FORMATION OF AMINOACYL-tRNA-GUANYLYL-5'-METHYLENE DIPHOSPHONATE-ELONGATION FACTOR COMPLEX

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Received February 11, 1975

SUMMARY

A procedure for the preparation of a ternary complex, guanylyl-5'-methylene diphosphonate-EF-Tu-Phe-tRNA is described. This complex is sufficiently stable to persist purification by gel filtration chromatography. Such a complex can bind to ribosomes in the presence of poly U.

INTRODUCTION

During protein synthesis binding of aminoacyl tRNA to ribosomes requires polypeptide elongation factors, EF-Ts and EF-Tu (See reviews 1-2). In this reaction the aminoacyl-tRNA first binds to a GTP-EF-Tu complex forming a ternary complex containing GTP-EF-Tu-aminoacyl-tRNA (3-10). Upon binding of the aminoacyl-tRNA to the A site of the ribosome, GTP is hydrolyzed yielding GDP-EF-Tu, which is released from the ribosomes (5,9,11-20). If the reaction is carried out in the presence of GDPCP, EF-Tu is not released from the ribosome (21).

While a stable ternary complex, GTP-EF-Tu-aminoacyl-tRNA has been isolated (5) and has become useful in studying the mechanism of action of EF-Tu, evidence for the formation of a similar stable intermediate involving GDPCP is weak. Presumably a complex involving GDPCP-EF-Tu-aminoacyl-tRNA is not stable enough to persist isolation. However it would be advantageous to use such a nonhydrolyzable analog of GTP. We therefore have investigated conditions necessary for the formation and isolation of a stable ternary complex containing GDPCP-EF-Tu-phe-tRNA. In this paper we describe a method for the preparation of such a complex sufficiently stable to persist purification by Sephadex gel filtration. While our work was in progress a different method for preparing such a complex was reported (22).

MATERIALS AND METHODS

^{3}HGDP and ^{14}C-phenylalanine (10 c/mmole and 460 mc/mmole respectively) were purchased from Schwarz-Mann. Guanylyl-5'-methylene diphosphonate (GDPCP) was the product of P-L Biochemicals. The purity of this nucleotide was checked by paper chromatography in two solvent systems and found to contain > 98% GDPCP and < 0.5% GTP. The solvent systems are isobutyric acid-ammonia-water (66-1-33 V/V) and isobutyric acid-ammonia-water (57-4-39 V/V). Purified tRNA of E. coli B was obtained from Schwarz-Mann. Poly U was obtained from Miles Lab. E. coli Q13 cells (mid log) were purchased from Truett Laboratories.

Elongation factor Tu was prepared from E. coli Q13 as described (23). EF-Tu activity was assayed by binding with ^{3}HGDP in the absence or presence of EF-Ts (23). The homogeneity of the enzyme was checked by electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulfate (24). The specific activity of the enzyme used for these studies was 12,000.

^{14}C-phenylalanyl-tRNA was prepared as described (23) and was purified by Sephadex G-25 column chromatography. The specific activity of the ^{14}C-phenylalanyl-tRNA used was 650 pmoles/mg tRNA. High salt washed ribosomes was isolated from E. coli Q13 as described (23).

To assay for aminoacyl-tRNA binding to ribosomes, the method of Ravel was used (23).

RESULTS AND DISCUSSION

EF-Tu free from bound guanine nucleotide was used for preparation of the ternary complex of GDPCP-EF-Tu-phe-tRNA. Such free EF-Tu was prepared by incubation of purified EF-Tu in the presence of 5mM EDTA, 10mM triethanolamine-HCl pH 7.4, 1mM DTT and 100mM KCl for 5 min at 25°. The reaction mixture was chromatographed on a column (0.6 x 36 cm) of Sephadex G-25 which had been equilibrated with the same buffer. Fractions containing EF-Tu activity were pooled, concentrated by Diaflo filtration and used for the preparation of the ternary complex. Free EF-Tu prepared in this manner migrated as a single

