AR-NE3A, BL-5A/2019G518

X-ray structure of *Arthrobacter globiformis* M30 ketose 3-epimerase

Hiromi Yoshida[1,2*], Akihide Yoshihara[2,3], Pushpa Kiran Gullapalli[4], Kouhei Ohtani[4], Kazuya Akimitsu[2,3], Ken Izumori[2,3] and Shigehiro Kamitori[1,2]
[1] Life Science Research Center and Faculty of Medicine, Kagawa University, Kagawa, Japan
[2] International Institute of Rare Sugar Research and Education, Kagawa University, Kagawa, Japan
[3] Faculty of Agriculture, Kagawa University, Kagawa, Japan
[4] Matsutani Chemical Industry Co. Ltd, Hyogo, Japan

1 Introduction

The ketose 3-epimerase from *Arthrobacter globiformis* M30 has been reported to be a D-allulose 3-epimerase (AgDAE), since its substrate specificity was similar to those of the known D-psicose 3-epimerases from *Clostridium cellulolyticum* (CcDPE) and *Agrobacterium fabrum* (*A. tumefaciens*; AtDPE) [1]. D-Allulose (or D-psicose) is a rare sugar, and its physiological functions (such as moderating blood glucose levels and fat accumulation) are being focused on for human healthcare. Therefore, the enzymes capable of D-allulose production are attractive. We determined here, the X-ray structure of AgDAE and found the structure is more similar to that of L-ribulose 3-epimerase from *Mesorhizobium loti* (MlLRE) than previously known structure of DPEs [2]. In addition, we found that AgDAE shows the highest enzymatic activity toward L-ribulose. Although AgDAE is considered to be L-ribulose 3-epimerase, it is useful for D-allulose production in the food industry, considering its enzymatic activity toward D-allulose, thermal stability and well-known microorganism recognized as safe.

2 Experiment

The recombinant AgDAE was used for crystallization. Crystals were obtained in a in a droplet consisting of 1.0 μl protein solution (10 mg/ml in 5 mM Tris–HCl buffer pH 8.0) and 1.0 μl reservoir solution (0.1 M sodium acetate pH 4.6, 3.0–4.0 M ammonium acetate) against 50 μl reservoir solution by the sitting-drop method at 293 K. X-ray diffraction data were collected on the PF-AR NE3A in the KEK, and processed using the programs HKL-2000 and the CCP4 suite. Initial phase was determined by molecular replacement using the structure of MlLRE (PDB code: 3vyl) as a search model.

3 Results and Discussion

The structure of AgDAE was refined at a resolution of 1.96 Å. Two molecules (Mol A and B) in an asymmetric unit (hexagonal space group $P6_522$) formed a homotetramer by a twofold symmetry operation (Fig.1). The monomer structure adopted a $(\beta/\alpha)_8$ barrel fold with the catalytic residues and metal ion located in the center of the barrel, as is observed in the structures of sugar-phosphate isomerases and epimerases (Fig. 2a).

The monomer structure and the overall structure of AgDAE was highly similar to that of the homotetrameric L-ribulose 3-epimerase from *Mesorhizobium loti* (Figs. 2a

Fig. 1: The overall structure of AgDAE.

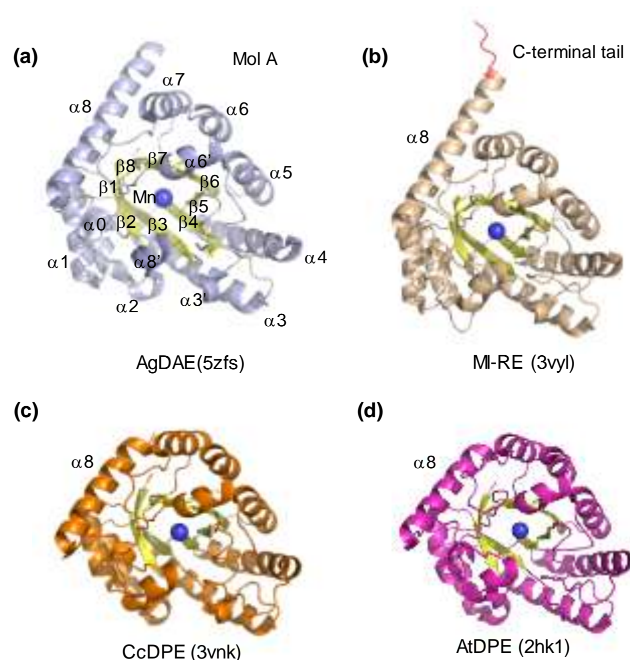

Fig. 2: The monomer structure comparisons among related enzymes. (a) *A. globiformis* D-allulose 3-epimerase (AgDAE), (b) *M. loti* L-ribulose 3-epimerase (MlLRE), (c) *C. cellulolyticum* D-psicose 3-epimerase (CcDPE), (d) *A. tumefaciens* D-psicose 3-epimerase (AtDPE).

and 2b). The common feature is in the lengths of their C-terminal helices (α8). The helices (α8) of AgDAE (Asp264–His289) and MlLRE (Asp265–Ala293) were longer than those of CcDPE (Asp268–Glu288) and AtDPE (Asp268–Gly288). The longer helices extend along the tetramerization interface and probably stabilize the tetrameric structure. According to the structure of MlLRE reported by Uechi et al., the longer C-terminal helix of MlLRE with an additional C-terminal tail (Arg294–Pro297) promoted the formation of additional intermolecular interactions and strengthened the stability of the enzyme compared with CcDPE and AtDPE [3].

Furthermore, we found that AgDAE showed a higher enzymatic activity towards L-ribulose, followed by D-allulose (63.5% of L-ribulose). AgDAE could be categorized as an L-ribulose 3-epimerase but showed a different substrate specificity to MlLRE that is specific for L-ribulose, followed by L-xylulose (42% of L-ribulose), D-tagatose (24% of L-ribulose) and D-ribulose (20% of L-ribulose) [4]. Therefore, AgDAE is useful enzyme for D-allulose production.

Acknowledgement

We thank the PF staff for the support of data collection.

References

[1] Yoshihara, A. et al., *J. Biosci. Bioeng*. **123**, 170-176 (2017).
[2] Yoshida, H. et al., *Acta Cryst.* F**74**, 669-676 (2018).
[3] Uechi, K. et al., *Acta Cryst.* D**69**, 2330-2339 (2013).
[4] Uechi, K. et al., *Biosci. Biotechnol. Biochem*. **77**, 511-515 (2013).

* h.yoshi@med.kagawa-u.ac.jp