J. Agric. Food Chem. 1996, 44, 1953–1959

# Oxidative Degradation of *in Situ* and Isolated Spruce Lignins by Water-Soluble Hydrogen Peroxide Resistant Pentafluorophenylporphyrin

Bernard Kurek,*,† Isabelle Artaud,‡ Brigitte Pollet,† Catherine Lapierre,† and Bernard Monties†

Laboratoire de Chimie Biologique, Institut National de la Recherche Agronomique (INRA),
Centre de Biotechnologie Agro-Industrielle, 78850 Thiverval-Grignon, France, and Laboratoire de Chimie et
Biochimie Pharmacologiques et Toxicologiques, Centre National de la Recherche Scientifique (CNRS),
Unité de Recherche Associée 400, Rue des Saint-Pères, 75270 Paris, France

The catalytic activity of a new oxidation-resistant Fe(III) *meso*-tetrakis(pentafluorophenyl)-$\beta$-tetrasulfonatoporphyrin [Fe(TF$_5$PS$_4$P)] on spruce milled wood lignin (MWL) and lignin in extractive free spruce wood (EFW) was investigated in the presence of $H_2O_2$. Size exclusion chromatography analysis shows that MWL was rapidly repolymerized by Fe(TF$_5$PS$_4$P)/$H_2O_2$ before undergoing further degradation. IR and $^1$H-NMR spectroscopy of oxidized lignins suggested that ring-opening reactions and demeth(ox)ylations occurred. Analysis by thioacidolysis of MWL revealed an 80−90% decrease in guaiacyl units involved in $\beta$-*O*-4 bonds after catalysis. The formation of some vanillin and vanillic acid structures in lignins was evidenced, confirming that C$\alpha$−C$\beta$ cleavages are promoted by the metalloporphyrin. Lignin in EFW was also degraded by Fe(TF$_5$PS$_4$P)/$H_2O_2$ in the same manner as MWL, but to a lesser extent. In conclusion, Fe(TF$_5$PS$_4$P)/$H_2O_2$ mimics the action of fungal ligninolytic peroxidases but is also able to penetrate within the compact structure of wood and degrade lignin to a significant extent.

**Keywords:** *Biomimetic; pentafluorophenylporphyrin; hydrogen peroxide; spruce wood; lignin; thioacidolysis; infrared spectroscopy; $^1$H NMR; size exclusion chromatography*

## INTRODUCTION

Catalysts based on porphyrins have been proposed since 1984 to mimic the catalytic action of fungal lignin-degrading enzymes lignin peroxidase (LiP) and manganese dependent peroxidase (MnP) (Shimada, 1991; Meunier, 1994). Several reactions such as the C$\alpha$−C$\beta$ bond cleavage of the propane side-chain, the formation of quinones, or the cleavage of aromatic rings into muconic derivatives were shown to be similarly catalyzed by hemin/tBuOOH and LiP/$H_2O_2$ on dimeric lignin model compounds (Shimada et al., 1987a,b). This analogy suggests close chemical pathways during iron porphyrin or LiP dependent oxidations, namely the occurrence of monoelectronic transfer between the catalyst and the substrate leading to the formation of cation radicals on aromatic lignin-like model compounds. However, the metalloporphyrins used were rapidly bleached by relatively low amounts of oxidants and the overall efficiency of the catalysis was low (Wood et al., 1986).

A second generation of water-soluble iron or manganese tetraarylporphyrins bearing electron-withdrawing substituents such as chloro and sulfonato groups on the phenyl rings were therefore synthesized to both increase reactivity and limit autoxidation. In this respect, Fe(TDCSPP) in the presence of mCPBA or tBuOOH (Cui et al., 1993), Fe(TSPP) with KHSO$_5$ (Labat and Meunier, 1985), or Fe(TF$_5$PS$_4$P) with $H_2O_2$ or MMPP (Artaud et al., 1991, 1993) were shown to be much more stable toward excess oxidant and to exhibit improved catalytic properties compared to LiP.

Considering the high reactivity of metalloporphyrins as well as their excellent diffusional properties, such catalysts were also proposed as possible alternative oxidants for new environmentally friendly bleaching technologies in the pulp and paper industry in place of fungal ligninolytic enzymes, which are unable to penetrate into the fiber structure (Wood et al., 1986; Srebotnik et al., 1990; Pettersson et al., 1988). The results obtained have shown that treatment of wood chips using a natural heme in the presence of tBuOOH leads to significant delignification (Paszczynski et al., 1988). Moreover, using the same oxidant, a strong delignification of pulps was achieved with the water-soluble catalysts Fe(TDCSPP) and Fe(TDCSPCl$_8$P). However, in both cases, under the refluxing or heating conditions used, the catalyst/oxidant couples were also shown to degrade the cellulose to a limited but undesirable extent (Paszczynski et al., 1988; Skerer et al., 1990). This points out that milder reaction conditions during the process have to be used to better control oxidative reactions for technological applications.

Today, the oxidation mechanisms of lignin model compounds by porphyrins are partly elucidated (Cui et al., 1993) and pulp bleaching experiments are performed on a laboratory scale (Wood et al., 1986; Paszczynski et al., 1988; Skerer et al., 1990). However, no description at the molecular level of the chemical modifications induced by metalloporphyrins on isolated or *in situ* macromolecular lignins is yet available. Furthermore, there are only a few studies on lignin model compound oxidation by metalloporphyrin and hydrogen peroxide, although the use of such a clean oxidant is recommended in the pulp and paper industry.

In this work, we have delineated the chemical modifications to purified and *in situ* spruce lignins oxidized by a new iron water-soluble porphyrin, Fe(TF$_5$PS$_4$P),

---

* Author to whom correspondence should be addressed [telephone (33-1) 3081 5464; fax (33-1) 3081 5373; e-mail kurek@cardere.grignon.inra.fr].