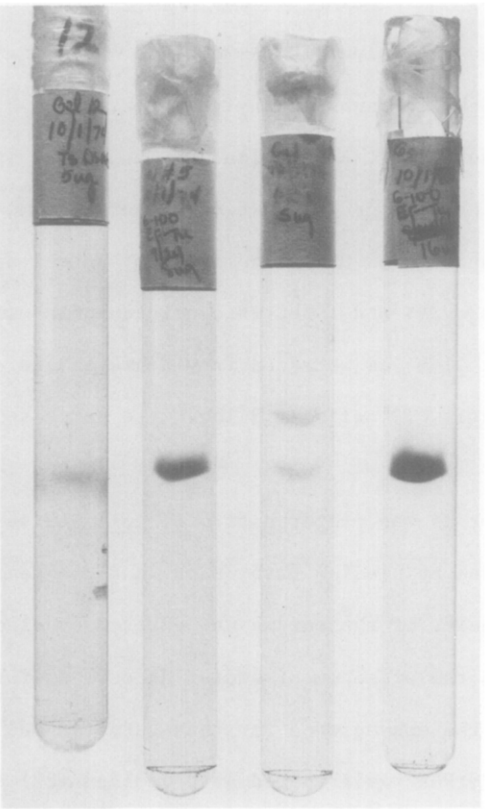

Figure 1. SDS-disc-gel electrophoresis of purified EF-Tu. Electrophoretic conditions are similar to those of Weber and Osborn (24). Amounts of protein used are 8 and 16 μg (b and d). The direction of migration is left to right. Electrophoretic patterns of EF-Ts (a) and pyruvate kinase and phosphocreatine kinase (c) are also shown for comparison.

component in SDS-containing polyacrylamide gel (Fig. 1).

For the preparation of GDPCP-EF-Tu-^{14}C-Phe-tRNA, approximately 4400 pmoles of free EF-Tu was incubated with 7000 pmoles of purified ^{14}C-phe-tRNA in a reaction mixture containing 10 mM triethanolamine-HCl pH 7.5, 5 mM DTT, 100 mM KCl, 10 mM $MgCl_2$ and 0.1 mM GDPCP. After incubation for 10 min at 0°, the reaction mixture was filtered through several Millipore filters (0.45 μ pore size). The filtrate was adjusted to contain 66% ethanol. Approximately 95% of the radioactivity appeared in the precipitate. The precipitate was then dissolved in 10 mM triethanolamine-HCl pH 7.5, 10 mM $MgCl_2$, 100 mM KCl,

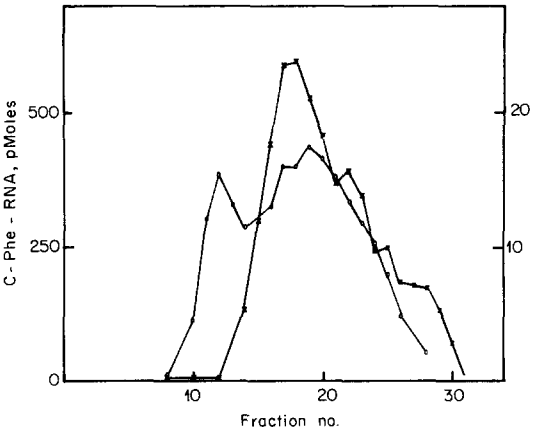

Figure 2. Elution profile of GDPCP-EF-Tu-phe-tRNA on a column of Sephadex G-100. A reaction mixture containing purified EF-Tu, ^{14}C-phe-tRNA and GDPCP was incubated for 10 min at 0° and passed through Millipore filters. The filtrate was adjusted to 66% ethanol. The precipitate was dissolved in buffer and applied to a Sephadex G-100 column equilibrated and developed with the same buffer described in the text. O.D. at 260 nm (o-o), ^{14}C-Phe-tRNA (x-x).

