ABSTRACTS (MASTER THESIS)

X-ray crystal structure analysis of *cis*-hinokiresinol synthase β subunit

(Division of Applied Life Sciences, Graduate School of Agriculture, Laboratory of Metabolic Science of Forest Plants and Microorganisms, RISH, Kyoto University)

Atsushi Azuma

Abstract

Norlignans are a natural phenolic compound with diphenylpentane carbon skeleton (C_6-C_5-C_6) and are found mainly in conifers and monocotyledons[1]. Norlignans and related compounds have various biological activities such as antifungal and antiprotozoal activities[2]. Moreover, norlignans are accumulated abundantly in the heartwood region of woody plants and known as substances attributed to heartwood coloration of *Cryptomeria japonica* and *Chamaecyparis obtusa*[2]. Hinokiresinols have the simplest structure among norlignans and *cis/trans*-isomers which are found in monocots/dicots and conifers, respectively[3, 4]. Both hinokiresinols have antifungal activity as phytoalexins to be produced in response to stresses[3]. The biosynthetic pathway of hinokiresinols and the existence of *cis*/*trans*-hinokiresinol synthases have been revealed by using *Asparagus officinalis* and *C. japonica* cells[5, 6]. In the biosynthetic pathway of *cis*/*trans*-hinokiresinols, it is demonstrated that *p*-coumaryl *p*-coumarate is catalyzed by *cis*-hinokiresinol synthase to form *cis*-hinokiresinol in *A. officinalis* and *trans*-hinokiresinol synthase to produce *trans*-hinokiresinol in *C. japonica* without any additional cofactors[5,6]. Previously, (*Z*)-hinokiresinol synthase, produced in *A. officinalis*, was found to be composed of two distinct subunits, HRSα and HRSβ. Interestingly, recombinant HRSα or HRSβ can catalyze the formation of (*E*)-hinokiresinol while the mixture of both recombinant subunits produces (*Z*)-hinokiresinol with >99% (+)-enantiomer excess[7]. To clarify these unique molecular mechanisms, elucidation of the structures of two subunits with X-ray crystallographic analysis is essential.

In this study, recombinant HRSβ expressed in *E. coli* BL21(DE3)pLysS was purified by IMAC and anionic exchanging chromatography, and crystalized. The diffraction data on the crystals were collected by CCD detector in SPring-8, and single-wavelength anomalous dispersion analysis with the heavy-atom replacement was performed to determine the structure of HRSβ. Calculating the figure of merit and the structure refinement were performed using the PHENIX, and the initial model was built by the program Coot. In addition, the crystal structures of HRSβ complexes with substrate analogs were compared with apo-HRSβ structure.

As a result, the crystal structure of recombinant HRSβ was solved at 2.6 Å by the single-wavelength anomalous dispersion, and the final model was refined at 2.2 Å for the first time. In addition, the crystal structures of complexes of HRSβ with substrate analogs were refined.

Acknowledgements

The author would like to thank Professor Dr. Bunzo Mikami, Graduate School of Agriculture, Kyoto University, for his warm supports. A part of this study was conducted using the facilities in the DASH/FBAS at RISH, Kyoto University.

References

[1] Suzuki S *et al*., *J Wood Sci* 53: 273-284 (2007).
[2] Suzuki S *et al*., *J Chem Soc, Perkin trans* 1, 3252-3257 (2001).
[3] Suzuki S *et al*., *Proc Natl Acad Sci USA* 104:21008-2113 (2007).
[4] Song MC *et al*., *Arch Pharm Res* 30:1392-1397 (2007).
[5] Suzuki S *et al*., *Chem Commun* 1088-1089 (2002).
[6] Suzuki S *et al*., *Chem Commum* 2838-2839 (2004).
[7] Yamamura M *et al*., *Org Biomol Chem* 8:1106-1100 (2010).