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Preliminary crystallographic analysis of D-*threo*-3-hydroxyaspartate dehydratase isolated from *Delftia* sp. HT23

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1 Introduction

3-Hydroxyaspartate and its derivatives are useful as competitive blockers of excitatory glutamate/aspartate transporters of the mammalian nervous system. 3-Hydroxyaspartate exists as four stereoisomers because it has two chiral centers, 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). They are difficult to synthesize individually, and thus, it is considered that the enzyme degrading 3-hydroxyaspartate might be useful for the enzymatic optical resolution of DL-racemic 3-hydroxyaspartate to produce optically pure 3-hydroxyaspartate isomers. D-*threo*-3-hydroxyaspartate dehydratase (D-THA DH; EC 4.3.1.27) [1] isolated from *Delftia* sp. HT23 is a novel enzyme that exhibits dehydratase activity towards D-THA, L-THA, L-EHA, and D-serine. To elucidate the structural mechanism of substrate stereoselectivity of D-THA DH, we have undertaken the crystallographic study of D-THA DH.

2 Methods

Recombinant D-THA DH was overexpressed by *Rhodococcus erythropolis*, and purified by Ni-affinity chromatography. Crystals of D-THA DH was obtained by hanging-drop vapor diffusion method at 293 K. Single crystals suitable for X-ray diffraction study were obtained in the solution containing 0.1 M Tris-HCl, pH 8.5, 0.2 M $MgCl_2$, and 10–14% PEG 3350. After the crystals were soaked into the cryoprotectant supplemented with 1 M NaBr, the Single-wavelength anomalous diffraction (SAD) data set was collected on the beamline NW-12A at Photon Factory (PF), by using Quantum 210r CCD detector. The wavelength was set at 0.91944 Å based on the results from the fluorescence scan around the *K* absorption edge of Br. The crystal of the D-THA DH belongs to the space group $I4_122$, with unit-cell dimensions $a = b = 157.5$, $c = 158.1$ Å. The total rotation range for the Br SAD data collection was 360°. The diffraction images were processed with the HKL2000 package. SAD phasing was performed with the program SHELXC/D/E. Data collection and phasing statistics were listed in Table 1.

3 Results and discussion

Structure solution of D-THA DH was attempted by Br-SAD method. Soaking procedure for preparation of Br-bound crystals was based on the method described by Dauter *et al* [2]. Br-SAD data were collected to a resolution of 2.0 Å. Total of 42 Br binding sites were determined by program SHELXC/D, using the data ranging 20-2.8 Å. SAD phasing and density modification were also performed by program SHELXE. The resultant electron density map was very clear and interpretable. The model building of D-THA DH is currently under way.

Table 1. Br SAD data collection and phasing statistics.

Data collection statistics	
Beamline	NW-12A
Wavelength (Å)	0.91944
Resolution (Å)	50–2.00 (2.03–2.00)
Unit-cell dimensions	
a, *b*, *c* (Å)	157.5, 157.5, 158.1
Space group	$I4_122$
Unique reflections	66,644
R_{merge}	0.140 (0.901)
Completeness (%)	100.0 (100.0)
Redundancy	29.7 (29.8)
Mean I/σ (I)	58.8 (8.6)
Phasing by SHELX	
No. of Br sites	42
Best CC All/Weak	41.8/20.2
FOM (after *SHELXE*)	0.64
Map contrast	0.66
Map connectivity	0.93

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References

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