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Crystallization and preliminary X-ray analysis of γ-hexachlorocyclohexane dehydrochlorinase LinA from *Sphingobium japonicum* UT26

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LinA from *Sphingobium japonicum* UT26 catalyzes two steps of dehydrochlorination from γ-hexachlorocyclohexane (γ-HCH) to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) *via* γ-pentachlorocyclohexene (γ-PCCH). LinA was crystallized by the sitting-drop vapour-diffusion method using PEG 3350 as the precipitant. The crystals belonged to space group $P4_1$ or $P4_3$, with unit-cell parameters $a = b = 68.9$, $c = 101.9$ Å, and diffracted X-rays to 2.25 Å resolution. The crystal contained three molecules in the asymmetric unit.

1. Introduction

Hexachlorocyclohexane (HCH) is a cyclic compound which exists in eight isomeric forms depending on the positions of the Cl atoms. The γ-isomer of hexachlorocyclohexane (γ-HCH) is a halogenated organic insecticide which at one time was used widely throughout the world. At present, however, the use of γ-HCH has been prohibited in most countries because of its toxicity and long persistence in soil.

Among the several sphingomonad strains which degrade α-, β-, γ- and δ-HCH (Kumari *et al.*, 2002; Dogra *et al.*, 2004; Ma *et al.*, 2005; Mohn *et al.*, 2006; Ceremonie *et al.*, 2006), *Sphingobium japonicum* UT26 is able to utilize γ-HCH as a sole source of carbon and energy under aerobic conditions (Imai *et al.*, 1989). The degradation pathway of γ-HCH in *S. japonicum* UT26 under aerobic conditions has been reported (Nagata, Miyauchi *et al.*, 1999; Endo *et al.*, 2005; Nagata *et al.*, 2007). LinA (accession code BAA144369) catalyzes the initial step in the degradation of γ-HCH and converts γ-HCH to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) *via* γ-pentachlorocyclohexene (γ-PCCH) (Imai *et al.*, 1991; Nagata, Futamura *et al.*, 1999). LinA consists of 156 amino-acid residues, with a predicted molecular weight of 17 341. The dehydrochlorination of γ-HCH proceeds by a 1,2-*anti* dehydrochlorination reaction (Trantírek *et al.*, 2001). The catalytic mechanism has previously been predicted (Nagata *et al.*, 2001) based on a homology model constructed using the structures of scytalone dehydratase (PDB code 1std; Lundqvist *et al.*, 1994), nuclear transport factor-2 (PDB code 1oun; Bullock *et al.*, 1996), 3-oxo-Δ^5-steroid isomerase (PDB code 1opy; Wu *et al.*, 1997) and naphthalene 1,2-dioxygenase (PDB code 1ndo; Kauppi *et al.*, 1998). To our knowledge, there is no information available about the structure of any γ-hexachlorocyclohexane dehydrochlorinase. In order to understand the catalytic mechanism of LinA from *S. japonicum* UT26, we have undertaken crystallographic analysis of LinA. Here, we report the expression, purification, crystallization and preliminary X-ray diffraction analysis of the enzyme.

2. Materials and methods

2.1. Expression

The *linA* gene was amplified by PCR using the pMYLA1 plasmid (Imai *et al.*, 1991) as the template. The PCR product was digested with *Nde*I and *Eco*RI and ligated into the T7 expression vector pET-28a(+) (Novagen) to generate recombinant protein with an

N-terminal His-tag fusion (MGSSHHHHHHSSGLVPRGSH). *Escherichia coli* strain Rosetta (DE3) was used as the host for protein expression. The *E. coli* cells were cultured in Luria–Bertani (LB) medium containing 30 µg ml^{-1} kanamycin until the OD_{600} reached 0.6. Protein expression was induced by the addition of 1.0 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) at 293 K.

2.2. Purification and crystallization

The harvested cells were suspended in solution *A* (50 m*M* Tris–HCl pH 7.5, 400 m*M* NaCl and 5 m*M* imidazole) and disrupted by sonication. After centrifugation at 40 000*g* for 30 min, the supernatant was loaded onto a 3 ml Ni Sepharose 6 Fast Flow (GE Healthcare) column. After a washing step with solution *B* (50 m*M* Tris–HCl pH 7.5, 400 m*M* NaCl and 50 m*M* imidazole), the protein was eluted with solution *C* (50 m*M* Tris–HCl pH 7.5, 400 m*M* NaCl and 200 m*M* imidazole). The purified protein was dialyzed against 20 m*M* Tris–HCl pH 7.5, 2 m*M* dithiothreitol (DTT) and 3%(*v*/*v*) glycerol and then concentrated to 25 mg ml^{-1}.

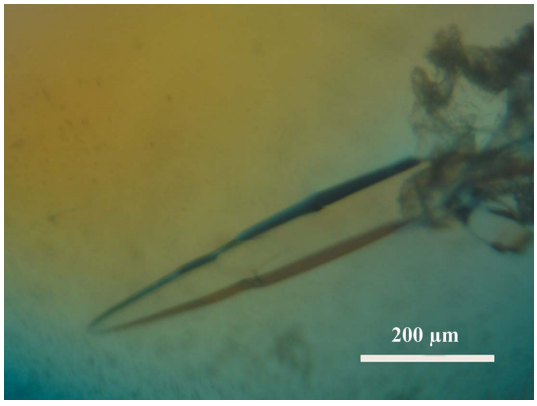

Figure 1
A crystal of LinA from *S. japonicum* UT26.

