THE TRANSLATIONAL SYSTEM FROM WHEAT EMBRYOS: SOME PROPERTIES OF THE POLYPEPTIDES ASSOCIATED IN $EF1_H$

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1. Introduction

The elongation factor EF1 is present in wheat embryos as heterogeneous forms of different mol. wts. [1–3]. We purified from wheat embryo cytoplasm a high molecular weight form of EF1 ($EF1_H$), and demonstrated that it is an aggregate of three polypeptides A, B and C [4]. During its functioning as elongation factor in peptide synthesis, the associated form disaggregates and only peptide A is found in the ternary complex (aminoacyl-tRNA·GTP·EF1) [3]. A type of EF1 ($EF1_R$), different from the cytoplasmic $EF1_H$, has been found to be linked to the ribosomes [5,6]; $EF1_H$ and $EF1_R$ resulted from associations of different polypeptides having in common only peptide A [6]. Whether these associations have a functional meaning or represent only different storage forms of EF1 is difficult to assess; a better insight into the nature and the functions of the different peptides which accompany A, either in $EF1_H$ or in $EF1_R$, could throw light on this question.

As we report in this letter, we could separate from cytoplasmic $EF1_H$ the polypeptides B, C, completely free from A. We describe some properties of B, C which seem to indicate a possible regulatory function of this portion of $EF1_H$.

2. Materials and methods

Viable wheat embryos were prepared according to the method of Johnston and Stern [7]. The elongation factor $EF1_H$ electrophoretically homogeneous was purified from wheat embryo postribosomal supernatant as already described [4]. EF2 was purified from the same source according to the method of Twardowski and Legocki [8]. [^{14}C]Phe-tRNA was prepared from partially purified $tRNA^{Phe}$ from wheat germ by the method of Vold and Sypherd [9]; the product contained 325 pmol of [^{14}C]Phe-tRNA per A_{260} unit. Low salt washed ribosomes were prepared from wheat embryos as already described [6] DEAE-chromatographed ribosomes were obtained by chromatography of low salt washed ribosomes on a DEAE-cellulose column, as already described [10]. Polyribonucleotide binding assay was effected in 0.1 ml mixtures, containing 20 mM Tris-HCl, pH 7.4, 50 mM KCl, 1 mM dithiothreitol, 0.2 A_{260} units of [^{3}H]poly(U) or [^{3}H]poly(A) (4000 c.p.m.), and the amounts of $EF1_H$ specified in table 1 or of chromatographic fractions indicated in fig.1. After incubation at 23°C for 5 min, reactions were stopped by dilution with 4 ml of buffer solution (20 mM Tris-HCl, pH 7.4; 50 mM KCl); mixtures were then filtered at 23°C on nitrocellulose filters which were washed 5 times with 4 ml of diluting buffer at 23°C, dried and counted. [^{3}H]GTP binding assay. 0.1 ml reaction mixtures contained: 10 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$; 50 mM NH_4Cl; 2 mM 2-mercaptoethanol; 2×10^{-5} M [^{3}H]GTP (spec. act. 5000 Ci/mole) and aliquots of chromatographic fractions of EF1 as described in the legend to fig.1. After 5 min at 0°C, the tests were diluted with 3 ml of ice-cold diluting buffer (10 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$; 50 mM NH_4Cl) and filtered on nitrocellulose filters. Filters were washed 5 times with 5 ml of diluting buffer, dried and counted. Protein concentration was determined according to the method of Lowry et al. [11].

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Table 1
Polynucleotide binding activity of $EF1_H$

$EF1_H$	Incubation conditions [^{3}H]poly(U)	[^{3}H]poly(A)	c.p.m. bound to nitrocellulose filters
–	+	–	110
–	–	+	98
+	+	–	1,190
+	–	+	180

The experimental conditions were as described for polynucleotide binding assay in Materials and methods section; 5 μg of purified $EF1_H$ and 4000 c.p.m. (0.2 A_{260} units) of labeled polynucleotides were used.

