*Phytochemistry*, 1976, Vol. 15, pp. 1607–1610. Pergamon Press. Printed in England.

# DIFFERENT FORMS OF EF1 AND VIABILITY IN WHEAT EMBRYOS

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(*Revised received* 12 *May* 1976)

**Key Word Index**—*Triticum durum*; wheat; regulation of protein synthesis at translation level; EF1 forms.

**Abstract**—Properties of heterogeneous forms of elongation factor (EF1) from cytoplasm of wheat embryos at different viability levels have been studied. The GTP-binding activity, the catalysis of the binding of Phe-[$^{14}$C]-*t*RNA to ribosomes and the complementation of EF2 for the synthesis of poly-U directed poly-Phe indicate that modifications of the EF1 activity occur as a result of seed ageing. The presence of a strong translational inhibitor was indicated in the cytoplasm of low viability embryos.

## INTRODUCTION

Wheat seeds, during the early stages of germination, synthesize proteins before any new synthesized RNA can be detected [1]. Previous studies demonstrated that protein synthesis becomes appreciable within 30 min of water imbibition [2], whereas new RNA and DNA are both synthesized later [3,4]. For this reason and since functioning *m*RNA has been isolated from dry seeds [5,6], long-lived *m*RNA, synthesized during late embryogenesis, is thought to be responsible for the early protein synthesis during germination.

A modification of the translational apparatus might be the reason for the differential translation of these long-lived *m*RNAs during maturation and germination of seeds. This modification can involve either ribosomal proteins (i.e. by phosphorylation [7]) or the translational soluble factors. The complexity and the molecular heterogeneity of EF1 from dormant wheat embryos [8–11] can be due to its predisposition to being changed. In fact it has been demonstrated that in order to function, EF1 from dormant wheat embryos has to be modified [10]. One of the facets of the ageing processes in wheat germs can be at the level of the occurrence of incorrect modifications of EF1. In rye embryos Roberts and Osborne [12] also demonstrated a correlation between the EF1 properties and the ageing phenomena.

In this paper we describe some molecular and functional properties of EF1 from wheat embryos at different ageing levels.

## RESULTS

Since the elongation factor EF1 from wheat embryo cytoplasm is heterogeneous with respect to molecular size [8–11], we have studied the elution patterns on a Sephadex G-150 column of EF1 preparations from wheat embryos at different viability (V), levels (85%–69%–48%–35% seed germination).

We first assayed EF1 by its ability to form the binary complex EF1–GTP. The GTP-binding activity from cytoplasm of embryos with 85% viability (85% V) [(85% germs)] was eluted in two different peaks: peak A and B (Fig. 1a). Peak A (fractions 80–110) (aggregated form of EF1) has an elution volume between those of blue dextran and catalase; peak B (fractions 110–170) corresponds to heterogeneous forms having elution volumes between those of catalase and serum albumin. Cytoplasm from 69% [$\mu$]V embryos shows a slightly different profile (Fig. 1b); the amount of activity contained in peak A diminishes and the elution position of peak B shifts towards that of smaller molecular sizes. Cytoplasm from 48% V embryos hardly contains EF1 activity in the elution zone of peak A (Fig. 1c), and aggregated EF1 has probably been nearly totally dissociated to forms of smaller MW. The cytoplasm from 35% V embryos (Fig. 1d), shows no GTP-binding activity in the A zone, and also the activity eluted between catalase and serum albumin is very disperse and without a distinct maximum. We examined the different GTP-binding peaks, obtained by gel-filtration, with regard to the other EF1 functions: catalysis of the binding of Phe-*t*RNA to ribosomes and complementation of EF2 for the synthesis of poly-U directed poly-phenylalanine. Thus we have precipitated at 80% $(NH_4)_2SO_4$ either peak A or peak B of Fig. 1a and Fig. 1c; peak B being divided into three parts $B_1$, $B_2$ and $B_3$.

