論文要約

Characterization of *O*-methyltransferases involved in lignan biosynthesis
（リグナン生合成に関与する *O*-メチルトランスフェラーゼの特性解明）

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Lignans are a group of phenolic compounds where two phenylpropanoid units are coupled by the central carbon (C8) of their side chains. These compounds have been gaining much interest due to their important biological activities, roles in plant metabolism, and unique stereochemical mechanisms for their biosynthesis. Albeit several reports on cDNAs encoding enzymes involved in lignan biosynthesis, little is known about *O*-methyltransferases (OMTs) in lignan biosynthesis (lignan OMTs). In addition, nothing has been known about enzymes involved in the biosynthesis of phenylpropanoid monomers which are dedicated to the biosynthesis of lignans, but not lignins. Furthermore, not much is known about the enantiomeric control of lignan OMTs. In the present study, the author isolated cDNAs that encode lignan OMTs and characterized the recombinant proteins in relation to regio- and enantiomeric selectivities for the first time. The author also studied the role of cinnamyl alcohol dehydrogenases (CADs), which are possibly involved in lignan biosynthesis.

First, a *Carthamus tinctorius* (safflower) cDNA encoding an OMT methylating a lignan was characterized for the first time. Its recombinant OMT catalyzed regioselective methylation of matairesinol to give rise to an antitumor lignan, arctigenin (4′-*O*-methylmatairesinol), and designated as *C. tinctorius* matairesinol OMT (CtMROMT). Gene expression analysis and biochemical characterization of the OMT indicated that CtMROMT is responsible for arctigenin biosynthesis in *C. tinctorius*. In addition, two other cDNAs encoding matairesinol-methylating OMT (MROMT), each from *Anthriscus sylvestris* (cow parsley) and *Forsythia koreana* (Korean forsythia), were obtained, which were designated as AsMROMT and FkMROMT, respectively. AsMROMT showed the same regioselectivity of matairesinol methylation as CtMROMT, whereas FkMROMT gave rise to only isoarctigenin, exhibiting the regioselectivity opposite to those of Ct and AsMROMTs.

Next, enantiomeric control of the three MROMTs was examined; all MROMTs exhibited strict enantioselectivity, giving rise to only (−)-enantiomers of their products. This is in sharp contrast to the previously proposed mechanism for matairesinol methylation by a *Forsythia intermedia* (golden bell) crude OMT preparation, which showed neither strict regioselectivity nor strict enantiomeric selectivity.

Third, a cDNA responsible for the first *O*-methylation step in the biosynthesis of podophyllotoxin (another antitumor lignan) from matairesinol was isolated from *A. sylvestris*. The recombinant OMT catalyzed regioselective *O*-methylation of thujaplicatin to afford 5-*O*-methylthujaplicatin. Based on the gene-expression and biochemical analyses, it was concluded that this is the first example of a cDNA encoding an enzyme in the podophyllotoxin biosynthetic pathway from matairesinol, and was designated as *A. sylvestris* thujaplicatin OMT (AsTJOMT).

In addition, characterization of CAD-encoding cDNAs was also conducted. CADs are responsible for the last reductive step in monolignol biosynthesis, which are precursors to lignin and lignans. Three *C. tinctorius* CADs were isolated and designated as *CtCAD1*, *CtCAD2* and *CtCAD3*. Gene expression analysis and biochemical characterization of plant and recombinant CADs suggested that CtCAD2 and CtCAD3 were involved in lignin and lignan biosynthesis, while CtCAD1 is most likely to be involved in plant defense mechanism.