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Crystal structure analyses of haloalkane dehalogenase LinB from *Sphingobium* sp. MI1205

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1 Introduction

Several β-HCH-degrading bacteria whose β-HCH degrading enzymes can be utilized for bioremediation have been identified. $LinB_{MI}$ and $LinB_{UT}$ are haloalkane dehalogenases isolated from *Sphingobium* sp. MI1205 and *Sphingobium japonicum* UT26, respectively, that can cleave the carbon-halogen bond in β-HCH. $LinB_{MI}$ and $LinB_{UT}$ share 98% sequence identity, with only 7 different amino acid residues (at positions 81, 112, 134, 135, 138, 247, and 253) out of 296 residues, but these enzymes exhibit different enzymatic properties. $LinB_{MI}$ catalyzes the two-step dehalogenation and converts β-HCH to 2,3,4,5,6-pentachlorocyclohexanol (PCHL) and further to 2,3,5,6-tetrachlorocyclohexane-1,4-diol (TCDL) [1], whereas $LinB_{UT}$ catalyzes only the first-step dehalogenation of β-HCH to PCHL [2] and cannot degrade PCHL further.

The crystal structure of $LinB_{UT}$ has been described, whereas the crystal structure of $LinB_{MI}$ has not. To investigate how the seven residues that are different between $LinB_{MI}$ and $LinB_{UT}$ contribute to their different enzymatic properties, we performed X-ray crystallographic studies of $LinB_{MI}$.

2 Experimental

The expression plasmids of wild-type $LinB_{MI}$ and the seven mutants (carrying T81A, V112A, V134I, T135A, L138I, H247A, and I253M) were constructed using the vector pAQNM. The best crystals of the wild-type $LinB_{MI}$ were obtained by mixing 1.0 μl of the protein solution (25 mg ml^{-1}) and 1.0 μl of the reservoir solution (100 mM Tris-HCl (pH 8.0), 20% (wt/vol) PEG 4000, and 200 mM $CaCl_2$) at 5°C. Similarly, the crystals of the seven mutants of $LinB_{MI}$ were obtained by mixing 1.0 μl of the protein solution (25 mg ml^{-1}) and 1.0 μl of the reservoir solution (100 mM Tris-HCl (pH 7.8 to 8.1), 17 to 20% (wt/vol) PEG 4000, and 200 mM $CaCl_2$) at 5°C. The crystal of wild-type $LinB_{MI}$ belonged to the space group $P2_12_12$ with the following unit cell dimensions: a = 50.4 Å, b = 72.1 Å, and c = 73.5 Å. It contained one $LinB_{MI}$ molecule per asymmetric unit. The Matthews coefficient and the solvent content were 1.96 $Å^3$ Da^{-1} and 37%, respectively. The crystals of the seven mutants had the same space group, $P2_12_12$, with unit cell dimensions similar to those of the crystal of wild-type $LinB_{MI}$.

3 Results and Discussion

We have solved the crystal structures of wild-type $LinB_{MI}$ at a 1.60-Å resolution and of the seven mutants at 1.75- to 2.10-Å resolutions by molecular replacement. The $LinB_{MI}$ molecule existed as a monomer in the crystal (Fig. 1) and consisted of two domains, the core domain and the cap domain. The core domain (residues 2 to 132 and 214 to 295) had a typical α/β-hydrolase fold, as seen in other haloalkane dehalogenases. Unlike the core domain, the cap domain varied in the number and orientations of helices among haloalkane dehalogenases, and the cap domain (residues 133 to 213) of $LinB_{MI}$ was composed of four 3_{10} and six α-helices. The crystal structures of the wild type and the seven mutants of $LinB_{MI}$ were very similar to one another, with root mean square deviations (RMSDs) for Cα atoms (residues 2 to 295) of 0.095 to 0.31 Å. However, the structural comparisons among wild-type $LinB_{MI}$, $LinB_{UT}$, and the seven mutants of $LinB_{MI}$ indicated that each mutant except the T81A mutant caused a small conformational change in the access tunnels or the active site that resulted in a reduction in the first and second-step dehalogenation activities of $LinB_{UT}$ compared with those of $LinB_{MI}$.

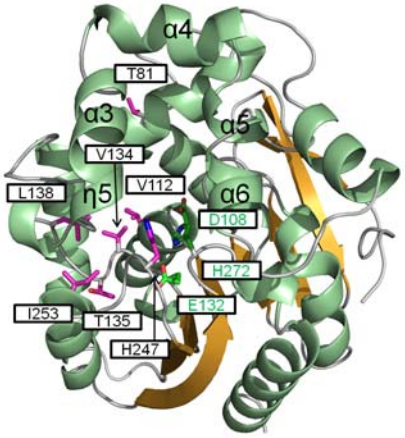

Fig. 1: Structure of wild-type $LinB_{MI}$.

References

[1] Ito *et al*., *Arch. Microbiol.* **188**, 313–325. (2007).
[2] Nagata *et al*., *Appl. Environ. Microbiol.* **71**, 2183–2185. (2005).

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