EVIDENCE FOR ACTIVITIES OF RABBIT RETICULOCYTE ELONGATION FACTOR 1 ANALOGOUS TO BACTERIAL FACTORS EF-Ts AND EF-Tu

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Summary: Preparations have been obtained from rabbit reticulocyte elongation factor 1 (EF-1) that exhibit activities analogous to the heat stable and heat labile factors, EF-Ts and EF-Tu, of Escherichia coli. The heat stable fraction, prepared by heating EF-1 in the presence of GTP, has virtually no activity in poly (U)-directed polyphenylalanine synthesis. The fraction exhibiting activity similar to bacterial EF-Tu is obtained by the interaction of EF-1 with GTP and phenylalanyl-tRNA followed by passage of the solution through a nitrocellulose filter. The filtrate, which alone has low activity in polyphenylalanine synthesis, when combined with the heat stable fraction gives high activity suggesting that the heat stable preparation catalyzes recycling of the filtrate component.

Previous investigations by several groups have indicated that protein biosynthesis in mammalian and bacterial systems differ in that the binding of aminoacyl-tRNA to ribosomes for chain elongation is catalyzed by a single factor, EF-1, in mammalian systems whereas two factors, EF-Ts and EF-Tu, are required in bacterial systems (1). As shown previously in this laboratory (2), the ability of rabbit reticulocyte EF-1 to catalyze the transfer of aminoacyl-tRNA to ribosomes greatly exceeds its ability to bind GTP. These data suggest that EF-1 might be composed of two moieties, one involved in the transfer of aminoacyl-tRNA to the ribosomes and the other involved in a recycling process. In the present investigation, two fractions have been prepared from EF-1 which respond in polymerization assays in the reticulocyte system in a manner similar to the factors EF-Ts and EF-Tu in the Escherichia coli system (3).

EXPERIMENTAL

Materials. Ribosomes were isolated from NaF-treated reticulocytes, washed, and treated with N-ethylmaleimide as described by Hardesty et al. (4).

[^{14}C]Phenylalanyl-tRNA (E. coli) and rabbit reticulocyte EF-2 were prepared by previously reported procedures (5, 6). Solutions of GTP were treated with pyruvate kinase and phosphoenol pyruvate to regenerate trace amounts of GDP as previously described (2, 7).

Preparation of EF-1. EF-1 was obtained from lysates of rabbit reticulocytes by ammonium sulfate precipitation, gel filtration, and hydroxylapatite chromatography as described by Hardesty et al. (4). The enzyme was further purified by chromatography on DEAE-cellulose by the following procedure. A 6 ml aliquot of the hydroxylapatite fraction containing a total of 16 mg of protein was dialyzed overnight against two 500 ml portions of Buffer A (0.05 M Tris-HCl, pH 7.5; 0.1 M NH_4Cl; and 1 mM dithiothreitol [DTT]). The enzyme solution was condensed to a volume of 1 ml in a Diaflo ultrafiltration cell equipped with a PM-10 membrane and applied to a DEAE-cellulose column (1.1 cm x 22 cm) previously equilibrated in Buffer A. The column was washed with approximately 25 ml of Buffer A at a flow rate of 30 ml per hour and 2 ml fractions were collected. The EF-1 was not adsorbed on the column under these conditions and emerged as a sharp peak at the void volume. The fractions containing EF-1 were pooled and condensed by ultrafiltration to a protein concentration of approximately 1.5 mg per ml. The concentration of protein was estimated by the method of Lowry et al. (8). A typical preparation of EF-1 by this procedure had a specific activity of 20,000 (pmoles of phenylalanine incorporated into polyphenylalanine per mg of protein in 10 minutes under the assay conditions described below). The enzyme prepared in this manner was used throughout this investigation.

