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# Crystallization and preliminary X-ray analysis of the haloalkane dehalogenase DatA from *Agrobacterium tumefaciens* C58

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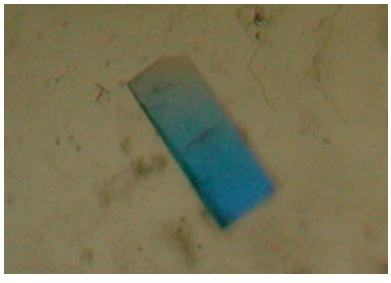
Haloalkane dehalogenases are enzymes that catalyze the hydrolytic reaction of a wide variety of haloalkyl substrates to form the corresponding alcohol and hydrogen halide products. DatA from *Agrobacterium tumefaciens* C58 is a haloalkane dehalogenase that has a unique pair of halide-binding residues, asparagine (Asn43) and tyrosine (Tyr109), instead of the asparagine and tryptophan that are conserved in other members of the subfamily. DatA was expressed in *Escherichia coli*, purified and crystallized using the sitting-drop vapour-diffusion method with a reservoir solution consisting of 0.1 *M* CHES pH 8.6, 1.0 *M* potassium sodium tartrate, 0.2 *M* lithium sulfate, 0.01 *M* barium chloride. X-ray diffraction data were collected to 1.70 Å resolution. The space group of the crystal was determined as the primitive tetragonal space group *P*422, with unit-cell parameters $a = b = 123.7$, $c = 88.1$ Å. The crystal contained two molecules in the asymmetric unit.

## 1. Introduction

Haloalkane dehalogenases (HLDs) are enzymes that catalyze the hydrolytic cleavage of carbon–halogen bonds in halogenated compounds to form the corresponding alcohol and halide-ion products (Janssen, 2004). Because halogenated compounds often cause environmental pollution, these enzymes have been suggested to be useful catalysts for biodegradation and bioremediation. HLDs have been found in a wide range of bacteria that can utilize halogenated organic compounds as their sole source of carbon and have broad substrate specificities (Janssen, 2007; Prokop *et al.*, 2010; Sato *et al.*, 2005). HLDs can be divided into three subfamilies (HLD-I, HLD-II and HLD-III) based on sequence similarities (Chovancová *et al.*, 2007). On the other hand, HLDs can be divided into four substrate-specificity groups (SSG-I, SSG-II, SSG-III and SSG-IV) based on their preferred and characteristic substrates (Koudelakova *et al.*, 2011). The structural architecture of HLDs consists of a core domain with a canonical $\alpha/\beta$-hydrolase fold and a cap domain with a principally helical structure (Franken *et al.*, 1991; Ollis *et al.*, 1992). The active site lies between the core domain and the cap domain and is formed by a catalytic pentad composed of a catalytic triad and two halide-binding residues (Silberstein *et al.*, 2007).

*Agrobacterium tumefaciens* is a plant pathogen with the characteristic ability to transfer a defined segment of DNA to a host plant, generating gall tumours (Wood *et al.*, 2001). The gene encoding DatA is located on the Ti plasmid of *A. tumefaciens* C58 as open reading frame *atu*6064. The haloalkane dehalogenase DatA from *A. tumefaciens* C58 (34 kDa) belongs to the HLD-II subfamily (Hasan *et al.*, 2011) and the SSG-IV subfamily (Koudelakova *et al.*, 2011). Both subfamilies have a characteristic catalytic pentad composed of Asp-His-Glu (the catalytic triad involved in the hydrolysis reaction) and Asn-Trp (the residues forming the halide-binding site that stabilizes the released halide ion). Interestingly, DatA differs from other known enzymes in these subfamilies in that one of the residues in its halide-binding site is a tyrosine (Tyr109) instead of the tryptophan that is conserved in the other members (Hasan *et al.*, 2011; Kennes *et al.*, 1995; Krooshof *et al.*, 1998; Schanstra *et al.*, 1996). There is no



structural information that shows that tyrosine contributes to the stabilization of the released halide ions in HLDs. To elucidate the role of the tyrosine residue (Tyr109) in the catalytic mechanism of DatA, we have crystallized and performed preliminary X-ray diffraction analysis of DatA from *A. tumefaciens* C58.

