Interaction of Eukaryote Elongation Factor EF 1 with Guanosine Nucleotides and Aminoacyl-tRNA

(protein synthesis/aminoacyl-tRNA binding/calf brain/Sephadex G-150)

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ABSTRACT Evidence for two species of elongation factor 1 (EF 1A and EF 1B) from calf brain has been obtained by molecular sieve chromatography on Sephadex G-150. A high molecular weight form, EF 1A, interacts with GTP to form an EF 1A-GTP complex. GDP also reacts with EF 1, but unlike the reaction with GTP, an EF 1A-GDP complex is formed that contains a lower molecular weight and labile species of EF 1. The results also indicate that EF 1A-GTP reacts with aminoacyl-tRNA to form an aminoacyl-tRNA-EF 1A-GTP complex. These results are discussed with regard to the role of EF 1 in aminoacyl-tRNA binding to ribosomes.

We have recently demonstrated that elongation factor 1 (EF 1) from calf brain can interact with GDP and GTP to form complexes that are retained on nitrocellulose filters (1). The EF 1-GTP complex was not retained on the filter in the presence of Phe-tRNA, suggesting that a Phe-tRNA-EF 1-GTP complex was formed (1). Similar results have been obtained with the bacterial elongation factor Tu (EF Tu), i.e., a reaction of EF Tu-GTP with aminoacyl-tRNA to form a ternary complex, aminoacyl-tRNA-EF Tu-GTP (2).

In the present experiments, evidence for the formation of a ternary complex with the eukaryotic elongation factor was sought by chromatographic procedures. It has now been possible to obtain more evidence for the formation of a PhetRNA-EF 1-GTP complex by molecular sieve chromatography. In addition, evidence will be presented that GDP and GTP react differently with EF 1. These results are discussed with respect to the role of EF 1 in aminoacyl-tRNA binding to ribosomes.

MATERIALS AND METHODS

Most of the materials used were obtained from commercial sources. The preparation of calf-brain ribosomes and an outline of the purification procedure of EF 1 from calf brain have been reported (1). The EF 1 preparation bound 800 pmol of GTP per mg of protein, of which EF 1 accounted for 70% of the GTP binding based on the interaction with aminoacyl-tRNA, as assayed on a nitrocellulose filter (1). EF 1 was calculated to be about 10% pure, if a molecular weight of the protein of 2 x 10^6 is assumed.

Incubations to study the interaction of EF 1 with guanosine nucleotides and aminoacyl-tRNA contained, in a total volume of 400 μl: 98 pmol of EF 1 (175 μg of protein), 50 mM Tris-HCl (pH 7.4), 10 mM MgCl2, 50 mM NH4Cl, 3.75 mM phosphoenolpyruvate (PEP), 20 μg of pyruvate kinase (PK), 5 mM dithiothreitol (DTT), and 1 nmol of [γ-32P]GTP. When [32P]GDP was used, the PEP and PK were omitted. The incubations were performed for 3 min at 37°C, then placed in ice. Where indicated, 200 pmol of [3H]Phe-tRNA (65% pure from Escherichia coli) was added, and the incubations were kept in ice for 3 min. The reaction mixtures were placed on a Sephadex G-150 column (0.8 × 30 cm), which was equilibrated with 50 mM Tris-HCl buffer (pH 7.4), 10 mM MgCl2, 1 mM DTT, and 60 mM NH4Cl; the column was eluted with this buffer. About 35 fractions of 0.5 ml each were collected; the chromatographic separation could be performed in less than 75 min. Aliquots from each fraction were assayed for radioactivity and for EF 1 activity by a polymerization assay. For this assay, the incubations contained in a total volume of 200 μl: 15 μl of the column fraction, 10 μg of poly(U), 7.5 μg of EF2 (3), 25 pmol of Phe-tRNA, 10 mM MgCl2, 50 mM NH4Cl, 50 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.25 mM GTP, and 0.5 A260 of calf-brain ribosomes. The incubations were for 10 min at 37°C, and the reactions were terminated by the addition of 5 μl of 5% Cl3CCOOH. The acidified reaction mixtures were heated at 90°C for 15 min and, after cooling, the incubations were filtered through nitrocellulose membranes (0.45 μm, Millipore Corp.) as described (1); the membranes were assayed for radioactivity. All radioactive measurements were performed in a Beckman LS-100 scintillation counter with a counting fluid described by Bray (4).

