BL-5A, AR-NW12A/2023G617, 2021G508, 2019G518

X-ray structure of L-rhamnose isomerase derived from *Lactobacillus rhamnosus* Probio-M9

Hiromi Yoshida[1,2*], Naho Yamamoto[3], Lin Hai Kurahara[4], Ken Izumori[2,3], and Akihide Yoshihara[2,3]
[1] Department of Basic Life Science, 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] Department of Cardiovascular Physiology, Faculty of Medicine, Kagawa University, Kagawa, Japan

1 Introduction

Lactobacillus rhamnosus Probio-M9 has been reported as probiotic bacterium [1] and it can grow using L-rhamnose and under acidic conditions. From the genome analysis of the Probio-M9 strain, several genes related to metabolized L-rhamnose, putative L-rhamnose mutarotase, putative L-rhamnose isomerase (L-RhI), putative L-rhamnulose kinase, and putative L-rhamnulose-1-phosphate aldolase have been identified.

L-RhIs catalyze isomerization between L-rhamnose and L-rhamnulose, and some L-RhIs are able to catalyze also between D-allulose and D-allose. In this study, we report the X-ray structure of recombinant L-RhI derived from *Lactobacillus rhamnosus* Probio-M9 (LrL-RhI), which shows enzyme activity toward rare sugars, D-allulose and D-allose [2].

2 Experiment

The recombinant LrL-RhI was expressed using a synthesized gene encoding the putative L-RhI derived from *Lactobacillus rhamnosus* Probio-M9 and used for crystallization. Crystals of C-terminally His-tagged LrL-RhI were obtained in a droplet containing a mixture of 0.8 μl protein solution (5.6 mg/ml in 5 mM Tris-HCl, pH 8.0) and 0.8 μl reservoir solution (0.1 M MES pH 6.5, 15% (v/v) PEG550MME) in a well containing 50 μl reservoir solution by the sitting-drop vapor-diffusion method at 293 K. To obtain the complex structures with substrates, each crystal was soaked in a solution containing 30% (w/v) L-rhamnose, D-allulose, or D-allose.

X-ray diffraction data were collected on the PF BL-5A and the AR-NW12A in the KEK, and processed using the programs XDS and the CCP4 suite. The initial phases of LrL-RhI were obtained by molecular replacement using the MOLREP program with the structure of L-RhI from *E. coli* (PDB ID 1DE5) as a probe model. Further model building was performed using the Coot program, and the structure was refined using the Refmac5 program.

3 Results and Discussion

Crystal structures, LrL-RhI alone and complexes with L-rhamnose (LrL-RhI/L-rhamnose), D-allulose (LrLRhI/ D-allulose), and D-allose (LrL-RhI/D-allose), were determined. A part of the flexible loop region (Asp46-Thr73) was almost missing in the structures of LrL-RhI alone (missing Asn54-Ser64) and complexes with L-rhamnose (missing Pro53-Ser64) and D-allose (missing Asn54-Gly65) due to the weak electron density maps, but the corresponding region in the structure of LrL-RhI/D-allulose (Mol-A and Mol-B) was relatively visible. Here we show the structure of LrL-RhI/ D-allulose.

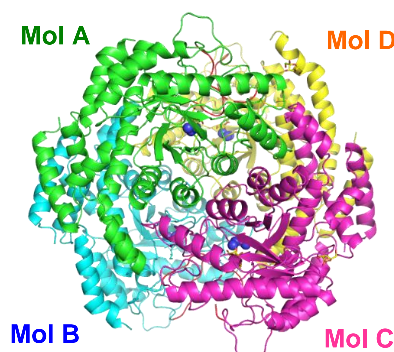

Fig. 1: Overall structure of homotetrameric recombinant LrL-RhI in complex with D-allulose.

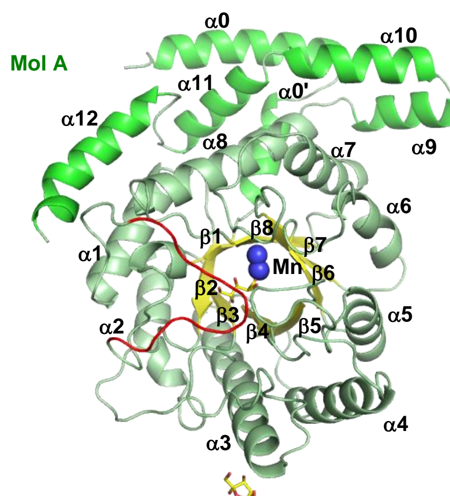

Fig. 2: Monomeric structure of recombinant LrL-RhI (Mol A) in complex with D-allulose.
The flexible loop region (Asn54-Gly64) is colored red. The bound D-allulose and metal ions are shown as yellow sticks and blue spheres, respectively.

Fig. 1 shows the overall structure of LrL-RhI/D-allulose. The overall structure is similar to the known structures of L-RhIs that form homotetramers. Each molecule adopts a $(\beta/\alpha)_8$ barrel fold with additional six α-helices (α0, α0', α9, α10, α11, and α12), as shown in Fig. 2 (Mol A of LrL-RhI/D-allulose). At the center of the beta barrel, there is an active site formed with two metal ions (structural metal and catalytic metal) and covered by a flexible loop region (Asn54-Gly65: loop region colored with red) like a lid. The flexible loop region is located between β1 and α1 (Asp46-Thr73). The dimer form (Mol-A and Mol-D) is shown in Fig. 3. The flexible loop region that is colored red (Asn54-Gly65) is visible in Mol-A, but the corresponding region with a red dotted line in Mol-D is missing (A56-V63) (Fig. 3). At the dimer interface of LrL-RhI, the flexible loop region (Asn54-Gly65) covers the active site in its own molecule (Figs. 2 and 3), as was observed in *E. coli* L-RhI (EcL-RhI), however, this corresponding loop region in *Pseudomonas stutzeri* L-RhI (PsL-RhI) covers the active of each neighboring molecule at the dimer interface. This swapping mode for covering the active site at the dimer interface has been observed in D-xylose isomerases, and the movement of the flexible loop region of PsLRhI is similar.

In this study, the flexible loop region of LrL-RhI was observed in the LrL-RhI/D-allulose complex; however, the corresponding regions in most of the LrL-RhI structures are also missing, suggesting that these regions are highly flexible [2].

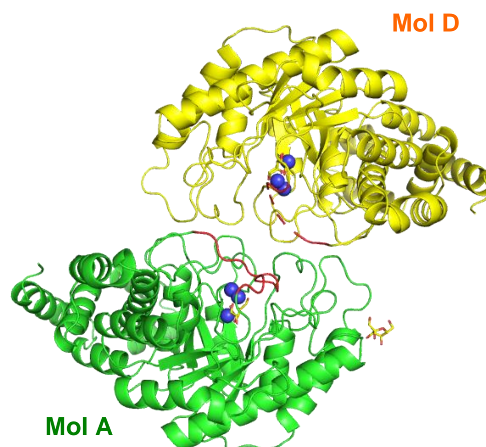

Fig. 3: Dimeric structure of recombinant LrL-RhI (Mol A and Mol D) in complex with D-allulose.

Acknowledgement

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

References

[1] Liu, W. *et al*., *J Dairy Sci*. **103**, 4013–4025. (2020)
[2] Yoshida, H. et al., *Appl Microbiol Biotechnol* **108**, 249 (2024).

*yoshida.hiromi@kagawa-u.ac.jp