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Crystallogrphic analysis of haloalkane dehalogenase DatA from *Agrobacterium tumefaciens* C58

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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.

Agrobacterium tumefaciens is a plant pathogen with the characteristic ability to transfer a defined segment of DNA to a host plant, generating gall tumors [1]. 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 and the SSG-IV subfamily. 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. 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 Experimental

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 [2] was transformed into *Escherichia coli* Rosetta (DE3) cells for protein expression. Recombinant DatA was 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.

3 Results and Discussion

The best crystal diffracted X-rays to 1.70 Å resolution. 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_M = 2.45 Å^3 Da^{-1}), corresponding to a solvent content of 49.9%. The data-collection statistics are summarized in Table 1. Molecular replacement searches were performed using the program MOLREP from the CCP4 program package. The coordinates of the haloalkane dehalogenase Rv2579 from *Mycobacterium tuberculosis* (PDB entry 2qvb), which showed 37% sequence identity to DatA, were used as a search model. Model building and refinement are in progress.

Table 1: Data collection statistics for DatA.

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)
Redundancy	20.8 (13.5)
R_{merge} †	0.053 (0.362)
$\langle I \rangle / \langle \sigma(I) \rangle$	43.7 (8.6)

Values in parentheses are for the highest-resolution shell.
† $R_{merge} = \Sigma_{hkl}\Sigma_i |I(hkl)_i - \langle I(hkl) \rangle| / \Sigma_{hkl}\Sigma_i I(hkl)_i$, where $I(hkl)_i$ is the intensity of reflection *hkl* and $\langle I(hkl) \rangle$ is the mean intensity of reflection *hkl*.

References

[1] Wood *et al*., *Science* **294**, 2317-2323 (2001).
[2] Hasan *et al*., *Appl. Environ. Microbiol.* **77**, 1881-1884 (2011).

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