RESULTS

The initial purpose of these experiments was to separate an aminoacyl-tRNA-EF 1-GTP complex from unreacted aminoacyl-tRNA and GTP, so as to study the interaction of the ternary complex with mammalian ribosomes. A Sephadex G-150 column was considered ideal for this purpose, since preliminary chromatographic experiments with a 0.8 × 30 cm column showed that it was possible to separate the bulk of EF 1, which emerged with the void volume in fractions 10–12, from [3H]Phe-tRNA, which emerged with a peak at fraction 23, and GTP (3H or 32P), which appeared with a peak at fraction 34. It was, therefore, expected that the formation of
a ternary complex would result in the appearance of the \([^{14}C]\)Phe-tRNA and \([\gamma-^{32}P, ^3H]GTP\) associated with EF 1 in the void volume. Fig. 1 shows the chromatographic patterns that were obtained after EF 1 was incubated with GTP and/or \([^{14}C]\)Phe-tRNA, as described in Methods. In part A the incubations contained EF 1 and \([\gamma-^{32}P, ^3H]GTP\). An EF 1–GTP complex is apparently formed, as evidenced by the appearance of \(^3H\) and \(^{32}P\) in the fractions (10–12) that contain the EF 1 activity (as measured by polymerization). It should be noted that in fractions 15–20 there is very little EF 1 activity, a very slight peak of \(^3H\) activity, but no evidence of a \(^{32}P\) peak. Fig. 1B shows the chromatographic pattern after incubation of EF 1 with \([^{14}C]\)Phe-tRNA in the absence of GTP. EF 1 again emerges with a peak in fraction 11, and the \([^{14}C]\)Phe-tRNA emerges with a peak in fractions 21–23. There is no \(^{14}C\) associated with the EF 1 activity, and no evidence of a \(^{14}C\)-labeled peak in fractions 15–20. Fig. 1C shows the chromatographic pattern obtained after incubation of EF 1 with \([\gamma-^{32}P, ^3H]GTP\) and \([^{14}C]\)Phe-tRNA. Two significant peaks of \(^3H\) and \(^{32}P\) appear, one at fraction 11 and the other at fraction 17. In addition, the \(^{32}P\) peak has shifted from fraction 23 to fractions 18–19. The EF 1 activity was greatest in fraction 11, but a significant, although low, activity was also observed in fractions 16–18. The data in Fig. 1C provide evidence that a ternary complex \([^{14}C]\)Phe-tRNA–EF 1–\([\gamma-^{32}P, ^3H]GTP\) has been formed. In addition, it appears that at least two species of EF 1 may exist; a high molecular weight form that emerges at the void volume and a species with a lower molecular weight that appears in fractions 15–20. The ternary complex that emerged from the column in the latter fractions appeared to contain the smaller molecular weight species of EF 1. There was no evidence of \([^{14}C]\)Phe-tRNA associated with EF 1 in peak fraction 11, although \(^3H\) and \(^{32}P\) were seen in the early fractions. Although the \(^3H\) to \(^{32}P\) ratio in both peaks was about one, there were two aspects of the results in Fig. 1C that were not clear. First, the \([^{14}C]\)Phe-tRNA only shifted from a peak at fraction 23 to fraction 19, and not to fraction 17 where the \(^3H\) and \(^{32}P\) peak was. This result was indicative that the peak at fraction 19 represented a mixture of \([^{14}C]\)Phe-tRNA in the complex and unreacted \([^{14}C]\)Phe-tRNA. Due to the incomplete shift, it was not possible to determine whether equivalent amounts of \([^{14}C]\)Phe-tRNA and GTP were present in the complex, since the unreacted \([^{14}C]\)Phe-tRNA peak overlapped with the \([^{14}C]\)Phe-tRNA in the complex. Second, although the \(^3H\) and \(^{32}P\) peak at fraction 17 was almost as prominent as the \(^3H\) and \(^{32}P\) peak at fraction 11, the EF 1 activity in fractions 16–18 was much lower than would have been expected from the GTP binding. These two points have been examined more closely below. In order to obtain a more significant shift of \([^{14}C]\)Phe-tRNA, the ratio of (pmol of EF 1/pmol of Phe-tRNA) was raised from a value of 0.49 (used in the experiments of Fig. 1C) to a value of about 1.0. The resultant chromatographic pattern on Sephadex G-150 is seen in Fig. 2. The concentration of GTP (only \(^3H\) labeled) and the other components of the incubation were kept the same as in Fig. 1. There are again two peaks of \([^{14}C]\)Phe-tRNA binding activity, similar to the results in Fig. 1C. In addition, under these conditions the \([^{14}C]\)Phe-tRNA emerges with a peak that coincides with the second \([^{14}C]\)Phe-tRNA peak (fraction 17), and in fractions 14–22 the \(^3H\) to \(^{14}C\) ratio is close to 1. It thus appeared that by elevation of
The amount of EF 1 relative to Phe-tRNA, it was possible to complex almost all of the Phe-tRNA.

