CRYSTALS OF A LARGE TRYPTIC PEPTIDE (FRAGMENT A) OF ELONGATION FACTOR EF-Tu FROM *ESCHERICHIA COLI*

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1. Introduction

We have recently described the crystallisation of native EF-Tu·GDP from *E. coli* [1] and of the product obtained by mild tryptic digestion of this protein [2] using polyethylene glycol (PEG) 6000 as a precipitant. The crystalline native protein is polymorphic with four interrelated trigonal and hexagonal crystal forms. The pseudo-tetragonal crystals of the trypsin treated protein were found to be essentially identical to those previously described by Sneden et al. [3] and were shown to contain proteolytically degraded protein.

A detailed study of the action of trypsin on EF-Tu·GDP by Arai et al. [4] supported our observation [2] that mild proteolysis did not destroy the nucleotide binding properties of the factor even though a number of scissions were made in the polypeptide chain. They also confirmed the observation made in this laboratory (Wittinghofer and Gast, unpublished results) that by incubating the reaction at 0°C the tryptic digestion of EF-Tu·GDP could be essentially limited to the production of a 39 000 molecular weight species, which they call fragment A. We describe here the crystallisation of this fragment from polyethylene glycol solutions in a form suitable for an X-ray diffraction study.

2. Materials and methods

EF-Tu·GDP was isolated from *E. coli* (MRE 600) by methods similar to those described by Arai et al. [5]. The protein had a molecular weight of 44 000 ± 2000 and bound 20 000 ± 2000 pmol GDP/mg.

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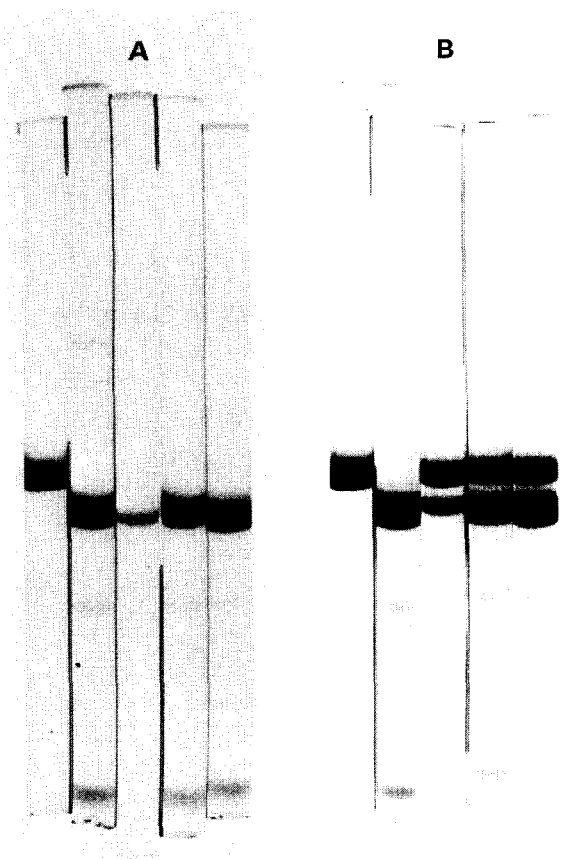

Fig.1. SDS–polyacrylamide gel electrophoresis (7.0% gels) of EF-Tu and products of trypsin digestion. (A) from left to right, starting material, product after digestion for 20 min at 0°C, 30%, 25%, and 20% ammonium sulphate fraction. (B) As (A) with starting material added to ammonium sulphate fraction.

Trypsin (TRL 355728) was obtained from Worthington (New Jersey) and soybean trypsin inhibitor from Boehringer (Mannheim). Polyethylene glycol was PEG 6000 from Serva (Heidelberg). All other reagents were of analytical quality. Analytical SDS–polyacrylamide electrophoresis was carried out as previously described [6].

