

Crystallization and preliminary X-ray diffraction studies of intact EF-Tu from *Thermus aquaticus* YT-1

Corinna Lippmann, Christian Betzel*, Zbyszek Dauter*, Keith Wilson* and Volker A. Erdmann

Institut für Biochemie, Freie Universität Berlin, Thielallee 63, D-1000 Berlin 33, Germany and *EMBL Outstation Hamburg, c/o DESY, Notkestr. 85, D-2000 Hamburg 52, FRG

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Many attempts have been made to elucidate the three-dimensional structure from elongation factor Tu, but so far the only crystals suitable for X-ray crystallography contained a partially degraded protein. Here, we report the crystallization of a fully active, intact EF-Tu from *Thermus aquaticus*. The crystals belong to hexagonal space group P6₃22 and diffract up to 2.6 Å. The cell dimensions are $a=b=178$ Å, $c=238$ Å and 6 molecules are contained per asymmetric unit.

Crystallization; X-ray diffraction; Synchrotron radiation; Elongation factor Tu

1. INTRODUCTION

Elongation factor Tu (EF-Tu) plays a central role in protein biosynthesis and during its function it has to interact with a number of different ligands such as GDP, GTP, aminoacyl tRNA, elongation factor Ts and ribosomes [1]. In addition, it has been shown that EF-Tu may also be a component of the bacteriophage Q β replicase [2]. Since it is known that there are extensive sequence homologies between EF-Tu and GDP/GTP-binding proteins [3], the elongation factor became a model for structure-function relationships of G-proteins [4], some of which play a crucial role in transmembrane signalling [5]. EF-Tu and its eukaryotic counterpart EF-1 α were isolated from different organisms. Their molecular masses have been determined to be around 50 kDa.

The first crystallization experiments with EF-Tu were carried out more than 15 years ago [6]. Dif-

ferent crystal forms were obtained [7], but only crystals from the partially degraded protein (EF-Tu from *E. coli*) [8], which lacked a fragment of 14 amino acids (residues 45–58) [4] were suitable for X-ray examination [9]. The high-resolution structure is still incomplete and has been solved only for the G-binding domain [10]. However, the trypsin-digested elongation factor is not fully active [11] and the tRNA-binding site is still obscure [12]. Since the thermal and chemical stability of *E. coli* EF-Tu is relatively poor, we attempted the crystallization of a thermophilic EF-Tu, namely from the eubacterium *Thermus aquaticus* YT-1. The present results show that this alternative enabled us to isolate and to crystallize the intact EF-Tu, so that its three-dimensional structure can be solved in the near future.

2. MATERIALS AND METHODS

Unless otherwise stated, all materials were purchased commercially. Culture media were from Gibco BRL, standard chemicals from Merck, and DEAE-Sepharose CL-6B and AcA-44 from Pharmacia-LKB. Nonradioactive nucleotides, tRNA^{Phe} (*E. coli*), phosphoenolpyruvate and pyruvate kinase were obtained from Boehringer. [³H]GPD (370 GBq/mmol) was purchased from New England Nuclear and [¹⁴C]phenylalanine (16.6 GBq/mmol) from Amersham.

We wish to dedicate this paper to Professor Friedrich Cramer on the occasion of his 65th birthday

Correspondence address: C. Lippmann, Institut für Biochemie, Freie Universität Berlin, Thielallee 63, D-1000 Berlin 33, Germany

T. aquaticus YT-1 cells harvested at late log phase were grown at 71°C in a medium containing 0.3% peptone 140, 0.3% yeast extract, 0.015% NaCl, 2.7% 0.5 M Na₂HPO₄ (adjusted with KOH to pH 7.6) and 0.05% essentials according to Brock and Freeze [13].

Ribosomes and the S-100 fraction were prepared as described [14]. After the removal of DNA with protamine sulfate, S-100 proteins were precipitated by adding solid ammonium sulfate up to 70% saturation. Elongation factors were isolated from this precipitate following the procedure of Lebermann et al. [15] with minor modifications. Enzyme activity of EF-Tu, EF-Ts and EF-G was monitored as in [6,16]. Charging of tRNA^{Phe} and ternary complex formation were carried out according to [17,18]. EF-Tu purification was analysed by SDS gel electrophoresis [19] and protein concentrations were determined by the method of Smith et al. [20]. The factor was in addition purified by equilibrium dialysis.