## 2. Materials and methods

### 2.1. Overexpression, purification and crystallization

A DatA expression plasmid constructed by inserting the *datA* gene (GenBank accession No. BAJ23993) under the control of the *tac* promoter into the pAQN vector (Hasan *et al.*, 2011) was transformed into *Escherichia coli* Rosetta (DE3) cells for protein expression. The transformants were cultivated at 310 K in LB medium containing 50 µg ml$^{-1}$ ampicillin. Overexpression was induced by adding 1.0 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) when the optical density at 600 nm reached 0.6 and the culture was continued overnight at 293 K. The cells were harvested by centrifugation at 5180*g* and 277 K for 15 min and were resuspended in 50 m*M* Tris–HCl pH 8.0, 400 m*M* NaCl, 5 m*M* imidazole. The cells were disrupted by sonication for 10 min using a Branson Digital Sonifier at power 7. After centrifugation at 40 000*g* and 277 K for 30 min, the supernatant was applied onto Ni-Sepharose and the DatA protein was eluted with 200 m*M* imidazole in 50 m*M* Tris–HCl pH 8.0, 400 m*M* NaCl. The eluted protein was dialyzed against 50 m*M* Tris–HCl pH 8.0, 1 m*M* DTT. As the final purification step, anion-exchange chromatography was performed with a 6 ml Resource Q column equilibrated with 25 m*M* Tris–HCl pH 8.0 and the protein was eluted with a 0–1 *M* NaCl gradient. The purified protein was dialyzed against 10 m*M* Tris–HCl pH 8.0, 1 m*M* DTT and concentrated to 10 mg ml$^{-1}$ by centrifugation at 6000*g* for approximately 30 min.

All crystallization experiments were performed at 293 and 277 K by the sitting-drop vapour-diffusion method. In the initial screening, each drop was formed by mixing 0.75 µl protein solution with 0.75 µl reservoir solution. Initial screening was performed using Wizard I and Wizard II (Emerald BioSystems), Crystal Screen HT and Index HT (Hampton Research). The reservoir-solution conditions that yielded protein crystals were optimized to produce crystals that were suitable for X-ray diffraction analysis. After refinement of the crystallization conditions, Additive Screen HT (Hampton Research) was also used. In the optimization step, the crystallization drops were prepared by mixing 2.0 µl protein solution and 2.0 µl reservoir solution.

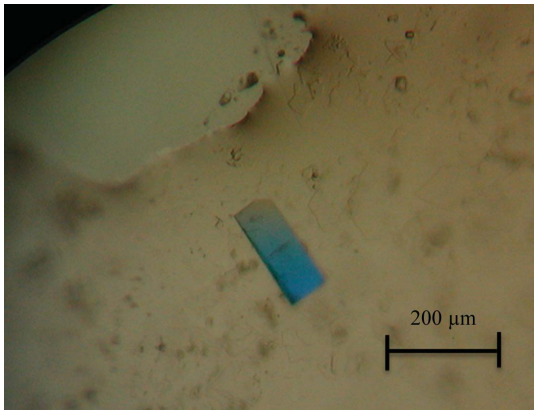



**Figure 1**
Image of a typical DatA crystal. The approximate dimensions of the crystal are 0.20 × 0.07 × 0.05 mm. The crystal was grown using reservoir-solution conditions consisting of 0.1 *M* CHES pH 8.6, 1.0 *M* potassium sodium tartrate, 0.2 *M* lithium sulfate, 0.01 *M* barium chloride. The colourless crystals typically grew in a few days at 293 K. The crystal appears blue in colour owing to the effect of the polarizing microscope.

### 2.2. X-ray data collection and processing

A crystal of DatA was picked up in a nylon loop, transferred into a cryoprotectant solution [0.1 *M* CHES pH 8.6, 1.0 *M* potassium sodium tartrate, 0.2 *M* lithium sulfate, 0.01 *M* barium chloride, 30%(*v*/*v*) glycerol] and flash-cooled using a nitrogen-gas stream cryodevice (100 K). X-ray diffraction data were collected on beamline NE3A at the Photon Factory (PF; Tukuba, Japan) using an ADSC Quantum 270 CCD detector with an X-ray beam size of 0.2 × 0.2 mm. The crystal diffracted X-rays to 1.70 Å resolution. Data set 1 (the low-resolution data set), consisting of 360 images, was collected with a crystal-to-detector distance of 243.88 mm to obtain data with 100% completeness in the low-resolution range. Data set 2 (the high-resolution data set), also consisting of 360 images, was collected with a crystal-to-detector distance of 150.67 mm to obtain high-resolution data. The two diffraction data sets were collected using a wavelength of 1.0000 Å, an oscillation angle of 0.5° and an exposure time of