† INRA.

‡ CNRS.

S0021-8561(95)00612-1 CCC: $12.00

**Table 1. Composition of the Reaction Media Used for MWL and EFW Oxidation by $Fe(TF_5PS_4P)$ and $H_2O_2$**

| | solvent mixture[a] | | | | |
|---|---|---|---|---|---|
| | dioxane/$H_2O$[b] | | | | water |
| | expt 1 | expt 2 | expt 3 | expt 4 | expt 5 |
| extractive free wood (mg/mL) | | | | 10 | 10 |
| lignin (mg/mL) | 1[c] | 1 | 1 | | |
| porphyrin[d] (mg/mL) | 0.36[e] | 0.036[f] | 0.007[g] | 0.18[h] | 0.18 |
| $H_2O_2$ supplied every 10 min (mM) | 5 | 0.5 | 0.1 | 5 | 5 |
| total $H_2O_2$ supplied (mM) | 100 | 10 | 5 | 100 | 100 |

[a] 5 mL final volume. [b] 9/1 v/v. [c] ≅5 mM. [d] In control experiments, no porphyrin was added. [e,f,g,h] 250, 25, 5, and 125 μM, respectively

known to be an efficient LiP biomimetic system on lignin model compounds in the presence of hydrogen peroxide (Artaud et al., 1993). The results obtained showed that this catalyst is an efficient lignin-degrading system which not only mimics the action of fungal LiP on the isolated polymer but also oxidizes it to a significant extent within the compact cell wall structure.

## EXPERIMENTAL PROCEDURES

**Safety.** Large-scale experiments involving peroxide intermediates and 1,4-dioxane must be performed with caution (explosion hazards) (Vogel et al., 1978).

**Preparation of Extractive Free Wood and Purification of Lignins.** Extractive free wood sawdust (EFW) from Sitka spruce (*Picea sitchensis*) was prepared by successive extractions with toluene/ethanol, ethanol/water, and water mixtures in a Soxhlet apparatus as previously described (Lapierre et al., 1986). Lignins were further isolated from 48 h ball-milled EFW by a dioxane/water (9/1 v/v) mixture and further purified by two successive precipitations in water and diethyl ether (Lapierre et al., 1986). The isolated and purified lignin will be called hereafter milled wood lignin (MWL).

**Synthesis of $Fe(TF_5PS_4P)$.** The water-soluble free base $TF_5PS_4PH_2$, prepared by sulfonation of $TF_5PPH_2$ in fuming sulfuric acid, was metalated by $FeCl_2 \cdot 4H_2O$ as previously described (Artaud et al., 1991, 1993). $TF_5PS_4PH_2$ was characterized by $^1H$ NMR, UV−visible spectroscopy and mass spectrometry (Artaud et al., 1991).

**Typical Procedure for Lignin Oxidation Catalyzed by $Fe(TF_5PS_4P)$.** Oxidations of purified lignin (MWL) and extractive free wood sawdust (EFW) were carried out in dioxane/water (9/1 v/v) medium or non-buffered water at room temperature (22 °C) under the conditions described in Table 1. The MWL and porphyrins were prepared as stock solutions [20 mg/mL for lignin in dioxane/water mixture (9/1 v/v); 3 mg/ml for $Fe(TF_5PS_4P)$ in dioxane/water mixture or water]. The peroxide (10 μL of a 3 or 1 M stock solution of aqueous hydrogen peroxide) initiated the reaction and was supplied periodically as described in Table 1. The reaction was stopped when $Fe(TF_5PS_4)$ was bleached, as indicated by the disappearance of the Soret band in the UV (see below). The whole reaction medium was then freeze-dried and stored at 4 °C before chemical analysis. In control experiments, no porphyrin was added to the reaction medium.

**Lignin Characterizations.** The molecular composition of the purified lignin and the polymer in EFW was determined by the thioacidolysis method. The main functional groups present in MWL were characterized using spectroscopic methods (UV, IR, and $^1H$ NMR). The hydrodynamic properties of MWL were determined by high-performance size exclusion chromatography (HP-SEC) in organic solvent.

*Thioacidolysis.* The procedure is suitable without restriction both for MWL analysis and for lignin characterization in EFW. Reactions were performed as described by Lapierre et al. (1986) and Rolando et al. (1992) directly on the whole lyophilized reaction media containing EFW or MWL (Kurek and Monties, 1994). The main monomers released (Table 2) were separated by capillary gas chromatography as trimethylsilyl (TMS) derivatives on a 30 m column SPB1-M (Supelco, France) using He as carrier gas, as previously described (Rolando et al., 1992). The detection was performed by flame ionization (FID). The products were quantified by adding tetracosane as an internal standard to each sample. The degradation and formation yields of each type of lignin monomer were determined relative to their absolute content in the nonoxidized MWL or EFW (control experiments incubated with all components of the reaction media but without porphyrin; Table 2). The proportion of condensed C−C-linked dimeric and oligomeric structures recovered after thioacidolysis of MWL and lignins in EFW was estimated by HP-SEC analysis on one 600 × 7 mm polystyrene−divinylbenzene column (100A PL-gel, Polymer Lab) using tetrahydrofuran as mobile phase (1 mL/min) at room temperature (Suckling et al., 1994). Detection was performed by UV spectroscopy at 280 nm.

