CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION DATA OF THE EF-Tu · EF-Ts (EF-T) COMPLEX OF *ESCHERICHIA COLI*

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1. Introduction

The bacterial polypeptide elongation factor T (EF-T) [1] was shown to be a complex of two proteins designated EF-Tu (heat-unstable) and EF-Ts (heat-stable) [2]. Elongation factor Tu, which represents ~5% of the total cell protein [3], functions in a complex with GTP in promoting the binding of aminoacyl-tRNA to the A-site of the ribosome–mRNA complex. The role of EF-Ts appears to be that of a catalyst for the exchange of guanine nucleotides bound to EF-Tu, facilitating the displacement of strongly bound GDP by the more weakly bound GTP (review [4]). The two proteins have a high affinity for each other and form a complex with a binding constant of $\sim 5 \times 10^8\ M^{-1}$ [5,6]. The two elongation factors have also been identified as subunits III and IV of Qβ-replicase [7], and although the individual functions of the two proteins in this system are not clearly defined their presence as a complex is required for activity (review [8]). Recently, a role for EF-Ts in the regulation of RNA synthesis and the process of stringent control has been proposed [9].

The structure of trypsin-modified [10,11] and proteolysed [12] EF-Tu · GDP complex is being analysed by X-ray diffraction in 3 laboratories. Crystals of the native protein are not presently being studied because of the inherent difficulties associated with polymorphic crystal forms and partial occupancy of the crystal lattice [13].

Despite many attempts no crystals of EF-Ts have been produced in this laboratory, most procedures which brought the protein out of solution just produced an 'oily' precipitate. However, the structure of the EF-Tu · EF-Ts complex provides a route to the study of both elongation factors and their interactions, as well as indicating a strategy that could be adopted in undertaking the structural analysis of a complex system such as Qβ-replicase.

2. Materials and methods

EF-Tu · GDP was isolated from *Escherichia coli* (MRE 600) as in [14]. The partially purified EF-Ts obtained by ion-exchange chromatography [14] was mixed with a slight excess of EF-Tu · GDP and dialysed against buffer A (0.05 ionic strength Tris–HCl, 0.5 mM dithioerythritol, 10 μM phenyl-methyl-sulphonyl fluoride, 1 mM NaN_3, pH 7.6) to remove GDP. The EF-Tu · EF-Ts complex was isolated from the dialysed solution by ion-exchange chromatography on DEAE–Sepharose (Pharmacia). Poyethylene glycol (PEG 6000) was from Roth (Karlsruhe). All other chemicals were of analytical quality.

After preliminary trials by the hanging-drop procedure, crystals were produced from 200 μl batch crystallizations. Crystals for X-ray analysis were transferred from the batch crystallization vials to a storage solution of 18% (w/w) PEG 6000 in buffer A. The search for isomorphous heavy atom derivatives was made by suspending crystals in solutions of heavy metal compounds in the storage buffer without dithioerythritol.

For the diffraction studies a precession camera (Enraf-Nonius) was used with a GX6 rotating anode X-ray generator (Elliott), and an automated diffractometer (Nicolet).

3. Results and discussion

3.1. *Crystallization*

Screening by means of the hanging-drop procedure indicated that crystallization from PEG 6000–NaCl solutions was most promising for the production of

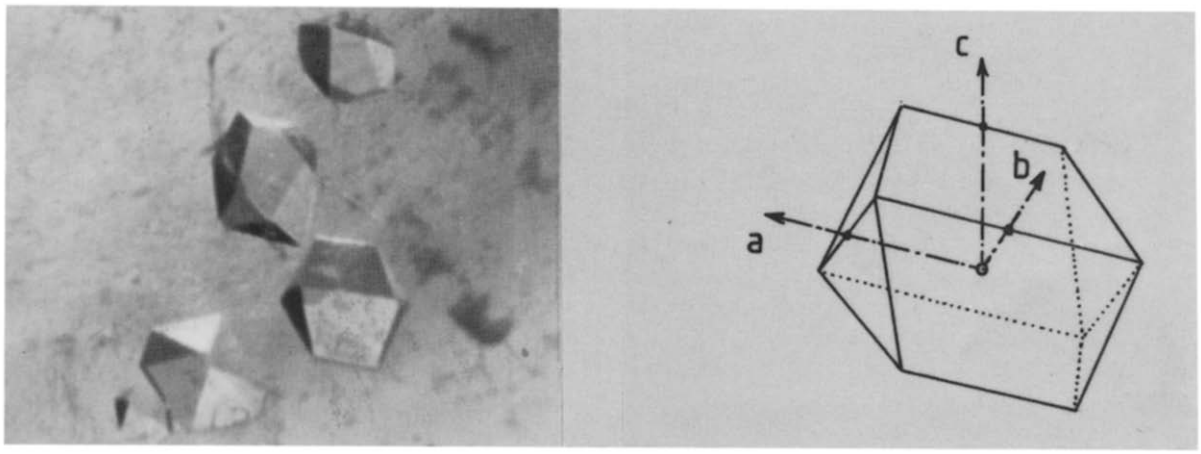

Fig.1. Left: crystals of *Escherichia coli* EF-Tu · EF-Ts complex: maximum dimension 0.6 mm. Right: a schematic drawing of the crystal shows the direction of the crystal axes in relation to the crystal morphology.

suitable crystals. The best crystals were grown from 0.2–0.3% protein solutions in buffer A containing 15% (w/w) PEG 6000, 0.2–0.3 M NaCl, at 18°C. Under these conditions crystals with a maximum dimension of 0.3–0.6 mm have been produced (fig.1). Substituting phosphate or pyrophosphate for NaCl at the same ionic strength we obtained crystals with a needle-like habit. Since these crystals belong to the same space group and have the same cell dimensions as the more chunky crystals (fig.1) a study of them was not pursued.