[^{14}C]Phenylalanine and [^{3}H]GTP were purchased from NEN; [^{3}H]poly(U), [^{3}H]poly(A), poly(U) and poly(A) from Miles; ATP and GTP from Boehringer; acrylamide, *N,N,N,N*-tetramethylendiamine and ammonium persulphate from Bio-Rad; Sephadex G-200 and G-25 from Pharmacia; DEAE-cellulose (DE23) from Whatmann; hydroxylapatite from Serva. The nitrocellulose filters were Millipore H.A.W.P., 0.45 μm pore diameter; the other chemicals were analytical grade from Merck.

3. Results

Besides its already known functions, we found that $EF1_H$ is able to form a complex with [^{3}H]poly(U), which is retained on nitrocellulose filters at 23°C (table 1). We performed the incubation at 23°C in order to eliminate non-specific aggregations, which may occur at 0°C [12]. The formation of this complex seems rather specific, since under the same conditions only very small amounts of [^{3}H]poly(A) were bound by $EF1_H$ (table 1).

To explore whether the poly(U) binding activity could be separated from the known property of $EF1_H$ of forming a complex with GTP, we chromatographed

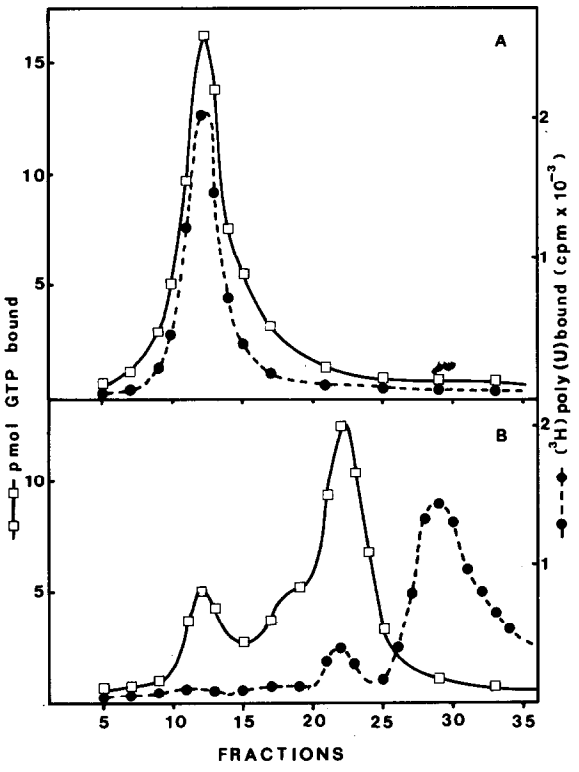

Fig.1. Hydroxylapatite column chromatography of wheat embryo $EF1_H$. (A) 10 mg of purified $EF1_H$ in 0.5 ml of buffer A (50 mM potassium phosphate, 1 mM dithiothreitol, pH 7.2) were applied to a hydroxylapatite column (1.5 × 20 cm), conditioned with buffer A. Elution was performed with a linear gradient formed by 100 ml of buffer A and 100 ml of buffer B (300 mM potassium phosphate, 1 mM dithiothreitol, pH 7.2). Fractions of 3 ml were collected; [^{3}H]GTP binding and [^{3}H]poly(U) binding were tested with aliquots of each fraction (20 μl and 80 μl respectively), according to the methods described in the Materials and methods section. (B) 10 mg of purified $EF1_H$ were incubated in 0.5 ml of buffer A containing 10^{-3} M GTP at 30°C for 2 minutes, applied to the same column as in (A), equilibrated in buffer A plus 10^{-4} M GTP. The elution was performed as in (A), except that buffer A and B were made 10^{-4} M in GTP. The fractions were collected and assayed as in (A).