*Spectroscopic Analysis of Purified Lignins. UV spectra* of MWL sample obtained after dilution of an aliquot of the reaction medium (10 μL, *ca.* 0.01 mg of lignin) into 1 mL of dioxane/water mixture (9/1 v/v) were recorded from 220 to 600 nm. The total UV contribution of the components of the reaction medium (lignin, active and bleached porphyrin) was determined separately. Without $H_2O_2$, the UV spectrum exhibited two maxima at 280 and 418 nm attributed, respectively to lignin and $Fe(III)(TF_5PS_4P)$ catalyst. Upon addition of $H_2O_2$, the Soret peak of the porphyrin shifts to 428 nm and the α bands shift from 584 to 570 nm. This new spectrum is typical of a (P)Fe(IV)O species (Bell et al., 1991). The bleaching of the catalyst was then followed by the disappearance of the 428 nm absorption band. The degradation of lignin was monitored by the spectral modifications occurring in the 220−360 nm region. *Infrared spectra* of the freeze-dried reaction medium were taken in solid KBr pellets in the 700−1800 $cm^{-1}$ region. Spectra of all the components of the reaction medium were also recorded individually and used as blanks. Band assignments for lignin were made with reference to the literature (Hergert, 1971). *$^1H$-NMR spectra* of lignin dissolved in DMSO-$d_6$ were recorded at 25 °C at 250 MHz. Chemical shifts were reported in parts per million (ppm) downfield from $Me_4Si$, and the peak assignment was based on the literature data (Lundquist, 1981). For NMR experiments, the lignin was first purified by thin layer chromatography (1 mm $SiO_2$-$F_{254}$, Merck) eluted with DMF. The solvent was removed by evaporation under reduced pressure. The stationary phase containing the lignin was then recovered and extracted with DMSO. The recovery yield of lignin was around 80% of the starting material. A control experiment checked that either the recovery yield and the $^1H$ NMR spectra of the nonoxidized lignin were not modified by this treatment.

*HP-SEC Analysis of MWLs.* An aliquot (≅100 μL) of the reaction medium containing *ca.* 0.1 mg of lignin was mixed with 1 volume of 0.05 M DMF/LiCl and chromatographed on two coupled 300 × 7 mm polystyrene−divinylbenzene columns (PL Gel linear mixed-bed, Polymer Lab). The elution was performed at room temperature with DMF containing 0.05 M LiCl at a flow rate of 0.4 mL/min. The products were detected spectrophotometrically at 280 nm.

## RESULTS

**Stability of the Iron Porphyrin toward $H_2O_2$ in the Reaction Medium.** The bleaching of $Fe(TF_5PS_4P)$ upon addition of $H_2O_2$ to the reaction mixture containing lignin was followed by the UV−visible absorption of the Soret band at 410−430 nm. The stability of the catalyst was shown to be dependent on the reaction medium used for reaction (dioxane/water mixture or pure water). In water, 10−15 equiv of $H_2O_2$ relative to the catalyst led to a complete destruction of the porphyrin, while in a dioxane/water mixture (9/1 v/v), more than 400 equiv of $H_2O_2$ could be added before bleaching occurred. It is also noteworthy that the catalyst was not destroyed

**Table 2. Composition Determined by Thioacidolysis of Nonoxidized MWL and Lignin in Nonoxidized EFW**

| lignin monomer | structure released by thioacidolysis[a] | abundance in MWL ($\mu$mol/g of lignin) | abundance in EFW ($\mu$mol/g of EFW) |
|---|---|---|---|
| guaiacyl | (**1**) G−CHR−CHR−$CH_2$R | 907 ± 40 | 415 ± 28 |
| 3,4-dihydroxybenzyl | (**2**) Cat−CHR−CHR−$CH_2$R | 7.5 ± 1.5 | 1.6 ± 0.05 |
| vanillin | (**3**) G−$CHR_2$ | 57.7 ± 4.3 | nd[b] |
| vanillic acid | (**4**) G−COOH | 32.0 ± 1.9 | nd |

[a] R = SEt; reference numbers of structure in text are in parentheses; see also Figure 4; GC response factor = 1.5 relative to tetracosane. G, 4-hydroxy-3-methoxyphenyl moiety; Cat, 3,4 dihydroxyphenyl moiety. [b] nd, not determined.

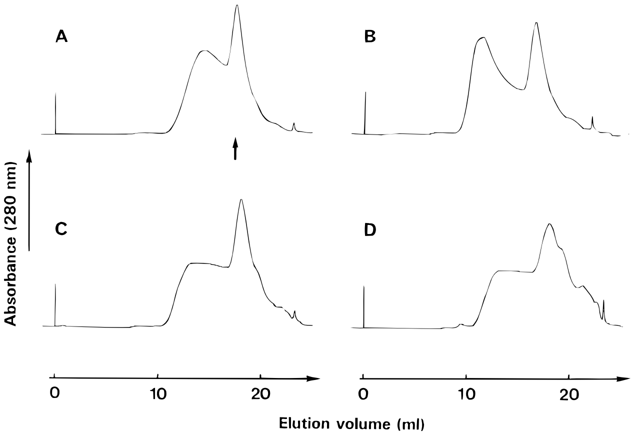

**Figure 1.** HP-SEC chromatograms of lignins oxidized by 250 $\mu$M Fe($TF_5PS_4P$). Lignins were analyzed after the progressive supply of 0 mM (A), 6 mM (B), 30 mM (C), and 90 mM (D) $H_2O_2$ in the reaction medium. In (A) the arrow indicates the elution volume of Fe($TF_5PS_4P$). Polystyrene standards were only used to check performance of the chromatographic system (void volume = 9.5 mL; total volume = 29 mL).

upon addition of $H_2O_2$ in the absence of lignin. In fact, the porphyrin was shown to oxidize the solvent itself in the presence of $H_2O_2$, as glyoxal formation from dioxane was directly detected by $^1$H NMR in $CD_3CN$ during catalysis (data not shown).

**Modification of the Hydrodynamic Properties of MWL by Fe($TF_5PS_4P$) and $H_2O_2$.** HP-SEC analysis in the DMF/LiCl system showed strong and progressive modifications of lignin macromolecular properties during Fe($TF_5PS_4P$)/$H_2O_2$ oxidation (Figure 1). During an initial phase of catalysis, a lignin fraction with high hydrodynamic volume was formed (6 mM $H_2O_2$ supplied). This new fraction was then further degraded (30 mM peroxide added) giving rise to the formation of some molecules with low hydrodynamic volumes (90 mM peroxide added).

