X-ray structure of *Cellulomonas parahominis* L-ribose isomerase

Yuji Terami\(^2\), Hiromi Yoshida\(^1\*, Keiko Uechi\(^2\), Kenji Morimoto\(^2\), Goro Takata\(^2\), Shigeo Kamitori\(^4\)

\(^1\) Life Science Research Center and Faculty of Medicine, Kagawa University, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

\(^2\) Rare Sugar Research Center, Miki-cho, Kita-gun, Kagawa 761-0795, Japan

1 Introduction

L-ribose is non-natural sugar, so-called rare sugar, and is not generally used in metabolic pathway as a carbon source. An enzyme, L-ribose isomerase (L-RI) from *Acinetobacter* sp. strain DL28 (AcL-RI) was reported as a new enzyme which can constitutively produce L-ribose isomerase [1], and had no significant sequence similarity to known protein structures [2, 3], implying an unique structure to recognize L-ribose as its ideal substrate. In previous study, we have determined X-ray structure of AcL-RI [4], and here we determined X-ray structure of L-RI from *Cellulomonas parahominis* MB426 (CpL-RI) that has a broader substrate specificity and more thermal stability than AcL-RI [5, 6].

2 Experiment

The expression and purification of CpL-RI were reported [5]. Crystals of his-tagged CpL-RI were grown in a droplet mixing 2 μl of protein solution (30 mg ml\(^{-1}\)) in 5 mM Tris-HCl, pH 8.0 and 2 μl of reservoir solution (3.9 M ammonium acetate, 0.1 M sodium acetate trihydrate pH 4.6) against 450 μl of the reservoir solution at 293 K. X-ray diffraction data were collected on the BL5A in the PF. Diffraction data were processed using the programs HKL2000 and the CCP4 program suite. The structure of CpL-RI was solved by molecular replacement using the structure of AcL-RI.

3 Results and Discussion

The subunit structure of CpL-RI was very similar to that of AcL-RI. CpL-RI adopted a cupin-type β-barrel structure, having four α-helices (H1, H3, H4, and H7), three 3₁₀-helices (H2, H5, and H6), and two large β-sheets (β-sheet 1 and β-sheet 2) formed by 11 β-strands (B1–B11) (Fig. 1a). Two molecules in the asymmetric unit (Mol-A and Mol-B) formed a homo-dimer with 2-fold symmetry, and this dimer formed a homo-tetramer with the symmetry-operated dimer (Mol-C and Mol-D) (Fig. 1b). The β-sheet 1 formed by six β-strands (B1, B2, B3, B10, B5, and B8) was located at the interface between Mol-A and Mol-B, contributing to the intermolecular interactions of the dimer.

In the structure of the catalytic site of CpL-RI, a bound metal ion was coordinated by His106, His108, Glu113, His188, and two waters (W1 and W2), giving an octahedral form of metal coordination (Fig. 1c). A large space for a substrate was identified between β-sheets 1 and β-sheet 2. Glu113 formed hydrogen bonds with W1 and W2, and Lys111 formed a hydrogen bond with W1. Glu204 directed its side chain to the catalytic center by a salt bridge with Lys93. It was expected that these charged residues were sequentially aligned on β-sheet 1 to recognize the hydroxyl groups of the substrate.

![Fig. 1: The structure of CpL-RI](image)

Acknowledgement

We thank the PF staff for the support of data collection. This research was performed with the approval of the Photon Factory Advisory Committee and the National Laboratory for High Energy Physics (2011G504 and 2013G506), Japan.

References


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