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Crystal structures of *Thermoplasma acidophilum* D-aldohexose dehydrogenase

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Introduction

The aldohexose dehydrogenase from the thermoacidophilic archaeon *Thermoplasma acidophilum* (AldT) is an enzyme that belongs to the short-chain dehydrogenase/reductase (SDR) superfamily, and catalyzes the oxidation of C1 hydroxyl of various monosaccharides such as D-mannose and D-xylose, with a preference of NAD^+ rather than $NADP^+$ as a cofactor. Most of the functionally related enzymes in the SDR superfamily are found in *Bacillus* species and display the highest activity for D-glucose. In contrast, AldT exhibits very low activity for D-glucose but the highest activity against D-mannose [1]. To date, AldT is the only enzyme known to display an efficient NAD^+-dependent dehydration activity against D-mannose. Although several crystal structures of the SDR family enzymes were determined, no structural information regarding the monosaccharide-recognition mechanism is available. It is of interest to investigate how these enzymes discriminate between various monosaccharides, particularly D-glucose and its C2 epimer D-mannose. Here we report the crystal structures of ligand-free, cofactor complex, and substrate complex of AldT.

Methods

The recombinant AldT was overexpressed by *Escherichia coli*, and purified by Ni affinity chromatography [1]. The crystals of AldT were obtained by the hanging-drop vapor-diffusion method at 20°C. All crystals used for this study were grown using reservoir solution containing 0.1 M Na acetate pH 4.2-5.2, 0.2 M ammonium sulfate, 14-18% PEG3350, 15-20% glycerol. The crystals belong to the space group $P3_221$ with unit-cell dimensions of $a = b = 82$ and $c = 139$ Å. The structures of AldT were determined by the molecular replacement method using *Bacillus megaterium* glucose dehydrogenase (BmGlcDH) as a search model (PDB code, 1GCO [2]). The model refinement was performed using the program CNS 1.1, and the model was fitted manually using the XtalView/Xfit. Atomic coordinates and structure factors have been deposited in Protein Data Bank under accession number 2DTD, 2DTE, and DTX. The detail experimental procedures were described elsewhere [3].

Results and Discussion

The crystal structures of AldT in ligand-free form, in complex with NADH and in complex with D-mannose have been determined to a resolution of 2.1 Å, 1.65 Å and 1.6 Å, respectively. The AldT forms a typical fold of the SDR enzymes, comprising nucleotide-binding Rossmann fold motif, and assembles tightly into tetramer having a 222 point-group symmetry (Fig. 1). Thanks to a high-resolution structure analyses (~1.6 Å), all interatomic interactions between enzyme and substrate or cofactor are identified. The D-mannose complex structure clearly showed that one glutamate residue (Glu84) interacts with the C2 hydroxyl group of the bound D-mannose. Structural comparison with BmGlcDH suggests that the side chain conformation of the glutamate residue is crucial for discrimination between D-mannose and its C2 epimer D-glucose. The structure of AldT also showed that C-terminal tail shuts the substrate-binding pocket of the neighboring subunit and preclude the access of substrate to the active site. The elaborate inter-subunit interactions between C-terminal tail and the entrance of substrate-binding pocket of the neighboring subunit indicate that the tail may play a pivotal role in enzymatic function of AldT.

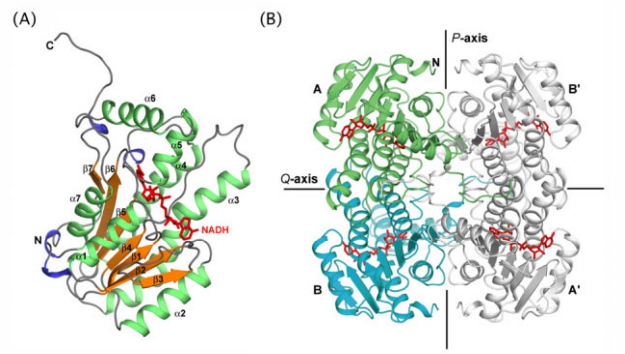

Fig.1 Ribbon representation of AldT structure. (A) Subunit structure. (B) Tetramer structure. Two subunits in the asymmetric unit is colored in green and blue. The bound NADH is colored in red.

References

[1] Y. Nishiya et al., Biosci. Biotechnol. Biochem. **68**, 2451-2456 (2004).
[2] K. Yamamoto et al., J. Biochem. 129, 303-312 (2001).
[3] Y. Yasutake et al., Acta Cryst. F**62**, 586-589 (2006).
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