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# Purification, crystallization and preliminary crystallographic analysis of DehI, a group I α-haloacid dehalogenase from *Pseudomonas putida* strain PP3

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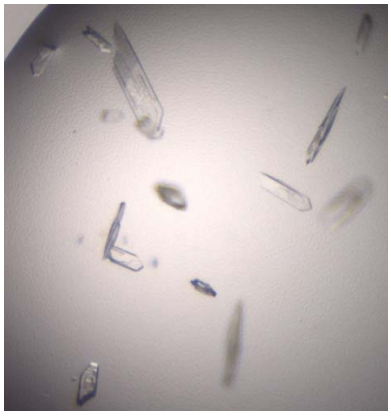
*Pseudomonas putida* strain PP3 produces two dehalogenases, DehI and DehII, which belong to the group I and II α-haloacid dehalogenases, respectively. Group I dehalogenases catalyse the removal of halides from D-haloalkanoic acids and in some cases also the L-enantiomers, both substituted at their chiral centres. Studies of members of this group have resulted in the proposal of general catalytic mechanisms, although no structural information is available in order to better characterize their function. This work presents the initial stages of the structural investigation of the group I α-haloacid dehalogenase DehI. The DehI gene was cloned into a pET15b vector with an N-terminal His tag and expressed in *Escherichia coli* Nova Blue strain. Purified protein was crystallized in 25% PEG 3350, 0.4 *M* lithium sulfate and 0.1 *M* bis-tris buffer pH 6.0. The crystals were primitive monoclinic (space group $P2_1$), with unit-cell parameters $a = 68.32$, $b = 111.86$, $c = 75.13$ Å, $\alpha = 90$, $\beta = 93.7$, $\gamma = 90^\circ$, and a complete native data set was collected. Molecular replacement is not an option for structure determination, so further experimental phasing methods will be necessary.

## 1. Introduction

α-Haloacid (αHA) dehalogenases are microbial enzymes which degrade short-chained organic acids substituted at the $C^\alpha$ position with one or more halogen atoms. They cleave the chemically stable carbon–halogen bond of these compounds, thus converting what are often toxic compounds into sources of carbon and energy. αHA dehalogenases are broken up into two distinct evolutionary groups that differ not only phylogenetically (Hill *et al.*, 1999) but also with respect to catalytic mechanism. Group II αHA dehalogenases are the better characterized of the two groups, with the X-ray crystallographic structures of four members having already been published (Arai *et al.*, 2006; Hisano *et al.*, 1996; Ridder *et al.*, 1997; Schmidberger *et al.*, 2007) and numerous biochemical studies having been conducted on this group of hydrolytic enzymes (Asmara *et al.*, 1993; Kurihara *et al.*, 1995; Pang & Tsang, 2001; Schneider *et al.*, 1993; Tsang & Pang, 2000). They are known to be specific for L-haloacid substrates and the reaction proceeds through an esterified intermediate, hydrolysis of which results in the inversion of configuration of the substrate, producing D-hydroxyacids.

Group I αHA dehalogenases, on the other hand, act on D-haloacids and in some cases L-haloacids as well. Unlike the members of group II, the reaction does not involve an ester intermediate state; rather, a hydrolytic water performs a direct nucleophilic attack on the substrate α-carbon, displacing the bound halogen (Nardi-Dei *et al.*, 1999). The catalytically important residues of a representative group I member have been identified (DL-DEX 113; Nardi-Dei *et al.*, 1997). To put these residues into context and gain a better understanding of the reaction mechanism of the group, structural information is necessary. A recent paper has reported the crystallization and preliminary X-ray diffraction analysis of a group I dehalogenase from *Methylobacterium* sp. CPA1 (Omi *et al.*, 2007), although as yet no structural information is available for any member of the group.



DehI is a group I dehalogenase from the bacterium *Pseudomonas putida* strain PP3, with activity towards both L- and D-enantiomers (Weightman *et al.*, 1979). This communication is a description of the methods of production, crystallization and preliminary X-ray crystallographic analysis of DehI.