**Chemical Characterization of Lignin Oxidized by Fe($TF_5PS_4P$).** *Spectroscopic Analysis of Oxidized Lignins.* $^1$H-NMR, IR, and UV spectra of lignins oxidized in dioxane/water medium show a strong modification of the chemical composition of the polymer after oxidation. Indeed, $^1$H-NMR signals at the 6.4−7.6 ppm region (peak 1, Figure 2) and at 3.81 ppm (peak 2, Figure 2), attributed to the aromatic rings and the methoxy groups, respectively (Lundquist et al., 1981), are severely decreased after Fe($TF_5PS_4P$)/$H_2O_2$ oxidation. IR spectra of oxidized and nonoxidized lignins are also clearly different (Figure 3). Thus, the intensity of the 1600, 1505, and 1420 cm$^{-1}$ bands attributed to aromatic ring carbon vibrations and CH in plane deformations (Hergert, 1971) (bands 1−3; Figure 3) are lower in oxidized lignins compared to the control experiment. The band at 1720 cm$^{-1}$, which could correspond to carbonyl stretching in unconjugated carboxyl groups (Hergert, 1971), also drastically increases in oxidized lignins (band 4, Figure 3), suggesting that aliphatic acids were produced during the reaction. Furthermore, the profile of the spectra in the 1140−700 cm$^{-1}$ region is very different in oxidized lignins. This dramatic change most likely reflects the degradation of lignin aromatic rings, even though a few percent of polysaccharides associated with the lignin preparation may interfere in this area (Hergert, 1971). The other unattributed IR signals in oxidized lignins are not those of bleached porphyrins or components of the reaction medium (spectra not shown). Finally, UV analysis showed the appearance versus time of a continual spectral drift from 220 to 380 nm progressively hiding the characteristic 280 nm band of lignin (data not shown). The chemical correspondence for these modifications remains unknown.

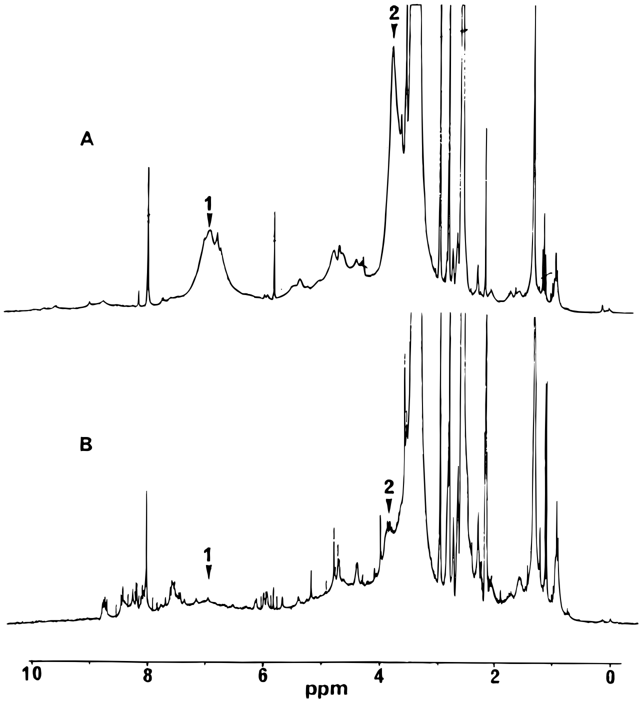

**Figure 2.** $^1$H-NMR spectra of MWL before (A) and after (B) treatment by 250 $\mu$M Fe($TF_5PS_4P$) supplied with a total amount of 96 mM $H_2O_2$. For signal assignments of bands 1 and 2, see text.

*Analysis by Thioacidolysis.* The degradation yield of the main lignin monomers in MWL and EFW was determined after thioacidolysis. This chemical depolymerization of lignin cleaves specifically the ether bonds in the polymer thus releasing a mixture of monomeric compounds and C−C-linked oligomeric structures as exemplified in Figure 4. A qualitative estimation of the C−C-linked structures released by thioacidolysis from the lignin polymer was also performed by HP-SEC.

The main monomer **1** (Table 2; Figure 4) recovered from the thioacidolysis of lignins originates from guaiacylglycerol units only involved in $\beta$-*O*-4 bonds. After

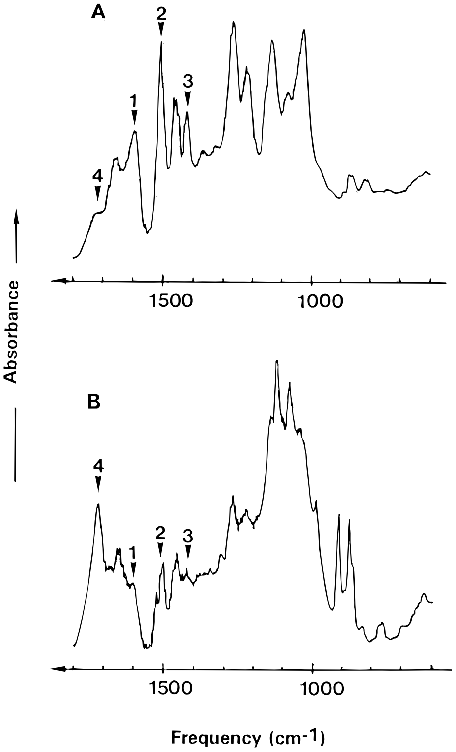

**Figure 3.** IR spectra of MWL before (A) and after (B) treatment by 250 $\mu$M $Fe(TF_5PS_4P)$ supplied with a total amount of 96 mM $H_2O_2$. For signal assignments of bands 1−4, see text.

oxidation of MWL by $Fe(TF_5PS_4P)/H_2O_2$ in dioxane medium, the recovery yields of compound **1** drastically decrease, depending on the porphyrin to lignin ratio used for the experiment (Table 3). The formation of some new structures in lignin was also observed, as indicated by the increase of the recovery yield of compounds **2**, **3**, and **4**, originating, respectively, from 3,4-dihydroxybenzyl, vanillin, and vanillic acid units of the polymer (Table 3; Figure 4).

