ABSTRACTS (MASTER THESIS)

Characterization of *O*-methyltransferases involved in antitumor lignan biosynthesis in *Anthriscus sylvestris*

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Lignans are typical plant secondary metabolites and distribute widely in vascular plants. Lignans are phenylpropanoid dimers that are linked at C8 position of their propyl side chain. Various lignans are known for having various biological effects, e.g., antitumor, antioxidative, and antiviral effects. Podophyllotoxin is known as an antitumor lignan and used as a precursor for the chemical synthesis of the anticancer drugs such as etoposide, teniposide, and etopophos. Podophyllotoxin for commercial use is mainly isolated from *Podophyllum hexandrum*. However, the overexploitation of the podophyllotoxin producing engendered plant species necessitates an alternative way to stably supply the lignan, that is, a biological production system of podophyllotoxin. To establish the biological production system, it is necessary to elucidate the genes involved in podophyllotoxin biosynthesis. Previous studies of podophyllotoxin biosynthesis used several plant species and some biosynthesis pathways were proposed [1]. There are several *O*-methylation steps in the podophyllotoxin biosynthetic pathways, while eight *O*-methyltransferases (OMTs) that are involved in *O*-methylation of various lignans have so far been identified from several plants [1-4]. Phylogenetic tree analysis using these lignan OMTs and plant OMTs (Pl-OMT II families) indicated that the lignan OMTs was widely spread in various clades, not grouped into a small clade, suggesting that the lignan OMTs were evolved by a convergent evolution [1]. Recently, the research group of the author found that four amino-acid residues are conserved among lignan OMTs by an evolutionary trace method, though homologies of whole amino acid sequences of the lignan OMTs was not high. However, effects of these amino-acid residues on lignan OMT activity have not been examined.

In this study, four mutant proteins of 5-*O*-methylthujaplicatin *O*-methyltransferase (5MTJOMT), where the four conserved amino acid residues were mutated individually, were prepared with single amino-acid residue substitution (K33H, K143R, A199S, and L318C) and subjected to the 5MTJOMT activity assay to examine whether each amino-acid residue affects lignan OMT activity or not. All mutant proteins showed 5MTJOMT activity, while their specific activities significantly differed among all mutant proteins and original 5MTJOMT (WT): 5MTJOMT specific activity was 1.58 ± 0.02 nmol/μg in WT, 0.93 ± 0.05 nmol/μg in K33H, 1.70 ± 0.11 nmol/μg in K143R, 0.09 ± 0.01 nmol/μg in A199S, and 0.36 ± 0.03 nmol/μg in L318C, respectively. Compared with the specific activity of WT (100%), those of K33H and K143R were slightly lower (59.1%) or almost same (107.5%), respectively. Interestingly, the specific activities of A199S and L318C significantly decreased to 5.6% and 23.0%, respectively, suggesting that amino-acid residues, A199 and L318, might contribute to an expression of lignan OMT activity.

References

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