FEBS Letters 356 (1994) 165–168

FEBS 14867

Purification and crystallization of the ternary complex of elongation factor Tu:GTP and Phe-tRNA^{Phe}

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Received 28 October 1994

Abstract Elongation factor Tu (EF-Tu) is the most abundant protein in prokaryotic cells. Its general function in protein biosynthesis is well established. It is a member of the large family of G-proteins, all of which bind guanosine phosphates (GDP or GTP) as cofactors. In its active GTP bound state EF-Tu binds aminoacylated tRNA (aa-tRNA) forming the ternary complex EF-Tu:GTP:aa-tRNA. The ternary complex interacts with the ribosome where the anticodon on tRNA recognises a codon on mRNA, GTPase activity is induced and inactive EF-Tu:GDP is released. Here we report the successful crystallization of a ternary complex of *Thermus aquaticus* EF-Tu:GDPNP and yeast Phe-tRNA^{Phe} after its purification by HPLC.

Key words: Protein biosynthesis; Elongation factor Tu; Aminoacylated tRNA; Ternary complex; Crystallization

1. Introduction

Protein biosynthesis is performed on the ribosome, which is a large complex of ribosomal RNA (rRNA) and proteins [1,2]. Ribosomes are in all species divided into two subunits. In bacteria these are the 50S and 30S particles of the 70S ribosome. The biosynthesis of proteins on the ribosome is divided into three phases: initiation, elongation, and termination. During initiation messenger RNA (mRNA) is bound to the 30S particle and the start codon on mRNA is recognised by initiator transfer RNA. Initiation factors thereafter catalyse the assembly of the 70S ribosome in the initiation complex. Termination is catalysed by one of several termination or release factors when ribosomes expose a stop codon.

Elongation is catalysed by three elongation factors [3,4]. Elongation factor Tu (EF-Tu) forms an active ternary complex with aminoacyl-tRNA (aa-tRNA) and guanosine triphosphate (GTP). This ternary complex assists the aa-tRNA in recognising an exposed codon on the ribosome and positions the aa-tRNA on the ribosomal A-site. Ribosomes induce hydrolysis of GTP to guanosine diphosphate (GDP), and the binary complex EF-Tu:GDP is ejected from the ribosome [2]. The nucleotide exchange factor Ts (EF-Ts) binds EF-Tu:GDP and catalyses the rapid exchange of GDP for GTP. A peptidyltransferase centre on the ribosome catalyses the transfer of the growing peptide on a peptidyl-tRNA in the P-site of the ribosome to the aminoacid on tRNA in the A-site, leaving a deacylated tRNA in the P-site. A translocation reaction is catalysed by elongation factor G (EF-G) whereby deacylated-tRNA is translocated to the E-site, peptidyl-tRNA is translocated to the P-site and in the A-site the next codon is exposed for recognition by a new ternary complex aa-tRNA:EF-Tu:GTP [3,4]. The E-site has been shown by Nierhaus and coworkers to have a negative allosteric effect on binding of the ternary complex to the A-site [1]. The ribosome is thus thought to be in one of two states: the pre-translocation state with aa-tRNA in the A-site and peptidyl-tRNA in the P-site or the post-translocation state with peptidyl-tRNA in the P-site and deacylated-tRNA in the E-site. Furthermore, it has been shown that tRNAs can be found in mixed states according to the state of the ribosome during elongation, i.e. after peptidyl transfer tRNA is at P-site on 50S but in A-site on 30S [2].

It has been proposed that two EF-Tu molecules participate in the recognition of aa-tRNA thus forming a pentameric complex, aa-tRNA:(EF-Tu:GTP)$_2$ [5,6] This model implies that two GTP molecules are hydrolysed when the tRNA is delivered to the ribosome, a result which is in agreement with the findings of Weijland and Parmeggiani [7]. However, they do point out that their results can also be explained by a sequential action of two ternary complexes. EF-Tu and EF-G are members of the GTP-binding protein family which includes *ras*-p21, transducin and the heterotrimeric G-proteins [8]. They all share common sequence motifs which are involved in binding of guanine nucleotides [9]. Crystal structures are known of EF-Tu:GDP from *E. coli* [10], of EF-Tu:GTP from *T. thermophilus* [11] and from *T. aquaticus* [12]. Crystal structures have been determined of nucleotide-free EF-G [13] and EF- G:GDP [14] from *T. thermophilus*. Crystal structures have also been determined of p21:GDP [15], p21:GTP [16], bovine transducin α:GDP [17] and bovine transducin α:GTP [18]. These proteins generally have two distinct states. An active GTP-bound form and an inactive form where GDP is bound. The active form will interact with its target effector whereafter GTP is hydrolysed to GDP and inorganic phosphate. The G-proteins constitute a superfamily of proteins with a conserved, common structural design [8]. Thus results obtained for one protein can to a certain degree be applied to other proteins in the superfamily.

