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

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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 3_{10}-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.

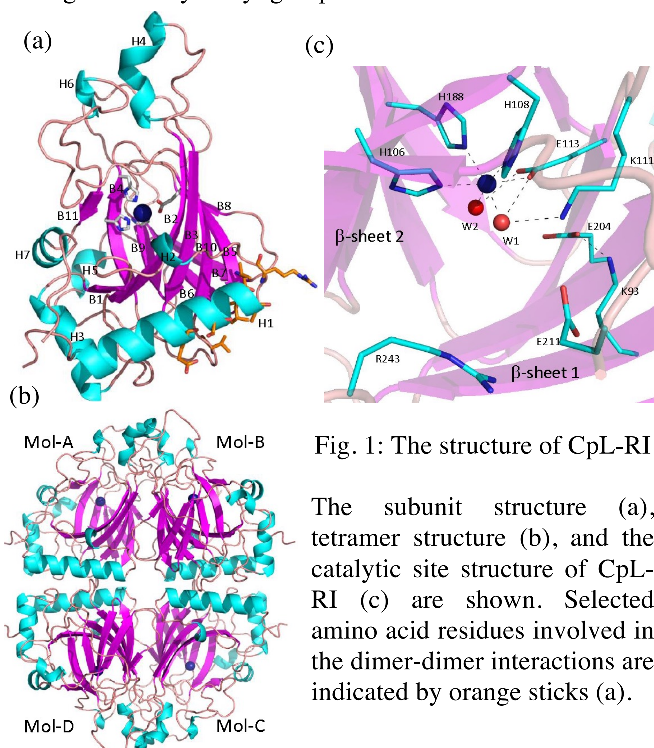

Fig. 1: The structure of CpL-RI

The subunit structure (a), tetramer structure (b), and the catalytic site structure of CpL-RI (c) are shown. Selected amino acid residues involved in the dimer-dimer interactions are indicated by orange sticks (a).

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