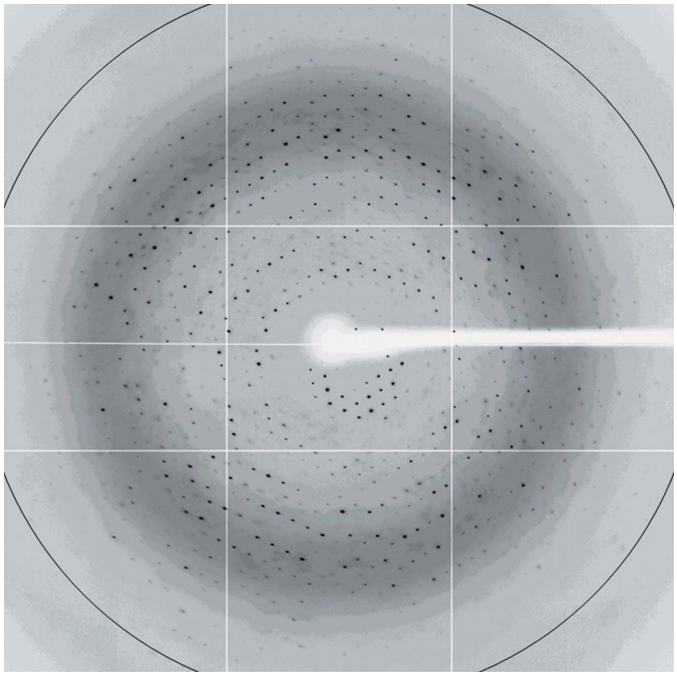

Figure 2
An X-ray diffraction image from a LinA crystal (0.5° oscillation). The X-ray diffraction extends to 2.25 Å resolution (indicated by the curved line).

Table 1
Data-collection statistics for the LinA crystal.

Values in parentheses are for the highest resolution shell.

Beamline	PF-BL5A
Wavelength (Å)	1.0000
Space group	$P4_1$ or $P4_3$
Unit-cell parameters (Å)	$a = b = 68.9$, $c = 101.9$
Resolution (Å)	50–2.25 (2.33–2.25)
No. of measurements	148454
No. unique reflections	22188
Completeness (%)	98.0 (89.0)
$R_{\rm merge}$†	0.027 (0.132)
$\langle I/\sigma(I)\rangle$	67.4 (11.3)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the average of individual measurements of $I_i(hkl)$.

Initial crystallization trials were performed by the sitting-drop vapour-diffusion method using the sparse-matrix screening kits Crystal Screen HT, Index HT (Hampton Research) and Wizard I and II (Emerald Biosystems). Drops containing equal volumes (0.7 µl) of protein solution and crystallization solution were equilibrated against 70 µl reservoir solution.

2.3. Data collection and processing

Crystals were transferred to a cryoprotectant solution containing 100 m*M* Na HEPES pH 7.0, 16%(*w*/*v*) PEG 3350, 200 m*M* ammonium acetate, 10 m*M* spermidine and 16%(*v*/*v*) glycerol. Data collections were performed in a nitrogen stream on beamline BL5A of Photon Factory (Tsukuba, Japan) using a wavelength of 1.0000 Å and an ADSC Quantum 315 detector. A data set was obtained by collecting 360 frames with an oscillation step of 0.5°. The diffraction data were indexed, integrated and scaled with *HKL*-2000 (Otwinowski & Minor, 1997).

3. Results

Poorly diffracting crystals were obtained using Index HT condition G8 at 293 K. This condition was optimized by varying the pH and the precipitant concentration and by using Additive Screen HT (Hampton Research). Crystals suitable for diffraction (0.6 × 0.1 × 0.1 mm; Fig. 1) were obtained after a few days by mixing 1.5 µl protein solution, 1.5 µl reservoir solution [100 m*M* Na HEPES pH 7.0, 16%(*w*/*v*) polyethylene glycol (PEG) 3350 and 200 m*M* ammonium acetate] and 0.3 µl additive solution (100 m*M* spermidine).

Data collections were performed on beamline BL5A of Photon Factory (Tsukuba, Japan) and the best crystal of LinA diffracted X-rays to 2.25 Å resolution (Fig. 2). Based on the molecular weight of 19 505 Da, the crystal should contain three LinA molecules per asymmetric unit. The Matthews coefficient (Matthews, 1968) and solvent content were calculated as 2.1 Å^3 Da^{-1} and 41%, respectively. The crystal belonged to space group $P4_1$ or $P4_3$, with unit-cell parameters $a = b = 68.9$, $c = 101.9$ Å. The data-collection statistics are shown in Table 1. Structure determination is in progress using the SeMet-derivative approach.

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