The structural analysis of lignin in oxidized spruce wood (EFW) by thioacidolysis showed that strong chemical modifications also occurred during catalysis, both in dioxane/water and in water media (Table 3). The extent of demethylation of $\beta$-*O*-4-linked guaiacyl units in EFW, leading to 3,4-dihydroxybenzyl products formation, was, however, independent of the amount of catalyst supplied (Table 3). Finally, under identical catalytic conditions (dioxane/water; 0.36 mg/mL porphyrin; 96 mM $H_2O_2$) the degradation yields of $\beta$-*O*-4-linked guaiacyl units of *in situ* lignin were lower than those of MWL (40 and 90%, respectively; data not shown; see also Table 3).

HP-SEC analysis of thioacidolysis products from oxidized MWL indicated a strong modification of the relative amount of C−C-linked structures, compared to control experiments (Figure 5). An increase of the intensity of the early eluting peaks corresponding to oligomeric condensed structures in lignin was detected after $Fe(TF_5PS_4P)$ oxidation. Interestingly, this phenomenon was not observed for thioacidolysis products originating from oxidized EFW. In both cases, however, the late eluting peak (marked X, Figure 5) was significantly higher in the thioacidolysates of both oxidized MWL and EFW, in water or in dioxane medium. This (these) low molecular weight UV absorbing compound(s) remain(s) to be further isolated and characterized.

## DISCUSSION

Various papers have been published on oxidation of lignin model compounds and pulp delignification by metalloporphyrinic systems (Shimada, 1991; Meunier, 1994; Cui et al., 1993). However, to the best of our knowledge, no data are currently available on the chemical characterization of lignins oxidized by such biomimetic agents *in situ* or in isolated state. This paper therefore reports several original features of lignin oxidation by $Fe(TF_5PS_4P)$ in the presence of hydrogen peroxide.

The solvent system used in this study was primarily selected because spruce milled wood lignin is completely soluble in dioxane/$H_2O$ (9/1 v/v) mixture, making it possible to perform degradation experiments in homogeneous phase. The high stability of iron porphyrin in this solvent system has further showed that this medium was particularly suitable for efficient catalysis on both isolated lignins (MWL) and extractive free wood (EFW). Only EFW oxidations were also performed in nonbuffered water medium.

The analysis of oxidized milled wood lignin in the dioxane medium revealed various intense chemical modifications of the polymer. First, a partial demethylation or demethoxylation of lignin was shown to be catalyzed by $Fe(TF_5PS_4P)/H_2O_2$. The disappearance of the methoxy groups in oxidized lignin was supported by $^1$H-NMR analysis, whereas the formation of 3,4-dihydroxybenzyl (catechol) structures linked in the polymer by $\beta$-*O*-4 bonds was confirmed by thioacidolysis. The amounts of these newly formed structures by $Fe(TF_5PS_4P)$ were independent of the amount of catalyst used. This suggested that demethylation was relatively easily performed by low amounts of $Fe(TF_5PS_4P)/H_2O_2$ on $\beta$-*O*-4-linked lignin substructures. In all cases, demeth(ox)ylation of lignin was observed after LiP or HRP treatment of the same spruce MWL (Kurek and Monties, 1994) and is also frequently reported to occur in ligninolytic cultures of various white- and brown-rot fungi [reviewed by Odier and Artaud (1991) and Kurek (1992)].

The second reaction evidenced by both NMR and IR spectroscopy was the partial degradation of the aromatic rings of the macromolecule. This reaction may contribute to the disappearance of the guaiacyl monomers in oxidized lignins, as observed by thioacidolysis analysis. According to the mechanisms of aromatic ring opening catalyzed by fungal LiP/$H_2O_2$ [reviewed in Odier and Artaud (1991) and Kurek (1992)], Fe(TDCSPP)/tBuOOH (Cui et al., 1993), $Fe(TF_5PS_4P)/H_2O_2$ (Artaud et al., 1991, 1993), or hemin/tBuOOH (Hattori et al., 1988), the formation of muconic compounds would be expected in our experimental conditions. These products were nevertheless not detected in the reaction medium in free soluble form by reversed phase HPLC analysis (data not shown). In this respect, the intense IR band observed at 1720 cm$^{-1}$ in oxidized lignins can be attributed to aliphatic carboxyl groups (Hergert, 1971) and could therefore be an indication that some muconic or related compounds remained trapped on the polymer, as already evidenced with oxidized dimeric lignin model compound (Cui et al., 1993). This point, however, requires further study for confirmation. Another reasonable explanation would be that the released molecules formed during catalysis could be further degraded into small water-soluble fragments, as described with $\beta$-*O*-4 lignin model compound oxidized with Mn or Fe porphyrins (Dolphin et al., 1988; Artaud et al., 1993; Cui et al., 1993).