The low EF 1 activity in fractions 15-20, where the ternary complex emerges (Fig. 1C) has not been fully explained, but appears to be due to a marked instability of the lower molecular weight species of EF 1 that emerges in these fractions. As an example, in one experiment the EF 1 activity in a Phe-tRNA polymerization assay was 2.8 pmol of Phe-tRNA polymerized per 10 min per 15 μl of fraction 17 when the assay was performed shortly after the fraction was collected. However, the activity of a similar aliquot of fraction 17, 2 hr later, was 0.9 pmol of polyphenylalanine formed per 10 min. This marked instability was not seen with the EF 1 present in fraction 11, and the reason for the enzyme lability is not clear. However, it is apparent that the amount of enzyme activity in fractions 15-20 is difficult to quantitate*, and the low activity seen in this region may not be representative of the EF 1 activity initially present. Other attempts to either restore or to increase the activity in fraction 17 by the addition of limiting amounts of fraction 11 were unsuccessful.

To gain further evidence that the change in the Phe-tRNA elution pattern (Figs. 1C and 2) in the presence of EF 1 and GTP was related to the formation of a Phe-tRNA-EF 1-GTP complex, similar incubations to those in Fig. 1 were performed with [3H]GDP in place of GTP. The results presented in a previous communication (1), in which a nitrocellulose filter assay was used, showed that although EF 1 could bind GDP to form an EF 1--GDP complex, this complex did not interact with Phe-tRNA to form a ternary complex. Fig. 3A shows the chromatographic pattern obtained on Sephadex G-150 after EF 1 is incubated with [3H]GDP. A tritium peak appears at fraction 17, but no [3H]GDP is seen at fraction 11. The pattern of EF 1 activity is similar to that seen in Fig. 1, namely, a large peak at fraction 11, with a minor but definite peak around fraction 17.

Fig. 3B shows the chromatographic pattern after incubation of EF 1 with [3H]GDP and [14C]Phe-tRNA. The presence of Phe-tRNA does not alter the pattern of EF 1 or [3H]GDP, as compared to the results seen in Fig. 3A, and there is no shift in the chromatographic pattern of the Phe-tRNA. By comparing these results to those seen in Figs. 1C and 2, one can conclude that GTP, not GDP, is required for formation of the ternary complex.

An unexpected observation was the difference in the chromatographic patterns when EF 1 was incubated with GTP (Fig. 1A) or GDP (Fig. 3A). In the former case, the GTP emerged with the bulk of the EF 1 activity in fractions 10-12, whereas GDP eluted with the labile EF 1 activity at fraction 17. These results are best explained by assuming that there are two species of EF 1, a high molecular weight species that interacts specifically with GTP, and a lighter species of EF 1 that binds GDP specifically. These two forms of the enzyme may normally exist in equilibrium, or their interconversion may require the presence of a specific guanosine nucleotide.

