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X-ray structure of *Bacillus pallidus* D-arabinose isomerase

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Introduction

D-Arabinose isomerase (D-AI) catalyzes the isomerization of D-arabinose (aldose) to D-ribulose (ketose). It is able to catalyze the isomerization of L-fucose to L-fuculose and also known as L-fucose isomerase (L-FI). *Bacillus pallidus* (*B. pallidus*) D-AI can catalyze the isomerization of D-psicose to D-altrose, as well as D-arabinose to D-ribulose and L-fucose to L-fuculose. D-psicose and D-altrose are so-called "rare sugars" which exist in small amounts in nature. Since rare sugars have various physiological functions, they have received much attention in the food, agricultural and medicinal industries. *B. pallidus* D-AI is expected to be used for rare sugar production. We have reported the X-ray structures of *B. pallidus* D-AI and its complex with an inhibitor, L-fucitol [1,2].

Materials and methods

A crystal of the complex of *B. pallidus* D-AI/L-fucitol was prepared by soaking in 3.0 μl of a reservoir solution containing 2.0 M L-fucitol for 48 h. X-ray diffraction data were collected on the NW-12A beam line in the Photon Factory with the wavelength of 1.0 Å. The data were processed using the programs HKL2000 and the CCP4 program suite. The initial phases were determined by a molecular replacement method with the program MOLREP in the CCP4 program suite, using the structure of *E. coli* L-FI (PDB code 1FUI) as a model probe. Further modeling was performed with the programs Coot in the CCP4 program suite, and X-fit in the XtalView program system, and the structure was refined using the programs Refmac5 and CNS.

Results and discussion

The structure of the subunit of *B. pallidus* D-AI including 23β-strands and 22α-helices can be divided into three domains. In a crystal, *B. pallidus* D-AI forms a homo-hexamer with a triangular prismatic shape as shown in Figure 1.

The structure of the active site with the bound L-fucitol is shown in Figure 2. Glu342(OE1), Asp366(OD1), Asp366(OD2), and O2 of L-fucitol coordinate Mn^{2+} with a planar structure, and O1 of L-fucitol coordinates the axial position. The distance between Glu342(OE2) and C2 of L-fucitol is 3.3 Å and H2 is located between them. On the opposite side, Asp366 forms a hydrogen bond with O1 and O2. This structure strongly supports the ene-diol mechanism, because Glu342 and Asp366 could be proposed to transfer a proton from C2 to C1 and O1 to O2, acting as acid/base catalysts.

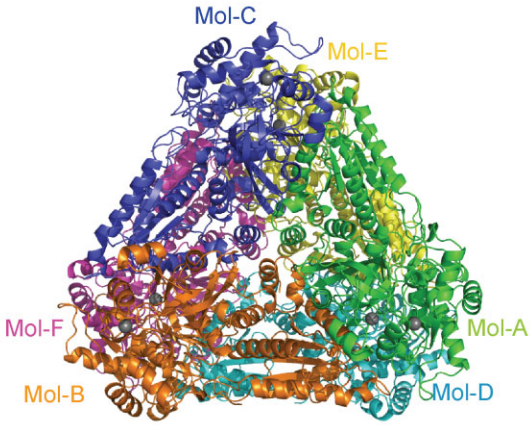

Figure 1. Overall structure of a hexamer of *B. pallidus* D-AI, viewed from two directions. The six molecules are colored in green (Mol-A, orange (Mol-B), blue (Mol-C), cyan (Mol-D), yellow (Mol-E) and magenta (Mol-F).

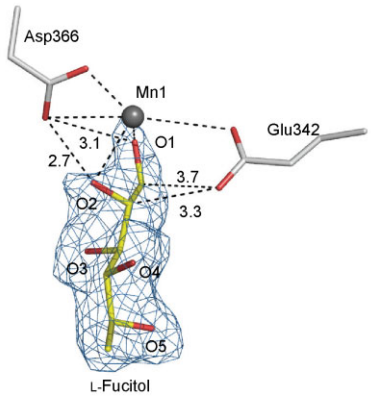

Figure 2. The structure of the active site of *B. pallidus* D-AI in a complex with L-fucitol. Simulated annealing omit maps of the bound ligand are shown at the 4.0 σ contour level. Selected interactions among amino acid residues, substrates, metal ions are indicated by dotted lines.

References

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