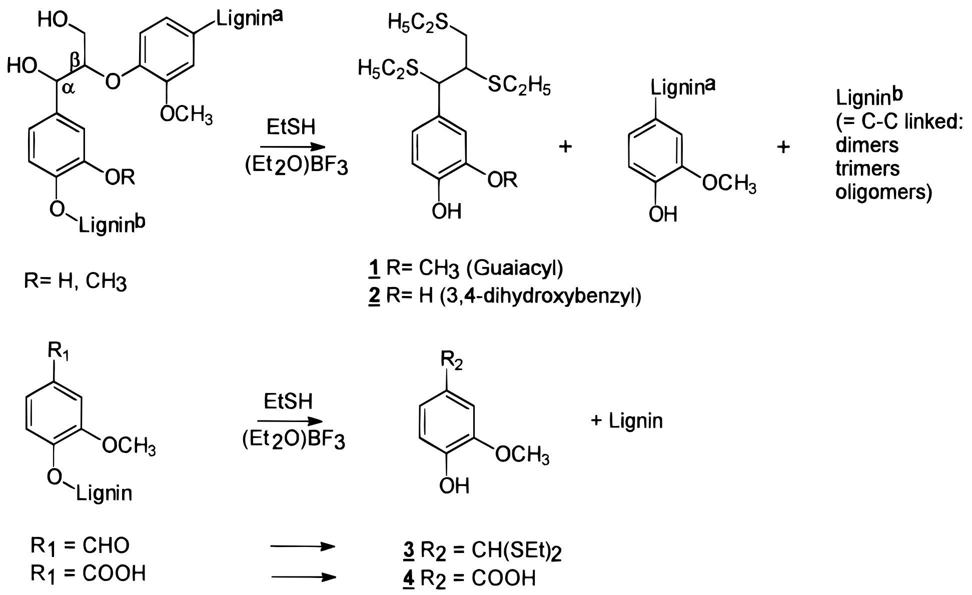

**Figure 4.** Thioacidolysis pivotal reaction. The abundance of the main monomers **1**−**4** originating from spruce lignin is given in Table 2; the relative abundance of C−C-linked oligomers can be estimated by HP-SEC analysis (see Experimental Procedures).

**Table 3. Relative Content of Monomers in Oxidized Samples**

| | lignin oxidized in solvent medium (% control experiment)[b] | | | EFW oxidized in solvent or water medium (% control experiment)[b] | |
|---|---|---|---|---|---|
| monomer | expt 1[a] | expt 2[a] | expt 3[a] | expt 4[a] | expt 5[a] |
| guaiacyl | 9 ± 0.4 | 21 ± 1.4 | 38 ± 1 | 68 ± 10 | 66 ± 9 |
| 3,4-dihydroxybenzyl | 164 ± 14 | 178 ± 7 | 192 ± 4 | 111 ± 3 | 120 ± 11 |
| vanillin | 66 ± 3 | 110 ± 5 | 110 ± 5 | nd[c] | nd |
| vanillic acid | 128 ± 7 | 95 ± 15 | 106 ± 13 | nd | nd |

[a] See Table 1 for details. [b] In molar percentage of each monomer in respective control experiments performed with peroxide but without Fe-porphyrin. [c] nd, not determined.

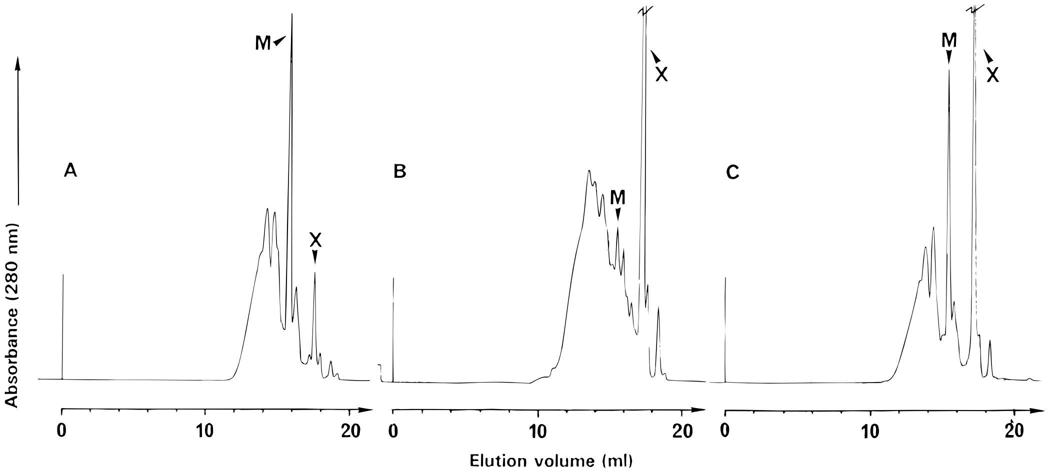

**Figure 5.** HP-SEC chromatograms of thioacidolysis products recovered from nonoxidized MWL and EFW (A) and from MWL and EFW oxidized by 250 μM Fe(TF$_5$PS$_4$P) with a total amount of 100 mM H$_2$O$_2$ (B and C, respectively). Arrow M indicates the elution volume of purified G−CHSEt−CHSEt−CH$_2$SEt. Arrow X is an unknown compound; see text. Polystyrenes of MW = 4000, 2000, and 800 are eluted in 10.6, 12, and 13.8 mL, respectively; syringaldazine (MW = 346) and guaiacol (MW = 124) eluted in 15 and 17.4 mL, respectively.

The third point shown in this study was the formation by Fe(TF$_5$PS$_4$P) of vanillin or vanillic acid structures in the polymer, as evidenced by thioacidolysis. The formation of such compounds indicates unambiguously that side-chain cleavage of guaiacyl units in lignin has occurred (see Figure 4 and Table 2). However, the amount of vanillic structures formed under our experimental conditions was always low. From Tables 2 and 3, it can be determined that the amount of these newly formed structures never represents more than 2% of the total monomers released by thioacidolysis of sound lignins. In all cases, reversed phase HPLC analysis indicated that vanillin was still not released in the reaction medium as a free soluble molecule. As proposed previously for muconates, a further degradation of these structures by the catalyst may occur. Our results also pointed out that only the lowest amount of catalyst used here led to some vanillin formation. With higher amounts of catalyst (0.36 mg/mL), the degradation of ≈100 nmol of vanillin was concomitant with the appearance of ≈65 nmol of vanillic acid, suggesting that a partial conversion of vanillin into the acidic derivative could have occurred.

