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Structural basis for substrate setereospecificity of D-*threo*-3-hydroxyaspartate dehydratase from *Delftia* sp. HT23

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1 Introduction

3-Hydroxyaspartate and its derivatives are known to be useful as competitive blockers of excitatory glutamate transporters of the mammalian central nervous system. 3-Hydroxyaspartate has two chiral carbon atoms, and thus there are four stereoisomers, i.e., D-*threo*-3-hydroxyaspartate (2*R*,3*R*; D-THA), L-*threo*-3-hydroxyaspartate (2*S*,3*S*; L-THA), D-*erythro*-3-hydroxyaspartate (2*R*,3*S*; D-EHA), and L-*erythro*-3-hydroxyaspartate (2*S*,3*R*; L-EHA). It is very difficult to synthesize individually by synthetic chemistry approach. D-*threo*-3-Hydroxyaspartate dehydratase (D-THA DH; EC 4.3.1.27) [1] isolated from *Delftia* sp. HT23 is a novel PLP-containing enzyme that shows dehydratase activity against D-THA, L-THA, and L-EHA [1]. Therefore, D-THA DH is useful to produce optically pure 3-hydroxyaspartate isomers from DL-racemic 3-hydroxyaspartate. To elucidate the substrate recognition and catalytic mechanism regarding enantio-selectivity, we have determined the crystal structures of D-THA DH and its inactive mutant in complex with various substrates and inhibitors [2, 3].

2 Experiment

Recombinant His-tagged D-THA DH and its mutants were overexpressed by *Rhodococcus erythropolis* L88 cells, and were purified by using Ni-affinity column. Bipyramid-shaped single crystals were obtained by hanging-drop vapor diffusion method at 20°C, using the reservoir solution containing 0.1 M Tris-HCl, pH 8.5, 0.2 M $MgCl_2$, and 10–18% PEG 3350. For the preparation of substrate/inhibitor complexes, molar excess of substrate/inhibitors were added to the crystallization sample before crystallization setup. X-ray diffraction data sets were collected on the beamline NW12A, NE3A and BL-17A at Photon Factory (PF), by using the CCD detector. All the crystals of the D-THA DH and its mutants were isomorphous: the space group $I4_122$ with unit-cell dimensions of approximately $a = b = 157$, and $c = 157$ Å. The diffraction images were processed with the HKL2000 program package. Singlewavelength anomalous diffraction (SAD) phasing was performed to build the first atomic model of D-THA DH with the program SHELXC/D/E, using the anomalous signal of bromide atom as previously described [2]. Model refinement was performed with the program REFMAC5. Structures of D-THA DH analyzed in this study was listed in Table 1.

Table 1: List of D-THA DH crystal structures reported in this study.

Enzyme	Ligand	Resolution (Å)	PDB ID
Wild-type	-	2.30	3WQF
Wild-type	Mg	1.50	3WQC
Wild-type	Mg, D-EHA	1.50	3WQD
C353A	-	1.55	3WQG
H351A	Mg	1.70	4PB3
H351A	Mg, 2-amino maleic acid (reaction intermediate)	1.80	4PB4
H351A	Mg, L-EHA	1.90	4PB5

3 Results and Discussion

Total of seven crystal structures of wild-type and poorly active mutant of D-THA DH were determined at resolution ranging 1.5-2.30 Å, with and without various substrate/inhibitors. The asymmetric unit contains a homodimer formed by a head-to-tail association of two polypeptide chains of D-THA DH. The structural analysis revealed that the D-THA DH belongs to fold type III family of pyridoxal enzymes (Fig. 1).

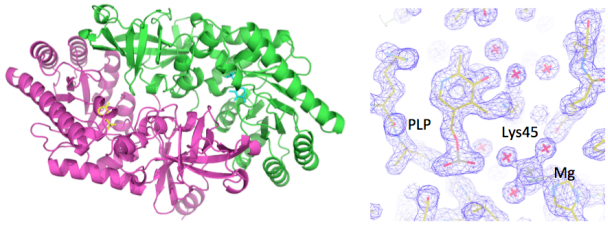

Fig. 1: Homodimeric structure of D-THA DH and its clear electron density for the cofactor PLP.

On the basis of the crystal structures of H351A complexed with D-THA/L-EHA, a possible mechanism for the catalytic reaction of D-THA DH could be proposed. The substrate covalently binds to the PLP molecule in each active-site pocket of the homodimeric state of D-THA DH. The bound Mg^{2+} is also in direct coordinated with the β-carboxyl and β-hydroxyl oxygen atoms, presumably playing an important role in the stabilization of the bound substrate during the catalytic reaction. After abstracting a proton from the α-carbon of

D-THA by Lys43 to generate a quinonoid resonance structure, the *β*-elimination reaction occurs by a proton donation to the substrate hydroxyl group. The substrate/inhibitor complex structures also provided important clues regarding substrate stereospecificity of the D-THA DH. The C*β*-hydroxyl group of the bound L-EHA (substrate) to the poorly active H351A mutant occupied a position close to the bound Mg^{2+} at the active site, clearly indicating a possibility of metal-assisted C*β*-hydroxyl elimination from the substrate as described above (Fig. 2). In contrast, the C*β*-hydroxyl of the D-EHA (inhibitor) was bound far from the Mg^{2+} (Fig. 2). These observations suggest that the substrate stereospecificity of D-THA DH depends on the orientation of the C*β*-hydroxyl group at the active site, and its spatial arrangement is compatible with the 3*R* configuration of 3-hydroxyaspartate.

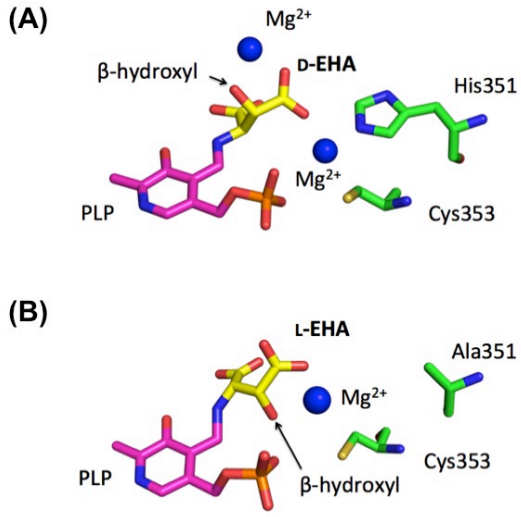

Fig. 2: Active-site snapshots for (A) the bound inhibitor D-EHA to the wild-type enzyme and (B) the substrate L-EHA bound to the poorly active H351A mutant.

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References

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