Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction studies of blasticidin S deaminase from *Aspergillus terreus*

Masayoshi Nakasako,[a] Makoto Kimura[b] and Isamu Yamaguchi[b]*

[a]PRESTO, Japan Science and Technology Corporation and The University of Tokyo, Yayoi 1-1-1, Bunkyoku, Tokyo 113-0032, Japan, and [b]The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama 351-0198, Japan

Correspondence e-mail: isyama@postman.riken.go.jp

Received 6 May 1998
Accepted 15 September 1998

Blasticidin S deaminase from *Aspergillus terreus* was crystallized with polyethylene glycol 8000. Two types of crystals were grown under the same crystallization conditions. One type grew as thin plates, while the other had a rhombic shape. The rhombic shaped crystal was suitable for high-resolution crystal structure analysis. Precession photographs and diffraction data showed that the crystal belonged to orthorhombic space group $P2_12_12_1$, with unit-cell dimensions $a = 70.33$, $b = 146.56$ and $c = 56.48$ Å. The calculated V_m value was acceptable when a tetramer of the enzyme was contained in an asymmetric unit. Preliminary diffraction data were collected to a resolution of 2.0 Å with good statistics.

1. Introduction

Blasticidin S (BS) is an antibiotic of the aminoacyl nucleoside group produced by *Streptomyces griseochromogenes* (Takeuchi *et al.*, 1958). This antibiotic inhibits the translocation reaction of peptides in protein biosynthesis for both eukaryotes and prokaryotes (Yamaguchi & Tanaka, 1966). Because of its potent antifungal activity, BS has been widely used as a fungicide against the phytopathogenic fungus causing rice blast disease (Misato *et al.*, 1959). In the course of a study on the metabolic fate of BS in soil, a fungus resistant to BS, *Aspergillus terreus* S-712, was discovered (Yamaguchi *et al.*, 1972). Through biochemical assays, blasticidin S deaminase (E.C. 3.5.4.23) was isolated from the fungus and identified as the cause of the detoxification (Yamaguchi *et al.*, 1975). BS deaminase is a hydrolase and converts BS to deaminohydroxyblasticidin S (Fig. 1) which is no longer toxic (Seto *et al.*, 1966). Because of this function, the BS deaminase gene has been utilized as a selectable marker for transformation and expression experiments in molecular biology (Kimura, Kamakura *et al.*, 1994; Kimura, Takatsuki *et al.*, 1994*b*; Kimura *et al.*, 1995; Tamura *et al.*, 1995)

BS deaminase is biochemically well characterized after the construction of an overexpression system in *Escherichia coli* (Kimura *et al.*, in preparation). BS deaminase is composed of 130 amino-acid residues and one zinc ion. The enzyme forms a tetramer in solution as probed by sedimentation equilibrium measurement. The zinc ion is chelated by three cysteine residues in the active site of the enzyme and has a determinant role in the hydrolase activity of the enzyme. The amino-acid sequences around the three cysteine residues are significantly conserved among enzymes in the cytidine deaminase family (Betts *et al.*, 1994). However, the overall similarity in the amino-acid sequences between BS deaminase and cytidine deaminase is very low. Because there is no protein having homologous amino-acid sequences to that in BS deaminase, the structure of the enzyme and its reaction mechanism are still obscure. Here, we report the crystallization of recombinant BS deaminase and a preliminary X-ray

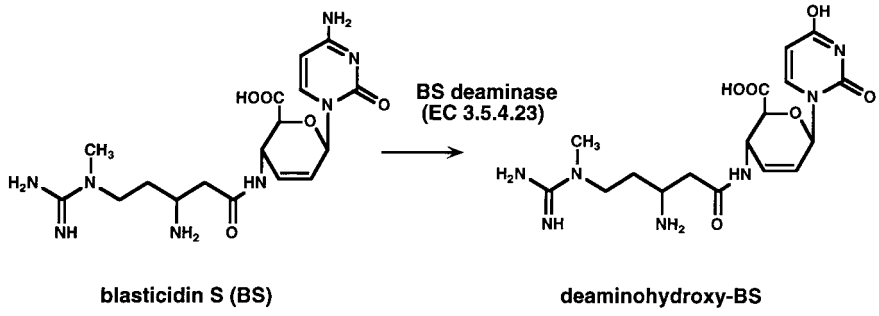

Figure 1
Inactivation of BS by BS deaminase.

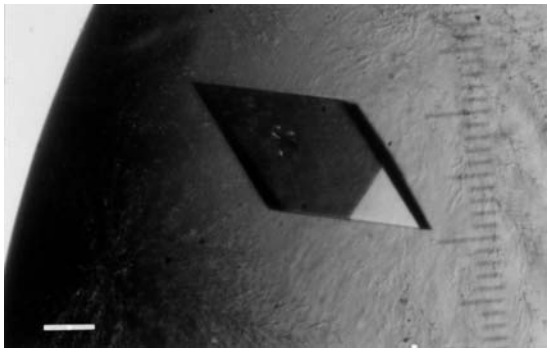

Figure 2
A photograph of a rhombic shaped crystal of BS deaminase. The white bar corresponds to 0.1 mm.

diffraction experiment on the crystals obtained.

