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Laccase catalyzed covalent coupling of fluorophenols increases lignocellulose surface hydrophobicity

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ABSTRACT

This work presents for the first time the mechanistic evidence of a laccase-catalyzed method of covalently grafting hydrophobicity enhancing fluorophenols onto *Fagus sylvatica* veneers. Coupling of fluorophenols onto complex lignin model compounds guaiacylglycerol β-guaiacyl ether and syringylglycerol β-guaiacyl ether was demonstrated by LC–MS and NMR. Laccase-mediated coupling increased binding of 4-[4-(trifluoromethyl)phenoxy]phenol (4,4-F3MPP) and 4-(trifluoromethoxy)phenol (4-F3MP) to veneers by 77.1% and 39.2%, respectively. XPS studies showed that laccase-catalyzed grafting of fluorophenols resulted in a fluorine content of 6.39% for 4,4-F3MPP, 3.01% for 4-F3MP and 0.26% for 4-fluoro-2-methylphenol (4,2-FMP). Grafting of the fluorophenols 4,2-FMP, 4-F3MP and 4,4-F3MPP led to a 9.6%, 28.6% and 65.5% increase in hydrophobicity, respectively, when compared to treatments with the respective fluorophenols in the absence of laccase, in good agreement with XPS data.

1. Introduction

Among the construction materials which are used by industry, wood holds a special place because of its impressive range of attractive properties, including low thermal extension, low density and high mechanical strength. Despite these attractive properties, wood is hygroscopic and susceptible to biodegradation, factors which are partly responsible for the continued search for better wood processing technologies. Traditionally two approaches, chemical and thermal wood processing technologies, have been developed to improve wood hydrophobicity. Chemical methods include use of silicon compounds (Mai and Militz, 2004), impregnation with hydrophobic oil (Ulvcrona et al., 2006), application of water repellants like waxes, oils, natural or synthetic resins (Borgin and Corbett, 1970; Feist and Mraz, 1978; Razzaque, 1982; Hyvönen et al., 2006) and using wood binders containing hydrophobic diluents. However, these physico-chemical methods are becoming increasingly unpopular as society becomes eco- and energy-sensitive. Water repellent substances seem to be bonded to the cell wall only by relatively weak Van der Waal forces which, over a long time and due to continuous exposure to water, are displaced and washed off (Razzaque, 1982). Although chemical modification of wood through for example acetylation (Rowell, 2006; Obataya et al., 2002) and thermal degradation of hemicellulose components (Viitaniemi and Jämsä, 1996) increases dimensional stability and hydrophobicity, it is also known that these treatments reduce mechanical properties such as tensile strength (Vick and Rowell, 1990; Ramsden et al., 1997). Modifications with silicon compounds usually involves complicated and expensive multi-step processes while, due to high chemical and weathering stability, such treatments are usually recommended for wood exposed to conditions of hazard class III (EN 335, outside exposure without soil contact) (Mai and Militz, 2004).

In response to shortcomings of chemical and physical methods, enzymes such as laccases have emerged as important biotechnological catalysts as they are both eco-friendly and work under mild conditions. Laccases (EC 1.10.3.2, *p*-diphenol:dioxygen oxidoreductase) are multi-copper glycoproteins that catalyze the monoeletronic oxidation of phenols and aromatic or aliphatic amines to reactive radicals in a redox reaction in which molecular oxygen is simultaneously reduced to water (Claus, 2004; Riva, 2006). The reaction usually involves oxidation of lignin moieties on the wood surface to create a radical-rich reactive surface to which oxidized (radical-containing) or non-oxidized molecules of interest can be

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grafted (Kudanga et al., 2008). Investigations into the possibility of using laccases to covalently attach molecules of interest to wood material are increasing (Grönqvist et al., 2006; Widsten and Kandelbauer, 2008). However, a review of the literature shows that only one attempt by Suurnakki et al. (2006) has been made to increase wood hydrophobicity by enzymatic grafting although no mechanistic proof was provided for covalent attachment and the type of bonding.

