BL-5A, BL-17A, AR-NW12A /2021G508, 2019G518

X-ray structure of a homodimeric L-ribulose 3-epimerase from *Methylomonas* sp.

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1 Introduction

D-Allulose is a rare sugar, and it shows physiological functions, such as suppressing postprandial blood glucose elevation and suppressing fat accumulation. To date, several enzymes capable of D-allulose production have been reported, and they belong to ketose 3-epimerase family, including D-tagatose 3-epimerases (DTEs, homodimer), D-allulose 3-epimerases (DAEs, homotetramer) and L-ribulose 3-epimerases (LREs, homotetramer). Based on the sequence similarity with the potential enzymes, a putative protein from *Methylomonas* sp. DH-1 (NCBI, WP_064020855.1, 286 a.a.) was selected as a putative sugar isomerase/epimerase. The recombinant putative protein exhibited the highest enzymatic activity towards L-ribulose. It was considered to be L-ribulose 3-epimerase (LRE), but also had relatively high enzymatic activity towards D-allulose. In this study, we determined the X-ray structure of LRE from *Methylomonas* sp. (MetLRE). The X-ray structure analysis of MetLRE revealed a homodimer different from previously reported homotetrameric LREs, and the most structurally similar protein to MetLRE was D-allulose 3-epimerase from *Arthrobacter globiformis* (AgDAE), which has a long C-terminal α-helix. The X-ray structure of MetLRE is the first known homodimeric L-ribulose 3-epimerase with a short C-terminal α-helix [1].

2 Experiment

The recombinant MetLRE was expressed using a synthesized gene encoding the putative sugar isomerase/epimerase from *Methylomonas* sp. DH-1 and used for crystallization. Crystals of C-terminal His-tagged MetLRE were obtained in a droplet containing a mixture of 0.8 µl protein solution (5.5 mg/ml in 5 mM Tris-HCl, pH 8.0) and 0.8 µl reservoir solution (0.2 M $MgCl_2$, 0.1 M Tris-HCl pH 8.5, 25 % (w/v) PEG3350) in a well containing 50 µl reservoir solution by the sitting-drop vapor-diffusion method at 293 K. As for the crystals of N-terminal His-tagged MetLRE, protein solution (22.3 mg/ml) in 5 mM Tris-HCl, pH 8.0 and the reservoir solution (0.1 M HEPES pH 7.5, 10 % (w/v) polyethylene glycol 8,000 and 8 % (v/v) ethylene glycol) were used with the above crystallization conditions.

X-ray diffraction data were collected on the PF BL-5A and the PF BL-17A in the KEK, and processed using the programs HKL2000, XDS and the CCP4 suite. The structure was determined by molecular replacement with the program MOLREP using the structure of AgDAE (PDB ID 5ZFS) .

3 Results and Discussion

MetLRE_his in complex with D-fructose was crystallized in the orthorhombic space group $P2_12_12_1$ with two molecules in an asymmetric unit. MetLRE forms a homodimer (Mol-A and Mol-B) (Fig. 1). In Mol-A, the C-terminus was refined to His293 including a linker and part of the His-tag, and the C-terminus of Mol-B was refined to Gly287, which is the beginning of a linker before the His-tag.

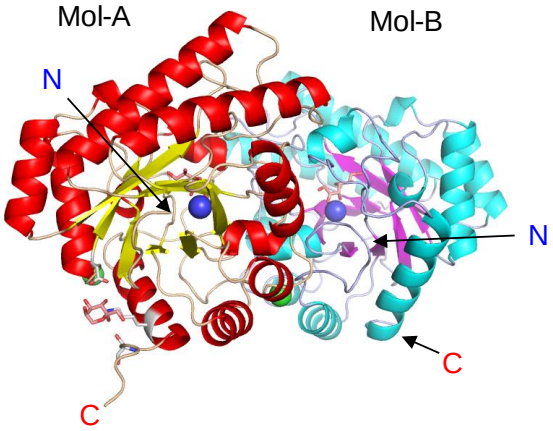

Fig. 1: Dimeric structure of MetLRE in complex with D-fructose. The bound metal ions are shown in blue spheres. N and C indicate the N terminus and C-terminus, respectively.

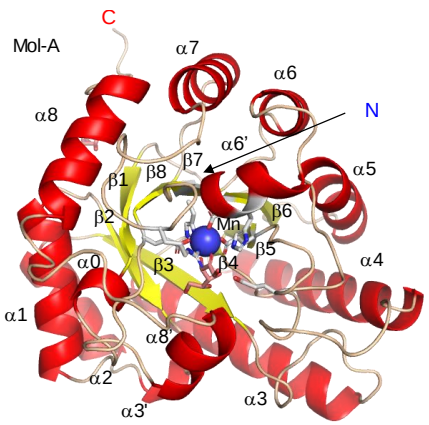

Fig. 2: Monomeric structure of MetLRE.

