Crystal structure of L-threonine 3-dehydrogenase from plant disease fungus *Phytophthora infestans*

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1 Introduction

A UDP-galactose 4-epimerase-like L-threonine 3-dehydrogenase (GalE-like L-ThrDH) homologue was identified in the plant disease fungus *Phytophthora infestans* using KEGG (Kyoto Encyclopedia of Genes and Genomes) database as a candidate gene for the creation of a new fungicide. The L-ThrDH gene was expressed in *Escherichia coli*, and its product was purified and characterized. The enzyme inhibitors on the enzyme activity were searched and N-acetylglycine was found to act as a competitive inhibitor of L-threonine (*Ki* = 0.18 mM). In addition, inhibition assay against *P. infestans* growth by N-acetylglycine (antifungal activity) was examined using disk diffusion method. The crystal structure of the unique hexameric L-ThrDH was determined using the molecular replacement method at a resolution of 2.3 Å, in the presence of NAD⁺ and a substrate analogue, citrate. The enzyme structure of the active site based on the structures of citrate and N-acetylglycine molecules was modeled. Consequently, the binding mode and inhibition mechanism of N-acetylglycine were estimated. This is the first report regarding the characteristics and three-dimensional structure of plant disease fungus L-ThrDH.

2 Experiment

Single-wavelength (1.0 Å) data for *P. infestans* L-ThrDH was collected on the beamline BL-5A at the Photon Factory. The data were processed using HKL2000 and the CCP4 program suite.

3 Results and Discussion

The structure of *P. infestans* L-ThrDH-NAD⁺-citrate ternary complex was determined using molecular replacement and was refined at a resolution of 2.3 Å to a crystallographic R-factor of 0.238 and a free R-factor of 0.242 [1]. The asymmetric unit of the enzyme crystal consisted of one hexamer (Fig. 1). Unfortunately, the electron density map for the N-terminal 24 residues of each subunit was not observed and it could not be modeled. Recently, the crystal structures of L-ThrDH from *Tr. brucei* have been reported. The amino acid sequence alignment showed that N-terminal parts of *P. infestans* and *Tr. brucei* L-ThrDH sequences were longer compared with those of *T. volcanium* and *F. frigidimarsh*. However, the detailed role of the N-terminal amino acid sequences of both enzymes is still unclear because of no structural analysis. Further investigation is needed to make it clear. The main-chain coordinates of the L-ThrDH monomer are basically the same as those of thermophilic archaeon *T. volcanium* L-ThrDH (RMSD = 0.98 Å for the Ca atoms of 305 residues; 3A1N) [2], psychrophilic bacterium *F. frigidimarsh* L-ThrDH (RMSD = 1.10 Å for the Ca atoms of 303 residues; 2YY7) [3] and *Tr. brucei* L-ThrDH (RMSD = 1.06 Å for the Ca atoms of 306 residues; 5K50) monomers (Fig. 2). Multiple oligomeric structures, including monomeric and dimeric forms have been observed for L-ThrDHs from bacteria and archaea. The enzymes from *T. volcanium*, *F. frigidimarsh* and *Tr. brucei* adopt a dimeric structure, while L-ThrDH from an uncultured archaeon reportedly forms a monomeric structure. In this regard, *P. infestans* L-ThrDH (hexameric one) differs substantially from previously described GalE-like L-ThrDHs.

Figure 3A presents the growth inhibitory activity of N-acetylglycine against *P. infestans*. The antifungal activity was measured by the diameter of the *P. infestans*-free area (inhibition zone). As negative control, the thinner fungal colonies of the *P. infestans* were used for comparison. N-Acetylglycine was found to exhibit antifungal effect against *P. infestans* at a concentration of 10 mM (No. 3). In contrast, the antifungal activity was not observed with the L-cysteine (Fig. 3B). Thus, N-acetylglycine, an inhibitor of *P. infestans* GalE-like L-ThrDH, is expected to be a potential lead compound for creation of new high specificity agrochemicals against *P. infestans*.

Fig. 1: Overall structure of *P. infestans* GalE-like L-ThrDH (6JYG). Overall structure of *P. infestans* GalE-like L-ThrDH. The hexameric structure of *P. infestans* GalE-like L-ThrDH. The six subunits are shown in green, cyan, yellow, white, lime green, and pink, respectively.
Fig. 2: The α-carbon trace superposition of *P. infestans* (magenta), *T. volcanium* (green), *F. frigidimaris* (cyan) and *Tr. brucei* (orange) L-ThrDH monomers.

Fig. 3: In vitro assay for inhibition of *P. infestans* growth by *N*-acetylglycine. (A) Paper disks containing sterilized distilled-water (No. 1; negative control), antibiotic G418 (No. 2; positive control), and *N*-acetylglycine (No. 3; 10 mM and No. 4; 1 mM) dissolved in sterilized distilled-water. (B) Paper disks containing sterilized distilled-water (No. 1; negative control), antibiotic G418 (No. 2; positive control), and L-cysteine (No. 3; 10 mM and No. 4; 1 mM) dissolved in sterilized distilled-water.

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**References**


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