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Expression, crystallization and preliminary X-ray crystallographic analysis of aldehyde dehydrogenase (ALDH) from *Bacillus cereus*

Aldehyde dehydrogenase (ALDH) catalyses the oxidation of aldehydes using NAD(P)^+ as a cofactor. Most aldehydes are toxic at low levels. ALDHs are used to regulate metabolic intermediate aldehydes. The *aldh* gene from *Bacillus cereus* was cloned and the ALDH protein was expressed, purified and crystallized. A crystal of the ALDH protein diffracted to 2.6 Å resolution and belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 83.5$, $b = 93.3$, $c = 145.5$ Å, $\beta = 98.05^\circ$. Four protomers were present in the asymmetric unit, with a corresponding V_M of $2.55 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 51.8%.

1. Introduction

In nature, various metabolic intermediate aldehydes are ubiquitous. Owing to their chemical reactivity, most aldehydes are toxic, even at low levels. It appears that most organisms utilize several kinds of aldehyde dehydrogenases (ALDHs) to regulate metabolic intermediate aldehydes (O'Brien *et al.*, 2005). Aldehyde dehydrogenases (EC 1.2.1.3), which are classed as oxidoreductases, catalyse the oxidation (dehydrogenation) of aldehydes using NAD(P)^+ as a cofactor. These enzymes exist as tetramers comprised of 54 kDa subunits.

The active site of aldehyde dehydrogenase is well conserved throughout the different classes of the enzymes (Perozich *et al.*, 1999). In the aldehyde dehydrogenase structure, the substrate aldehyde and the NAD(P)^+ cofactor are bound to the active site (Gonzalez-Segura *et al.*, 2009). The catalytic cysteine residue (Cys300 in *Bacillus cereus*) forms a thiohemiacetal covalent intermediate with the aldehyde substrate *via* nucleophilic attack (Cobessi *et al.*, 2000). Because of the free thiol in the catalytic cysteine, ALDH is unstable. Thus, the enzyme must be kept in a reducing-agent [dithiothreitol (DTT)]-containing buffer solution or be stored at low temperature (Jo *et al.*, 2008; Rothacker & Ilg, 2008).

B. cereus is a mesophilic, facultative anaerobic bacterium (Kim *et al.*, 2000) and is found in a wide range of habitats, *e.g.* in soil and vegetation (Tayabali & Seligy, 2000). In *B. cereus*, *aldedh* is one of the three reported genes that encode ALDH. The *aldedh*-encoded ALDH contains 494 amino-acid residues and shares 53.8% sequence identity with human ALDH. Even though the DNA sequence of *B. cereus* aldehyde dehydrogenase has been reported, the structure of ALDH is still lacking. As the first step to understanding the detoxification mechanism of ALDH from *B. cereus*, we studied the cloning and expression of the *aldedh* gene and the purification, crystallization and preliminary X-ray crystallographic analysis of the *aldedh*-encoded ALDH.

2. Materials and methods

2.1. Cloning

The genomic DNA from *B. cereus* ATCC 10876 was extracted using a genomic DNA extraction kit (Qiagen, Hilden, Germany). The *aldedh* gene (1485 bp) encoding for an aldehyde dehydrogenase was amplified by PCR using the genomic DNA isolated from *B. cereus* as a template. The sequences of the oligonucleotide primers used for

Table 1

Data-collection statistics.

Values in parentheses are for the outer shell.

X-ray source	Beamline 5C SBII, PLS
Wavelength (Å)	0.97951
Unit-cell parameters (Å, °)	$a = 83.5$, $b = 93.3$, $c = 145.5$, $\alpha = \gamma = 90^\circ$, $\beta = 98.05^\circ$
Total rotation (°)	360
Mosaicity (°)	0.73
Multiplicity	7.4 (7.1)
Space group	$P2_1$
Resolution (Å)	50.0–2.6 (2.64–2.6)
No. of observations	500404
No. of unique reflections	67464
Completeness (%)	99.2 (97.7)
$R_{\text{merge}}^\dagger$ (%)	4.6 (7.5)
$\langle I/\sigma(I) \rangle$	69.7 (46.9)