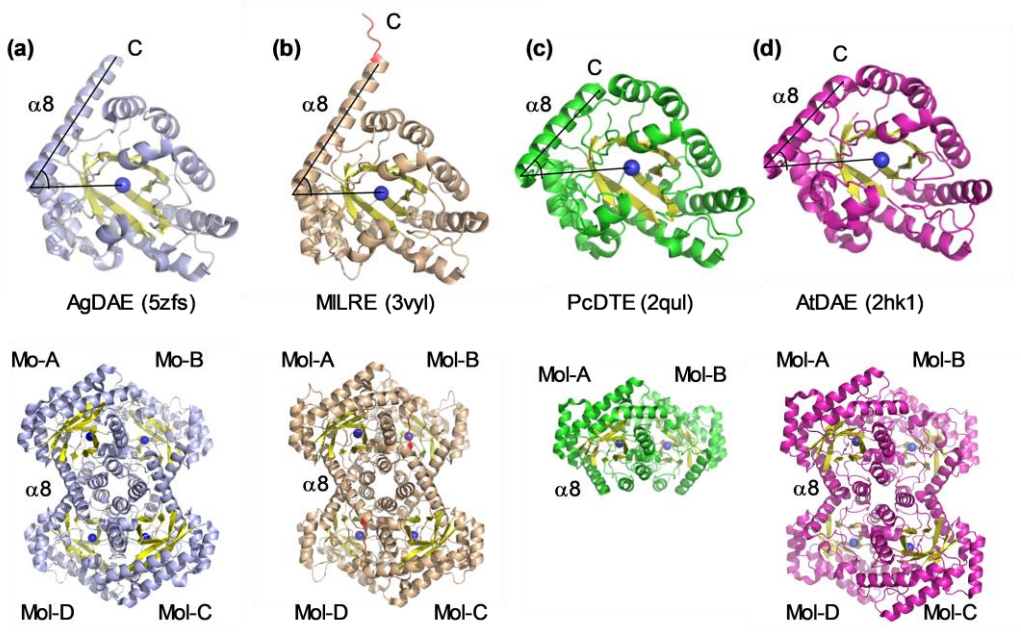

Fig. 3: Overall structure of the structurally similar enzymes.
The monomeric structure (up) and overall structure (down) of structurally similar enzymes are represented. (a) *A. globiformis* ketose 3-epimerase (AgDAE), (b) *M. loti* L-ribulose 3-epimerase (MlLRE), (c) *P. cichorii* D-tagatose 3-epimerase (PcDTE), and (d) *A. tumefaciens* D-allulose 3-epimerase (AtDAE). The C-terminal α-helix is labeled as α8.

The monomeric structure has twelve α-helices and eight β-strands, forming a $(\beta/\alpha)_8$ barrel fold with four additional short α-helices (α0, α3', α6' and α8') (Fig. 2). There is a metal ion at the center of the barrel, which is coordinated by Glu152, Asp185, His211 and Glu246 to form the catalytic site and bind the substrate.

The most structurally similar protein was ketose 3-epimerase from *Arthrobacter globiformis* (previously known as AgDAE, L-ribulose 3-epimerase, PDB code 5zfs), with 54 % identity, 1.6 Å r.m.s.d. and 45.6 Z-score by Dali search. Other similar proteins were L-ribulose 3-epimerase from *Mesorhizobium loti* (MlLRE, 3vyl, 40 %, 1.3 Å, 42.5), D-tagatose 3-epimerase from *Pseudomonas cichorii* (PcDTE, 2qul, 30 %, 1.7 Å, 37.3) and D-allulose 3-epimerase from *Agrobacterium tumefaciens* (AtDAE, 2hk1, 28 %, 1.8 Å, 36.8). The monomeric structures of these proteins are compared in Fig. 3. The structures adopt $(\beta/\alpha)_8$ barrel fold, and a metal ion is bound at the center of the each barrel. Although the features of LREs are a long C-terminal helix (α8, 26-29 residues) and formation of stable homotetramers, MetLRE has a shorter C-terminal helix (α8, 16 residues) than previously reported LREs and forms a homodimer like homodimeric PcDTE. A similar feature of MetLRE with LRE was the angle of the catalytic metal – N-terminus of α8 – C-terminus of α8 (metal-α8N-α8C), which was 54 º, being between MlLRE (58 º) and AgDAE (52 º), and different from PcDTE (49 º) and AtDAE (43 º) (Fig. 3).

In this study, a novel homodimeric L-ribulose 3-epimerase from *Methylomonas* sp. was expressed and its X-ray structure was determined. The structure showed a unique short C-terminal α-helix compared to the known structures of the structurally similar homotetrameric L-ribulose 3-epimerases and D-allulose 3-epimerases.

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References

[1] Yoshida, H. et al., *FEBS Open Bio*. **11**, 1621-1637 (2021).

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