BL-5A/2020G502, 2019G015

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

Kazunari Yoneda*[1], Rina Nagano[1], Haruhiko Sakuraba[2], Toshihisa Ohshima[3]
[1]Department of Bioscience, School of Agriculture, Tokai University, Kumamoto, 862-8652, Japan
[2]Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-cho, Kita-gun, Kagawa761-0795, Japan
[3] Department of Biomedical Engineering, Osaka Institute of Technology, Osaka, Japan

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. frigidimaris*. 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 Cα atoms of 305 residues; 3A1N) [2], psychrophilic bacterium *F. frigidimaris* L-ThrDH (RMSD = 1.10 Å for the Cα atoms of 303 residues; 2YY7) [3] and *Tr. brucei* L-ThrDH (RMSD = 1.06 Å for the Cα 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. frigidimaris* 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*.

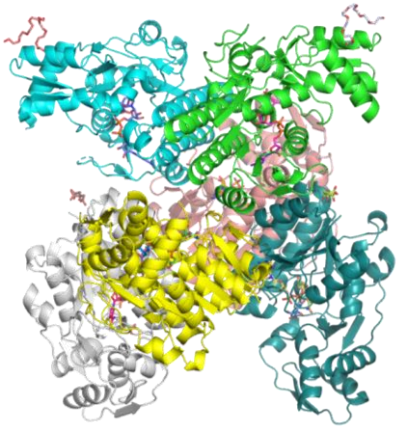

Fig. 1: Overall structure of *P. infestans* GalE-like L-ThrDH (6JYG). Overall structure of *P. infestans* GalE-like L-ThrDH. The hexameric structure of *P. infestans* GalE-like L-ThrDH. The six subunits are shown in green, cyan, yellow, white, lime green, and pink, respectively.

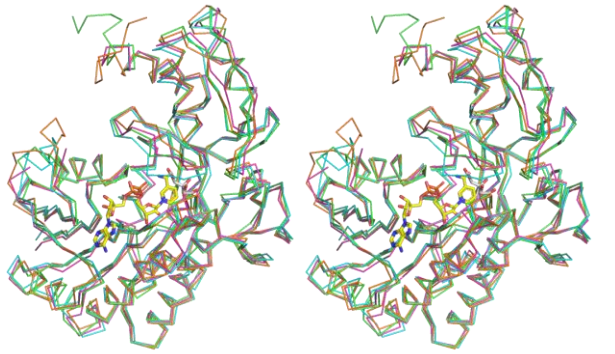

Fig. 2: The α-carbon trace superposition of *P. infestans* (magenta), *T. volcanium* (green), *F. frigidimaris* (cyan) and *Tr. brucei* (orange) L-ThrDH monomers.

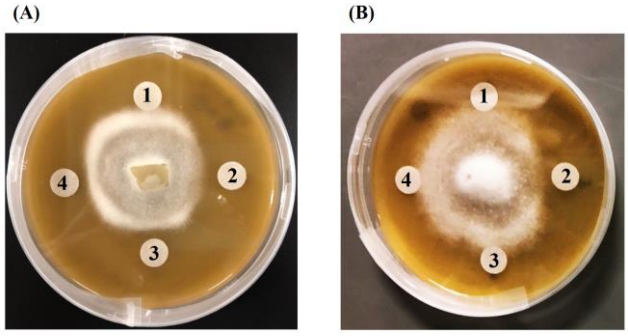

Fig. 3: In vitro assay for inhibition of *P. infestans* growth by *N*-acetylglycine. (A) Paper disks containing sterilized distilled-water (No. 1; negative control), antibiotic G418 (No. 2; positive control), and *N*-acetylglycine (No. 3; 10 mM and No. 4; 1 mM) dissolved in sterilized distilled-water.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

Acknowledgement

We thank members of the Photon Factory Synchrotron Radiation Facility (KEK-PF, in Tsukuba, Japan) for their assistance during X-ray data collection. This work was supported in part by a Grant-in-Aid for Young Scientists (B, No. 16K18689) of JSPS KAKENHI (to K.Y.) and a grant from the Tokai University Educational System (to K.Y.).

References

[1] K. Yoneda *et al*., Enzyme Microb Technol. **140** (2020) 109627.
[2] K. Yoneda *et al*., J. Biol. Chem. **287** (2012) 12966-12974.
[3] K. Yoneda *et al*., FEBS J. **277** (2010) 5124-5132.

* kyoneda@agri.u-tokai.ac.jp