For crystallization experiments [21] a 100% saturated ammonium sulfate solution [50 mM Tris-HCl (pH 7.6 at 20°C),

10 mM MgCl₂, 1 mM DTE, 10 μM GDP, 0.1 mM NaN₃, 10 μM PMFS) was prepared and used to obtain different salt concentrations. All dialysis steps were carried out at 0°C. EF-Tu·GDP-containing fractions from the AcA-44 column were combined and protein concentration was adjusted to 15 mg/ml. The solution was then dialysed vs 40% ammonium sulfate. Thus, contaminating proteins were precipitated and removed by centrifugation at 5500 × g and 4°C for 1 h. The supernatant was dialysed again as described above. Crystal growth was achieved by increasing the precipitant slowly (0.5% per day) to a final concentration of 51%. During dialysis vs 46% ammonium sulfate the solution was allowed to warm to room temperature and then cooled again to 0°C [22]. After this step the first crystals appeared. Most of the protein had crystallized after 4 weeks.

Single crystals were obtained by the 'hanging-' and 'sitting-drop' vapor diffusion technique [22,23]. Numerous crystallization conditions were tested by varying temperature, precipitant, pH and concentration of buffer and protein.

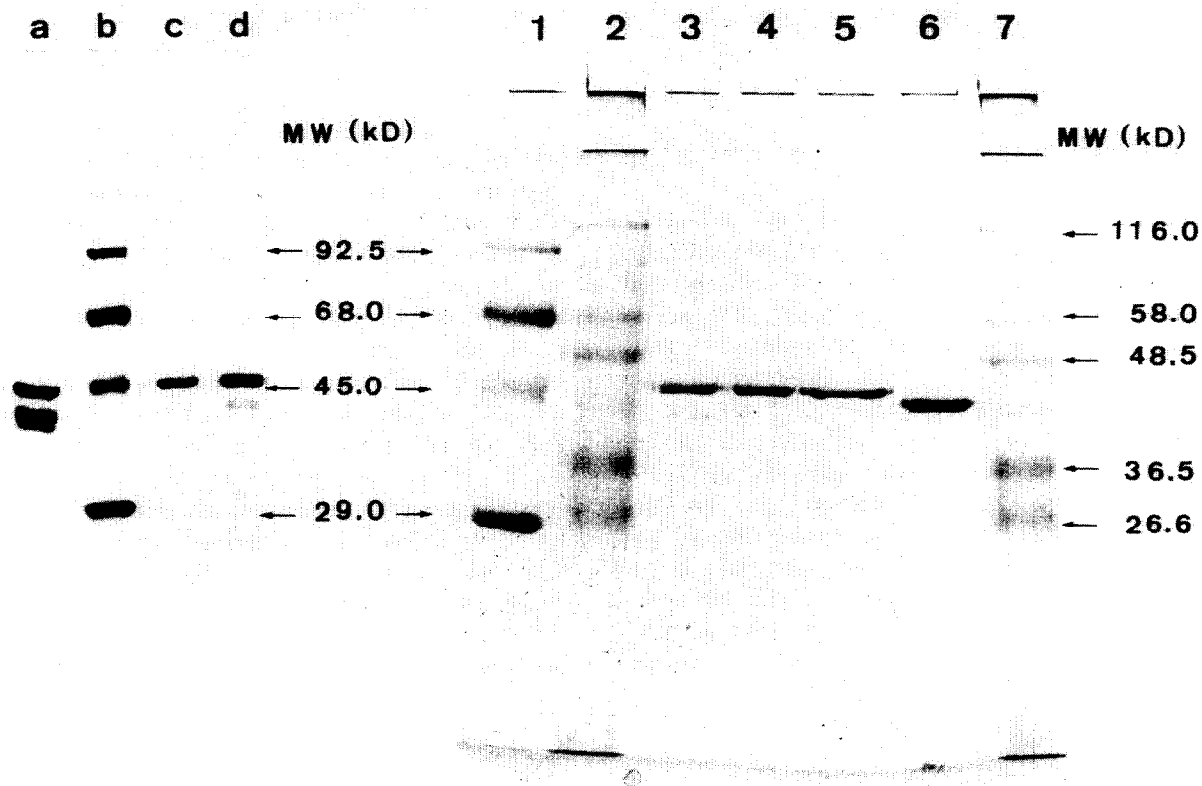


Fig.1. SDS gel electrophoresis from different preparation stages of *T. aquaticus* EF-Tu. Lanes: (a) after AcA-44 column; (b) molecular mass markers (phosphorylase *b*, 92.5 kDa; bovine serum albumin, 68 kDa; ovalbumin, 45 kDa; carboanhydrase, 29 kDa); (c,d) redissolved crystals after first crystallization step; (1) same as (b); (2) prestained molecular mass marker proteins (β -galactosidase, 116 