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Crystallographic Study of Elongation Factor G from *Thermus thermophilus* HB8

Crystals of elongation factor G with a molecular mass of about 76,000 from *Thermus thermophilus* HB8 were grown. The crystals belong to space group $P2_12_12_1$ with unit cell dimensions: $a = 76{\cdot}2$ Å, $b = 107{\cdot}6$ Å and $c = 117{\cdot}1$ Å. The assumed number of protein molecules per asymmetric part of a unit cell is one.

Polypeptide chain elongation factor G (EF-G) is a large monomeric protein. In the process of biosynthesis, EF-G promotes the translocation of the peptidyl-tRNA·mRNA complex from the A to the P site on the ribosome (Lucas-Lenard & Lipmann, 1971). Knowledge of the three-dimensional structure of EF-G could provide an understanding of the mechanism of EF-G binding with nucleotides and ribosome. Factor G from *Escherichia coli* has been crystallized by Kaziro & Inoue (1968) but large crystals suitable for X-ray analysis have not been obtained. The large tryptic N and C-terminal fragments of EF-G from *E. coli* have been crystallized by us recently (Reshetnikova *et al.*, 1982). However, the large crystals obtained did not have a good radiation stability.

In this letter we report preliminary data on the crystallographic study of elongation factor G isolated from the thermophilic bacteria *Thermus thermophilus* HB8 (TEF-G). It has a molecular mass of about 76,000 as estimated by us with the use of gel electrophoresis. The factors TEF-G and EF-G from *E. coli* have similar catalytic and molecular properties (Arai *et al.*, 1978*a*,*b*; Nakamura *et al.*, 1978).

The isolation of TEF-G was carried out as described by Garber & Reshetnikova (1982). During this procedure the protein molecule undergoes some proteolytic modification. The polypeptide chain breaks at a distance of about 10 to 15 amino acid residues from the N-terminal end and the modified factor retains its biological activity. The native and modified forms of TEF-G seem to be very similar. Practically the same crystals have been obtained from a homogeneous modified form of TEF-G as well as from a mixture of native and modified forms. Crystals were grown by the hanging drop microdiffusion technique at 4°C. A drop of TEF-G solution at a concentration of 10 mg/ml in 20 mM-imidasol·HCl (pH 7·8), 3 mM-sodium azide was equilibrated with 20% (v/v) 2-methyl-2,4-pentanediol as a precipitant. The crystals were grown for three weeks and had dimensions of about 0·5 mm × 0·5 mm × 0·15 mm. Other details are described by Reshetnikova & Garber (1983).

X-ray diffraction measurements were carried out on an Elliott GX-6 rotating anode generator operated at 35 kV and 35 mA. The size of the focal spot was 0·2 mm × 0·2 mm. Precession photographs were taken with a Nonius camera. Unit cell dimensions taken from the photographs were increased by 0·15% due to film

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shrinkage. The root-mean-square errors of the unit cell dimension measurements were no worse than 0·25% as calculated from several precession photographs.

The analysis of diffraction pattern symmetry was done from precession photographs of the main zones at about 5 Å resolution. The crystals displayed a diffraction limit up to 4 Å during 30 to 40 hours at 18°C. The crystals belong to space group $P2_12_12_1$ as seen from the absences along three orthogonal axes observed in the zones *hko*, *okl* and *hkh*. In addition a 2-fold screw pseudo-axis was found along the diagonal direction [101] of a unit cell. The absences along this direction were observed to about 6·5 Å resolution.

According to the symmetry of the given space group the unit cell has four asymmetric parts. We can estimate the number of molecules in the unit cell using volume packing parameter $V_m = V_{\text{unit cell}}/nM$, where V is the volume, n is the number of molecules in the unit cell and M is the molecular mass of a protein molecule (Matthews, 1968). For a large number of proteins the V_m value for their crystals varied from 1·68 to 3·53 Å^3/dalton; the most frequently occurring value was 2·15 Å^3/dalton. For TEF-G crystals the possible number of molecules in the unit cell is assumed to be four or eight. In these cases the V_m value is 3·08 and 1·54 Å^3/dalton, respectively. Therefore, the assumed number of protein molecules in the unit cell appears to be four and the asymmetric part of the unit cell contains one molecule. Taking into account a low resolution non-crystallographic symmetry along the diagonal [101] in the *xoz* plane we can suppose the internal molecular symmetry is supplied by the 2-fold screw pseudo-axis. Therefore, a molecule of TEF-G seems to consist of two semi-equal domains. An X-ray crystal structure analysis of TEF-G is now in progress.

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