In addition to chemical modifications, the macromolecular properties of MWL were also dramatically changed during Fe($TF_5PS_4P$) oxidation in the presence of $H_2O_2$. The progressive enrichment of lignin in molecules of high hydrodynamic volume during catalysis suggested that repolymerization occured at this time. This was further demonstrated by HP-SEC analysis of the products released after thioacidolysis. Indeed, the proportion of molecules corresponding to C−C-linked dimers, trimers, and oligomers (MW > 700) was qualitatively higher in oxidized lignins than in control experiments. We therefore propose that formation of new biphenyl bonds by phenoxy radical coupling was catalyzed by Fe($TF_5PS_4P$). Such repolymerization is also frequently reported to occur with fungal peroxidases (LiP, MnP) (Wariishi et al., 1991; Kurek and Monties, 1994) and could also directly explain the strong decrease after oxidation of the lignin content in guaiacyl units linked by *β*-*O*-4 bonds. However, further partial depolymerization and degradation occurred as molecules of lower hydrodynamic volumes were also formed at the end of catalysis. It is likely that the progressive depletion through repolymerization of the highly reactive phenolic structures is followed by a second degradation stage, maybe involving the aromatic ring cleavages and demethylation reactions evidenced in this study. The different sets of reactions that could be catalyzed by the porphyrin during time would thus depend on the chemical structures available for oxidation within the polymer.

The oxidation of MWL by Fe($TF_5PS_4P$)/$H_2O_2$ was shown to be very efficient in our experimental conditions. However, lignin degradation was performed in homogeneous phase in organic solvent, a condition obviously not suitable for the various applications proposed in the pulp and paper industry. Therefore, the demonstration here that lignin could be oxidized *in situ* within extractive free spruce wood in dioxane/water or pure water and with comparable efficiency is of utmost importance in view of further technological uses. The results obtained have clearly shown that the oxidized species generated from Fe($TF_5PS_4P$)/$H_2O_2$ couple were able (i) to penetrate into the porous structure of EFW and (ii) to degrade lignin in a way similar to that observed for MWL. According to the particular constraints of catalysis in solid media, the overall efficiency of lignin degradation in EFW was lower than that obtained in homogeneous phase. Nevertheless, a particular aspect of lignin degradation in EFW was that the macromolecule was not repolymerized by Fe($TF_5PS_4P$), as shown by the HP-SEC profile of the thioacidolysis products obtained after reaction. This suggested that secondary C−C coupling did not occur, probably due to immobilization of lignin radicals within the cell wall structure. This absence of repolymerization during catalysis was never obtained either with fungal LiP or with Fe($TF_5PS_4P$) acting on isolated lignins (B. Kurek, unpublished results). Lignin was also never reported to be oxidized in EFW by LiP (Odier and Artaud, 1991; Kurek, 1992), even in the presence of veratryl alcohol, frequently proposed in the literature to act as a diffusing mediator during enzymatic catalysis (Harvey et al., 1986).

## CONCLUSION

This study demonstrated the high efficiency and the high oxidizing power of Fe($TF_5PS_4P$) catalyst associated with $H_2O_2$ for the degradation of natural lignin in homogeneous conditions. A detailed chemical characterization of this lignin oxidized by different methods pointed out that the Fe($TF_5PS_4P$)/$H_2O_2$ system mimics quite well the LiP catalysis. Moreover, as in lignin degradation by LiP, lignin was first repolymerized before undergoing partial depolymerization and further degradation.

However, this water-soluble catalyst is more than a model for LiP, as strong degradation of lignin in extractive free wood was obtained in water, a result never obtained with LiP. This pointed out its high potential as a diffusible delignifying agent that could be used to bleach wood fibers in the pulp and paper industry under mild catalytic conditions.

## ABBREVIATIONS USED

tBuOOH, *tert*-butyl hydroperoxide; *m*-CPBA, *m*-chloroperoxybenzoic acid; MMPP, magnesium monoperoxyphthalate; Fe(TSPP), iron(III) *meso*-tetrakis(4-sulfonatophenyl)porphyrin; Fe(TDCSPP), iron(III) *meso*-tetrakis(2,6-dichloro-3-sulfonatophenyl)porphyrin; Fe($TDCSPCl_8P$), iron(III) *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)-*β*-octachloroporphyrin; Fe($TF_5PS_4P$), *meso*-tetrakis(pentafluorophenyl)-*β*-tetrasulfonatoporphyrin.

## LITERATURE CITED

Artaud, I.; Ben-Aziza, K.; Chopard, C.; Mansuy, D. A new easy access to quinones from iron porphyrin-catalyzed oxidation of methoxyarenes by magnesium monoperoxyphthalate. *J. Chem. Soc., Chem. Commun.* **1991**, 31−33.

Artaud, I.; Ben-Aziza, K.; Mansuy, D. Iron porphyrin-catalyzed oxidation of dimethoxyarenes: a discussion of the different reactions involved and the competition between the formation of methoxyquinones or muconic dimethyl esters. *J. Org. Chem.* **1993,** *58,* 3373−3380.

Bell, S. E. J.; Cook, P. R.; Inchley, P.; Leanord, D. R.; Lindsay-Smith, J. R.; Robbins, A. Oxoiron(IV) porphyrins derived from charged iron(III) tetraarylporphyrins and chemical oxidants in aqueous and methanolic solutions. *J. Chem. Soc., Perkin Trans.* **1991**, 549−559.