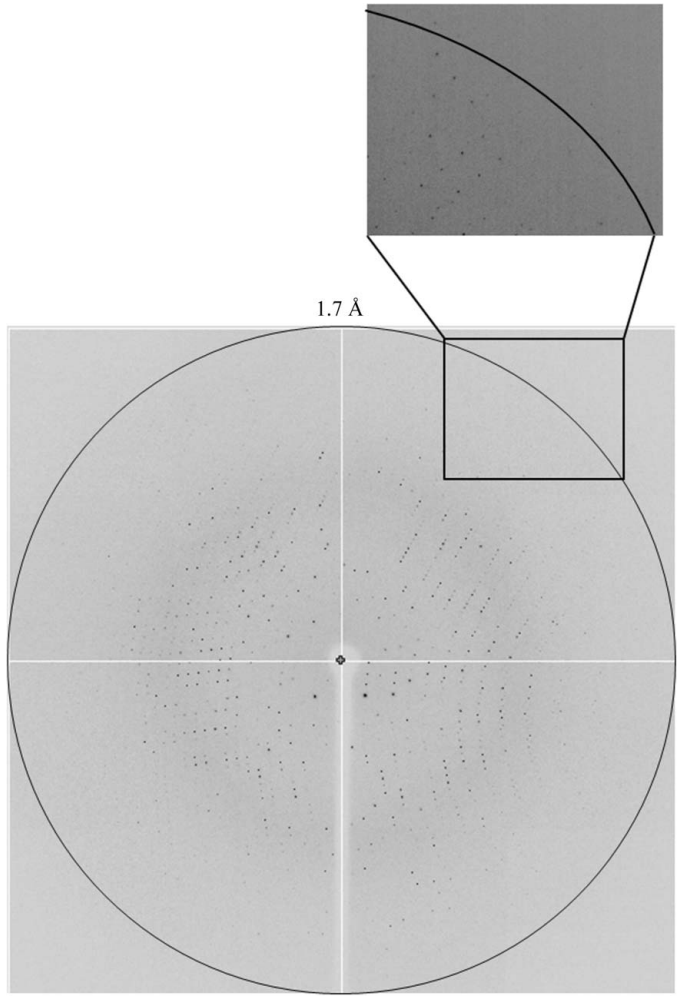



**Figure 2**
An X-ray diffraction image of a typical crystal. The edge of the detector corresponds to a resolution of 1.70 Å. Data sets were collected at a wavelength of 1.0000 Å, an oscillation angle of 0.5° and an exposure time of 1.0 s.

**Table 1**
Merged data-collection statistics for DatA.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Beamline | Photon Factory NE-3A |
| Space group | *P*422 |
| Unit-cell parameters (Å) | $a = b = 123.7$, $c = 88.1$ |
| Wavelength (Å) | 1.0000 |
| Resolution (Å) | 50–1.70 (1.75–1.70) |
| No. of observations | 1563053 (72718) |
| No. of unique reflections | 75138 (5375) |
| Data completeness (%) | 99.8 (97.7) |
| Multiplicity | 20.8 (13.5) |
| $R_{\rm merge}$† | 0.053 (0.362) |
| $\langle I/\sigma(I)\rangle$ | 43.7 (8.6) |

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection $hkl$ and $\langle I(hkl)\rangle$ is the mean intensity of reflection $hkl$.

1.00 s. The data were indexed, integrated, scaled and merged using *XDS* (Kabsch, 2010).

## 3. Results and discussion

Recombinant DatA was expressed in *E. coli* and purified by two column-chromatography steps. In the initial trials, crystals of DatA appeared after a few days in a drop consisting of equal volumes of protein solution and solution No. 38 of Wizard I (0.1 *M* CHES pH 9.5, 1.0 *M* potassium sodium tartrate, 0.2 *M* lithium sulfate) at 293 K. The best crystal of DatA was obtained using a reservoir solution consisting of 0.1 *M* CHES pH 8.6, 1.0 *M* potassium sodium tartrate, 0.2 *M* lithium sulfate, 0.01 *M* barium chloride at 293 K. Fig. 1 shows a typical crystal (0.2 × 0.07 × 0.05 mm). No crystals of DatA were obtained at 277 K.

The best crystal diffracted X-rays to 1.70 Å resolution (Fig. 2). The crystal belonged to the primitive tetragonal space group *P*422, with unit-cell parameters $a = b = 123.7$, $c = 88.1$ Å. The crystal contained two molecules per asymmetric unit according to the Matthews coefficient ($V_{\rm M} = 2.45$ Å$^3$ Da$^{-1}$), corresponding to a solvent content of 49.9% (Matthews, 1968). The data-collection statistics are summarized in Table 1. Molecular-replacement searches were performed using the program *MOLREP* (Vagin & Teplyakov, 2010) from the *CCP*4 program package (Winn *et al.*, 2011). The coordinates of the haloalkane dehalogenase Rv2579 from *Mycobacterium tuberculosis* (PDB entry 2qvb; Mazumdar *et al.*, 2008), which showed 37% sequence identity to DatA, were used as a search model and the *R* factor after *MOLREP* was 0.556. The *R* factor and $R_{\rm free}$ after five cycles of refinement by *REFMAC*5 (Murshudov *et al.*, 2011) were 0.498 and 0.523, respectively. Further model building and refinement are in progress.

The synchrotron-radiation experiments were performed on beamline NE3A at the Photon Factory, Tsukuba, Japan (Proposal No. 2010G082). This work was supported in part by the National Project on Targeted Proteins Research Program (TPRP) of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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