3.2. *X-ray diffraction*

Data collected by precession photography and using the automated diffractometer showed that the crystals belong to the space group $P2_12_12_1$ with cell dimensions of $a = 81.7$ Å, $b = 110.5$ Å, $c = 206.0$ Å, corresponding to a unit cell volume of 1.86×10^6 Å^3. No deviations from these dimensions larger than the limits of error of ~1% were observed in the 6 native crystals examined. The diffraction pattern of the 0*kl*-plane is shown in fig.2. Using the largest crystals available, reflections were observed out to a resolution of 4.5 Å.

Dividing the unit cell volume by the molecular weight (or M_r-value) of the EF-Tu · EF-Ts complex of ~80 000, the values obtained for the volume/M_r unit, V_m, are 5.80 Å^3, 2.90 Å^3, and 1.94 Å^3 for 1, 2 and 3 complex molecules/asymmetric unit, respectively. Among these options only 2 molecules/asymmetric unit fits the observed distribution for other proteins [15]; this corresponds to 8 EF-Tu · EF-Ts molecules in the unit cell.

For the diffraction analysis it is crucial to obtain heavy atom derivatives of the crystals. Preliminary screening led to the detection of two such derivatives:

(i) That prepared by soaking crystals in 10 μM methyl mercury acetate for 3 days at 18°C. As the diffraction pattern changed no variation in the cell dimensions exceeding the limits of error was observed. Since there are 5 thiol groups in the protein complex, binding of methyl mercury is to be expected;

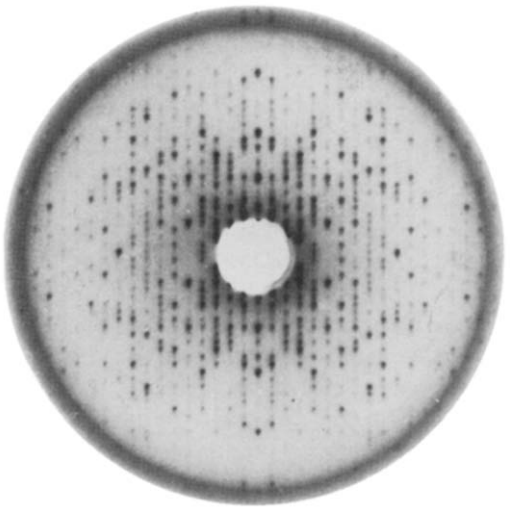

Fig.2. X-ray precession photograph of the 0*kl*-plane of native crystals of the EF-Tu · EF-Ts complex as taken using CuK_α radiation from a GX6 (Elliott, London) rotating anode X-ray generator: the resolution is 6 Å.

(ii) That produced by soaking the crystals for 6 days in 1 mM uranyl sulphosalicylate. There were clear differences in the diffraction pattern compared to those of both the native and methyl mercury soaked crystals. Again, within the limits of error there were no changes in the crystal axes.

These observations are strong indications that both these derivatives will be useful in the structural analysis.

At a maximum resolution of 4.5 Å the crystals described could lead to a low resolution structure determination based on 13 000 crystallographically-independent reflections. Such an anaylsis will yield the general outline and some prominent features of the EF-Tu · EF-Ts complex. Since the low resolution structure of the EF-Tu moiety is known [10–12], the analysis could be used to reveal the low resolution structure of the EF-Ts molecule. Furthermore, it could provide a general description of the interactions between the component molecules. Hopefully, further biochemical studies in conjunction with the crystallographic analysis might provide more detailed structural information.

3.3. *A high resolution crystal form*

When trying to produce a methyl mercury acetate derivative under mild conditions by soaking crystals in a 1 μM solution, the two crystals examined had changed to a new crystal form with improved melecular order. Since other, unsoaked, crystals from the same crystallization batch retained the original crystal form, this molecular rearrangement must have been induced by binding methyl mercury.

During this transition the crystals did not show any macroscopic changes, such as cracking. The space group of the new form remained $P2_12_12_1$ but the cell dimensions were markedly different. With a = 74.3 Å, b = 108.7 Å and c = 198.5 Å, the axes had shortened by 10%, 2% and 4%, respectively. In general the diffraction pattern of the contracted crystal form was quite different from that of the original crystals. Below 10 Å resolution, however, both patterns were very similar showing that only slight changes with respect to the molecular packing had occurred. On shrinking, the volume/M_r unit decreased from V_m = 2.9 Å^3 to V_m = 2.5 Å^3. This value still lies within the observed distribution for other proteins [15] and corroborates the assumption of two complex molecules in the asymmetric unit.

More importantly, the new crystal form showed a definite improvement in molecular order within the crystal lattice; its X-ray diffraction pattern extending to a resolution of ⩾3 Å. Such a resolution would permit the location of the polypeptide chain in an electron density map [16]. Consequently, these crystals may provide the possibility to study the structures and interactions between EF-Tu and EF-Ts in detail.

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