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Pinus taeda phenylpropenal double-bond reductase: Purification, cDNA cloning, heterologous expression in *Escherichia coli*, and subcellular localization in *P. taeda* ☆

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Dedicated to Rodney Croteau in honor of his 60th birthday.

Abstract

A phenylpropenal double-bond reductase (PPDBR) was obtained from cell suspension cultures of loblolly pine (*Pinus taeda* L.). Following trypsin digestion and amino acid sequencing, the cDNA encoding this protein was subsequently cloned, with the functional recombinant protein expressed in *Escherichia coli* and characterized. PPDBR readily converted both dehydrodiconiferyl and coniferyl aldehydes into dihydrodehydrodiconiferyl and dihydroconiferyl aldehydes, when NADPH was added as cofactor. However, it was unable to reduce directly either the double bond of dehydrodiconiferyl or coniferyl alcohols in the presence of NADPH. During this reductive step, the corresponding 4-*proR* hydrogen was abstracted from [4*R*-^{3}H]-NADPH during hydride transfer. This is thus the first report of a double-bond reductase involved in phenylpropanoid metabolism, and which is presumed to be involved in plant defense. *In situ* mRNA hybridization indicated that the PPDBR transcripts in *P. taeda* stem sections were localized to the vascular cambium, as well as to radial and axial parenchyma cell types.

Additionally, using *P. taeda* cell suspension culture crude protein extracts, dehydrodiconiferyl and coniferyl alcohols could be dehydrogenated to afford dehydrodiconiferyl and coniferyl aldehydes. Furthermore, these same extracts were able to convert dihydrodehydrodiconiferyl and dihydroconiferyl aldehydes into the corresponding alcohols. Taken together, these results indicate that in the crude extracts dehydrodiconiferyl and coniferyl alcohols can be converted to dihydrodehydrodiconiferyl and dihydroconiferyl alcohols through a three-step process, i.e. by initial phenylpropenol oxidation, then sequential PPDBR and phenylpropanal reductions, respectively.

Keywords: *Pinus taeda*; Pinaceae; Loblolly pine; Phenylpropenal double bond reductase; Lignans; Lignins; Dihydroconiferyl aldehyde; Dihydrodehydrodiconiferyl aldehyde

1. Introduction

The lignans are an ubiquitous class of phenylpropanoid metabolites present in vascular plants, ranging in size from dimers to oligomers. They are conveniently classified on the basis of their structural interunit linkages, e.g. 8–8′, 8–*O*–4′, 8–5′, etc. (Lewis and Davin, 1999). Their primary function in plants appears to be in defense (e.g. as biocides, antioxidants, etc.) (Ayres and Loike, 1990; Lewis and Davin, 1999; Davin and Lewis, 2005). In certain families,

Abbreviations: CAD, cinnamyl alcohol dehydrogenase; DDC, dehydrodiconiferyl alcohol; DDCAL, dehydrodiconiferyl aldehyde; DDDC, dihydrodehydrodiconiferyl alcohol; DDDCAL, dihydrodehydrodiconiferyl aldehyde; ENR, enoyl acyl carrier protein reductase; ER, enoate reductase; GST, glutathione *S*-transferase; IDDDC, isodihydrodehydrodiconiferyl alcohol; IPTG, isopropyl β-D-thiogalactoside; PCBER, phenylcoumaran benzylic ether reductase; PLR, pinoresinol/lariciresinol reductase; PPDBR, phenylpropenal double-bond reductase; SDH, secoisolariciresinol dehydrogenase; TDDC, tetrahydrodehydrodiconiferyl alcohol.

☆ Data deposition: The sequence reported in this paper has been deposited in the GenBank database (Accession No. DQ829775).

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such as the Pinaceae, they also typically help determine the characteristic color and properties of heartwood tissue. For example, over the last two decades, a range of dimeric and oligomeric lignans have been reported in the Pinaceae (Sakakibara et al., 1987; Barrero et al., 1993, 1996; Kawamura et al., 1997).

Recently, the biosynthetic pathways to the 8–8′ linked lignans (see Fig. 1) have been defined at the metabolic, enzymatic, and molecular levels (Chu et al., 1993; Ozawa et al., 1993; Dinkova-Kostova et al., 1996; Davin et al., 1997; Fujita et al., 1999; Gang et al., 1999a; Xia et al., 2000, 2001; Halls and Lewis, 2002; Kim et al., 2002; Min et al., 2003; Halls et al., 2004; Moinuddin et al., 2006). This demonstrated the existence of two distinct pathways using the monolignol, coniferyl alcohol (**1**), namely either directly into the various non-structural lignans or partitioned for polymerization into the lignins (Gang et al., 1999a; Burlat et al., 2001). For the 8–8′ linked lignans, a biosynthetic pathway (Fig. 1) from *E*-coniferyl alcohol (**1**) through stereoselective coupling to give (+)-pinoresinol (**2a**) has been established (Davin et al., 1997; Gang et al., 1999a; Halls and Lewis, 2002; Halls et al., 2004), with the latter being enantiospecifically metabolized into various bioactive compounds (Ozawa et al., 1993; Dinkova-Kostova et al., 1996; Fujita et al., 1999; Xia et al., 2000, 2001). These, depending upon the plant species, can include formation of molecules such as secoisolariciresinol (**4**), matairesinol (**5**), α-conidendrin (**6**), plicatic acid (**7**), podophyllotoxin (**8**) and their (oligomeric) congeners (Lewis and Davin, 1999). That is, pinoresinol (**2**) is a common precursor of the 8–8′ lignans and can be metabolized into a very broad range of natural products.