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl , $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl .

gene cloning were based on the DNA sequence of *B. cereus* aldehyde dehydrogenase (GenBank accession No. ACLT01000061.1). Forward (5'-**GGATCCGATGTTAAAGACAAATATTGAGCTG**-3') and reverse primers (5'-**GCGGCCGCTTACTTTATATTTACCCAAACA**-3') were designed to introduce the *Bam*HI and *Not*I restriction sites (bold), respectively. The PCR-amplified DNA fragments were purified using a QIAquick gel extraction kit (Qiagen, Hilden, Germany), inserted into the pRSFDuet-1 vector digested with the same restriction enzymes and ligated into the *Bam*HI and *Not*I sites of pRSFDuet-1. The resulting plasmid was transformed into *Escherichia coli* ER2566 and plated on Luria–Bertani (LB) agar containing 20 $\mu\text{g ml}^{-1}$ kanamycin. A kanamycin-resistant colony was selected and plasmid DNA from the transformant was isolated using a plasmid purification kit (Promega, Madison, Wisconsin, USA). DNA sequencing to confirm the cloning was carried out at the Macrogen facility (Seoul, Republic of Korea).

2.2. Overexpression and purification

The recombinant *E. coli* cells containing aldehyde dehydrogenase/pRSFDuet-1 gene, coding for residues 1–494, were cultivated in a 2 l flask containing 500 ml LB medium and 20 $\mu\text{g ml}^{-1}$ kanamycin at 310 K with shaking at 200 rev min $^{-1}$. When the optical density of bacteria reached 0.6 at 600 nm, isopropyl β -D-1-thiogalactopyranoside was added to a final concentration of 0.1 mM to induce ALDH expression. The culture was incubated with shaking at 150 rev min $^{-1}$ at 288 K for an additional 12 h to obtain the highest expression level. The cells were harvested by centrifugation for 20 min at 6000g at 277 K, washed twice with 0.85% NaCl and resuspended in 50 mM phosphate buffer containing 300 mM KCl and 10 mM imidazole. The resuspended cells were disrupted on ice by using a sonicator (Sonic Vibra Cell, Sonics & Material Inc.). The unbroken cells and cell debris were removed by centrifugation at 13 000g for 10 min at 277 K and the supernatant was filtered through a 0.45 μm filter. The filtrate was applied onto an immobilized metal-ion affinity chromatography cartridge (Bio-Rad, Hercules, California, USA) equilibrated with 50 mM phosphate buffer pH 8.0. The cartridge was washed extensively with the same buffer, and the bound protein was eluted with a linear gradient from 10 to 250 mM imidazole at a flow rate of 1 ml min $^{-1}$. The eluate was collected and immediately loaded onto a Bio-Gel P-6 desalting cartridge (Bio-Rad), equilibrated with 50 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES) buffer pH 7.5. The loaded protein was eluted with 50 mM PIPES buffer pH 7.5 at a flow rate of 1 ml min $^{-1}$ and the fractions containing eluted protein

Figure 1

Purified *B. cereus* ALDH is shown on a 10% SDS-PAGE gel (lane P). Lane M contains molecular-mass markers (labelled in kDa).

Figure 2

Crystals of *B. cereus* ALDH. (a) Initial crystals obtained after 3 d using a reservoir solution consisting of 0.15 M DL-malic acid pH 7.0, 20% PEG 3350 from Index (Hampton Research). (b) Optimized crystal of ALDH with dimensions of 0.25 $\times$ 0.2 $\times$ 0.05 mm obtained using 0.15 M DL-malic acid pH 7.0, 18% PEG 3350. The scale bar represents 0.05 mm.

were collected. The homogeneity of the purified protein was analysed via SDS-PAGE (Fig. 1). The molecular weight of purified ALDH is

55.6 kDa instead of 54 kDa because 13 additional residues from the pRSF Duet-1 vector remained at the N-terminus of ALDH (Supplementary Table 1[†]). For crystallization, purified *B. cereus* ALDH was dialysed for 4 h in buffer A (25 mM Tris, 15 mM NaCl, 3 mM β -mercaptoethanol) and concentrated to a final concentration of 10 mg ml⁻¹.