Table 1 reports the comparison of the Phe-*t*RNA bound to ribosomes by the different fractions and their GTP-binding activities. The ratio: Phe-*t*RNA binding to ribosomes activity/GTP-linking activity contained in peak $B_2$ from 85% V embryos is extremely high in contrast to the very low value of this ratio for the corresponding $B_2$ from 35% V embryos. Peak A appears to contain both EF1 and EF2 activities (Table 1) because it can form poly-Phe without addition of EF2. This fact partly explains why only a small amount of Phe-*t*RNA was bound to ribosomes by peak A (Table 1); a substantial portion of the bound Phe-*t*RNA was transformed

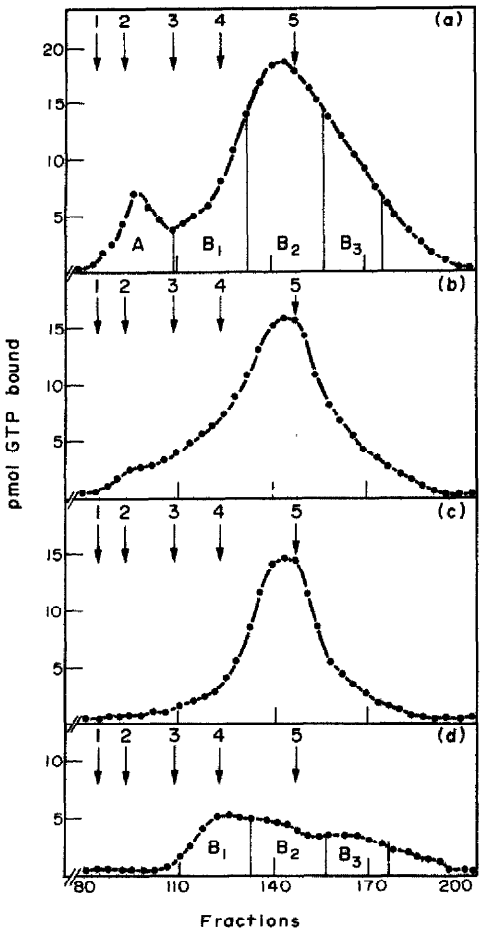


Fig. 1. Sephadex G-150 gel-filtration of 100000 *g* supernatant from embryos of wheat seeds at different viability levels, 85% germination (a), 69% germination (b), 45% germination (c), and 35% germination (d). The profile represent pmol of GTP bound per mg of protein determined (described in Experimental). The elution of blue-dextran (arrow 1), ferritin (arrow 2) catalase (arrow 3), aldolase (arrow 4) and serum albumin (arrow 5) was determined in separated runs.

to poly-Phe. Peak $B_2$ from 85% V embryos which shows the greatest capacity to catalyze Phe-*t*RNA binding to ribosomes, is also the most active in complementing EF2 for poly-Phe synthesis. In contrast, $B_2$ from 35% V embryos, has nearly lost the capacity to complement EF2.

Table 1. Comparison of the Phe-[$^{14}$C]-*t*RNA binding to ribosomes, and GTP-[$^{3}$H] linking activities of the various peaks from Sephadex G-150 gel-filtration from embryos of 85% and 35% viability

| Sephadex G-150 peak (100 μg protein) | GTP-[$^{3}$H] linked (pmol) | Phe-[$^{14}$C]-*t*RNA bound to ribosomes (pmol) |
|---|---|---|
| A 85% viability | 12.3 | 1.2 |
| $B_1$ | 17.9 | 8.1 |
| $B_2$ | 12.3 | 32.8 |
| $B_3$ | 8.3 | 9.1 |
| $B_1$ 35% viability | 6.5 | 9.9 |
| $B_2$ | 8.1 | 2.6 |
| $B_3$ | 5.1 | 5.4 |

Table 2. Comparison of the ability of the different Sephadex G-150 peaks from embryos of 85% and 35% viability to complement EF2 for the poly-Phe synthesis

| Sephadex G-150 peak (100 μg protein) | EF2 (10 μg) | Poly-Phe formed (pmol) |
|---|---|---|
| A 85% viability | – | 37.2 |
| A | + | 36.8 |
| $B_1$ | – | 0.9 |
| $B_1$ | + | 1.1 |
| $B_2$ | – | 0.7 |
| $B_2$ | + | 42.8 |
| $B_3$ | – | 0.7 |
| $B_3$ | + | 6.4 |
| $B_1$ 35% viability | – | 0.6 |
| $B_1$ | + | 0.7 |
| $B_2$ | – | 0.7 |
| $B_2$ | + | 3.1 |
| $B_3$ | – | 0.5 |
| $B_3$ | + | 1.2 |

In order to get a better insight into the differences between peaks $B_2$ from 85% and 35% V embryos, we further purified them by means of hydroxyapatite chromatographies. In Fig. 2a the profile of GTP-binding activities of $B_2$ from 85% V embryos is represented. Three peaks of activity (I, II, III) are separated. As one can see in Fig. 2b a more complicated profile of GTP-binding activities is obtained by chromatography of peak $B_2$ from 35% V embryos: a long trail follows peak I, and peak II is preceded by peak IIa. The ability of the different fractions to catalyse the binding of labelled Phe-*t*RNA to ribosomes was compared with their GTP-binding activity (Table 3); only peak II from 85% V embryos is able to catalyse the binding of Phe-*t*RNA to ribosomes, whereas no peak from 35% V embryos was active.

