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Amino Acid Sequence Homology of Hybrid Poplar O-methyltransferase Involved in Lignin Biosynthesis

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Abstract

In λ-Zap II vector system, a cDNA library was constructed for the developing secondary xylem mRNA from hybrid poplar, *Populus nigra* × *maximowiczii*. A cDNA clone of 1.5 kb in size, pOMTB1.4 encoding a lignin-bispecific O-methyltransferase was screened by plaque hybridization using a probe of 540 bp cDNA amplified by polymerase chain reaction from the cDNA library and identified by nucleotide sequencing. Its nucleotide sequence contains one open reading frame of 366 amino acids. The deduced amino acid sequence in comparison with that of *Populus tremuloides* showed the differences of 9 amino acids and revealed 85-99% homology among alfalfa, poplar and aspen.

Introduction

Lignins, aromatic heteropolymer of vascular plant cell wall that provides mechanical support to the stem behaves as a water-impermeable seal for the xylem vessel and protects cellulose fibers from degradation in any means (Sarkenen, 1971). They represent 30% of the earth's woody biomass and are considered to have a crucial role in the adaptation of land plants to the terrestrial habitat. However, many aspects concerning their biosynthesis and its regulation have not yet been elucidated and it appears that the dehydrogenative polymerization of lignin monomer could be accomplished through a number of different enzymes. Lignins are three dimensional phenolic structures to be synthesized by the dehydrogenative, free-radical polymerization of monolignols (cinnamyl alcohols) formed via the phenylpropanoid pathway which produces intermediates required for synthesis of a wide range of aromatic compounds including flavonoids, phytoalexins, stilbenes, suberin and lignin (Higuchi, 1990). The first reaction of the phenylpropanoid pathway is the deamination of phenylalanine to cinnamic acid by phenylalanine ammonia-lyase. Subsequent hydroxylations, methylation, and reduction step lead to the synthesis of lignin monomers called monolignols such as 4-coumaryl, coniferyl, and sinapyl alcohols (Grisebach, 1981). Gymnosperms contain guaiacyl lignin produced from the polymerization of coniferyl alcohol, whereas angiosperm dicots contain guaiacyl-syringyl lignin formed by the copolymerization of coniferyl and sinapyl alcohols. However, angiosperm monocots synthesize 4-coumaryl alcohol present in small amounts in both guaiacyl and guaiacyl-syringyl lignin (Gowri et al., 1991). And also, the monomeric composition of lignin varies with the plant family, the developmental stage, the tissue, and the subcellular localization (Bugos, 1991). The monolignols like coniferyl and sinapyl alcohols have methyl groups transferred to the level of caffeic and 5-hydroxyferulic acids by S-adenosyl-L-Met: *o*-diphenol-O-methyltransferase, EC 2.1. 1.6 (OMT). Therefore, OMT plays an important role in the synthesis of lignin precursors by catalyzing the meta-specific methylation of caffeic acid and 5-hydrox-

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yferulic acid to ferulic acid and sinapic acid, respectively (Dumas et al., 1992). Recently, OMT cDNAs have been isolated from alfalfa (Gowri et al., 1991), *Populus tremuloides* (Bugos et al., 1991), *Populus deltoides* × *Populus trichocarpa* (Dumas et al., 1992), maize (Collazo et al., 1992), and tobacco (Jaeck et al., 1992; Pellegrini et al., 1993). And also, the cDNAs for cross-linking enzymes (peroxidases and laccases) have been reported (LaFayette et al., 1995; Kiefer-Meyer et al., 1996; Ranocha et al., 1999). More detailed studies of these enzymes in lignin-related tissues have been performing by many researchers. Here, we report an OMT cDNA from hybrid poplar, *Populus nigra* × *maximowiczii* and its amino acid sequence comparison with other OMT genes.

Materials and Methods

Plant materials

Plant materials were obtained from the nursery of Kyungpook Forest Environment Institute in Kyungju. Stems with 1.5 to 2 cm in diameter were collected and the bark was peeled to expose the developing secondary xylem. The layer of developing secondary xylem was scraped from the stem with a razor blade and immediately frozen in liquid nitrogen.

cDNA library construction

Total RNA was prepared from leaves or xylem of poplar tree by several successive extraction with the saturated phenol-chloroform (Draper et al., 1988) and then precipitated with 3 M NaOAc and ethanol. It was further purified to isolate poly(A)$^+$ RNA using oligo (dT)-cellulose chromatography (Sambrook et al., 1989). Poly(A)$^+$ RNA was used to synthesize an oligo (dT)-primed cDNA. Double-stranded cDNA was synthesized using cDNA synthesis kit (Stratagene) and ligated to *Eco*RI adaptor. cDNAs larger than 500 bp were ligated to λZapII vector and packaged using Gigapack *in vitro* packaging extracts (Stratagene).

