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Crystallization and preliminary X-ray analyses of desulfurization enzyme DszB and its C27S mutant complexed with biphenyl-2-sulfinic acid

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DszB is a hydrolase involved in the biodegradation of dibenzothiophene in the soil bacterium *Rhodococcus* sp. IGTS8. DszB catalyzes the hydrolysis of 2′-hydroxybiphenyl-2-sulfinic acid to sulfite and biphenyl-2-ol. DszB and DszB C27S mutant complexed with biphenyl-2-sulfinic acid were crystallized and preliminary X-ray crystallographic analyses were conducted. The crystals of DszB were found to belong to the orthorhombic $P2_12_12_1$ space group, with unit-cell parameters $a = 36.7$, $b = 82.6$, $c = 139.6$ Å, and to contain one molecule of DszB in the asymmetric unit. Crystals of DszB C27S complexed with biphenyl-2-sulfinic acid belong to space group $C2$, with unit-cell parameters $a = 153.4$, $b = 45.9$, $c = 112.9$ Å, $\beta = 115.93^\circ$. The calculated Matthews coefficient V_M for the $C2$ crystals is approximately 2.3 Å^3 Da^{-1} if two molecules of DszB are present in the asymmetric unit.

1. Introduction

Sulfur oxides emitted from the combustion of fossil fuels are the primary causes of acid rain and restrictions are becoming stricter regarding the sulfur content of fuel oil. A large number of organic sulfur compounds in the higher boiling fraction of crude oil have the basic structure of dibenzothiophene (DBT) and efforts have been made to remove DBT in the oil-refinery process by microbial activity. The *dsz* (desulfurization) operon, one of the microbial systems capable of degrading DBT, has been isolated from the soil bacterium *Rhodococcus erythropolis* (Gallagher *et al.*, 1993) and biochemically characterized (Gray *et al.*, 1996). The *dsz* operon contains three enzymes: DszA, DszB and DszC. In the *dsz* pathway, DBT is first monooxygenated to DBT sulfoxide and then to DBT sulfone by DszA. One of the carbon–sulfur bonds of DBT sulfone is cleaved by DszC to form 2′-hydroxybiphenyl-2-sulfinic acid (HBPS). DszB catalyzes the hydrolysis of HBPS to biphenyl-2-ol and sulfite. Sulfite is further oxidized to sulfate and utilized by the microbe, while biphenyl-2-ol can be reutilized as fuel. In the biochemical characterization study of DszA, DszB and DszC it was shown that the reaction catalyzed by DszB is the slowest *in vitro* and is a possible rate-limiting step in biodesulfurization.

The DszB polypeptide is 365 amino acids long and a *BLAST* search of the National Center for Biotechnology Information (NCBI) databases suggested that DszB belongs with the nitrate-, sulfonate- and bicarbonate-binding proteins of the ABC transporter family (Altschul *et al.*, 1997). DszB has been over-expressed and purified for biochemical study (Nakayama *et al.*, 2002). According to the study, the reaction catalyzed by DszB is not dependent on metals or cofactors. DszB can also catalyze hydrolysis of an artificial substrate, biphenyl-2-sulfinic acid (BPS), in addition to HBPS. DszB contains only one S atom, in residue Cys27, and mutation of Cys27 to Ser completely abolishes the activity. Other than Cys27, no information regarding the catalytic residues of DszB is available. DszB is heat-labile and easily inactivated by incubation over 303 K, in contrast to DszA and DszC which are relatively stable up to ∼313 K.

As the reaction step of DszB is rate-limiting in biodesulfurization, understanding its reaction mechanism is essential to the industrial application of biodesulfurization. The atomic structure of DszB should be very helpful in explaining this unique reaction, but no known structure of a protein homologous to DszB is yet available. To obtain the crystal structures of DszB and DszB complexed with substrates, we crystallized DszB and the DszB C27S mutant in complex with the substrate BPS. These crystals were of sufficient quality to collect high-resolution data sets and preliminary X-ray crystallographic analyses were conducted on these data sets.

2. Materials and methods

2.1. Purification and crystallization

The DszB solution was prepared as a recombinant protein from *Escherichia coli* BL21(DE3) by co-expression of chaperonin and was purified as described previously (Nakayama *et al.*, 2002). After the final purifi-

cation step, the protein solution was dialyzed against 2 m*M* potassium phosphate buffer pH 7.0 and concentrated to 30 mg ml^{-1} using Centriprep (Amicon, USA) prior to crystallization experiments. Initial sparse-matrix crystal screening was conducted using Crystal Screen I (Hampton Research, USA). Crystallization was conducted by the hanging-drop method in which 1 µl DszB solution was mixed with the same volume of crystallization buffer and incubated at 277 K.

2.2. Crystallization of DszB C27S mutant in complex with BPS

The DszB C27S mutant was over-expressed and purified in the same way as native DszB. DszB C27S was dialyzed against 20 m*M* Tris pH 8.0, 10%(*v*/*v*) glycerol and concentrated to 15 mg ml^{-1}. The sodium salt of BPS was added to the DszB C27S solution to a final concentration of 20 m*M*, 50 times the molar concentration of DszB C27S, and incubated on ice for several hours prior to crystallization. The crystallization of DszB C27S in complex with BPS was conducted using Crystal Screen HT (Hampton Research, USA).