Elongation factor Tu from *Thermus aquaticus* is a monomeric protein of 405 amino acid residues and a molecular weight of 44.6 kDa. In *T. aquaticus* [19] two genes encode EF-Tu: *tufA* and *tufB*. The exsistence of two genes coding for EF-Tu is likely to be part of a complicated bioregulatory mechanism [20]. Yeast tRNA^{Phe} has 76 nucleotides and a molecular

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SSDI 0014-5793(94)01254-7

weight of 25 kDa. Here we report the successful crystallization of the ternary complex of yeast Phe-tRNAPhe with EF-Tu:GDPNP from *Thermus aquaticus*. The eventual determination of the crystal structure of the ternary complex will undoubtedly give new important views on one part of the central elongation phase in protein biosynthesis and on biomolecular recognition between proteins and nucleic acids.

2. Materials and methods

EF-Tu was purified from a cellpaste of *Thermus aquaticus* YT-1 (DSM, Germany) and EF-Tu:GDPNP prepared essentially as described earlier [12] except that a soluble alkaline phosphatase (Böhringer, Germany) was used. Phe-tRNA^{Phe} was formed by incubation of tRNA^{Phe} from brewers yeast (Böhringer, Germany) with yeast Phe-RS, [^{14}C]Phe and ATP [21]. Gel filtration of the ternary complex (TC) was carried out on a TosoHaas G-3000SW HPLC column (in 50 mM Tris-HCl, 100 mM KCl, 40 mM NH_4Cl, 10 mM $MgCl_2$, 0.5 mM NaN_3, 0.5 mM DTT, 10 μM GDPNP, pH 7.0) at room temperature. The TC-pool was concentrated after addition of 30% ammonium sulphate (AS) in Centricon-30 tubes to a concentration exceeding 1 mg/ml and precipitated in 65% AS. Reverse fractional extraction was performed in 100 μl volumes of AS containing buffer solutions (60–40% AS, 30 mM Tris-HCl, 5 mM MES, 7 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM NaN_3 and 0.2 mM GDPNP, pH 7.0) with a 2.5% AS decrease per step. The concentration of EF-Tu:GDPNP was measured by colorimetric methods with a BSA standard and by counting of the EF-Tu bound [^{3}H]GDPNP. The concentration of Phe-tRNA^{Phe} was measured by counting of [^{14}C]Phe-tRNA^{Phe}.

3. Results and discussion

An earlier attempt at the crystallization of the ternary complex was reported by Kaziro et al. [22]. In unpublished experiments, using powder diffraction on crystals produced under similar conditions to the ones reported, it was shown that these crystals most likely contained EF-Tu:GDP (T.F.M. la Cour and J. Nyborg, 1974, unpublished).

Many laboratories have since then tried to crystallize the ternary complex. The attempts have been hampered by the fact that the complex contains two sources of instability. One is the ester bond between the amino acid and tRNA which is easily hydrolysed in aqueous solutions. This, and the fact that EF-Tu:GTP protects aa-tRNA against RNAse hydrolysis, have been used to study stabilities of ternary complexes [23].

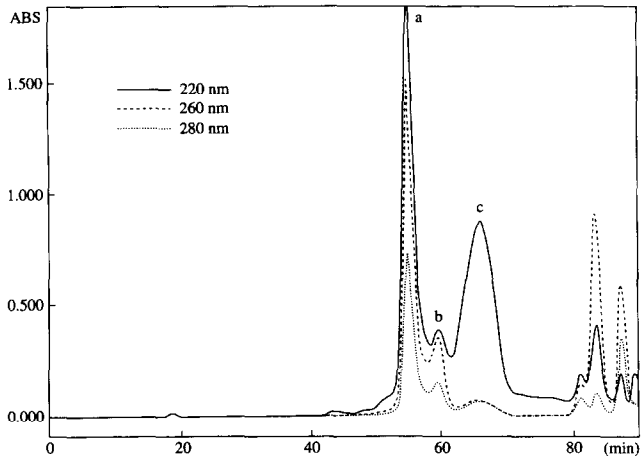

Fig. 1. Purification of ternary complex by HPLC gel filtration. (a) Peak of EF-Tu:GDPNP:Phe-tRNA^{Phe}. (b) Peak of uncomplexed tRNA^{Phe}. (c) Peak of uncomplexed EF-Tu:GDPNP.