Preparation of Filtered EF-1. The reaction mixture contained in a total volume of 1 ml: Buffer B (0.05 M Tris-HCl, pH 7.5; 0.16 M NH_4Cl; 10 mM $MgCl_2$; and 5 mM DTT); 20 μM GTP (treated as indicated above); 1.8 mg tRNA charged with 1200 pmoles of phenylalanine; and 200 μg of EF-1. The solution was incubated at 0° for 10 minutes and then passed through a stack of 2 Millipore nitrocellulose filters (25 mm diameter, 0.45 μ pore size), and the filters

were washed with 1 ml of Buffer B. The filtrate and wash were collected in a test tube at 0^{o}. In a typical preparation, the filtrate exhibited 15-20% of the activity of unfiltered EF-1 as measured by polyphenylalanine synthesis described below. The activity of the filtrate was relatively unstable; therefore, when the solution was not used immediately, it was divided into 0.45 ml aliquots and the activity was precipitated by the addition of two volumes of ethanol (5). The pellets were stored at -80^{o} and were resuspended in Buffer B just prior to use. Precipitation of the protein and storage at -80^{o} for several weeks caused no detectable loss in activity.

Assay for Polyphenylalanine Synthesis. The assay mixture contained in a total volume of 0.5 ml: Buffer C (0.05 M Tris-HCl, pH 7.5; 0.07 M KCl; 8 mM $MgCl_2$; and 10 mM DTT); 0.25 mg of rabbit reticulocyte ribosomes (treated as described above); 50 μg of poly (U); 0.2 mM GTP; 4 μg of EF-2; 0.1 mg of tRNA charged with 65 pmoles of [^{14}C]phenylalanine; and EF-1 preparations as indicated. The solution was incubated at 37^{o} for the time indicated; the reaction was stopped by the addition of 5% tricholoracetic acid (TCA); and the radioactivity incorporated into hot TCA insoluble material was measured as previously described (5).

RESULTS AND DISCUSSION

As mentioned above, it has been reported that for several different preparations of rabbit reticulocyte EF-1 the ratio of the amount of GTP bound to the enzyme to the amount of phenylalanyl-tRNA (Phe-tRNA) transferred to ribosomes by EF-1 averaged about 0.15 (2). These results indicated that EF-1 might be reutilized in a catalytic process and suggested that the factor might be composed of two activities, one involved in the transfer of aminoacyl-tRNA to the ribosome and one involved in catalysis of a recycling process.

Similarly, the 80 to 85% decrease in activity observed when EF-1 was incubated with GTP and Phe-tRNA and passed through a Millipore filter as described in the Experimental section could be ascribed to a loss of the

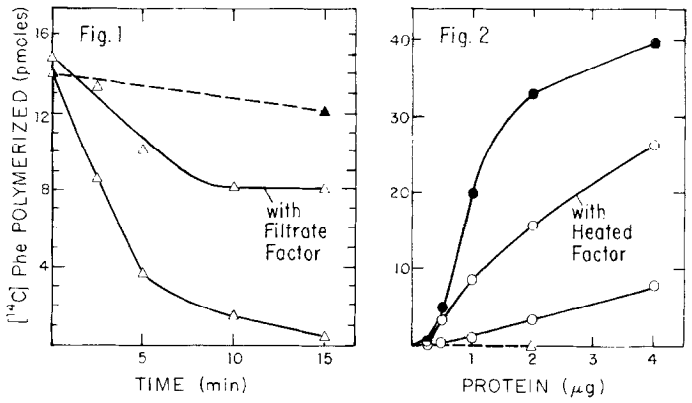

Fig. 1. The effect of heating EF-1 on its ability to stimulate polyphenylalanine synthesis. EF-1 (30 μg) was heated at 42° for the times indicated in 0.05 ml of Buffer B without GTP (▲---▲) and with 0.2 mM GTP (△—△). Aliquots containing 1 μg of protein were tested in the polymerization assay as described in Experimental. The assay was incubated for 5 min at 37°. The enzyme heated with GTP was also assayed in the presence of filtrate derived from 4 μg of EF-1. The amount of polymerization obtained with filtrate alone was subtracted from the total incorporation.

Fig. 2. The effect of heated EF-1 on the activity of the filtrate factor. The standard polymerization assay was supplemented in amounts indicated with untreated EF-1 (●—●); EF-1 heated with 0.2 mM GTP for 15 min at 52° (△---△); or filtrated derived from EF-1 as described in Experimental (o—o) assayed with and without the heated EF-1 (2 μg). The assay was incubated for 10 min at 37°.

ability of the filtrate to catalyze the recycling process. If so, such preparations would contain the component corresponding to the heat labile EF-Tu of E. coli and would allow the detection of the activity analogous to the more heat stable EF-Ts of the bacterial system.