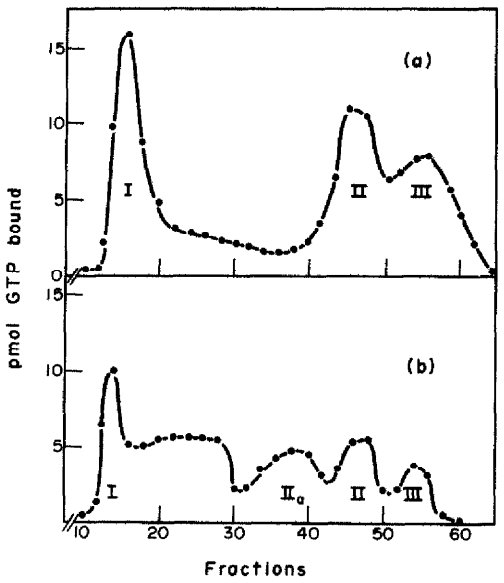


Fig. 2. Hydroxy apatite chromatography of $B_2$ fractions from Sephadex G-150 gel-filtration column of cytoplasm from embryos of (a) 85% viability and (b) 35% viability. The profiles represent the pmols of GTP bound per 100 μg protein determined.

Table 3. Comparison of the Phe-[$^{14}$C]*t*RNA binding to ribosomes, and GTP-[$^{3}$H] linking activities of the different hydroxyapatite chromatography peaks from embryos of 85% and 35% viability

| Hydroxyapatite peak (10 μg protein) | GTP-[$^{3}$H] linked (pmol) | Phe-[$^{14}$C]-*t*RNA bound to ribosomes (pmol) |
|---|---|---|
| I 85% viability | 8.3 | 0.2 |
| II | 7.1 | 58.9 |
| III | 6.5 | 0.3 |
| I 35% viability | 4.1 | 0.2 |
| IIa | 3.2 | 0.1 |
| II | 3.5 | 0.7 |
| III | 2.1 | 0.1 |

Table 4. Comparison of the influence of the different hydroxyapatite peaks from embryos of 85% and 35% viability on the poly-Phe synthesis

| Hydroxyapatite peak (10 μg protein) | EF2 (10 μg) | $EF1_R$ (10 μg) | Poly-Phe formed (pmol) |
|---|---|---|---|
| I (85% viability | + | − | 0.2 |
| I | + | + | 60.2 |
| II | + | − | 60.1 |
| II | + | + | 60.1 |
| III | + | − | 0.1 |
| III | + | + | 21.5 |
| I 35% viability | + | − | 0.2 |
| I | + | + | 61.2 |
| IIa | + | − | 0.1 |
| IIa | + | + | 0.2 |
| II | + | − | 3.1 |
| II | + | + | 40.3 |
| III | + | − | 0.1 |
| III | + | + | 26.3 |

We have further compared the influence of the different hydroxyapatite fractions on the poly-Phe synthesis either in the presence of purified EF2 or in the presence of a complete system, including a highly purified preparation of $EF1_R$ from viable wheat embryos [16] (Table 4). It is evident that only one of the peaks of GTP-binding activity from 85% embryos, i.e. peak II, is able to complement EF2. The peak I of the same germs does not influence the poly-Phe synthesis in the complete system, whereas the peak III partially inhibits it. As far as peaks from 35% V embryos are concerned, peak I does not influence the poly-Phe synthesis; peak IIa, behaved as strong inhibitor, in that it inhibited at all poly-Phe formation when added to the complete $EF1_R$–EF2 system. Peak II neither activates nor inhibits poly-Phe synthesis while peak III acts as a partial inhibitor, like the peak III from 85% germs.

## DISCUSSION

Remarkable modifications are observed in the elution patterns on Sephadex G-150 of the GTP-binding activity from the cytoplasm of embryos of different degrees of viability. Peak A diminishes in 69% V embryos and disappears completely in 35% embryos. From its elution position from Sephadex G-150 (Fig. 1a) it is clear that in peak A high MW proteins are present. As far as the translational functions contained in this peak are concerned, both EF1 and EF2 activities were found in it (Table 2). The two functions EF1 and EF2 in peak A are strongly linked to a supporting lipoprotein and are separated only with difficulty from this aggregate [13]. Other authors have also found that aggregated forms of EF1 from calf brain and from wheat embryos contain phospholipids [14]; a complex containing linked forms of EF1 and EF2 has been found in wheat embryos by Twardoski and Legocki [15]. The fact now observed by us that this aggregated form of EF1 is not present in the aged embryos indicates that during the ageing process some change of this aggregation occurred.

