BL-5A, 17A, NW12A, NE3A/ 2011G504, 2011A1873, 2013G506

X-ray structure of *Acinetobacter* sp. L-ribose isomerase in complex with non-natural sugars

Hiromi Yoshida[1*], Akihide Yoshihara[2], Misa Teraoka[1], Yuji Terami[2], Goro Takata[2], Ken Izumori[2], Shigehiro Kamitori[1]

[1]Life Science Research Center and Faculty of Medicine, Kagawa University, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

[2]Rare Sugar Research Center, Kagawa University, 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 was reported as a new enzyme [1], and had no significant sequence similarity to known protein structures [2, 3]. Since *Acinetobacter* sp. L-RI uses L-ribose as the most favorable substrate for isomerization reaction, it is expected to have the unique three-dimensional structure to recognize L-ribose as its ideal substrate. In previous study, we have determined crystal structure of his-tagged *Acinetobacter* sp. L-RI (AcL-RI) by using selenomethionin-substituted AcL-RI. In this study, we determined the crystal structure of AcL-RI in complexes with substrate L-ribose and inhibitor ribitol [4].

2 Experiment

The expression, purification, and crystallization of AcL-RI were done as was previously reported [3]. A crystal of AcL-RI mounted in a cryoloop was soaked in 50 % (w/v) L-ribose or 50 % (w/v) ribitol solution and directly flash-cooled in the cryo gas stream at 100 K. X-ray diffraction data of AcL-RI were collected on the PF-AR NW12A, PF BL-5A in the KEK, on the BL26B1 in SPring-8, and on a Rigaku RA-Micro7HF rotating anode (CuKα) X-ray generator with ValiMax optics (40 kV, 30 mA) in house. Diffraction data were processed using the programs HKL2000, CrystalClear and the CCP4 program suite. All structures of AcL-RI in complex with ligands were solved by molecular replacement. Further model building was performed with the programs Coot in CCP4 program suite, and X-fit in the XtalView program system, and the structure was refined using the programs Refmac5 and CNS.

3 Results and Discussion

The overall structure of AcL-RI adopts a cupin-type β-barrel structure, which is shown to be widely distributed from non-enzymatic proteins to enzymes. The DALI server showed that AcL-RI had significant structural homology to D-lyxose isomerases from *Bacillus subtilis* strain 168 (PDBID:2Y0O) and from pathogenic *E. coli* O157: H7 (PDBID: 3MPB) with a r.m.s.d. of 2.4 and 2.8 Å, and Z-scores of 17.4 and 15.8, respectively.

The structure of the catalytic site with a bound ribitol is shown in Fig. 1. O1 and O2 of the bound ribitol coordinate to the metal ion, giving an octahedral form of metal coordination. Glu113 forms hydrogen bonds with the O1 and O2 of ribitol, and Glu204 directs its side chain carboxyl group to the hydrogen atoms attached to C1 and C2 (H1 and H2), which is assisted by the salt bridge from Lys93. These two Glu residues are considered to be responsible for the aldose-ketose isomerization reaction. Lys111 forms hydrogen bonds with the O2, O3 and O4 of ribitol to fix the proper orientation of a substrate for the isomerization reaction. Glu211 and Arg243 form hydrogen bonds with the O4 and O5 of ribitol, respectively, and they are expected to contribute to substrate recognition.

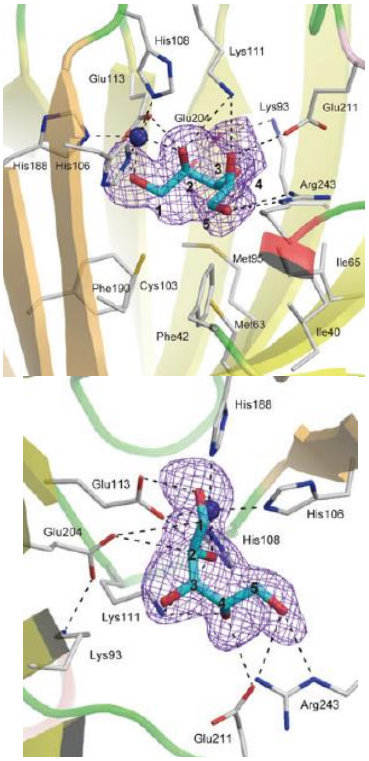

Fig. 1: The catalytic site structures with a bound ribitol.

Fig. 2 shows bound L-ribose at the catalytic site in AcL-RI /L-ribose (left) and bound L-ribose forms on the surface of the enzyme (right). The electron density indicated that bound L-ribose was in a $^4C_1$ pyranose ring form (L-ribopyranose) with axial O1. The O5 of L-ribose coordinates to the metal ion, and an additional water

molecule (W1) is introduced to coordinate to the metal ion. W1 forms hydrogen bonds with the O1 and O5, indicating that W1 may transfer a proton between them to help the ring opening of L-ribopyranose. Lys111 and Glu211 form hydrogen bonds with O2 and O4, respectively, to recognize the substrate. Crystal structure analysis revealed that D-ribose adopted a $^4C_1$ pyranose ring form, meaning that L-ribose should be in a $^1C_4$ pyranose ring form. However, the conformational conversion of L-ribose between the $^4C_1$ and $^1C_4$ pyranose rings likely occurs by interactions with the enzyme because L-ribose, a pentose, has no group at the 5-position of the pyranose ring. L-Ribose in the $^1C_4$ pyranose ring form, as well as the furanose ring form, was found on the surface of the enzyme.

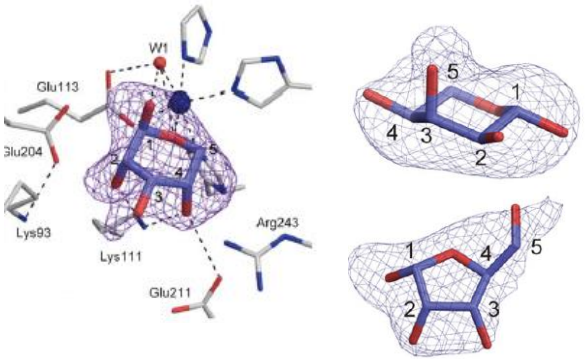

Fig. 2: The bound L-ribose to AcL-RI in a $^4C_1$ pyranose ring form (left), the bound L-ribose in a $^1C_4$ pyranose ring form (right up) and the bound L-ribose in a furanose ring form (right down).

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