2.3. Crystallization and X-ray data collection

Initial crystallization was carried out at 287 K by the sitting-drop vapour-diffusion method in 96-well IntelliPlates (Art Robbins) using a Hydra II e-drop automated pipetting system (Matrix) and screening kits from Hampton Research (Index, Crystal Screen, Crystal Screen Cryo, Crystal Lite and PEGRx 1 and 2), Emerald Biosystems (Wizard Classic 1 and 2) and Molecular Dimensions (Morpheus MD1-46 kit). 0.5 μ l protein solution was mixed with 0.5 μ l reservoir solution and equilibrated against 70 μ l reservoir solution. After 3 d, small crystals were observed from four conditions. Crystals were reproduced and optimized using condition H7 from Index (Hampton Research) [0.15 M DL-malic acid pH 7.0, 20% (w/v) PEG 3350] by the hanging-drop method, in which the drops consisted of 0.9 μ l protein solution mixed with 0.9 μ l reservoir solution (Fig. 2a). Each hanging drop was positioned over 1 ml reservoir solution. Optimization was achieved by varying the concentration of PEG 3350 (10–22%) and DL-malic acid pH 7.0 (0.12–0.18 M). In total, 48 optimization drops were set up. Rhombohedral-shaped crystals with adequate dimensions were obtained after 1 week using a reservoir solution consisting of 0.15 M DL-malic acid pH 7.0, 18% (w/v) PEG 3350 (Fig. 2b). The fully grown crystals (0.25 $\times$ 0.2 $\times$ 0.05 mm) were flash-cooled at 100 K in liquid nitrogen using 20% (v/v) glycerol, 0.15 M DL-malic acid pH 7.0 and 20% (w/v) PEG 3350 as a cryoprotectant. X-ray diffraction data were collected from the cryoprotected crystal (at 100 K) using 1° oscillations with a crystal-to-detector distance of 400 mm, using an ADSC Q315r detector on beamline 5C SBII of the Pohang Light Source (PLS), Republic of Korea. The crystals diffracted to 2.6 Å resolution. Diffraction data were integrated and scaled using the *HKL-2000* program package (Otwinowski & Minor, 1997).

3. Results and discussion

The crystals belonged to the crystallographic space group *P2*₁. The unit-cell parameters were $a = 83.5$, $b = 93.3$, $c = 145.5$ Å, $\beta = 98.05^\circ$. The space group was assigned by auto-indexing (Otwinowski & Minor, 1997) and data-collection statistics are provided in Table 1. According to the Matthews coefficient calculation (Matthews, 1968), there are probably four molecules in the asymmetric unit, corresponding to a V_M of 2.55 Å³ Da⁻¹ and a solvent content of 51.8%. Self-rotation functions were calculated at $\chi = 180^\circ$, 120° , 90° and 60° to detect twofold, threefold, fourfold and sixfold symmetry, respectively. The self-rotation function was calculated using data from 50 to 4 Å using *MOLREP* (Vagin & Teplyakov, 2010). Based on this self-rotation calculation (Fig. 3), the crystal contains additional twofold and threefold noncrystallographic symmetry different from crystallographic twofold symmetry. Molecular replacement (MR) using *Phaser* from the *CCP4* program package (McCoy *et al.*, 2007) with aldehyde dehydrogenase from human (PDB entry 4fr8; Lang *et al.*, 2012; 53.8% sequence identity) as a search model was successful and showed four protomers in the asymmetric unit. The initial *R* value from the molecular-replacement solution was 30.9%. The resulting

Figure 3
Self-rotation functions calculated using the ALDH crystal. (a) $\chi = 120^\circ$. (b) $\chi = 180^\circ$.

electron-density maps were clear and fitted the model well. No clashes were found between molecules. After rigid-body and restrained refinement by *REFMAC5* (Murshudov *et al.*, 2011), the *R* value decreased to 24.3% and the R_{free} was 30.1%. Currently, the structure is being refined. Final structural details will be described in a separate paper.

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[†] Supplementary material has been deposited in the IUCr electronic archive (Reference: NJ5148).

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