## 2. Materials and methods

### 2.1. Cloning of DehI

The DehI gene from *P. putida* strain PP3 was originally cloned by ligation of an ∼11.6 kbp *Eco*RI fragment from a pWW0 *DEH* recombinant with plasmid vector pHG327 to construct the derivative pAWT6 (Thomas *et al.*, 1992). The 891 bp *dehI* gene was then subcloned into a pET15b vector (Novagen) for bacterial expression with an N-terminal hexa-His tag. This was performed using *Nde*I and *Xho*I sites incorporated through PCR primer extension at the N- and C-terminal ends, respectively. The resulting construct was termed pDEHI15b.

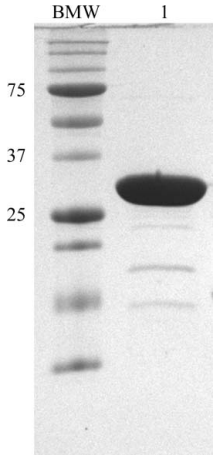



**Figure 1**
SDS–PAGE monitoring of the purification process of DehI. Lane 1 is the eluted DehI sample after Ni-IMAC affinity binding. The percentage purity was ∼95%. The lane marked BMW is a broad molecular-weight protein marker (labelled in kDa; Bio-Rad).

**Table 1**
Purification of DehI from overexpression in *E. coli* strain Nova Blue.

Purity was estimated by SDS–PAGE analysis.

| Step | Purity (%) |
|---|---|
| Lysate | 5 |
| Ni-IMAC | 95 |
| Q-Sepharose | 97 |
| Superdex 75 | 99 |

### 2.2. Overexpression and purification of DehI

pDEHI15b was transformed into *Escherichia coli* strain Nova Blue (DE3) and the bacteria were cultured in three 2 l flasks, each containing 800 ml Luria Broth medium at 310 K, until the $OD_{600}$ reached 0.8. At this point, IPTG was added (to 1 m*M*) to induce expression of the *dehI* gene. The culture-growth temperature was not modified. Cells were left to induce for 4 h, after which they were harvested by centrifugation at 4000*g* for 15 min. Cells were collected and resuspended in 40 ml 50 m*M* Tris–HCl pH 8.0, 25%(*w*/*v*) sucrose and 1 m*M* EDTA and frozen at 193 K for storage.

PMSF was added to a final concentration of 1 m*M* and frozen cell samples were lysed by sonication (four cycles of 30 s on/30 s off). The lysate was spun down at 20 000 rev $min^{-1}$ for 20 min and the supernatant was applied directly to immobilized metal-affinity chromatography beads (IMAC, BioRad) charged with nickel. The equilibrating buffer was composed of 20 m*M* Tris–HCl with 300 m*M* NaCl and 10 m*M* imidazole, while the elution buffer was the same except with a higher imidazole concentration (400 m*M*). Binding was very specific and resulted in a sample that was ∼95% pure (Fig. 1). The sample was dialysed overnight into 20 m*M* Tris–HCl pH 8.0 with 25 m*M* NaCl and then applied onto a Q-Sepharose (Pharmacia/Pfizer, New York, USA) anion-exchange chromatography column pre-equilibrated with 10 m*M* Tris–HCl pH 8.0, 25 m*M* NaCl. DehI was eluted with a linear gradient from 0 to 500 m*M* NaCl at a flow rate of 1.0 ml $min^{-1}$ in the same Tris–HCl buffer.

Samples containing DehI as indicated by SDS–PAGE (Fig. 1) were combined, concentrated to ∼1 ml and then loaded onto a Hiload 16/60 Superdex 75 (Pharmacia/Pfizer, New York, USA) size-exclusion column and run at 1 ml $min^{-1}$ (Table 1). The sample eluted at 55.8 min. The purified DehI was concentrated to ∼8 mg $ml^{-1}$ (6 ml Vivaspin concentrator tube with 3500 Da molecular-weight cutoff; Vivascience, Hanover, Germany) in 20 m*M* Tris–HCl pH 8.0 and