In contrast to the 8–8′ linked lignans, the biosynthetic pathways (metabolic steps, enzymes and genes involved) to the structurally diverse 8–5′ linked lignans are only now yielding to inquiry. The initial coupling step leads to dehydrodiconiferyl alcohol (**9**, DDC) which can, depending upon the species, be found in either racemic (Cutillo et al., 2003) and/or optically active (Yoshikawa et al., 2003) forms. Some of these enantiomerically pure metabolites have relatively low optical rotations, e.g. (+)- and (−)-dihydrodehydrodiconiferyl alcohols (**10a** and **10b**, $[\alpha]_D^{25} = +5.1$ and −9.18, respectively) (see Section 4) (Lewis and Davin, 1999).

Loblolly pine (*Pinus taeda* L.) is a popular and commercially important softwood species, and has been used as a model plant for studies on lignin biosynthesis and monolignol regulation (Eberhardt et al., 1993; Nose et al., 1995; Anterola et al., 1999, 2002). Moreover, its cell suspension

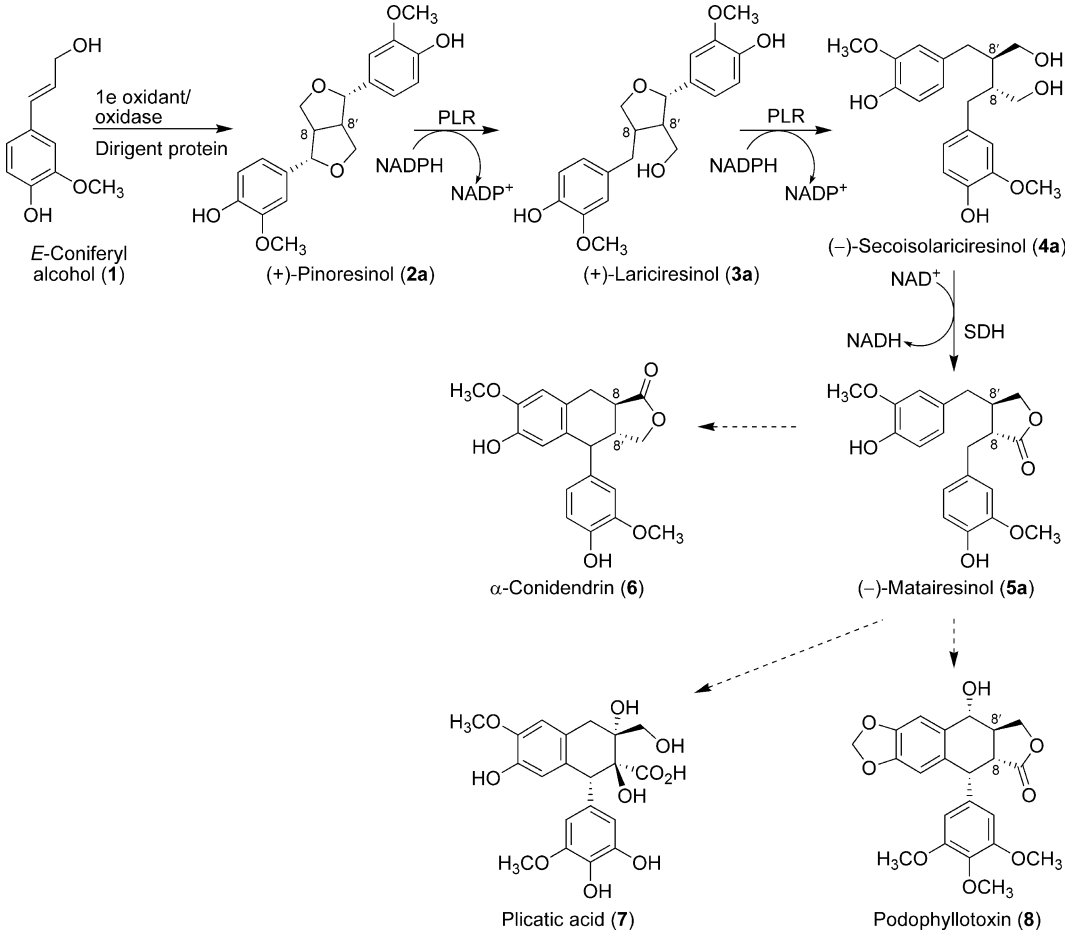

Fig. 1. Biosynthetic pathway to (−)-matairesinol (**5a**) and proposed conversions to α-conidendrin (**6**), plicatic acid (**7**) and podophyllotoxin (**8**).