Biological Science

17A/2006G165

Mutational and crystallographic analyses of *Thermoplasma acidophilum* D-aldohexose dehydrogenase C-teminal deletion mutants

Taiki NISHIOKA[1], Yoshiaki YASUTAKE[2*], Yoshiaki NISHIYA, Noriko TAMURA[1], and Tomohiro TAMURA[1,2,]

[1]Graduate School of Agriculture, Hokkaido University, N9W9, Kita-ku, Sapporo 060-8589, Japan
[2]Bioproduction research institute, National Institute of Advanced Industrial Science and Technology (AIST), 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan
[3]Tsuruga Institute of Biotechnology, Toyobo Co., Ltd., 10-24, Toyo-cho, Tsuruga 914-0047, Japan

Introduction

The D-aldohexose dehydrogenase from the thermoacidophilic archaeon *Thermoplasma acidophilum* (AldT) is a homotetrameric enzyme that catalyzes the oxidation of several D-aldohexoses, especially D-mannose [1]. AldT is a unique enzyme that effectively acts on D-mannose rather than D-glucose. Crystal structures of AldT have been determined with and without substrate and have shown the sugar recognition mechanism [2]. In addition, the structure also revealed that AldT possesses a unique C-terminal tail motif (residues 247-255), which is located at the entrance of the substrate-binding pocket of the neighboring subunit. The functional role of the C-terminal tail of AldT has been investigated using mutational and crystallographic analyses. A total of four C-terminal deletion mutants Δ254 (1-254), Δ253 (1-253), Δ252 (1-252), and Δ249 (1-249)) and two site-specific mutants (Y86G and P254G) were constructed and characterized. The crystal structure of Δ249 was also solved to investigate the structural differences by comparing with the structure of wild-type AldT. Here we report the X-ray structure of C-terminal deletion mutant Δ249 in substrate-free form at 2.7 Å resolution.

Methods

The AldT Δ249 was overexpressed by *Escherichia coli*, and purified by Ni-affinity chromatography [1]. The crystals of Δ249 were obtained by the hanging-drop vapor-diffusion method at 20°C, using reservoir solution containing 0.1 M imidazole pH 8.0, 0.8 M Na^+/K^+ tartrate, and 0.1 M NaCl. The crystal belongs to the hexagonal system $P6_122$ with unit-cell dimensions $a = b = 68.2$ Å and $c = 337.5$ Å. The structure of Δ249 was solved by the molecular replacement using wild-type AldT as a search model. The model refinement was performed using the program REFMAC5. Atomic coordinates and structure factors have been deposited in Protein Data Bank under accession code 2ZK7. The detail experimental procedures were described elsewhere [3].

Results and discussion

The enzyme assay using C-terminal deletion mutants revealed that the lack of both Glu255 and Pro254 drastically decreases the catalytic activity. The structure of the wild-type AldT indicated the van der waals interaction occurs between Pro254 and the Tyr86 side chain at a distance of ~4.0 Å. To investigate the importance of the interaction, two mutants P254G and Y86G were constructed and characterized. The activities of these mutants were also drastically decreased as well as the results of C-terminal deletion mutants. The results suggested that the hydrophobic interaction between Tyr86 and Pro254 is critical for enzyme activity. The Δ249 did not show catalytic activity. To elucidate why the C-terminal tail deletion mutant becomes inactive, crystallographic studies of the Δ249 was performed. The crystal structure of Δ249 was determined at 2.7 Å resolution. The structure showed that the active-site loops undergo a significant conformational change, which leads to the structural deformation of the substrate-binding pocket. It is concluded that the conformation of the loops observed in the wild-type structure is altered by C-terminal deletion, which consequently distorts the active site structure and almost inactivates the enzyme.

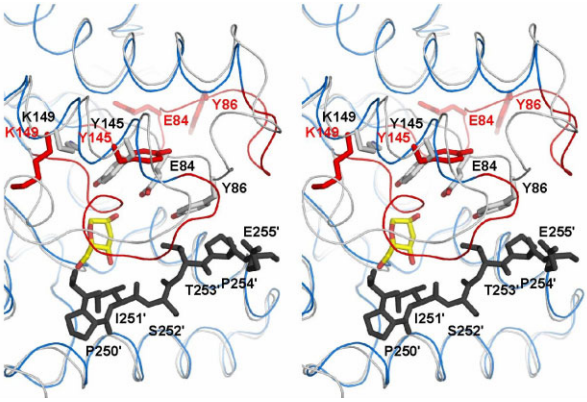

Fig. 1 Stereo view active-site structure of Δ249 and wild-type AldT. The model is colored in blue and red for Δ249 and in gray and dark gray for wild-type AldT

References

[1] Y. Nishiya *et al.*, *Biosci. Biotechnol. Biochem.* **68**, 2451-2456 (2004).
[2] Y. Yasutake *et al.*, *J. Mol. Biol.* **367**, 1034-1046 (2007).
[3] T. Nishioka *et al.*, *Proteins*, **74**, 801-807 (2009).

*y-yasutake@aist.go.jp