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X-ray structures of the *Pseudomonas cichorii* D-Tagatose 3-epimerase mutant C66S recognizing deoxy sugars as substrates

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1 Introduction

D-Tagatose 3-epimerase (DTE) catalyzes epimerization between D-tagatose and D-sorbose (Fig.1 (a)), and DTE family enzymes are widely found in various microorganisms. DTE from *Pseudomonas cichorii* (PcDTE) has a broad substrate specificity and catalyzes the epimerization of not only D-tagatose to D-sorbose, but also D-fructose to D-allulose that is one of rare sugars. Therefore, PcDTE is used to produce D-allulose from the more abundant sugar D-fructose. We have already determined the X-ray structures of PcDTE in complexes with D-tagatose and D-fructose, and proposed catalytic reaction mechanism via a cis-enediolate intermediate [1]. In these X-ray structures, the hydroxyl groups (O1 and O3) and ketone group (O2) of the substrate were important to be recognized by the enzyme with specific interactions, while the interactions at the 4-, 5-, 6-positions of substrate seemed to be recognized weakly by the enzyme. In recent years, we found PcDTE can also recognize deoxy sugars as ligand, such as 1-deoxy D-, L-tagatose, 6-deoxy L-allulose, and 1-deoxy 3-keto D-galactitol (D3G) (Fig.1(c)). The last ligand, D3G has no hydroxyl group at 1- and 3- positions and no ketone group at 2- position.

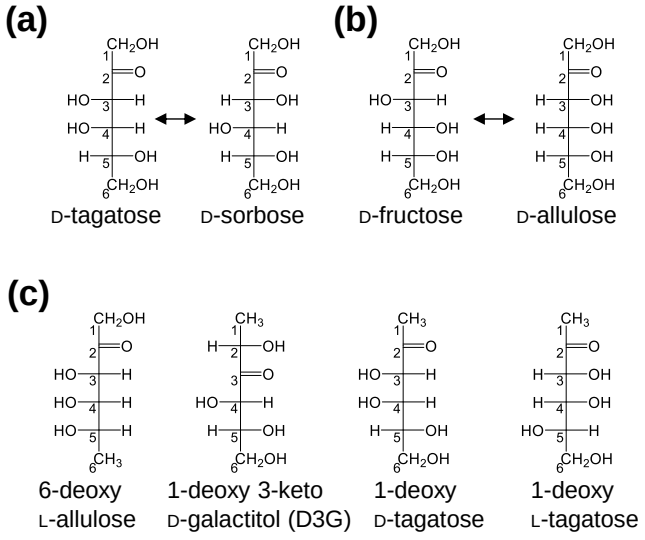

Fig. 1: Chemical reaction catalyzed by PcDTE and ligand molecules. (a, b) The epimerization reaction of substrate. (c) The chemical structure of deoxy sugars.

In this study, we report the X-ray structure of PcDTE mutant Cys66Ser (PcDTE C66S) in complexes with D3G, 1-deoxy D-tagatose, and 1-deoxy L-tagatose, and show the unique recognition mode of PcDTE to deoxy sugars. Since crystals of PcDTE C66S diffracted to a higher resolution than wild-type PcDTE crystals without significant differences in their structures, we used the mutant enzyme.

2 Experiment

PcDTE C66S was expressed as His-tagged protein and purified by affinity chromatography [2]. The purified protein solution was concentrated to 6 - 7 mg/ml for crystallization. Crystals were obtained in a droplet containing 2.0 μ L of protein solution (6 - 7 mg/mL in 5 mM of Tris-HCl, pH 8.0) and 2.0 μL of reservoir solution 6.0 – 11.0 % (w/v) PEG 4000 and 100 mM CH_3COONa, pH 4.6) against 450 μ L of the reservoir solution by the hanging-drop method at 293 K.

In addition, a crystal was grown in microgravity by utilizing the crystallization facilities of the JAPAN Aerospace Exploration Agency (JAXA) on board the Japanese Experiment Module Kibo in the International Space Station (ISS) under a high-quality protein crystal growth project (JAXA-PCG). In order to obtain crystals grown in a microgravity environment in the ISS, crystallization was established using a counter-diffusion method in solution containing 15 % (w/v) PEG 4000 and 100 mM CH_3COONa, pH 4.6.

For obtaining a ligand-complexed structure, a single crystal was soaked in 25 – 50 % (w/v) ligand molecules in reservoir solution. X-ray diffraction data were collected on the PF BL-5A PF-AR NW12A, NE3A, and a Rigaku R-AXIS VII imaging system on a Rigaku RA-Micro7HF rotating anode (CuKα) X-ray generator with ValiMax optics (40 kV, 30 mA). Diffraction data were processed using the programs HKL2000, the CrystalClear system and CCP4 program suite.

Initial phase determination of PcDTE C66S was performed by molecular replacement using the structure of PcDTE (PDB ID:2QUL) as a probe model.

3 Results and Discussion

The overall structure of PcDTE C66S was equivalent to the previously reported structure of wild-type PcDTE.