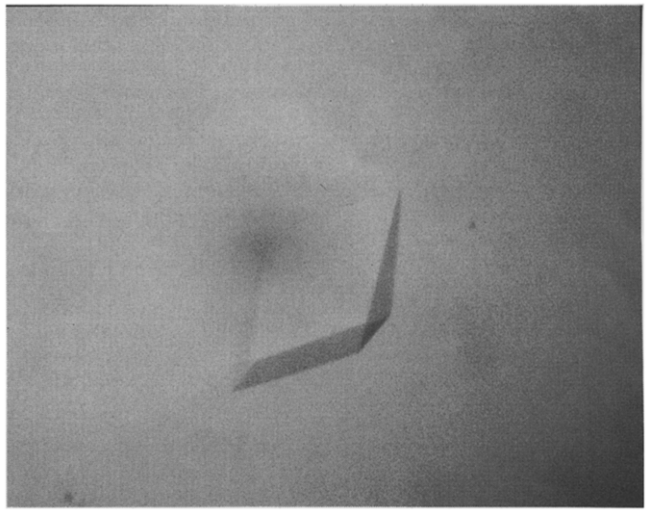

Fig. 2. Single monoclinic crystal of ternary complex. Crystal dimensions are 0.7, 0.5 and 0.3 mm.

The other instability is the intrinsic hydrolysis of GTP and the fact that the affinity of EF-Tu for GDP is two orders of magnitude higher than its affinity for GTP. These problems have spurred many biochemical experiments with the aim of identifying regions or residues on tRNA or EF-Tu that are involved in binding each other. Several recent reviews summarize these experiments [24–26].

The crystal structures of the components of the ternary complex are known. The first well diffracting crystals of yeast tRNA^{Phe} were reported in 1968 [27]. Crystallization of EF-Tu:GDP was reported in 1973 [28]. The structure of yeast tRNA^{Phe} has been known since 1974 [29–30]. The stable but inactive EF-Tu:GDP from *E. coli* has been the subject of several crystallographic investigations over more than 15 years (see [10] and references therein). Recently the structures of the active EF-Tu:GDPNP from *T. thermophilus* [11] and from *T. aquaticus* [12] have been determined. Attempts at getting information on the overall structure and possible conformational changes of aa-tRNA and/or EF-Tu during ternary complex formation include small angle neutron diffraction experiments. The results are confusing as one report indicates a rather elongated complex [31] while the other suggests a compact one [32]. These structural results together with the wealth of biochemical data have resulted in different attempts at predicting the structure of the ternary complex. Owing to the large conformational change of EF-Tu in the transition from EF-Tu:GDP to EF-Tu:GTP earlier predictions have been erroneous [24–26]. More recent predictions are in this respect more reliable [25].

It was found rather early that the ternary complex with Val-tRNA^{Val} shows increased stability in high concentrations of ammonium sulphate (AS) and ammonium citrate [33]. These experiments were repeated, showing that this was generally true for other ternary complexes [34]. In the same paper it was reported that ternary complexes were also more stable in polyethylene glycol (PEG). Purification of milligram quantities of ternary complexes, free of deacylated tRNA and inactive EF-Tu, was shown to be possible using HPLC gel filtration [21]. The same report showed that ternary complexes could be

isolated by a fractional extraction in the presence of AS or PEG and that concentrated samples of ternary complex exhibits increased stability.

In our laboratory the ternary complex of EFTu:GDPNP:Phe-tRNA^{Phe} was formed by incubation of *Thermus aquaticus* EF-Tu:GDPNP with a preparation of yeast Phe-tRNA^{Phe}. The complex was separated from other components of the reaction mixture by HPLC silica gel filtration in a high ionic strength buffer (Fig. 1). The high ionic strength increases the elution volume of free EF-Tu due to unspecific binding. The ternary complex in the precipitated pool was further purified by reverse fractional extraction. Excess tRNA and EF-Tu appeared at 60% and 45% AS, respectively. The ternary complex was extracted in fractions from 55% to 47.5% AS. Fractions of ternary complex, at a molar ratio of EF-Tu:GDPNP over Phe-tRNA^{Phe} equal to 1 were used for crystallization by the vapor diffusion method using hanging or sitting drops. The final yield of pure EF-Tu:GDPNP:Phe-tRNA^{Phe} was about 30% of the starting materials (Phe-tRNA^{Phe}).

Single crystals of rhombohedral shape appeared within three to five days of equilibration at 4°C (Fig. 2). Crystallization conditions were: 20 mM Tris-HCl (pH 7.6), 3 mM MES (pH 2.7), 35% AS, 7 mM $MgCl_2$, 0.5 mM NaN_3, 0.5 mM DTT and 0.4 mM GDPNP with a final concentration of 15 mg TC per ml in the drop and 47–49% AS, pH 6.7–7.0 in the reservoir. It was noted that a concentration of GDPNP in a 2- to 4-fold excess over the concentration of EF-Tu was critical for the growth of single crystals. Crystals of size $0.7 \times 0.5 \times 0.3$ mm^3 were obtained within 14 days . The crystals were stable in a buffer with 65% AS and could be kept for at least two months. Carefully washed and redissolved crystals showed the unique presence of EF-Tu and Phe-tRNA^{Phe} in the initial molar ratio on a silverstained SDS-PAGE (Fig. 3).

