5A, 17A, NW12A, NE3A/ 2011G502, 2011G002

Crystal structure of UDP-galactose 4-epimerase-like L-threonine dehydrogenase

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Introduction

L-Threonine dehydrogenase (L-ThrDH) catalyzes NAD^+-dependent dehydrogenation at the β-carbon (C3) position of L-threonine. The primary structure of L-ThrDH was initially determined using the enzyme from *Escherichia coli*; it revealed that the enzyme belongs to the medium-chain alcohol dehydrogenase family. Recently, a novel L-ThrDH that shows no sequence similarity to the *E. coli* enzymes were identified in a psychrophilic bacterium *Flavobacterium frigidimaris* KUC-1. This enzyme exhibits notable sequence identity with UDP-galactose 4-epimerase (GalE) homologues which, belongs to the short-chain dehydrogenase-reductase family. We have previously solved the first three-dimensional structure of the GalE-like L-ThrDH from *F. frigidimaris* [1]. However, the catalytic mechanism of GalE-like L-ThrDH is unknown. To clarify the structural basis of the catalytic mechanism, we solved the four crystal structures of archaeal GalE-like L-ThrDH in the presence of NAD^+, a pyruvate (inhibitor) and two substrates were determined. This is the first description of the molecular basis for the substrate recognition of a GalE-like L-ThrDH.

Materials and Methods

Single-wavelength (1.0 Å) data for *Thermoplasma volcanium* L-ThrDH-NAD^+, L-ThrDH in complex with NAD^+ and pyruvate, Y137F inactive mutant in complex with NAD^+ and L-threonine, and Y137F in complex with NAD^+ and L-3-hydroxynorvaline were collected on the beamline 5A, 17A, NW12A and NE3A at the Photon Factory. The data were processed using HKL2000 and the CCP4 program suite.

Results and Discussion

Given the structure of pyruvate, we sought to predict the structure of the L-threonine molecule within the active site of *T. volcanium* L-ThrDH (Fig. 1A) [2]. Based on the structure of pyruvate, we tried the modeling of L-threonine binding into the active site of *T. volcanium* L-ThrDH. However, we could not postulate a single binding mode of L-threonine molecule. On the other hand, using the inactive *T. volcanium* L-ThrDH Y137F mutant, which trapped the substrate within the active site, we were able to determine the substrate-bound structures of the enzyme in complex with L-threonine or L-3-hydroxynorvaline (Fig. 1B, 1C).

Within the structures of the *T. volcanium* L-ThrDH Y137F/NAD^+/L-threonine and L-3-hydroxynorvaline, we observed that the Oη of Tyr^{137}, lies within hydrogen-bonding distance (2.4-2.6 Å) of the β-hydroxyl group of the L-threonine or L-3-hydroxynorvaline (Fig. 1D). This means that Tyr^{137} could serve directly as the active-site base. Taken together, this finding suggest the catalytic mechanism underlying the *T. volcanium* L-ThrDH reaction likely proceed through the following steps: 1) abstraction of the β-hydroxyl hydrogen of L-threonine via direct proton transfer driven by Tyr^{137}; and 2) transfer of a hydrogen from the β-carbon (C3) of the L-threonine to C4 of the NAD^+ (*si*-face), forming L-2-amino-3-oxobutyrate and NADH (Fig. 2).

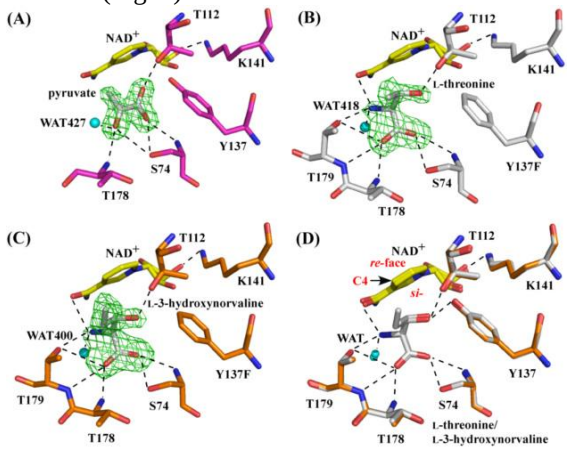

Figure1 *A*, The NAD^+/pyruvate-bound wild-type enzyme (*magenta*). *B*, The NAD^+/L-threonine-bound Y137F mutant enzyme (*white*). *C*, The NAD^+/L-3-hydroxynorvaline-bound Y137F mutant enzyme (*gold*). *D*, Superposition of the active site pocket of the NAD^+/L-threonine-bound Y137F mutant with that of the NAD^+/L-3-hydroxynorvaline-bound Y137F mutant.

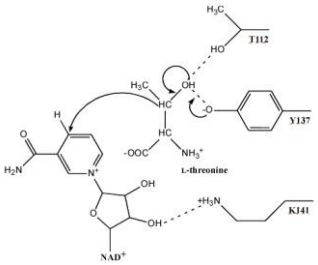

Figure 2 Proposed catalytic mechanism of *T. volcanium* L-ThrDH.

References

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