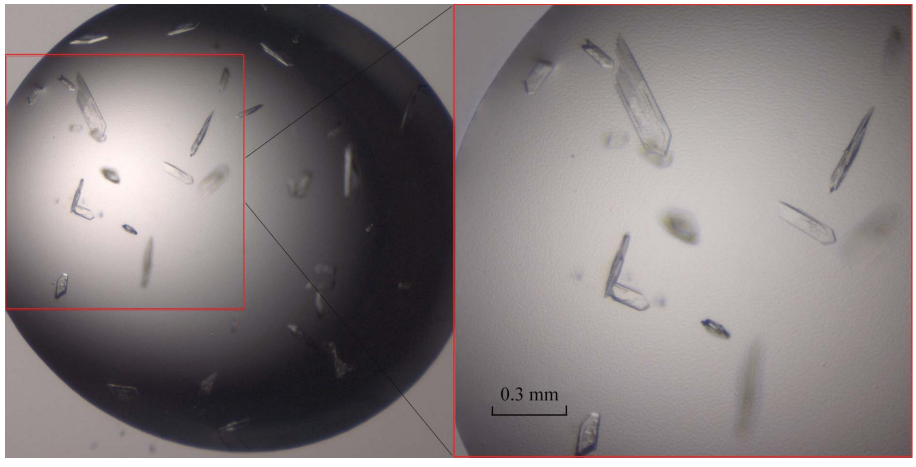



**Figure 2**
Plate-like crystals of DehI grown in 25% PEG 3350, 0.4 *M* lithium sulfate and 0.1 *M* bis-tris–HCl pH 6.0. The image on the right is an enlarged section of the left image.

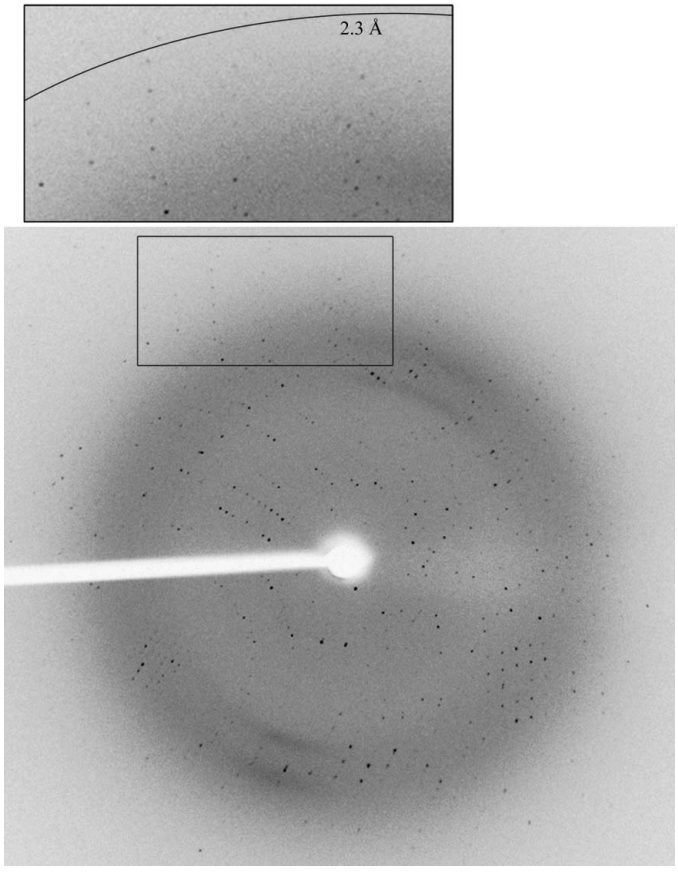



**Figure 3**
Picture of a diffraction image collected in-house. The exposure time was 8 min, with an oscillation range of 0.25°; the crystal-to-detector distance was 200 mm.

25 m*M* NaCl. Recombinant protein activity was confirmed against monochloroacetate using isothermal titration calorimetry (data not shown).

### 2.3. Crystallization

Crystallization screens were set up using a HoneyBee crystallization robot (Genomic Solutions, Michigan, USA) and 96-well sitting-drop Intelliplates (Hampton Research, California, USA). A number of different screens gave crystals with a similar plate-like morphology, each of which involved lithium sulfate as the salt additive. The condition that gave the best diffracting crystals originated from Index Screen (condition No. 75; Hampton Research, California, USA). The optimized condition contained 25%(*w*/*v*) PEG 3350, 0.4 *M* lithium sulfate and 0.1 *M* bis-tris pH 6.0 and gave irregular plate-like crystals (Fig. 2). All screens were conducted at room temperature (~293 K). Crystals grew to average dimensions of ~0.15 × 0.1 × 0.25 mm over a two-week period.

