Crystallization of elongation factor G from an extreme thermophile, *Thermus thermophilus* HB8

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1. INTRODUCTION

Elongation factor G is an important component of the protein-synthesizing system; its functional and structural aspects have been studied intensively. The G factor from *Escherichia coli* is the most studied, but due to its structural instability no satisfactory crystals of this protein have been obtained yet. We have managed to grow single crystals of tryptic fragments of the G factor from *E. coli* [1]. Unfortunately, these crystals have a low radiation stability and a special approach should be developed for their study.

The G factor from *Thermus thermophilus* HB8 (TEF-G) was first isolated and crystallized in [2]. TEF-G is very similar to the G factor from *E. coli*, but has a more compact structure and is strongly resistant to trypsin [3,4]. In the process of purification, due to natural proteolysis, the polypeptide chain of TEF-G breaks at a distance of 10–15 amino acid residues from the N-end, and the modified factor retains its biological activity. The modified and native forms of TEF-G have very similar properties and cannot be separated from each other by gel filtration or ion-exchange chromatography. Only adsorption chromatography on an hydroxylapatite column is effective for the separation of these two forms of TEF-G [5]. On gel electrophoresis in Tris–borate buffer without denaturation agents, the native and the modified TEF-G moves as a single band. It is possible that this is a result of an association of the cleaved N-terminal peptide with the remaining part of the molecule. If this is the case the modified TEF-G differs from the native one only by a break in the polypeptide chain. A similar modification of TEF-G can be also produced by trypsin treatment [4,5].

Here, we describe the growing of large crystals of TEF-G suitable for X-ray structure determination.

2. MATERIALS AND METHODS

The *Thermus thermophilus* strain HB8 was kindly provided by Professor Tairo Oshima (Mitsubishi-Kasei Institute of Life Science, Tokyo). Cells were cultured at 75°C, harvested at the mid-log phase, and stored frozen at −30°C. The purification of the TEF-G was done as in [5]. Crystallization was performed at 4°C or 20°C using the hanging-drop microdiffusion technique with ammonium sulfate, sodium citrate and 2-methyl-2,4-pentanediol (MPD) as precipitants. Protein solution (10 μl) on siliconized glass was placed over a vial with 1 ml of the precipitant solution. SDS–polyacrylamide gel electrophoresis was done as in [6].

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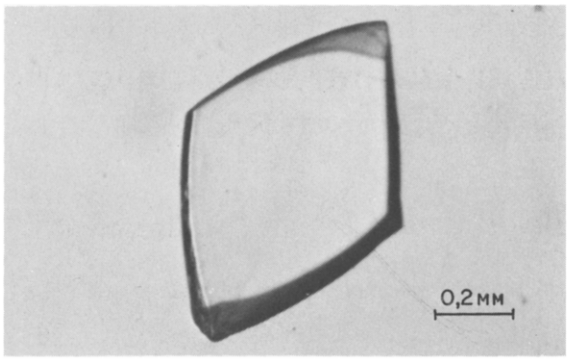

Fig.1. Microphotograph of the crystal of the G factor from *Thermus thermophilus* HB8.

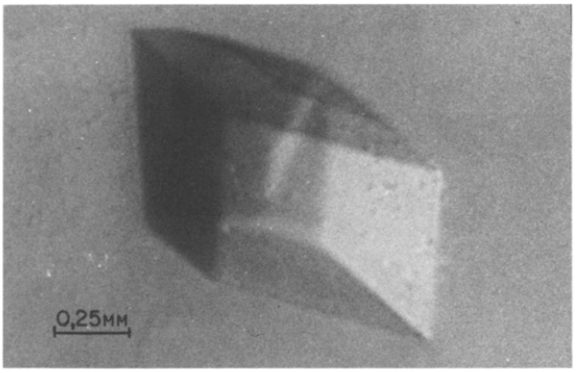

Fig.2. Microphotograph of the crystal of the trypsin-modified G factor from *Thermus thermophilus* HB8.

3. RESULTS AND DISCUSSION

Preparations of native and naturally modified TEF-G were crystallized with MPD. The best crystals were obtained when 10 μl of the TEF-G solution (7–10 mg/ml) in 20 mM imidazole–HCl buffer (pH 7.8) with 3 mM sodium azide were equilibrated by vapour diffusion with 20% (v/v) MPD. Crystals appeared after 1–2 weeks and had the form of parallelepipeds with rounded angles of 0.15 × 0.5 × 0.6 mm (fig.1). A preliminary crystallographic study has shown that the TEF-G crystals obtained in the above conditions are stable in X-ray beam and suitable for X-ray analysis at ~3.5 Å resolution [7].

TEF-G modified by trypsin [5] was crystallized with ammonium sulfate and sodium citrate as precipitants. Rhombic crystals grew up to 0.4 × 0.6 × 0.8 mm in 10 μl drops of the protein solution (20–30 mg/ml) in 50 mM Tris–(or imidazole)HCl buffer (pH 7.8–8.2) with 1 mM DTT in the presence of sodium citrate: 500 mM in the drop and 650–750 mM in the vial (fig.2). Crystallization occurs both at 5°C and at 20°C. At 5°C the sodium citrate in the vial should be 750 mM and at 20°C 650 mM. The result of crystallization depends strongly on pH of the solution. In the above conditions, at pH 8.0–8.2 we obtained rhombic crystals and at pH 7.5 thin needle-like crystals or spherolites. However, in spite of large dimensions and well-defined form, the crystals of TEF-G modified by trypsin were very unstable. They were damaged at any mechanical or radiation action and therefore could not be investigated with X-ray.

The obtainment of stable and well-ordered crystals of the G factor opens a possibility for studying the three-dimensional structure of this protein which is of primary importance in the process of translocation. Crystallographic studies of TEF-G crystals (see fig.1) have been initiated in our Institute.

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