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Structure of L-rhamnose isomerase in complex with L-rhamnopyranose demonstrates the sugar-ring opening mechanism

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1 Introduction

Pseudomonas stutzeri L-rhamnose isomerase (L-RhI) catalyzes a reversible isomerization of L-rhamnose to L-rhamnulose. We have reported the X-ray structure of L-RhI with two metal ions and L-rhamnose (linear form) at the catalytic site, and proposed that *P. stutzeri* L-RhI adopts a metal-mediated hydride-shift mechanism as was proposed in *Escherichia coli* L-RhI [1]. In previous study, the X-ray structure of an inactive mutant *P. stutzeri* L-RhI with a substitution of Asp327 with Asn (D327N) was determiend, in which substrate in a furanose-ring form (L-rhamnulofranose) bound to the catalytic site, suggesting that Asp327 is responsible for furanose-ring opening [2]. However, the pyranose-ring opening mechanism for L-rhamnopyranose was unclear.

Here we report the X-ray structure of a mutant *P. stutzeri* L-RhI H101N in complex with L-rhamnose, in which substrate was partly in a pyranose-ring form.

2 Experiment

Construction of mutant, and its expression, purification and crystallization were done as was reported previously [1,2]. A crystal of the mutant H101N in complex with L-rhamnose was obtained by quick soaking method using 33 % (w/v) L-rhamnose solution as a cryoprotectant. X-ray diffraction data including preliminary data were collected on the PF-AR NE3A and NW12A beam line in the Photon Factory, and the BL26B1 beam line in SPring-8. Diffraction data process, structure determination and refinement were performed as previously reported [1,2].

3 Results and Discussion

The overall structure of the mutant *P. stutzeri* L-RhI H101N in a complex with L-rhamnose was almost the same as that of wild-type enzyme, which was homo-tetramer composed of four molecules (Mol-A, B, C, D) [1]. The bound substrates at the catalytic site were found to be in a linear form in Mol-A and C, while these bound substrates adopted two alternative conformations, a linear form and pyranose-ring form, with the same occupancy of 0.5 in Mol-B and D.

The catalytic site structure with a bound L-rhamnose in a linear form (RNS) in Mol-A was very similar to the previously reported structures [1,2]. Structural Mn^{2+} (Mn1) was coordinated by six coordination bonds from Glu219, Asp254, His281, Asp327, and the O2/O3 of RNS, and catalytic Mn^{2+} (Mn2) was coordinated by His257, Asp289, two water molecules (W1 and W2), and the O1/O2 of RNS. W1 was the catalytic water molecule responsible for the proton transfer between O1 and O2 (Fig. 1A). In the wild-type enzyme, His101 also formed a hydrogen bond with RNS (Fig. 1A).

In the catalytic site structure with a bound β-L-rhamnopyranose (β-RPS) in Mol-B (Fig. 1B), the conformation of C1–C3 of β-RPS was almost equivalent to that of RNS, giving the same metal coordination structure as Mol-A. A difference was found between β-RPS and RNS for the torsion angle of C3–C4, −52◦ (β-RPS) and 171◦ (RNS), meaning that rotation around C3–C4 by +223◦ (or −137◦) gave the inter-conversion between β-RPS and RNS. The catalytic water molecule (W1) formed a hydrogen bond with the O1 and O5 of β-RPS, likely acting as an acid/base catalyst in pyranose-ring opening. The substituted Asn101 formed a hydrogen bond with the O4 of β-RPS via a water molecule (W3), which contributed to the stabilization of the enzyme/β-RPS complex. These structures provide us a putative ring-opening mechanism of *P. stutzeri* L-RhI [3].

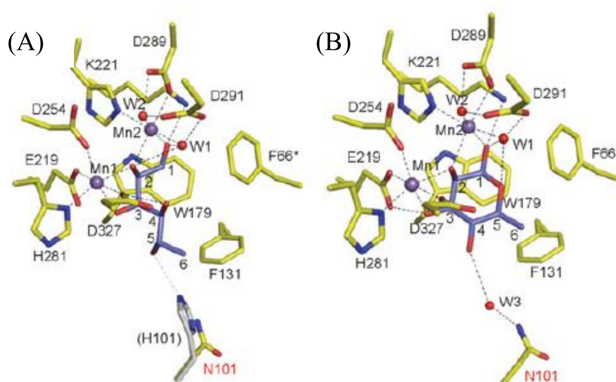

Fig. 1: The catalytic site with bound L-rhamnose, (A) RNS in Mol-A and (B) β-RPS in Mol-B.

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