$EF1_H$ on a hydroxylapatite column. The two functions of $EF1_H$ were eluted as coincident peaks and the curves representing the two activities were symmetrical (fig.1 A). Also a gel filtration on Sephadex G-200 and a chromatography on a DEAE-cellulose column gave similar results (data not shown). It has been reported that $EF1_H$, incubated with GTP, dissociates into molecular species of lower molecular size [1]. We chromatographed on the same hydroxylapatite column as for untreated $EF1_H$ a sample of $EF1_H$ which had been incubated in a 10^{-3} M GTP solution (fig.1 B): a first peak containing only GTP binding activity, a second peak containing both functions and a third peak containing only poly(U) binding activity were obtained. As demonstrated by SDS polyacrylamide disc gel electrophoresis, the third peak contained only two peptides (fig.2), which were identified as B and C by comparison of their mobilities with those of the peptides A, B and C obtained from SDS-treated $EF1_H$ and with markers of known molecular weights. From these data we deduce that the ability to bind GTP pertains only to polypeptide A; this fact is in agreement with the observation that only peptide A forms the ternary complex aminoacyl-tRNA·GTP·EF1 [4]. Since we could not separate peptide B from C, we are not able to decide if the ability to bind poly(U) is a property of one of the two polypeptides B and C, of both, or of a dimer B–C.

The influence on poly(U) directed poly-Phe synthesis of the moiety B, C of $EF1_H$ was tested by adding a preparation of B, C to incorporation mixtures containing $EF1_R$, in which peptide A is associated with peptides different from B, C [6]. As is shown in table 2, 10 μg per test of B, C inhibited poly-Phe synthesis nearly completely when DEAE-chromatographed ribosomes were used, whereas in the presence of low salt washed ribosomes no inhibition of peptide synthesis was observed; the inhibitory effect