In contrast to the less damaged embryos, the cytoplasm from embryos of the extreme degree of germination (35% V embryos) contains GTP-binding activities, which are very heterogeneously eluted from Sephadex G-150; the lack of a distinct peak B (Fig. 1d), as in the profiles of the GTP-binding activities from the other types of embryos, indicates that this activity is associated in proteins of very different MWs. As we demonstrated [11] $EF1_H$ from wheat embryos is an association of three different polypeptides (polypeptide A (MW 52000), B (MW 47000) and C (MW 27000). Distinct molecular species formed by association of a common polypeptide (polypeptide A) with different polypeptides have been purified from cytoplasm ($EF1_H$) and from ribosomes ($EF1_R$) [16].

The existence of a large heterogeneity in the GTP-binding activities from 35% V embryos, as demonstrated by the flat elution profile of Fig. 1d can be caused by a disassociation and a disordered reassociation of the polypeptides composing $EF1_H$ and $EF1_R$, during the ageing process. The hydroxyapatite fractions from peak $B_2$ of 35% V embryos, though having GTP-binding activity, show very low Phe-*t*RNA binding activity and are not able to complement EF2 for the poly-Phe synthesis.

This fact might be due to the presence of inhibitors of poly-Phe synthesis in these fractions. Indeed from the hydroxyapatite chromatography of 35% V embryos peak B, a fraction containing a strong inhibitor of polypeptide synthesis was separated, which does not exist in peak B from 85% V embryos. We demonstrated [17] that the disassociation of $EF1_H$ gives rise to polypeptides, which inhibit the poly-Phe synthesis; peak IIa from hydroxyapatite chromatography of 35% V embryos peak B might contain these polypeptides deriving from the dissociation of active $EF1_H$.

We believe that the dynamics of associations and dissociations of the peptides composing $EF1_H$ and $EF1_R$ is part of the process of modification of the translational machinery, which were hypothesized to occur for regulation in wheat embryos. Some data seem to indicate that these modifications are regulated by the level of cyclic nucleotides [18]. We postulate that the effects of ageing on the molecular properties of EF1, are the result of an incomplete and disordered occurrence of these changes.

## EXPERIMENTAL

Stocks of wheat seeds at various percentage-viability levels (*Triticum durum* cv Lamia, 1974 harvest) were obtained by storage at moisture contents between 15–12% and at 38°, according to the method of Ref. [19]. In these conditions the percentage of viability (V) decreased from 90 to 20% within 35 days. Embryos used in these experiments were prepared by mass-isolation technique of Ref. [20].

*Preparation of post-ribosomal supernatant.* 200–230 g dry embryos were homogenized with 500 ml buffer A (0.25 M sucrose, 50 mM Tris–HCl, 10 mM $MgCl_2$, 25 mM KCl, 5 mM 2-mercaptoethanol, pH 7.5) 3 × for 2 min at low speed. The homogenate was centrifuged 2 × for 30 min at 12000 *g*. Combined supernatants were centrifuged for 3 hr at 100000 *g*. The 100000 *g* supernatant (250 ml) was passed through a Sephadex G-25 column (80 × 6 cm), conditioned with 10 mM Tris–HCl and 1 mM 2-mercaptoethanol. The protein peak, eluted in the void vol., was precipitated by 56.1 g of $(NH_4)_2SO_4$ per 100 ml soln. After centrifugation at 12000 *g* for 20 min, the pellet was dissolved in 50 ml of 20 mM Tris, 1 mM 2-mercaptoethanol, 8 mM $MgCl_2$, 200 mM KCl buffer, pH 7.5 (buffer B), and dialyzed for 18 hr against buffer B; this constituted the dialyzed post-ribosomal supernatant (DPRS).

*Sephadex G-150 gel-filtration.* 70 ml DPRS from embryos of the various viabilities, indicated in Fig. 1, and containing similar amounts of protein (from 550 to 600 mg) were put on a Sephadex G-150 column (80 × 10 cm), conditioned with buffer B. The same buffer B was used for the elution. Fractions of 15 ml were collected.

