MnO_2 and Oxalate: An Abiotic Route for the Oxidation of Aromatic Components in Wheat Straw

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Oxidants formed through the interaction between MnO_2 and oxalate accumulated in rotten wood have been evaluated as abiotic agents possibly involved in wheat straw ligninolysis. The hemicellulose and cellulose content of straw remained unchanged, and no release of free soluble materials from lignin or polysaccharides could be evidenced. Structural analysis of oxidized lignin in situ has revealed up to 30% and 10% decrease of β-O-4 linked Guaiacyl and Syringyl units, respectively. Lignin phenolic moieties only were directly oxidized by MnO_2/oxalate as no structural alteration was observed within extensively permethylated wheat straw. Modifications were also evidenced at the level of the cell wall linked cinnamic acids present in wheat straw. Esterified phenolic acids were more readily oxidized by Mn complexes than ethers analogues, and disappearance of ferulic moieties was always more pronounced than that of *p*-coumaric acids. The abiotic Mn oxidants generated from MnO_2 and oxalate may therefore significantly contribute to the decay and humification process of lignocellulosic material in Nature.

Keywords: *Manganese dioxide; oxalate; abiotic oxidation; wheat straw; lignin; cinnamic acid; hemicellulose; cellulose*

INTRODUCTION

Although the role of enzymatically generated manganese III in the biological degradation of lignin has been extensively studied, the only species identified so far in decayed wood is Mn(IV)O_2 (Daniel and Bergman, 1997; Blanchette, 1984; Glenn and Gold, 1985; Glenn et al., 1986; Wariishi, 1991). We have recently demonstrated that the interaction between solid MnO_2, accumulated in rotted wood and oxalic acid, a metabolite produced by wood-rotting fungi, results in the formation of Mn complexes able to oxidize lignin within poplar sawdust (Hames and Kurek, 1997). Such abiotic reactions in ligninolysis are important as they may provide a natural treatment process of the lignified material which could occur in wood as well as in soil, prior to or in complement of microbial and enzymatic decay.

The purpose of the present study was to investigate the effect of the chelate(s) formed by the interaction of MnO_2 and oxalate on the macromolecular components of wheat straw within the cell wall. The modifications undergone by lignin, cinnamic acids, cellulose, and hemicellulose in wheat straw are presented, and the alteration of the cross links between the different cell wall components are discussed.

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EXPERIMENTAL PROCEDURES

Plant Material and Substrates. Wheat straws (*Triticum aestivum* sp.) were harvested by hand at full maturity and air-dried. Internodes were separated, collected, and reduced into ~2 mm particle size. The substrate was homogenized by a short time ball milling before oxidation. Permethylation of in situ lignin was carried out by diazomethane generated from Diazald (Sigma Aldrich) (Lapierre et al., 1988).

Chemicals. Activated MnO_2 (85%; particle size <5 μm Aldrich (France). Other reagents and solvents used were of analytical grade.

Oxidative Treatment with MnO_2. Two hundred milligrams of straw (21.0 ± 1.3% Klason lignin content) were incubated for 20 h with stirring at room temperature (20−25 °C) in 20 mL of Na-oxalate buffer containing MnO_2. Three reaction conditions were studied. **A**: 100 mM oxalate buffer pH 2.5; MnO_2 50 mM. **B**: 20 mM oxalate buffer pH 2.5; MnO_2 10 mM. **C**: 2 mM oxalate buffer pH 2.5; MnO_2 1 mM.

The control sample consisted of wheat straw incubated in 20 mL of 100 mM oxalate buffer pH 2.5.

At the end of the reaction period, the straw was recovered by vacuum filtration and washed (i) with oxalate buffer (100 mM pH 2.5) to remove black MnO_2 deposits and (ii) with hot water to remove white precipitates which were formed during the reaction. The samples were freeze-dried before chemical analysis.

Chemical Analysis. *Determination of Mass Variation in Oxidized Samples.* Mass variation generated by incomplete removal of Mn- and oxalate-precipitates in oxidized samples after washing was determined with reference to the starting material incubated in oxalate buffer alone (control sample). For this purpose, wheat straw was recovered quantitatively on a previously weighed Gooch crucible and washed with oxalate and water, as described above. The sample was then freeze-dried in the crucible and weighed. In this way, the mass ratio of precipitate contaminating the lignocellulosic material in the oxidized sample was determined. This value was then

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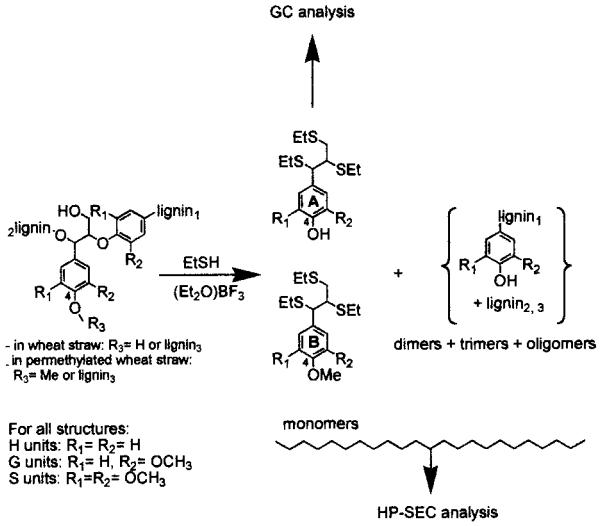

Figure 1. Lignin depolymerization by thioacidolysis and strategy for product analysis. In original lignins (R_3 = H or lignin$_3$), only monomers with ring type A are released by thioacidolysis. In lignins which have been permethylated within wheat straw (R_3 = Me or lignin$_3$), monomers with ring type A are released from 4-O ether linked internal structures (R_3 = lignin$_3$), whereas monomers with ring type B are released exclusively from methylated structures (R_3 = Me), originally bearing free 4-OH group.

used as a correction factor for all subsequent calculations of the lignin, polysaccharide, and cinnamic acid content in wheat straw.