### 2.4. X-ray diffraction analysis

The DehI crystals had a tendency to grow on top of each other, so care had to be taken to select single crystals. A complete data set was collected to 2.3 Å resolution on an in-house Rigaku RUH2R X-ray source with a rotating copper anode equipped with Osmic confocal optics, an R-AXIS IV detector and an Oxford Cryosystems 700 Series cryostream. The crystal was transferred to a 6 µl drop of mother liquor with 15% glycerol and left to equilibrate in air for 1 min prior to relooping and flash-freezing in the liquid-nitrogen cryostream. An example diffraction image is shown in Fig. 3.

**Table 2**
Crystallographic diffraction data and processing statistics.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Resolution range (Å) | 30–2.2 |
| Observed reflections | 127313 |
| Unique reflections | 52524 |
| Completeness (%) | 91.9 (63.1) |
| Redundancy | 2.4 (1.7) |
| $R_{\rm merge}$ (%) | 12.2 (58.9) |
| $I/\sigma(I)$ | 8.8 (1.4) |
| No. of images | 250 |
| $\varphi$ rotation angle (°) | 0.25 |

Data were processed using *MOSFLM* (Leslie, 1992) and *CCP*4 (Collaborative Computational Project, Number 4, 1994). A summary of the data collection is presented in Table 2. The crystals belonged to space group $P2_1$, with unit-cell parameters $a$ = 68.32, $b$ = 111.86, $c$ = 75.13 Å, $\alpha$ = 90, $\beta$ = 93.7, $\gamma$ = 90°. An analysis of the MTZ file led to the conclusion that there are likely to be four monomers of DehI (molecular weight 32 784 Da) in the asymmetric unit (Matthews coefficient 2.31 Å$^3$ Da$^{-1}$). Unfortunately, no structural homologues exist in the Protein Data Bank, so molecular replacement was not available for phase determination. Efforts to prepare selenomethionyl-substituted DehI are currently under way in order to solve the structure of this very interesting enzyme through MAD phasing.

## References


Arai, R., Kukimoto-Niino, M., Kuroishi, C., Bessho, Y., Shirouzu, M. & Yokoyama, S. (2006). *Protein Sci.* **15**, 373–377.
Asmara, W., Murdiyatmo, U., Baines, A. J., Bull, A. T. & Hardman, D. J. (1993). *Biochem. J.* **292**, 69–74.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Hill, K. E., Marchesi, J. R. & Weightman, A. J. (1999). *J. Bacteriol.* **181**, 2535–2547.
Hisano, T., Hata, Y., Fujii, T., Liu, J. Q., Kurihara, T., Esaki, N. & Soda, K. (1996). *J. Biol. Chem.* **271**, 20322–20330.
Kurihara, T., Liu, J. Q., Nardi-Dei, V., Koshikawa, H., Esaki, N. & Soda, K. (1995). *J. Biochem.* **117**, 1317–1322.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **26**.
Nardi-Dei, V., Kurihara, T., Park, C., Esaki, N. & Soda, K. (1997). *J. Bacteriol.* **179**, 4232–4238.
Nardi-Dei, V., Kurihara, T., Park, C., Miyagi, M., Tsunasawa, S., Soda, K. & Esaki, N. (1999). *J. Biol. Chem.* **274**, 20977–20981.
Omi, R., Jitsumori, K., Yamauchi, T., Ichiyama, S., Kurihara, T., Esaki, N., Kamiya, N., Hirotsu, K. & Miyahara, I. (2007). *Acta Cryst.* F**63**, 586–589.
Pang, B. C. & Tsang, J. S. (2001). *FEMS Microbiol. Lett.* **204**, 135–140.
Ridder, I. S., Rozeboom, H. J., Kalk, K. H., Janssen, D. B. & Dijkstra, B. W. (1997). *J. Biol. Chem.* **272**, 33015–33022.
Schmidberger, J. W., Wilce, J. A., Tsang, J. S. & Wilce, M. C. J. (2007). *J. Mol. Biol.* **368**, 706–717.
Schneider, B., Muller, R., Frank, R. & Lingens, F. (1993). *Biol. Chem. Hoppe-Seyler*, **374**, 489–496.
Thomas, A. W., Slater, J. H. & Weightman, A. J. (1992). *J. Bacteriol.* **174**, 1932–1940.
Tsang, J. S. & Pang, B. C. (2000). *Appl. Environ. Microbiol.* **66**, 3180–3186.
Weightman, A. J., Slater, J. H. & Bull, A. T. (1979). *FEMS Microbiol. Lett.* **6**, 231–234.