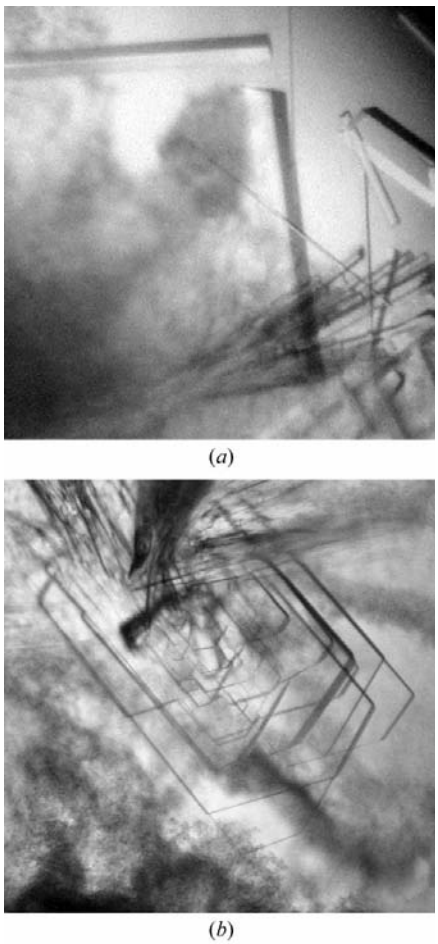

Figure 1
Crystals of (*a*) DszB and (*b*) the DszB C27S mutant in complex with BPS.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Data set	DszB	DszB C27S complexed with BPS
Wavelength (Å)	1	0.978
Space group	$P2_12_12_1$	$C2$
Resolution range (Å)	28.8–1.8 (1.9–1.8)	31.0–1.8 (1.9–1.8)
No. measured reflections	139971	236875
No. unique reflections	39991	65706
Completeness	99.4 (98.6)	99.4 (96.3)
$I/\sigma(I)$	6.8 (3.2)	9.2 (4.8)
$R_{\rm merge}$†	0.072 (0.214)	0.054 (0.138)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

2.3. X-ray diffraction data collection

Prior to each data collection, crystals were transferred to a cryoprotectant composed of reservoir buffer containing 30% glycerol. Native data sets were collected from DszB and DszB C27S mutant complexed with BPS using a Quantum ADSC CCD detector at beamlines 18B and 6A of the Photon Factory, Japan, respectively. The diffraction images were integrated using the *MOSFLM* program (Leslie, 1992) and scaled using the *SCALA* program (Evans, 1993).

3. Results and discussion

From the initial sparse-matrix crystal screening, needle crystals of DszB grew from condition No. 18 [0.2 *M* magnesium acetate, 0.1 *M* sodium cacodylate pH 6.5, 20%(*w*/*v*) PEG 8000] in a few days. Sodium cacodylate buffer was replaced with 0.1 *M* HEPES pH 7.0 and the concentration of DszB was changed to 15 mg ml^{-1} to optimize the crystal size. The typical widths and lengths of the DszB crystals were approximately 0.04 and 0.3 mm, respectively (Fig. 1*a*). The native data set from DszB was collected to a resolution of 1.8 Å at a temperature of 90 K. The crystal was found to belong to a primitive orthorhombic lattice with unit-cell parameters $a = 36.66$, $b = 82.50$, $c = 139.64$ Å; the space group was $P2_12_12_1$. Detailed data statistics are given in Table 1.

Small spherulite crystals of DszB C27S in complex with BPS grew in a week from condition D10 (0.2 *M* calcium acetate, 0.1 *M* sodium cacodylate pH 6.5, 18% PEG 8000) of Crystal Screen HT. The spherulites were composed of small plate crystals. The protein solution was diluted to 10 mg ml^{-1} to obtain larger crystals, but it took more than a month for crystals of sufficient size for data collection to grow. Crystals grew as stacks of plates (Fig. 1*b*) and the thickness and width of each plate were approximately 0.02 and 0.4 mm, respectively. A diffraction data set was collected from a single plate crystal to a resolution of 1.8 Å at a temperature of 90 K (Fig. 2). The lattice of the crystal was found to be centred monoclinic with unit-cell parameters $a = 153.37$, $b = 45.86$, $c = 112.9$ Å, $\beta = 115.93^\circ$. The Matthews coefficient $V_{\rm M}$ of the $C2$ crystals was calculated to be 2.3 Å^3 Da^{-1}, assuming two molecules of DszB C27S in the asymmetric unit. To obtain the phase information for DszB native data sets, a heavy-atom derivatization study is under way.

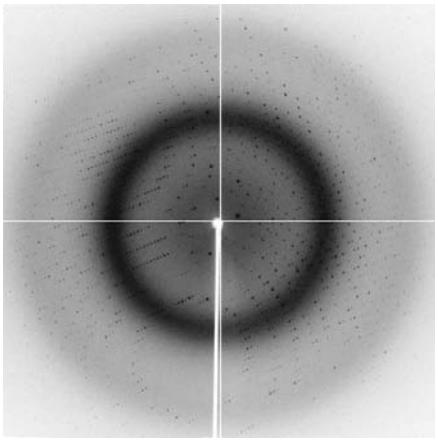

Figure 2
The X-ray diffraction image of the $C2$ crystal of DszB.

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References

Altschul, S. F., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). *Nucleic Acids Res.* **25**, 3389–3402.
Evans, P. R. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Proces-*

sing, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 114–122. Warrington: Daresbury Laboratory.
Gallagher, J. R., Olson, E. S. & Stanley, D. C. (1993). *FEMS Microbiol. Lett.* **107**, 31–35.
Gray, K. A., Pogrebinsky, O. S., Mrachko, G. T., Xi, L., Monticello, D. J. & Squires, C. H. (1996). *Nature Biotechnol.* **14**, 1705–1709.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EAMCB Newsl. Protein Crystallogr.* **26**, 27–33.
Nakayama, N., Matsubara, T., Ohshiro, T., Moroto, Y., Kawata, Y., Koizumi, K., Hirakawa, Y., Suzuki, M., Maruhashi, K., Izumi, Y. & Kurane, R. (2002). *Biochim. Biophys. Acta*, **1598**, 122–130.