In an effort to use green chemistry technology to increase wood surface hydrophobicity, this work presents for the first time a laccase-mediated method of covalently grafting fluorophenols onto beech (*Fagus sylvatica*) veneers. Further, evidence for covalent binding of fluorophenols to complex lignin model substrates is provided by LC–MS and NMR in order to gain mechanistic information on the coupling reaction.

2. Methods

2.1. Chemicals

The lignin model compounds guaiacylglycerol β-guaiacyl ether **E** (erol) and syringylglycerol β-guaiacyl ether **G** (Fig. 1) were synthesized following the procedure described by Sipilä and Syrjänen (1995). All other chemicals including the fluorophenol molecules 4-fluoro-2-methylphenol (4,2-FMP), 4-[4-(trifluoromethyl)phenoxy]phenol (4,4-F3MPP) and 4-(trifluoromethoxy)phenol (4-F3MP) were purchased from Sigma–Aldrich.

2.2. Veneers

The wood veneers used in the present investigation were prepared from European beech (*F. sylvatica*) and measured 10 mm × 10 mm × 1 mm. Prior to their use in grafting experiments, they were Soxhlet-extracted with acetone overnight to remove lipophilic extractives which could interfere with oxidized molecules and also affect analysis of modified surface (Gutiérrez et al., 1998; Nzokou and Kamdem, 2004).

2.3. Laccases

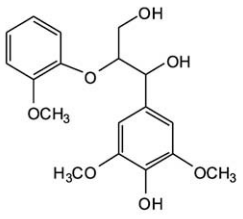
The 62 kDa laccase from *Trametes hirsuta* was produced and purified as previously reported by Almansa et al. (2004). The activity of the laccase was determined spectrophotometrically by monitoring the oxidation of 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulphonate (ABTS) (ε_{436} = 29,300 M^{-1} cm^{-1}) as substrate at 436 nm in 50 mM succinate buffer at pH 4.5 and 37 °C as described by Niku-Paavola et al. (1988), with some modifications. Briefly, the reaction mixture contained 30 μl laccase, 350 μl ABTS (1 mM) and 50 mM succinate buffer pH 4.5 to make a final volume of 1.5 ml. A blank was set in the same way as the sample experiment except that the laccase was initially heat denatured at 100 °C for 10 min. The spectrometric measurements were done by recording the absorbance in the time scan mode for 2 min.

2.4. Oxidation of fluorophenols

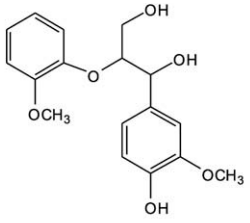
The ability of the *T. hirsuta* laccase to oxidize the three fluorophenols (4,2-FMP, 4-F3MP and 4,4-F3MPP) was monitored by UV/Vis-spectrophotometry and confirmed by HPLC analysis. To start the reaction, laccase with a final activity of 13.4 nkat ml^{-1} was added to a reaction mixture containing 0.1 mM fluorophenol in 50 mM succinate buffer pH 4.5. The reactions were monitored by UV/Vis-spectrophotometry in the wavelength scan mode from 900 to 200 nm at 3 min per cycle for a total of 18 min to identify oxidation products.

2.5. Coupling of fluorophenols to lignin model compounds

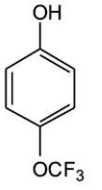
The reaction mixture contained a fluorophenol (2.0 mM) and one of the lignin model compounds **E** and **G** (4.0 mM) in the molar



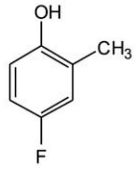
Syringylglycerol β-guaiacyl ether (G)



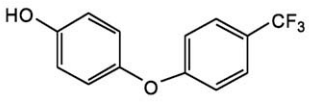
Guaiacylglycerol β-guaiacyl ether (E)



4-(Trifluoromethoxy)phenol (4-F3MP)



4-Fluoro-2-methylphenol (4,2-FMP)



4-[4-(Trifluoromethyl)phenoxy]phenol (4,4-F3MPP)

Fig. 1. Chemical structures of the lignin model compounds and fluorophenols used in the coupling reactions.