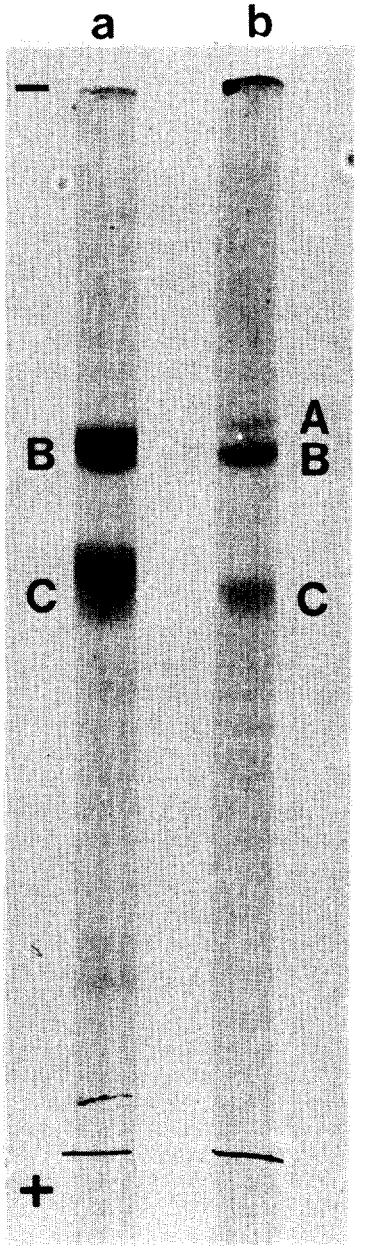

Fig.2. SDS polyacrylamide disc gel electrophoresis (a) the fractions 25–35 forming the peak III of hydroxylapatite column chromatography (fig.1 B) were pooled and precipitated at 80% ammonium sulphate saturation; the pellet obtained by centrifugation at 9000 *g* for 20 minutes was dissolved in 0.5 ml of 50 mM potassium phosphate, 1 mM dithiothreitol, pH 7.2 buffer. 50 μl of the solution containing 115 μg of protein were used for the electrophoresis. (b) 50 μl of an $EF1_H$ solution containing 20 μg of protein were used. The polypeptide composition was determined by electrophoresis according to the method of Weber and Osborn [14]. The protein solutions were brought to 1% sodium dodecyl sulphate and 1% 2-mercaptoethanol, incubated at 37°C for 2 h, applied to 10% polyacrylamide gels (0.5 × 9.5 cm) and electrophoresed in the presence of 0.1% sodium dodecylsulphate. Gels were run at 2 mA/tube at 13°C, until Bromophenol blue reached the bottom of the gel. Gels were stained with Coomassie Blue in 1% trichloroacetic acid.

Table 2
Effect of B, C on polyphenylalanine synthesis

Type of ribosomes	poly(U) (μg)	B, C	polyPhe synthetized (pmoles)
low salt washed ribosomes	15	–	11.3
low salt washed ribosomes	15	+	12.7
low salt washed ribosomes	60	+	12.6
DEAE-chromatographed ribosomes	15	–	10.4
DEAE-chromatographed ribosomes	15	+	0.2
DEAE-chromatographed ribosomes	30	+	6.1
DEAE-chromatographed ribosomes	60	+	12.4

0.1 ml mixtures contained: 50 mM Tris-HCl pH 7.8, 7.5 mM $MgCl_2$, 25 mM KCl, 2 mM dithiothreitol, 10^{-3} M ATP, 0.5×10^{-3} M GTP, 0.2 A_{260} units of [^{14}C] Phe-tRNA 10 μg of $EF1_R$, purified as described [6], 10 μg of EF2, 3 A_{260} units of DEAE-cellulose chromatographed ribosomes or low salt washed ribosomes, the indicated amounts of poly(U) and 10 μg of B, C when indicated in the table. After incubation at 37°C for 20 min, 0.1 ml of 10% trichloroacetic acid were added; the mixtures were maintened for 20 min at 90°C; the hot trichloroacetic acid-insoluble material was collected and washed with 5% trichloroacetic acid on nitrocellulose filters, which were dried and counted.

of B, C in the presence of DEAE-chromatographed ribosomes was relieved by increasing the concentration of poly(U).

4. Discussion

The functional reasons underlying the subunit structure of wheat embryo $EF1_H$ receive some clarification from these new data, but they are not completely elucidated.

The moiety of $EF1_H$ constituted by A fulfills known functions of EF1: formation of a binary complex with GTP and a ternary complex with aminoacyl-tRNA·GTP [4], which are the intermediates in the enzymic binding of aminoacyl-tRNA to ribosomes; the other moiety constituted by B, C has a property, which is not, as yet, known to be related with EF1 function: ability to bind poly(U). The transformation of $EF1_H$ during its functioning is, nevertheless, more complicated than if a mere dissociation of a blocking moiety B, C were necessary for A to become active. Indeed the moiety B, C interferes with some parts of the translational machinery; in the presence of low salt washed ribosomes, the moiety B, C slightly increases the poly-Phe formed, whereas using DEAE-chromatographed ribosomes, it completely inhibits the polymerization of phenylalanine (table 2). Since its action depends on the type of ribosomes and on the concentration of poly(U), and as it is able to link this polynucleotide, we believe that the moiety B, C affects the interactions between ribosomes and poly(U).

The hypothesis that the subunit structure of $EF1_H$ from wheat embryos might be a characteristic of the resting state of wheat embryos is reinforced by the data obtained in another dormant organism by Slobin and Möller [13]. They found that in the dehydrated dormant cysts of *Artemia salina* a high molecular weight $EF1_H$, formed by different peptides, was present, while in the free swimming hatched embryos only a light molecular weight $EF1_L$ existed.

The possible regulatory functions of the moiety

B, C of wheat embryo $EF1_H$ might be related to the resting state of wheat embryos, but for the physiological meaning of B, C to be clarified, further studies are needed.

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