kDa; pyruvate kinase, 58 kDa; fumarase, 48.5 kDa; lactate dehydrogenase, 36.5 kDa; triosephosphate isomerase, 26.6 kDa); (3) after hanging drop crystallization, under nonreducing conditions; (4) same as (3), but under reducing conditions; (5) same as (c,d); (6) EF-Tu from *E. coli* MRE 600; (7) same as (2). Gels are of 12% acrylamide concentration and stained with Coomassie blue. Probes were dissolved in sample buffer containing β -mercaptoethanol unless otherwise indicated and were heated to 100°C for 5 min prior to electrophoresis.

3. RESULTS

About 50 mg purified elongation factor Tu were isolated from 200 g wet cells as determined after the first crystallization, which was used as a purification step. EF-Tu from *T. aquaticus* YT-1 has an estimated molecular mass of 45 kDa as deduced from SDS gel electrophoresis (fig.1) and HPLC on Bio-Sil TSK 3000 SW (not shown). The isoelectric point was estimated to be 5.4. SDS gel electrophoresis from redissolved crystals under reducing and denaturing conditions clearly showed the factor to be a pure and intact protein (fig.1), which was fully active in GDP binding and ternary complex formation. 1 pmol protein bound approx. 1 pmol GDP.

After the first crystallization (final purification step) the protein concentration was adjusted to 10 mg/ml. The factor was then dialysed vs 20% $(\text{NH}_4)_2\text{SO}_4$ by microdialysis according to [24].

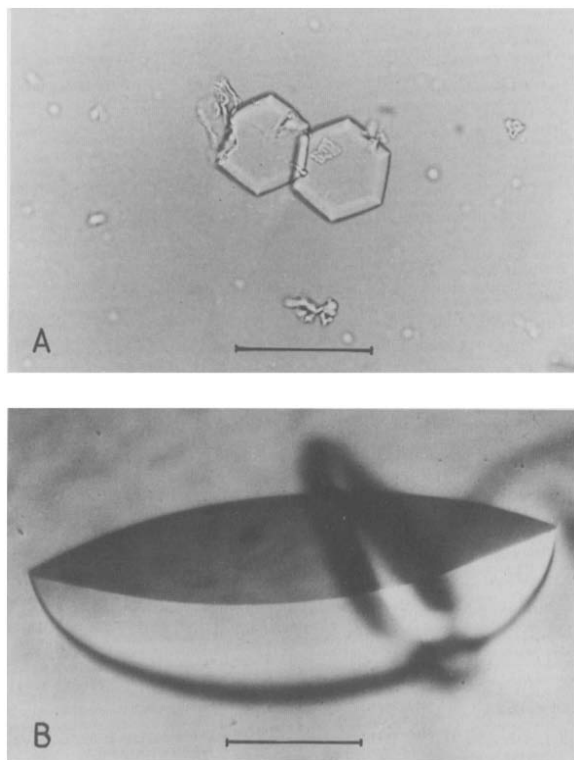


Fig.2. Crystal habit after first crystallization step (A) and from hanging drop (B). B shows a typical crystal used for X-ray investigation. Scale bars represent 0.15 mm (A) and 0.35 mm (B).