Cui, F.; Wijesekera, T.; Dolphin, D.; Farrell, R.; Skerer, P. Biomimetic degradation of lignin. *J. Biotechnol.* **1993,** *30,* 15−26.

Dolphin, D. H.; Nakano, T.; Kirk, T. K.; Maione, T. E.; Farrell, R. L.; Wijesekera, T. *Pat. PCT Int. Appl.* WO 88/07988, 1988.

Harvey, P. J.; Schoemaker, H. E.; Palmer, J. M. Veratryl alcohol as a mediator and the role of radical cation in lignin biodegradation. *FEBS Lett.* **1986**, *195*, 242−246.

Hattori, T.; Shimada, M.; Umezawa, T.; Higuchi, T.; Leisola, M. S. A.; Fiechter, A. New mechanism for oxygenative ring cleavage of 3,4-dimethoxybenzyl alcohol catalyzed by the ligninase model. *Agric. Biol. Chem.* **1988**, *52*, 879−880.

Hergert, H. L. In *Lignins. Occurence, Formation, Structure and Reactions*; Sarkanen, K. V., Ludwig, C. H., Eds.; Wiley-Interscience: New York, 1971; pp 43−94.

Kurek, B. In *Plant peroxidases 1980−1990*; topics and detailed literature on molecular, biochemical and physiological aspects; Penel, C., Gaspar, Th., Greppin, H., Eds.; University of Geneva, 1992; pp 139−186.

Kurek, B.; Monties, B. Oxidation of spruce lignin by fungal lignin peroxidase and horseradish peroxidase: comparison of their action on the molecular structure of the polymer in colloidal solution. *Enzyme Microb. Technol.* **1994**, *16,* 125−130.

Labat, G.; Meunier, B. Factors controlling the reactivity of a ligninase model based on the association of potassium monopersulfate to manganese and iron porphyrin complexes. *J. Org. Chem.* **1989**, *54,* 5008−5011.

Lapierre, C.; Monties, B.; Rolando, C. Thioacidolysis of poplar lignins: identification of monomeric syringyl products and characterization of guaiacyl-syringyl lignin fraction. *Holzforschung* **1986**, *40*, 113−118.

Lundquist, K. NMR studies of lignins. 5. Investigation on non-derivatized spruce and birch lignin by $^1$H-NMR spectroscopy. *Acta Chem. Scand.* **1981**, *B35*, 497−501.

Meunier, B. In *Metalloporphyrins in catalytic oxidations*; Sheldon, R. A., Ed.; Dekker: New York, 1994; pp 133−155.

Odier, E.; Artaud, I. In *Microbial Degradation of Natural Products*; Winkelmann, G., Ed.; VCH: Weinheim, 1991; pp 162−191.

Paszczynski, A.; Crawford, R. L.; Blanchette, R. A. Delignification of wood chips and pulps by using natural and synthetic porphyrins: model of fungal decay. *Appl. Environ. Microbiol.* **1988**, *54*, 62−68.

Pettersson, B.; Yang, J. L.; Eriksson, K. E. Biotechnical approaches to pulp bleaching. *Nordic Pulp Paper Res. J.* **1988**, *4*, 198−202.

Rolando, C.; Monties, B.; Lapierre, C. In *Methods in Lignin Chemistry*; Lin, S. Y., Dence, C. W., Eds.; Springer-Verlag: Berlin, 1992; pp 334−349.

Shimada, M. Biomimetic chemistry in lignin degradation. *Mokuzai Gakkaishi* **1991**, *37* (12), 1103−1114.

Shimada, M.; Habe, T.; Higuchi, T.; Okamoto, T.; Panjipan, B. Biomimetic approach to lignin degradation. II. The mechanisms of oxidative C-C bond cleavage reaction of lignin model compounds with natural iron(III) porphyrinchloride as a heme-enzyme model system. *Holzforschung* **1987a**, *41*, 277−285.

Shimada, M.; Hattori, T.; Umezawa, T.; Higuchi, T.; Okamoto, T. In *Lignin Enzymic and Microbial Degradation*; Odier, E., Ed; INRA: Paris, 1987b; pp 151−156.

Skerer, P. S.; Farrell, R. L.; Dolphin, D.; Cui, F.; Wijesekera, T. In *Biotechnology in the Pulp and Paper Manufacture*; Kirk, T. K., Chang, H. M., Eds.; Butterworth-Heinemann: Stoneham, MA, 1990; pp 203−210.

Srebotnik, E.; Messner, K. In *Biotechnology in the Pulp and Paper Manufacture*; Kirk, T. K., Chang; H. M., Eds.; Butterworth-Heinemann: Stoneham, MA, 1990; pp 111−119.

Suckling, I. D.; Pasco, M. F.; Hortling, B.; Sundquist, J. Assessment of lignin condensation by GPC analysis of lignin thioacidolysis products. *Holzforschung* **1994**, *48*, 501−503.

Vogel, A.; Furniss, B. S.; Hannaford, A. J.; Rogers, V.; Smith, P. W. G.; Tatchell, A. R. In *Textbook of Practical Organic Chemistry*, 4th ed.; Longman Group Limited: London, England, 1978; pp 1−24.

Wariishi, H.; Valli, K.; Gold, M. H. *In vitro* depolymerization of lignin by manganese peroxidase of *Phanerochaete chrysosporium*. *Biochem. Biophys. Res. Commun.* **1991**, *176* (1), 269−275.

Wood, J. M.; Folsom, B. R.; Eudy, W. E. Biomimetic oxidation of a lignin model compound by substituted porphyrins. *Proc. 3rd Int. Conf. Biotechnol. Pulp Paper Ind.* **1986**, 31−35.

Received for review September 13, 1995. Revised manuscript received February 20, 1996. Accepted April 26, 1996.⊗

JF950612G

---

⊗ Abstract published in *Advance ACS Abstracts,* June 1, 1996.