(1) 1-Deoxy D-tagatose and 1-deoxy L-tagatose

The electron density of the bound 1-deoxy D-tagatose at the catalytic site indicated that the bound substrate adopts two alternative conformations, Form A and Form B, as shown in Fig. 2(a, b). In Form A, O2 and O3 coordinate to the metal ion, and O5 and O6 interact with Ser66 directly or via a water molecule. In Form B, the direction of the bound substrate molecule is opposite to that of Form A, in which O6 interacts with Glu158, His188, and Arg217. O5 and O4 coordinate to the metal ion. O2 forms a water-mediated hydrogen bond with Ser66 (Cys66 in wild-type PcDTE). The ketone group of the substrate is required to coordinate to the metal ion for the epimerization reaction. The binding mode of Form A is suitable for the epimerization reaction, while that of Form B cannot lead to the epimerization reaction, which is assumed to be the inhibitor-binding mode. The molecule of 1-deoxy L-tagatose is found to bind to the catalytic site in the inhibitor-binding mode (Fig. 2(c)), as found in Form B of 1-deoxy D-tagatose in which O6 interacts with Glu158, His188, and Arg217, O5 and O4 coordinate to the metal ion, and O2 forms a hydrogen bond with Glu152.

(2) 1-Deoxy 3-keto-D-galactitol (D3G)

The catalytic site structure with the bound 1-deoxy 3-keto-D-galactitol (D3G) is shown in Fig. 2(d). O3 (ketone group) and O4 (hydroxyl group) of D3G coordinate to the metal ion, forming hydrogen bonds with His188 and Glu152. Glu246 directs its OE2 atom to the hydrogen atom attached to C4 with a distance of 2.3 Å. O2 forms hydrogen bonds with Glu158, His188, and Arg217. C1 (methyl group) is located in the hydrophobic pocket formed by Trp113, Phe248, and Val259. O5 does not form a hydrogen bond with the enzyme, and O6 interacts with Ser66 by a water-mediated hydrogen bond. This structure shows that the epimerization reaction occurs at the 4-position of D3G to give 1-deoxy 3-keto D-allitol as a product. The positon of a ketone group coordinating to the metal ion (O3 of D3G or O2 of D-tagatose) is strictly fixed by interactions between the hydroxyl group (O2 of D3G or O1 of D-tagatose) and enzyme.

PcDTE catalyzes the epimerization of ketohexoses at the 3-position adjacent to the ketone group at the 2-position (Fig. 1 (a, b)). The ketone group (O2) and hydroxyl group at the 3-position (O3) are required to coordinate to the metal ion at the catalytic site of the enzyme for the epimerization reaction, and this correct metal coordination by the substrate is performed by the recognition of a hydroxyl group at the 1-position (O1) with Glu158, His188, and Arg217. Thus, PcDTE strictly recognizes the substrate at the 1-, 2-, and 3-positions. On the other hand, PcDTE loosely recognizes the substrate at the 4-, 5-, and 6-positions with a few specific interactions between the substrate and enzyme. The substrate recognition at the 1-, 2-, and 3-positions is responsible for enzymatic activity, and substrate-enzyme interactions at the 4-, 5-, and 6-positions are not essential for the catalytic reaction of the enzyme; they merely adjust substrate to the proper orientation. This may be the reason why PcDTE efficiently catalyzes the epimerization of various ketoses including deoxy sugars as substrates.

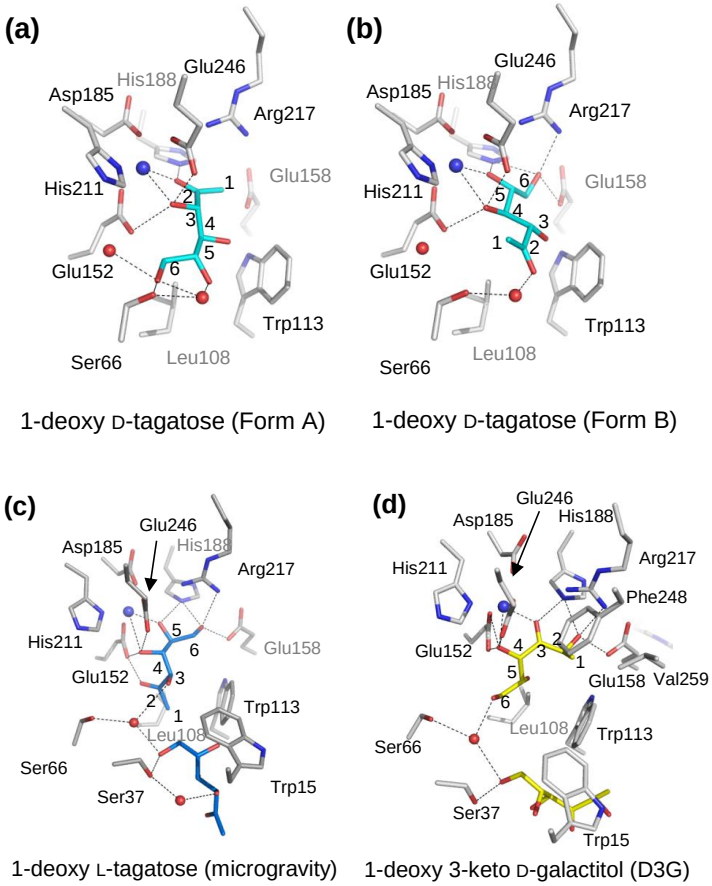

Fig. 2: Structure of the catalytic site of PcDTE C66S.

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