2. Experimental and results

2.1. Crystallization

Recombinant BS deaminase was produced from an overexpression system in *E. coli* and was isolated using affinity chromatography with BS and anion-exchange chromatography (Kimura *et al.*, manuscript in preparation). The purified enzyme was concentrated to 10 mg ml^{-1} for crystallization trials. Crystallization were carried out with the hanging-drop vapour-diffusion technique at 293 K. A 3 µl droplet of the protein solution and the same volume of a precipitant solution were mixed and equilibrated against 1 ml of the precipitant solution in a reservoir. BS deaminase was crystallized from precipitant solutions containing polyethylene glycol (PEG) 8000 and magnesium ions in the pH range 6.0–7.5. The magnesium ion concentration was one of the critical factors for reproducing crystals. In refined crystallization conditions, the precipitant solution contained 20%(*w*/*v*) PEG 8000, 50 m*M* $MgCl_2$ and 0.1 *M* sodium cacodylate at pH 7.0. Usually, two types of crystals appeared under these crystallization conditions; thin plates and rhombic shaped crystals (Fig. 2). These two crystals grew in different batches under the same crystallization conditions. The thin plate-shaped crystals stacked on top of each other and were not suitable for X-ray crystal structure analysis. Thus, the subsequent crystallographic characterization studies were carried out on a rhombic shaped crystal. The crystal was stable for 3 months in a stabilization buffer.

2.2. Data collection

Precession photographs taken for a rhombic shaped crystal showed *mmm* symmetry, and the space group of the crystal was determined to be $P2_12_12_1$ from systematic absences of reflections. Further crystallographic characterization was carried out by the oscillation method. Intensity data were collected at room temperature using an R-AXIS IV diffractometer (Rigaku, Japan) with Cu $K\alpha$ radiation from an Ultrax18 rotating-anode generator (Rigaku, Japan) operated at 4.1 kW load. The indexing of reflections, calculation of integrated intensities, scaling and post-refinement procedures were performed using the program *PROCESS* in the R-AXIS IV system. The approximate size of the crystal used was 0.75 × 0.40 × 0.40 mm.

Diffraction data were collected as a series of 1.2° oscillation frames with an overlapping angle of 0.2°. The exposure time in each frame was 8 min. The BS deaminase crystal diffracted X-rays beyond a resolution of 2.0 Å and was stable during an X-ray exposure of more than 10 h. A total of 86261 reflections [$I \geq \sigma(I)$] were observed and 34331 unique reflections were obtained between the Bragg spacings 50.0 and 2.0 Å. The completeness of the set of the unique reflections was 85.1% to 2.0 Å resolution. The overall merging *R* factor of the data was 0.049 for reflections with $I \geq \sigma(I)$ and an averaged ratio of $I/\sigma(I)$ for all the reflections was 19.5. In the highest resolution shell (2.25–2.00 Å), the completeness, the averaged ratio of $I/\sigma(I)$ and the merging *R* factor were 73%, 4.0 and 0.127, respectively.

The lattice constants of the crystal were determined to be a = 70.33, b = 146.56 and c = 56.48 Å. Assuming one tetramer of BS deaminase per asymmetric unit, the V_m value was calculated to be 2.8 Å^3 Da^{-1}, which is within the reasonable range for protein crystals (Matthews, 1968). These results were consistent with the fact that BS deaminase existed as a tetramer in solution (Kimura *et al.*, in preparation).

Preparation of heavy-atom derivatives was attempted using more than 30 heavy-atom reagents for multiple isomorphous replacement analysis; however, no derivatives were obtained. As BS deaminase possesses one zinc ion chelated to the active-centre cysteine residues, the structure determination of this enzyme is under way using the multiwavelength anomalous dispersion method with synchrotron X-rays.

This work was supported in part by the Biodesign Research group and the SR structural biology research group of RIKEN.

References

Betts, L., Xiang, S., Short, S. A., Wolfenden, R. & Carter, C. W. Jr (1994). *J. Mol. Biol.* **235**, 635–656.
Kimura, M., Izawa, K., Yoneyama, K., Arie, T., Kamakura, T. & Yamaguchi, I. (1995). *Biosci. Biotechnol. Biochem.* **59**, 1177–1180.
Kimura, M., Kamakura, T., Tao, Q.-Z., Kaneko, I. & Yamaguchi, I. (1994). *Mol. Gen. Genet.* **242**, 121–129.
Kimura, M., Takatsuki, A. & Yamaguchi, I. (1994). *Biochim. Biophys. Acta*, **1219**, 653–659.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Misato, T., Ishii, M., Asakawa, M., Okimoto, Y. & Fukunaga, K. (1959). *Ann. Phytopathol. Soc. Jpn*, **24**, 302–306.
Seto, H., Otake, N. & Yonehara, H. (1966). *Agric. Biol. Chem.* **30**, 877–886.
Takeuchi, S., Hirayama, K., Ueda, K. & Yonehara, H. (1958). *J. Antibiot. Ser. A*, **11**, 1–5.
Tamura, K., Kimura, M. & Yamaguchi, I. (1995). *Biosci. Biotechnol. Biochem.* **59**, 2336–2338.
Yamaguchi, H. & Tanaka, N. (1966). *J. Biochem.* **60**, 632–634.
Yamaguchi, I., Shibata, H., Seto, H. & Misato, T. (1975). *J. Antibiot.* **28**, 7–14.
Yamaguchi, I., Takagi, K. & Misato, T. (1972). *Agric. Biol. Chem.* **36**, 1719–1727.