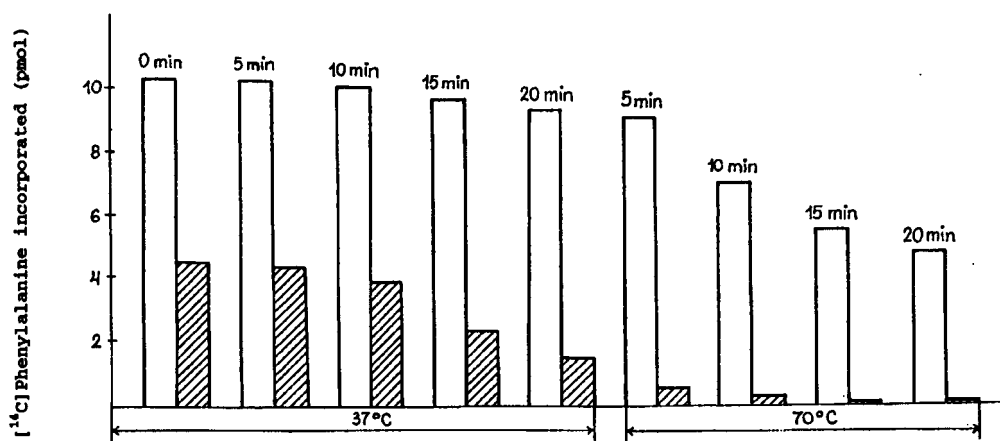
Fig. 8. Polyphenylalanine synthesis in the presence of thermally inactivated EF-1B (☐) and EF-1A (▧). Both factors were previously exposed to 37°C and then to 70°C.
Thermal inactivation of elongation factors was performed in the incubation mixture: 0.05 M Tris/HCl buffer, pH 8.0, containing 10 mM 2-mercaptoethanol and 25% glycerol. The samples (10 µg of protein) were collected from the incubation mixture at the indicated time intervals and their activity in polyphenylalanine synthesis was assayed [9]

of the lower eukaryotic organisms [14, 15]. In the larval stage of *Artemia salina* [14] and *Turbatrix aceti* [15] EF-1 exists as a high molecular complex EF-1H and, during the growth season, is transformed into the light form EF-1L (EF-1α). However, EF-1H differs from Guerin epithelioma EF-1 aggregates. EF-1H has low activity and it represents some kind of storage form of EF-1L, which can be activated only after separation from other proteins. EF-1 from Guerin epithelioma is highly active in all aggregate forms.

That part of EF-1A which was isolated in the free form [11] may exist in physiological excess with respect to EF-1C.

EF-1C from rat liver did not show any tendency to aggregation. In preliminary experiments (not shown) EF-1C was isolated from rat liver cells without the GTP-dependent dissociation of EF-1. It is possible that, in those cells, EF-1C can exist in the free form or it can be aggregated with other proteins. This idea was confirmed by reports from other laboratories working on calf brain [2], pig liver [16], rabbit reticulocytes [1], yeasts [3], *A. salina* [4] and wheat seeds [17].

The concentrated fraction EF-1C obtained after dissociation of the EF-1B preparation and chromatography on DEAE-Sephadex A-50, was separated into two polypeptides (Fig. 3c). After reversible denaturation with 6 M urea [1, 4] these two peptides were separated by chro-

matography on DE-52 cellulose (Fig. 4). The first fraction, not adsorbed onto the column, after renaturation did not show any EF-1C activity, while the second fraction, eluted with 0.2 M KCl, distinctly stimulated EF-1A activity. Both fractions behaved as single homogeneous polypeptides: the first of 32000 Da (Fig. 3d) may be a homologue of EF-1β, while the second of 51000 Da (Fig. 3e), could correspond to EF-1γ of other eukaryotic cells. These results were confirmed (Fig. 5) by two-dimensional IEF/SDS-PAGE [13].

The stimulating effect of EF-1C on the phenylalanyl-tRNA binding to ribosomes and on polyphenylalanine synthesis is shown in Fig. 6. When the content of EF-1C in the incubation mixture was increased (Fig. 6A), both phenylalanyl-tRNA binding to ribosomes and polyphenylalanine synthesis run faster. The increase of the amount of EF-1A in the mixture accelerated distinctly only the initial rate of [^{14}C]Phe-tRNA binding to the ribosomes, while polyphenylalanine synthesis was linearly dependent on EF-1A concentration (Fig. 6B).

The participation of EF-1C in the exchange of GDP to GTP in the EF-1A · GDP complex is presented in Fig. 7.

The amount of [^{14}C]GTP in the active EF-1A·GTP complex was increased when EF-1C concentration in the reaction mixture was raised. The polypeptide of 32000 Da showed similar properties while the polypeptide of

51 000 Da was inactive in these conditions. The hydrophobic character of the latter polypeptide was evidenced and is in agreement with the results described by Janssen & Möller [4].

Fraction EF-1B, isolated from Guerin epithelioma [11] was thermostable (Fig. 8). The EF-1B preparation preserved approximately 50% of its activity in polyphenylalanine synthesis after 20 min incubation at 70°C, while EF-1A was quickly inactivated even when the temperatures were lower. Similarly, Slobin & Möller [18] 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. These observations could suggest that EF-1C protects EF-1A against thermal inactivation. It would be interesting to know whether the polypeptide γ, the function of which is till now unknown, is responsible for the thermostability of EF-1C.

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