Crystals forming hexagonal plates were obtained of a ternary complex of *E. coli* EF-Tu:GDPNP and yeast Phe-tRNA^{Phe}. The main difference was in the use of lower concentrations of AS in precipitation and in fractional extraction. However, these crystals were less stable and further work on them was not carried out. Attempts to crystallize a pentameric complex resulted in crystals of EF-Tu:GDPNP.

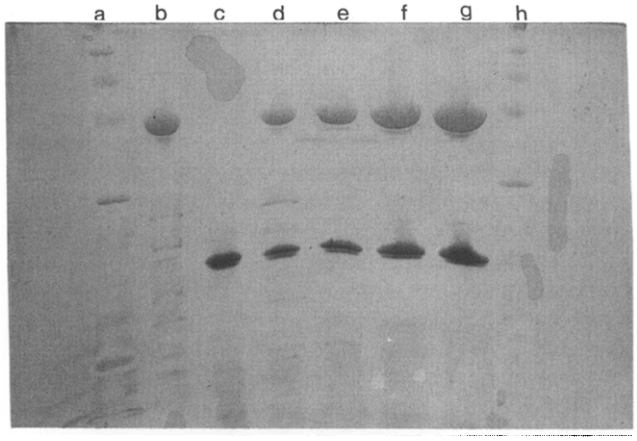

Fig. 3. Silver stained SDS-PAGE in 18% polyacrylamide of redissolved crystals of the ternary complex. Lane a, molecular weight markers (90, 65, 43, 30, 21 kDa); lane b, EF-Tu:GDPNP preparation; lane c, tRNA^{Phe} from brewers yeast (Böhringer); lane d, a 1:1 molar mixture of EF-Tu and tRNA^{Phe}; Lane e, washed and redissolved crystal of TC; Lane f, mother liquor of TC crystals; lane g, Purified TC (1:1) in crystallization buffer; lane h, molecular weight markers.

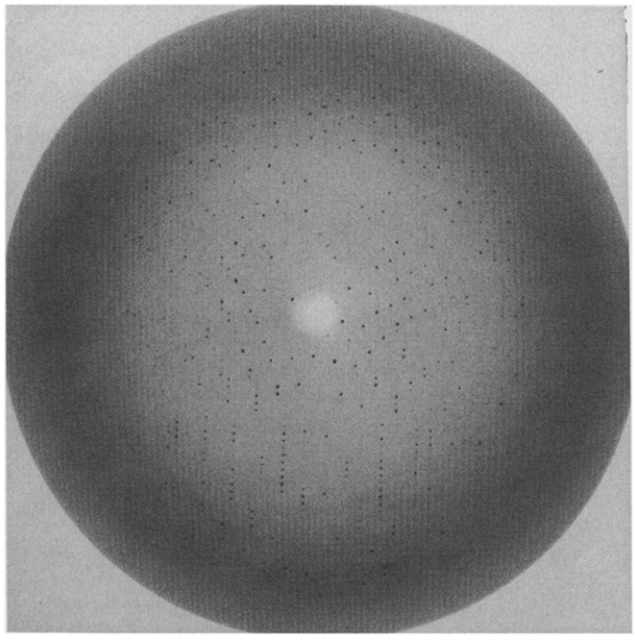

Fig. 4. Oscillation photograph of crystals of the ternary complex obtained at SRS station 9.6. The wavelength was 0.88 Å. The oscillation range was 1.0°. The maximum resolution is 2.8 Å.

A sucrose concentration of 20% was found suitable for data collection at cryogenic temperatures [35]. Diffraction data were collected to 2.8 Å resolution at SRS, Daresbury, station 9.6 (Fig. 4). The crystal system is monoclinic, spacegroup C2, with cell dimensions $a = 208.28$ Å, $b = 122.30$ Å, $c = 151.81$Å, $\beta = 126.70°$. Calculations give three to six ternary complexes per asymmetric unit assuming a normal range of solvent content.

The crystallization of the ternary complex described above is the first crucial step in the successful elucidation of its spatial structure by X-ray crystallographic analysis.

Acknowledgements: We thank Svetlana Khodyreva and Ove Wiborg for help with *T. aquaticus* fermentation. We thank the staff at ESRF/EMBL and SRS for help with datacollection. This work is supported by the Danish Biotechnology Research Programme through its Protein Engineering Research Centre and through its special support to Synchrotron Radiation Research. The work has also been supported by the Network for Protein Crystallography funded by the EEC Programme Human Capital and Mobility.

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