* EF 1 was assayed by its ability to catalyze amino-acid polymerization because of the sensitivity of this procedure. However, this assay in our hands does not give a linear response to EF 1 concentration, but instead we obtain a pronounced sigmoidal curve. The inherent difficulty with the assay, plus the lability of the enzyme in the column fractions, has made it impractical to attempt to calculate the actual amount of enzyme in the individual fractions.

DISCUSSION

The data presented above indicate that there are two species of EF 1 activity that can be separated by molecular sieve chromatography. The difference in the elution patterns appears to be consistent with the known information on the structure of EF 1. The enzyme from liver has been reported to exist in multiple forms that have molecular weights ranging from 100,000 to greater than 300,000 (5). Reticulocyte EF 1 has a molecular weight of 186,000, and this protein has been dissociated into three subunits that were enzymatically inactive (6). In the present studies, the EF 1 emerging at fraction 11 could represent a high molecular weight species, whereas the EF 1 that appears with a peak at fraction 17 may represent a labile, low molecular weight form, perhaps one or more subunits of the larger form. For the sake of discussion, the two species of EF 1 will be referred to as EF 1A (high molecular weight species emerging at fraction 11) and EF 1B (low molecular weight species emerging at fraction 17). The results presented above suggest that the following interactions may occur.

(a) EF 1A + GTP \(\rightarrow\) EF 1A--GTP (Fig. 1A)
(b) EF 1A--GTP + Phe-tRNA \(\rightarrow\) Phe-tRNA-EF 1B--GTP (Fig. 1C)
(c) EF 1A + GDP \(\rightarrow\) EF 1B--GDP (Fig. 3A)

Thus, EF 1A does not dissociate in the presence of GTP (Reaction a) but is converted to a lower molecular weight species in the presence of both GTP and aminoacyl-tRNA (Reaction b), and also in the presence of GDP (Reaction c). An alternative explanation for the results that have been obtained is that EF 1A is in equilibrium with EF 1B (Reaction d).

(d) EF 1A \(\Leftrightarrow\) EF 1B

Thus, GTP, by interacting with EF 1A, may pull the equilibrium of Reaction d to the left, whereas GTP, in the presence of aminoacyl-tRNA or GDP, would shift the equilibrium to the right by reacting with EF 1B.

Elongation factor T in prokaryote systems is composed of equimolar amounts of EF Tu and EF Ts. EF Tu is involved

† Preliminary studies of the molecular weight of EF 1B suggest that the protein(s) in this fraction has a molecular weight in the 60,000-80,000 range, since bovine serum albumin (molecular weight 68,000) appears with a peak at fraction 17 under the conditions of chromatography used. In similar experiments, it was determined that EF 1A, appearing at fraction 11, had a molecular weight of over 150,000.
in reactions with guanosine nucleotides and aminoacyl-tRNA (2), whereas EF Ts is required for the interconversion of EF Tu–GDP → EF Tu–GTP (7–11). It will be of interest to see whether a factor comparable to EF Ts is a component of EF 1.

The marked lability of EF 1B in these experiments has thus far hindered studies on its nature, and on the interaction of the Phe-tRNA–EF 1B–GTP complex with ribosomes. Until it is stabilized, any interpretation of the present results must be considered preliminary. However, it is tempting to speculate that the interconversions between EF 1A and EF 1B may be involved in the overall process of aminoacyl-tRNA binding to ribosomes, as shown in Fig. 4. In this sequence of reactions the interaction of the ternary complex containing EF 1B with the messenger RNA–ribosome complex may result in GTP hydrolysis, with the formation of EF 1B–GDP. The reaction of EF 1B–GDP with GTP is pictured as yielding EF 1A–GTP. The EF 1A–GTP thus formed interacts with aminoacyl-tRNA, and the EF 1A could be converted back to EF 1B during the formation of the ternary complex, aminoacyl-tRNA–EF 1B–GTP. It should be stressed that direct evidence for some of the proposed interconversions in Fig. 4 has not been obtained.