Incubation of rabbit reticulocyte EF-1 with GTP for 10 minutes at 37° has been reported to diminish the ability of the enzyme to interact with Phe-tRNA and to transfer Phe-tRNA to ribosomes (2). As shown in Figure 1, the activity of EF-1 in poly (U)-directed polyphenylalanine synthesis is not appreciably diminished by heating at 42° for 15 minutes in the absence of GTP, but heating in the presence of GTP decreases the activity more than 90%. If, however, the EF-1 heated with GTP is assayed in the presence of the filtrate factor, it exhibits approximately 60% of its original activity. The possibility that a heat labile component of EF-1 may have been destroyed

with retention of activity of a heat stable component was further investigated as shown in Figure 2.

In poly (U)-directed polyphenylalanine synthesis, EF-1 heated with GTP has essentially no activity and the Millipore filtrate of the products of the interaction of EF-1, GTP and Phe-tRNA has about 15% of the activity of untreated EF-1; but the combination of the two act synergistically. A level of the heat inactivated EF-1, which possesses negligible activity alone, increases the response of the filtrate factor 3 to 4 fold reaching levels of 50 to 60% that of untreated EF-1. The polyphenylalanine synthesis assay was chosen to demonstrate the stimulation of filtrate activity by heat treated EF-1; however, similar results were obtained by measuring Phe-tRNA binding to ribosomes. These responses are analogous to those observed with the E. coli factors, EF-Ts and Ef-Tu, as previously reported (3), and indicate that the reticulocyte EF-1 complex is composed of two moieties and may be similar to the EF-Ts·EF-Tu complex in bacterial systems.

Although this represents the first indication of these similarities of bacterial and reticulocyte systems, Weissbach and co-workers (9) have reported that calf brain EF-1 exists in two forms: a heavy species, Ef-1A, with a molecular weight of over 150,000 and a lighter species, EF-1B, with a molecular weight of 60,000 to 80,000. Data obtained by gel filtration indicated that when EF-1A interacts with GTP and aminoacyl-tRNA, the ternary complex formed contained the lighter (EF-1B) species of the enzyme.

In our work, the factor in the filtrate obtained from EF-1 that had been incubated with GTP and Phe-tRNA appears to be of low molecular weight, approximately 50,000 as determined by SDS polyacrylamide gel electrophoresis (to be published). This result suggests the separation of a subunit of the EF-1 complex which corresponds to the heat labile component, EF-Tu, of the bacterial system.

Since this work was completed, Iwasaki et al. (10), have reported the resolution of pig liver preparations into two components, EF-1α and EF-1β,

having activities which may correspond to our filtrate and heat stable factors respectively. Their results in combination with the above data strengthen the indication that reticulocyte EF-1 is a very stable complex containing two activities which may, under the proper conditions, be separated from one another.

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REFERENCES

1. Haselkorn, R. and Rothman-Denes, L. B., Ann. Rev. Biochem. 42, 397 (1973).
2. Ravel, J. M., Dawkins, R. C., Lax, S., Odom, O. W., and Hardesty, B., Arch. Biochem. Biophys. 155, 332 (1973).
3. Ravel, J. M., Shorey, R. L., and Shive, W., Biochem. Biophys. Res. Comm. 32, 9 (1968).
4. Hardesty, B., McKeehan, W., and Culp, W., Methods in Enzymology XX, 316 (1971).
5. Ravel, J. M. and Shorey, R. L., Methods in Enzymology XX, 306 (1971).
6. Hardesty, B. and MeKeehan, W., Methods in Enzymology XX, 330 (1971).
7. Cooper, D. and Gordon, J., Biochemistry 8, 4289 (1969).
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).
9. Moon, H. M., Redfield, B., and Weissbach, H., Proc. Nat. Acad. Sci. USA 69, 1249 (1972).
10. Iwasaki, K., Mizumoto, K., Tanaka, M., and Kaziro, Y., J. Biochem. (Japan) 74, 849 (1973).