Table I

Binding of Ternary Complex to E. coli Ribosomes

Ternary Complex	^{14}C-Phe-tRNA bound (pmoles/100 µg ribosomes)
GTP-EF-Tu-^{14}C-Phe-tRNA	10.9
GDPCP-EF-Tu-^{14}C-Phe-tRNA	10.4

The reaction mixture contained 10 mM triethanolamine-HCl, pH 7.5, 10 mM $MgCl_2$, 0.4 mg poly U, 1.0 mg ribosomes, 0.5 mg tRNA and about 1000 pmoles of the preformed complex.

and 1 mM DTT, and applied to a column (1 x 41 cm) of Sephadex G-100 which was pre-equilibrated with the same buffer. Half-ml fractions were collected. Radioactivity, absorbance at 260 nm and EF-Tu activity in each fraction were determined. The elution profile is shown (Fig. 2). Approximately 70% of the ^{14}C radioactivity eluted with EF-Tu activity. Materials from this peak was pooled and precipitated with ethanol.

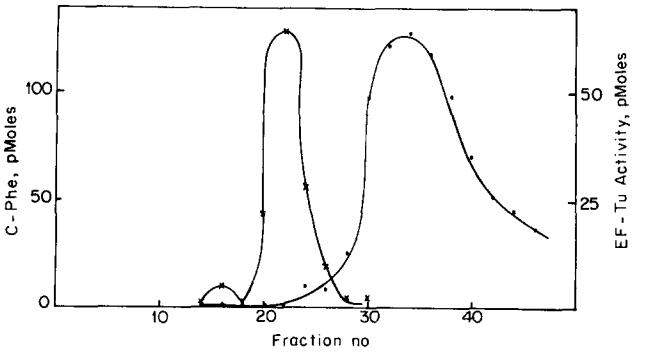

Figure 3. Elution profile of GDPCP-EF-Tu-Phe-tRNA on Sephadex G-100 in the presence of EDTA. About 900 pmole (^{14}C) of the complex was applied to a Sephadex G-100 column equilibrated and developed with the same buffer containing EDTA as described in the text. Fractions of 1.0 ml were collected at a flow rate of 15 ml per hr. EF-Tu activity was followed by measuring the binding of ^{3}H GDP (o-o). ^{14}C-Phe-tRNA was determined by direct counting of an aliquot of each fraction (x-x).

To examine whether such a complex was biologically active, the complex obtained after chromatography on Sephadex G-100 was used for binding of phe-tRNA to ribosome-poly U complex. As shown in the experiments described in Table I, both ternary complexes containing either GTP or GDPCP form a complex with ribosomes to comparable extent. Shorey et al. (21) reported similar values for GDP- and GDPCP-stimulated enzymatic binding of phe-tRNA to ribosomes not using preformed ternary complexes.

To examine the molar ratio of ^{14}C-phe-tRNA and EF-Tu in this ternary complex, GDPCP-EF-Tu-^{14}C-phe-tRNA, the complex was subjected to chromatography on a column (0.6 x 36 cm) of Sephadex G-100. Elution was effected with 10 mM triethanolamine-HCl pH 7.5, 100 mM KCl, 1 mM DTT and 5 mM EDTA. Radioactivity and EF-Tu activity present in each fraction were determined. As shown in Fig. 3, EF-Tu activity was well resolved from the ^{14}C-phe-tRNA material. The molar ratio of EF-Tu to phe-tRNA was approximately 0.4. It is unclear at the present why the ratio is not unique.

Arai et al. (22) reported the isolation of GDPCP-EF-Tu-phe-tRNA complex by ammonium sulfate precipitation. Whether the complex isolated by their procedure will bind to ribosomes cannot be ascertained.

In this communication we describe a procedure for the preparation of a ternary complex, GDPCP-EF-Tu-phe-tRNA. The complex is stable enough to persist gel filtration. Such a complex is capable of binding to ribosome-poly U complex. Similar to the complex containing GTP, this complex is not retained by Millipore filters. Both ternary complexes bind to ribosomes-poly U to similar degree.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. Joanne Ravel for supplying us initially with samples of purified elongation factors. This work was supported in part by a grant from the Robert A. Welch Foundation (AQ 521).

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