Probe DNA preparation

To prepare the probe DNA for screening a full-length OMT cDNA, total cDNAs extracted from the library were used as a template. Oligonucleotide primers were designed by referring with the nucleotide sequence of *Populus tremuloides;* sense and antisense primer, GTCGACAAGATGGG TTCAACA and GGATCCGTGCCATGATATTC, respectively. PCR was performed for 29 cycles of reaction (95°C, 1 min; 40°C, 30 sec; 72°C, 2 min) followed with postreaction at 72°C for 5 min. The 540 bp-DNA fragment of PCR product was cloned into pGEM-T cloning vector. The clone was confirmed by nucleotide sequencing and used as a OMT probe. Probe DNA was labelled with [α-^{32}P]dCTP (3,000 Ci/mmole) using Prime-a-Gene Labelling system from Promega.

cDNA library screening

About 500,000 pfu from the hybrid poplar λZapII cDNA library were screened by the plaque hybridization method according to a laboratory manual of Molecular Cloning (Sambrook et al., 1989). The results were monitored by autoradiography. Several positive plaques obtained were further identified by subsequent secondary and tertiary plaque hybridization processes. DNA sequencing was carried out by the dideoxy chain termination methods on double-stranded template (Sanger et al., 1977). The DNA sequence was analyzed using PC-Gene v6.7 (Intelligenetics).

Results and Discussion

Populus nigra × *maximowiczii* secondary xylem λZapII cDNA library was constructed to be titered 8×10^6 pfu μg^{-1}. It was amplified for once in *E. coli* XL1-blue MRF′ cells to result in approximately 1×10^8 pfu. A 540 bp cDNA fragment amplified by PCR which was identified by nucleotide sequencing was used as a probe DNA to screen the cDNA library. After 3 subsequent rounds of screening, five positive λ clones were isolated for rescuing and analyzed by restriction enzyme digestion with *Eco*RI and *Xho*I. Restriction mapping showed that they were actually identical, but heterogeneous in length. From this, one clone of greater length with two internal *Eco*RI sites was determined for further analysis. This clone, pOMTB1.4, which consists of 1.5 kb cDNA insert in pBluescript SK was completely sequenced on both strands. The cDNA insert is 1,503 nucleotides in length and contains one open reading frame of 1098 nucleotides encoding a protein of 366 amino acids. The 3′-end consists of a untranslated region (UTR) of 322 nucleotides, including a 19 bp poly(A)$^+$ tail. Within this UTR, three putative polyadenylation signals (AAUAAA) are located 12,100, and 146 bases upstream of the poly(A)$^+$ tail, respectively. The deduced amino acid sequence of pOMTB 1.4 was compared with the consensus OMT sequence derived from various OMT sequences of various poplar plant sources as shown in Figure 1. Basically, the OMT enzyme consists of three to five well-conserved

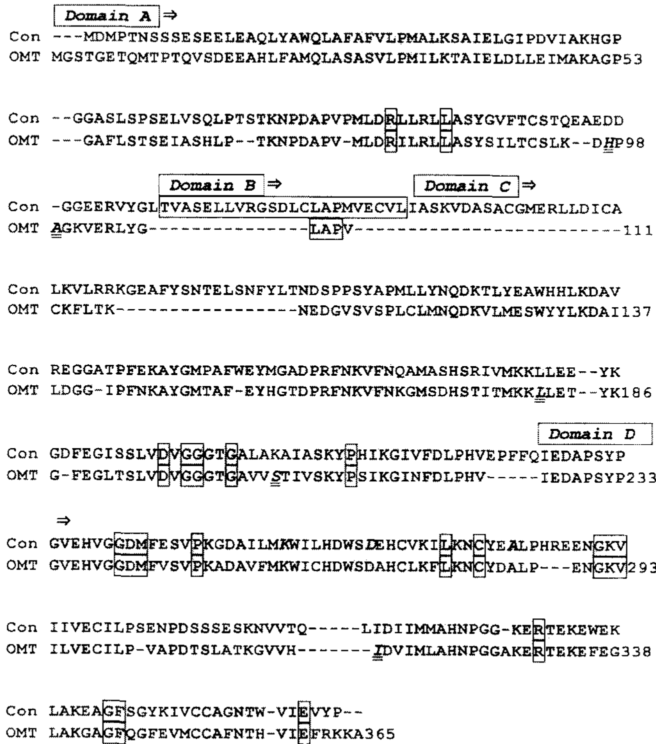

Figure 1. Homologous comparison of the deduced amino acid sequence of OMTB1.4 (OMT) to the conserved amino acid sequence output derived by computational homology analysis (Con) for various OMT sequences.

domains, but three in most of poplar plants. Domains A, C and D are well conserved for OMTB1.4 as represented well from bispecific caffeic acid/5-hydroxyferulic acid O-methyltransferase. Isoliquiritigenin-O-methyltransferase in *Pinus radiata* is shown to have an additional short domain ahead of domain A. Domain B which is retained in such as isoflavone-O-methyltransferase of *Medicago sativa* is almost deficient in OMTB1.4. In OMTB1.4, most of strictly conserved amino acid sequences throughout the domains A, C and D are well conserved as in the consensus one, but not for the domain B consisting of 23 amino acids, implying a barely functioning divergence of OMT for specificity during lignin biosynthesis among different plant species. In the domain C, its N-terminal region in short of the first 37 amino acids is continued to the C-terminus being relatively well conserved in OMTB1.4 as seen well in other poplar species. At the amino acid level of homology, the poplar OMT sequence of *Populus nigra* × *maximowiczii* is in homology of 85 and 99% with the alfalfa and aspen OMT, respectively. Meanwhile, the poplar OMT sequence contains an ATP-binding site (GxGxxG), suggesting its role possibly in S-adenosylmethionine binding activity.