Crystallisations were carried out at room temperature by mixing suitably buffered solutions of protein and PEG 6000. Photographs were obtained from crystals mounted in thin-walled quartz capillaries and exposed to CuK_α X-rays produced by a rotating anode tube (GX-6, Elliott Brothers Ltd.). Crystal density was measured in a gradient of xylol–carbon tetrachloride calibrated with CsCl solutions.

3. Results

EF-Tu·GDP (10 mg/ml) in a buffer containing 50 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.1 mM NaN_3, 10 μm phenylmethylsulphonyl fluoride, pH 7.6 (buffer A) was incubated for 20 min at 0°C with trypsin (1% w/w). The reaction was terminated by the addition of excess soybean trypsin inhibitor (3% w/w). The digested protein in the reaction mixture was precipitated by the addition of an equal volume of saturated ammonium sulphate solution and collected by centrifugation. The precipitate was extracted successively with ammonium sulphate solutions of decreasing concentration [7] from 45% to 20% saturation in 5% steps. GDP binding activity and SDS–polyacrylamide gel electrophoresis located the 39 000 molecular weight product (fragment A) in the 30, 25 and 20% saturated ammonium sulphate fractions. Gel electrophoresis (fig.1) of these fractions alone and with starting material indicated that at least 90% of the tryptic product was fragment A.

The fragment A containing fractions were dialysed against a buffer having the same compositions as buffer A, but pH 7.0, made 2×10^{-3} M in GDP and

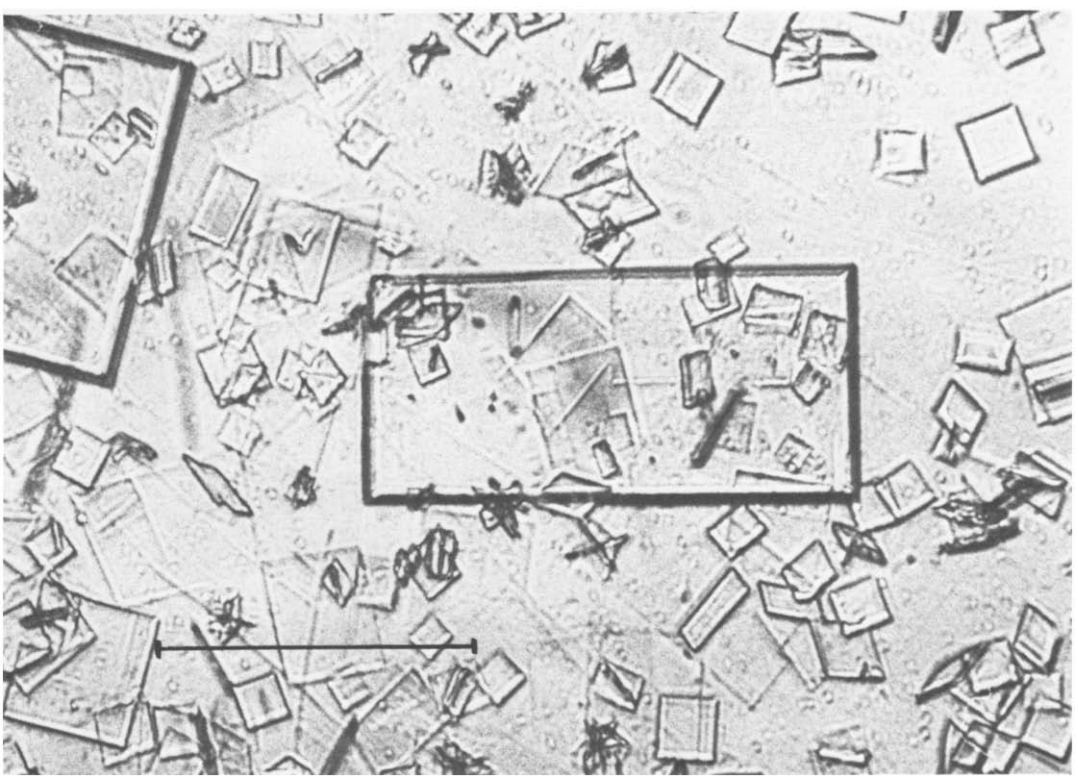

Fig.2. Crystals of EF-Tu-fragment A. Bar represents 0.5 mm.

set up to crystallise as described. Within 24 h crystals were obtained at 3 mg fragment A/ml and 8–9% (w/w) PEG 6000, these grew to about 1 mm in the largest dimension in the course of 3 days.

