BL-5A, AR-NE3A/2022G538

X-ray structures of *Enterobacter cloacae* allose-binding protein in complexes with D-allose and D-ribose

Shigehiro KAMITORI*
Research Facility Center for Science & Technology and Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

1 Introduction

D-Allose is an aldohexose of the C3-epimer of D-glucose, existing in very small amounts in nature, called a rare sugar. The operon responsible for D-allose metabolism, the allose operon, was found in several bacteria, which consists of seven genes: *alsR, alsB, alsA, alsC, alsE, alsK*, and *rpiB*. To understand the biological implication of the allose operon utilizing a rare sugar of D-allose as a carbon source, it is important to clarify whether the allose operon functions specifically for D-allose or also functions for other ligands. It was proposed that the allose operon can function for D-ribose, which is essential as a component of nucleotides and abundant in nature. Allose-binding protein, AlsB, coded in the allose operon, is thought to capture a ligand outside the cell, and is expected to show high affinity for the specific ligand. X-ray structure determinations of *Enterobacter cloacae* AlsB (EtcAlsB) in ligand-free form, and in complexes with D-allose and D-ribose were performed.

2 Experiment

The purified sample was concentrated to 21 mg/mL by Amicon Ultra-4 (Millipore, Billerica, MA, USA). Crystals of EtcAlsB were grown in a droplet mixed with 1 μL of protein solution and 1 μL of reservoir solutions, against 50 μL of the reservoir solutions, by the sitting drop method at 20°C. X-ray diffraction data were collected at 100 K using a PILATUS3 S6M detector system on PF-BL5A and PILATUS 2M-F detector system on AR-NE3A beam lines in KEK (Tsukuba, Japan). Diffraction data were processed using the program XDS, and the CCP4 program suite. The structures were determined by molecular replacement with the program MOLREP using the structure of *E.coli* AlsB/D-allose (PDB code: 1RPJ) as a probe model. Further model-building was performed with the program Coot, and the structure was refined using the program Refmac5.

3 Results and Discussion

EtcAlsB has two domains (D1 and D2) to form a deep cleft for the ligand-binding, and the cleft is closed through the binding of D-allose. The loops linking two domains act as a hinge for conformational change with a rotation angle of 26° (Fig. 1A, B). The bound D-allose is recognized by many amino acid residues (Fig. 1C). However, through the binding of D-ribose, D1 rotates by 26° relative to D2 around a hinge region, but Lys32 and Glu65, which are important to recognize D-allose, are still far from D-ribose. Thus, this state with the bound D-ribose is thought to be an intermediate form (Fig. 1D).

These results demonstrated that EtcAlsB has a unique recognition mechanism for high affinity to D-allose by changing its conformation from an open to a closed form depending on D-allose-binding, and that the binding of D-ribose to EtcAlsB could not induce a completely closed form but an intermediate form, explaining the low affinity for D-ribose.

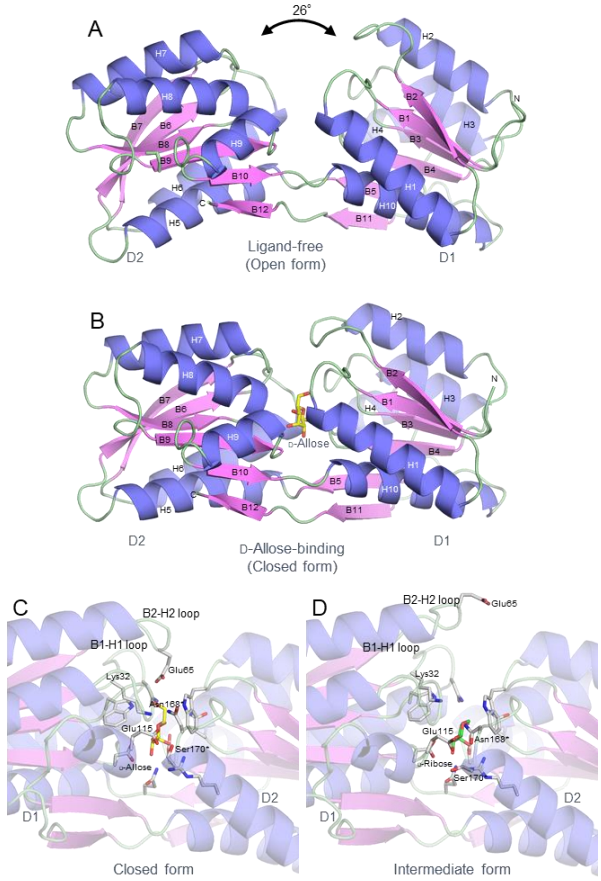

Fig. 1: Structures of EtcAlsB. (A) Overall structures of ligand-free EtcAlsB (open form) and (B) EtcAlsB/D-allose (closed form) are illustrated. (C) Structure of the closed form of EtcAlsB/D-allose and (D) structure of the intermediate form of EtcAlsB/D-ribose are illustrated.

Acknowledgement

I thank the PF staff for support of data collection.

References

[1] S. Kamitori, *Biochem. Biophys. Res. Commun.* **682**, 187-192 (2023).

* kamitori.shigehiro@kagawa-u.ac.jp