In our previous paper, seasonal expression patterns of the OMT gene from the hybrid poplar were observed using aspen OMT cDNA probe in developing secondary xylem tissue from March to September (Park et al., 1998). It shows that the OMT gene is actively expressed from late April to late September, different from the two expression peaks in early June and late July reported by Bugos et al. (1991) who suggested the biphasic pattern of gene expression probably due to the characteristics of xylem tissue development of earlywood and latewood. This biphasic pattern was not observed in our study, suggesting that it might be caused by the climate difference between locations. From this observations, OMTB1.4 clone can be utilized to demark the initial differentiation of xylem from cambium into monolignol-producing cells, which eventually lignify and form wood. In addition to this, this clone could be used to transform softwoods to modify lignin biosynthesis by expressing the antisense-oriented and its transferring into the hybrid poplar plants. Regulation of the level of OMT expression by an antisense mechanism could be a useful tool for genetically engineering plants with modified lignin without altering normal growth and development. Accordingly, we are examining the effect of the hybrid OMT antisense sequence under the control of the strong constitutive cauliflower mosaic virus 35S promoter. For further study, modification of the lignin content in the xylem structure will be attempted in accordance with antisense suppression of the hybrid OMT gene expression.

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References

Bugos RC, Chiang VL, Campbell WH (1991) cDNA cloning, sequence analysis and seasonal expression of lignin-bispecific caffeic acid/5-hydroxyferulic acid O-methyltransferase of aspen. Plant Mol Biol 17: 1203-1215.

Collazo P, Montoliu L, Puigdomenech P, Rigau J (1992) Structure and expression of the lignin O-methyltransferase gene from Zea mays L. Plant Mol Biol 20: 857-867.

Draper J, Scott R, Armitage P, Walden R (1988) Plant genetic transformation and Gene expression. Oxford, The Alden Press.

Dumas, B, Van Doorsselaere J, Gielen J, Legrand M, Fritig B, Van Montagu M, Inze D (1992) Nucleotide sequence of a complementary DNA encoding O-methyltransferase

from poplar. Plant Physiol 98: 796-797.

Gowri G, Bogos RC, Campbell WH, Maxwell CA, Dixon R (1991) Stress responses in alfalfa (*Medicago sativa* L.). X. Molecular cloning and expression of S-adenosyl-L-methionine: caffeic acid-O-methyl-transferase, a key enzyme of lignin biosynthesis. Plant Physiol 97: 7-14.

Grisebach H (1981) Lignins. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 7., pp 457-478, Academic Press, New York.

Hahlbrock K, Scheel D (1989) Physiology and molecular biology of phenylpropanoid metabolism. Annu Rev Plant Physiol Plant Mol Biol 40: 347-369.

Higuchi T (1985) Biosynthesis of lignin. In: Higuchi, T (ed.) Biosynthesis and Biodegradation of Wood Components. Academic Press pp. 141-160.

Higuchi T (1990) Lignin biochemistry: biosynthesis and biodegradation. Wood Sci Technol 24: 23-63.

Jaeck E, Dumas B, Geoffroy P, Favet N, Inze D, Van Montagu M, Fritig B, Legrand M (1992) Regulation of enzymes involved in lignin biosynthesis: Induction of O-methyltransferase mRNAs during the hypersensitive reaction of tobacco to tobacco mosaic virus. Mol Plant-Microbe Interact 4: 294-300.

Kiefer-Meyer MC, Gomord V, O'Connell A, Halpin C, Faye L (1996) Cloning and sequence analysis of laccase-encoding cDNA clones from tobacco. Gene 178: 205-207.

LaFayette PR, Eriksson KEL, Dean JFD (1995) Nucleotide sequence of a cDNA clone encoding an acidic laccase from sycamore maple (*Acer pseudoplatanus* L.) Plant Physiol 107: 667-668.

Lewis NG, Yamamoto E (1990) Lignin: occurrence, biogenesis and biodegradation. Annu Rev Plant Physiol Plant Mol Biol 41: 455-496.

Park YG, Park HS, Choi JW, Sul IW, Chung IK, Shin DI (1998) Seasonal expression of OMT gene in relation to lignin biosynthesis in two poplar species. Korean J Life Science 8: 443-448.

Ranocha P, McDougall G, Hawkins S, Sterjiades R, Borderies G, Stewart D, Cabanes-Macheteau M, Boudet AM, Goffner D (1999) Biochemical and characterization, molecular cloning and expression of laccase- a divergent gene family- in poplar. Eur J Biochem 259: 485-495.

Sarkanen KV, Hergert HL (1971) Lignins in the plant kingdom. Classification and distribution. In: Sarkanen KV, Ludwig S (eds), Lignins: Occurrence, Formation, Structure and Reactions. pp 43-94. Wiley, New York.