*Hydroxyapatite chromatography.* Tubes containing GTP-binding activity from Sephadex G-150 gel-filtration were pooled as indicated in the Fig. 1, precipitated at 80% $(NH_4)_2SO_4$ saturation and the ppt from each peak dissolved in 10 ml of a 50-mM KPi, 1 mM dithiothreitol buffer, pH 7.5 (buffer C), and dialyzed for 18 hr against buffer C. 12 ml of this soln of peaks $B_2$ from 85 and 35% V embryos (130 and 100 mg protein respectively) were passed through a hydroxyapatite column (20 × 2 cm), conditioned with buffer C, and eluted with a linear gradient (formed with 150 ml of a buffer C and 150 ml of 300 mM KPi, 1 mM dithiothreitol buffer, pH 7.5). Fractions of 5 ml were collected.

*Preparation of DEAE chromatographed ribosomes (DEAE ribosomes).* Ribosomes completely free from elongation factors were obtained by chromatography of low salt washed ribosomes on a DEAE–cellulose column as already described [21].

*Preparation of $EF1_R$.* Preparation of electrophoretically homogeneous $EF1_R$ from wheat embryos ribosomes was made as described [16].

*Phe-[$^{14}$C]-tRNA preparation.* Phe-*t*RNA was prepared from partially purified $tRNA^{Phe}$ from wheat germ by the method of Ref. [22]. The product contained 471 pmol of Phe-[$^{14}$C]-*t*RNA per $A_{260\,nm}$ unit.

*Preparation of EF2.* The elongation factor EF2 was purified according to the method of Ref. [23].

*EF1 activity* was measured either as GTP-[$^3$H]-binding activity, or as Phe-[$^{14}$C]-*t*RNA binding to DEAE-ribosomes. The GTP-[$^3$H]-binding activities were measured in 0.1 ml reaction mixtures containing: 10 mM Tris–HCl, pH 7.8; 10 mM MgCl; 50 mM $NH_4Cl$; 2 mM 2-mercaptoethanol; $2 \times 10^{-6}$ M, GTP-[$^3$H] (sp. act. 1000 Ci/mol) and 100 $\mu$l of each chromatographic fraction. After 5 min incubation at 0°, the tests were diluted with 3 ml cold diluting buffer (10 mM Tris–HCl/10 mM $MgCl_2$ 50 mM $NH_4Cl$, pH 7.8) and filtered on nitrocellulose. Filters were washed 4 × with 5 ml of diluting buffer, dried and counted. The binding of Phe-[$^{14}$C]-*t*RNA to the ribosomes was measured by the method of Ref. [24], at 7.5 mM $Mg^{2+}$ conc, at which the nonenzymic binding was negligible [21]. The 0.1 ml reaction mixtures contained: 50 mM Tris–HCl pH 7.8, 7.5 mM $MgCl_2$, 25 mM KCl, 2 mM 2-mercaptoethanol, 0.5 mM GTP, poly (U), (0.02 $\mu$mol P), 0.2 $A_{260\,nm}$ units of Phe-[$^{14}$C]-*t*RNA, 3 $A_{260\,nm}$ units of DEAE-ribosomes, and the amounts of peaks indicated in the Table. After incubation at 32° for 20 min, the tests were added to 2 ml of diluting buffer (10 mM Tris–HCl pH 7.8/7.5 mM $MgCl_2$/25 mM KCl) at 0°, passed through nitrocellulose filters and washed 3 × with 2 ml of diluting buffer. The filters were dried and counted. One unit of EF1 is defined as the amount of enzyme which binds 1 pmol of Phe-*t*RNA to DEAE ribosomes in 30 min. All the quantitative data of EF1 unities in the result are reported as Phe-[$^{14}$C]-*t*RNA binding to DEAE-ribosomes.

*Poly (U) directed poly (Phe) synthesis.* The reaction mixture (0.1 ml) contained: 50 mM Tris–HCl pH 7.8, 7.5 mM $MgCl_2$, 25 mM KCl, 2 mM 2-mercaptoethanol, $10^{-3}$ M ATP, 0.5 × $10^{-3}$ M GTP, poly (U) (0.02 $\mu$mol P), 0.2 $A_{260\,nm}$ units of ribosomes, 10 $\mu$g of purified EF2 and either 10 $\mu$g of $EF1_R$ or the amounts of peaks indicated in the Tables. After incubation at 37° for 30 min, the hot 5% TCA-insoluble material was collected on nitrocellulose filters; the filters were washed ×4 with 5 ml of 5% TCA dried and counted.

*Protein concentration* was determined utilizing the method of Ref. [25] using crystalline bovine serum albumin as a standard.

*Acknowledgements*—We should like to thank Dr. G. Savino for supplying wheat stocks and for valuable support.

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