Large single crystals as shown in fig.2B were obtained from a 46% ammonium sulfate solution under the conditions described in section 2. Crystal growth was achieved in hanging drops after 6–8 weeks. Decrease in pH and increase in temperature gave a shower of small crystals. At concentrations higher than 55% ammonium sulfate EF-Tu precipitated, although crystal growth could be observed in the precipitates. Seeding with small crystals results mostly in the growth of polycrystallites and not single crystals.

Crystals were first characterised by synchrotron radiation using a modified Arndt-Wonacott oscillation camera on EMBL beam line X31 at the storage ring DORIS (DESY, Hamburg) during main user time. For these studies single crystals were mounted in 1 mm diameter glass capillaries. They were aligned along the a^* axis and oscillated by 2° per photograph. Exposure times were 14 min using a wavelength of 1.488 Å and a crystal-to-film distance of 80 mm. The oscillation photographs were digitised with an Optronics P1000 drum scanner in the 0–2 Å range and processed with the MOSCO film integration package [25].

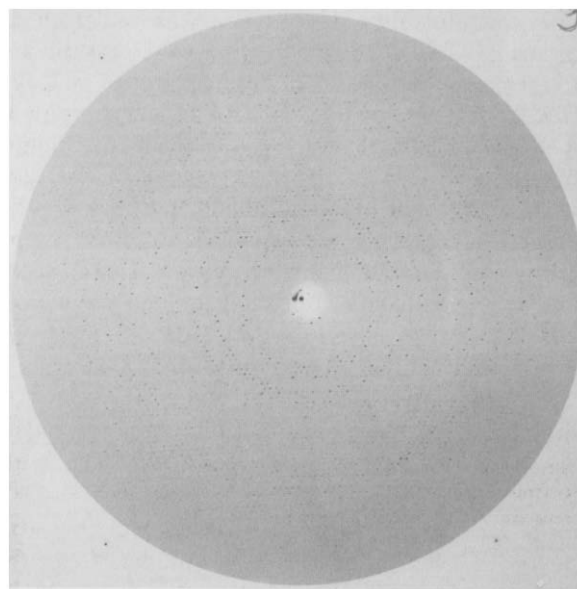


Fig.3. Oscillation photograph of intact EF-Tu with a crystal-to-film distance of 80 mm, rotation range 2° and exposure time 14 min. The 6-fold axis is almost perpendicular to the film plane.

The diffraction extends to about 2.6 Å resolution for fresh crystals. From the processing of oscillation photographs the space group was primarily assigned to P₆₃22 with cell dimensions $a = b = 178$ Å, $c = 238$ Å. These yield a unit cell volume of 6.5×10^6 Å³ and a packing density parameter $V_M = 2.3$ Å³/Da assuming 6 molecules in the asymmetric unit [26].

4. DISCUSSION

Recently, the structure of another G-protein, c-H-ras p21, was reported [27]. A detailed comparison with the G-binding domain from a modified *E. coli* EF-Tu showed remarkable homology not only in primary [3], but also in tertiary structure [4]. One of the major differences between the structure of p21 and EF-Tu is the loop from residue 25 to 39 in p21, whose counterpart is missing in the modified EF-Tu, i.e. residues 45–58. For p21 this loop contains positions which are essential for biological activity. In EF-Tu this loop seems to be responsible for tRNA binding [11]. The loop also appears to be in close contact with the ribose-phosphate part of the GDP in p21 and in EF-Tu [28].

The crystals described here are derived from an intact EF-Tu, as shown in fig.1. The crystals are relatively insensitive to radiation damage and diffract to 2.6 Å or better. With the crystals obtained, it should be possible in the future to resolve all ambiguities concerning the structure of EF-Tu, not only in the GDP-binding domain, but also for the rest of the molecule, for which the structure is currently only known at low resolution. Heavy-atom derivation and data collection at high resolution are in progress.

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