Table 1
Crystallographic parameters of crystals of fragment A from EF-Tu·GDP

Parameters	Value/Description
Habit	Rectangular prism
Density	1.19 g/cm^3
Space group	$P2_12_12_1$
Cell dimensions	a = 144 Å
	b = 93 Å
	c = 69 Å
Cell Volume	9.24 × 10^5 Å^3
V_m	2.98 Å^3/dalton
Molecules/asymmetric unit	2

The relevant crystallographic parameters are summarised in table 1 and a precession photograph of the Okl-plane is shown in fig.3. The X-ray diffraction pattern extends to a resolution of at least 3.0 Å. The crystals have a lifetime of more than 30 h in the X-ray beam at 40 kV and 40 mA.

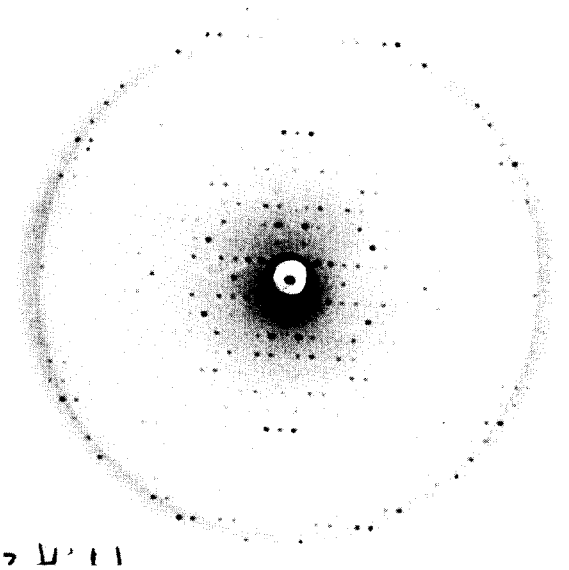

Fig.3. Precession photograph of Okl plane of EF-Tu-fragment A X-tal, μ = 9°, exposure time 20 h at 40 kV and 40 mA.

4. Discussion

Arai et al. [4] have shown that, unlike EF-Tu, fragment A cannot form a complex with aminoacyl-tRNA but still possesses other properties of the native protein such as complex formation with GDP, GTP and EF-Ts. The maintenance of these properties would indicate that a large part of the conformation of the native protein is preserved so that a structural study of this fragment should be undertaken.

The crystals we have described here appear to be eminently suitable for such a study. They are of a single polypeptide chain corresponding to about 90% of the native protein. They diffract to a resolution of at least 2.5 Å, are mechanically stable and are relatively insensitive to radiation damage.

Acknowledgements

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References

[1] Leberman, R., Wittinghofer, A. and Schulz, G. E. (1976) J. Mol. Biol. 106, 951–961.
[2] Gast, W. H., Leberman, R., Schulz, G. E. and Wittinghofer, A. (1976) J. Mol. Biol. 106, 943–950.
[3] Sneden, D., Miller, D. L., Kim, S. H. and Rich, A. (1973) Nature (London) 241, 530–531.
[4] Arai, K. I., Nakamura, S., Arai, T., Kawakita, M. and Kaziro, Y. (1976) J. Biochem. 79, 68–83.
[5] Arai, K. I., Kawakita, M. and Kaziro, Y. (1972) J. Biol. Chem. 247, 7029–7037.
[6] Ziegler, A., Harrison, S. C. and Leberman, R. (1974) Virology 59, 509–515.
[7] Jacoby, W. (1968) Anal. Biochem. 26, 295–298.