Lignin Content. The Klason lignin content was estimated according to a procedure similar to that described by Effland (Effland, 1977). The wheat straw (100 mg) was treated with 72% (w/v) H_2SO_4 at 20 °C for 2 h, followed by a 5% (w/v) H_2SO_4 hydrolysis under reflux for 3 h. The acid insoluble fraction was recovered by filtration on glass fiber in a Gooch crucible and weighed after 16 h drying at 110 °C. The ash content in this fraction was determined after mineralization (3 h, 600 °C). The lignin fraction is accounted as the acid insoluble fraction depleted from its ashes.

Lignin Characterization. The lignin content in β-O-4 ether linked units was determined by the thioacidolysis method as described by Rolando et al. (1992) (Figure 1). The analysis was performed on a 10 mg sample with 2 mL of [EtSH/$BF_3(Et_2O)$] reactant per mg of straw; *n*-docosane in CH_2Cl_2 was chosen as internal standard.

The main monomers released were separated by capillary gas chromatography as trimethylsilyl (TMS) derivatives on a 30 m × 0.32 mm column SPB1-M (Supelco, France), using a temperature gradient of 160−260 °C at 2 °C/min, with He as the carrier gas (0.5 bar) (Rolando et al., 1992). Products were detected by flame ionization.

The depletion of Guaiacyl (G) and Syringyl (S) monomers in the oxidized sample was determined as the percentage loss of each structures relative to the control sample.

The lignin content in C−C linked structures of the thioacidolysis products was estimated qualitatively by high performance size exclusion chromatography (HP−SEC) analysis on a 600 × 7 mm polystyrene-divinylbenzene column (100A PL-gel, Polymer Lab) (Figure 1). Tetrahydrofuran was used as the mobile phase (1 mL/min) at room temperature (Suckling et al., 1994; Kurek et al., 1996). Detection of products was performed spectrophotometrically at 280 nm.

Phenolic Acid Analysis. The content in ester and ether linked *p*-coumaric (pCA) and ferulic (FA) acids was determined on 40 mg of wheat straw sample after successive alkaline hydrolysis and acidolysis, respectively, according to Scalbert (Scalbert et al., 1985). Total cinnamic acids were determined after strong alkaline hydrolysis at 170 °C on 40 mg of sample (Iiyama et al., 1990). 3,4,5-Trimethoxycinnamic acid was used as an internal standard.

Free cinnamic acids were extracted by diethyl ether, evaporated to dryness, and recovered in 1 mL of methanol. Analysis was performed by HPLC on a Lichrospher 100RP-18, 5 μm, 250 × 4 mm column (Merck). Separation was obtained with a linear gradient from 10% to 40% CH_3CN in [H_2O:H_3PO_4, 1000:1] at a flow rate of 1 mL/min. Detection was monitored at 280 and 313 nm.

The degradation extent is expressed as the percentage loss of esterified and etherified pCA and FA structures relative to a nonoxidized reference control sample.

Sugar Analysis. Hydrolysis of wheat straw polysaccharides was performed according to Blakeney et al. (1983) on 10 mg of samples. Fucose was added as an internal standard just after the prehydrolysis step. Hydrolysis was performed 2 h at 100 °C. After reaction, the samples were diluted 50-fold, filtered (0.45 μm, Millipore, France), and injected to a carboPac PA1 anion-exchange column (4 × 250 mm, Dionex). Monosaccharides were separated in 4 mM NaOH at a flow rate of 1 mL/min. Detection was performed by pulsed amperometry (PAD 2, Dionex) after mixing of eluant with 300 mM NaOH (1 mL/min) in a postcolumn reactor.

Analysis of Extractives. Low molecular weight aromatic substances which could be released from lignin or cinnamic acids in the reaction mixture were tentatively extracted by 3 volumes of ethyl acetate after acidification of the reaction medium with HCl to pH 1. Extracts were concentrated to dryness and dissolved in 1 mL of methanol or tetrahydrofuran (THF). The analyses were performed by HP−SEC or reverse-phase HPLC as described above.

The release of low molecular weight sugars and oligosaccharides in solution was checked by direct injection of 100 μL to 500 μL of the reaction medium to a carbopac PA1 column (Dionex) in the condition described above.

RESULTS

Reaction between MnO_2, Oxalate, and Wheat Straw. The black MnO_2 powder mixed to the oxalate and wheat straw suspension was rapidly solubilized with appearance of a dark red solution. Simultaneously, a gentle bubbling could be observed. As the reaction proceeded, the pH began to increase from 2.5 up to 3, 6, or 7, depending on the reaction conditions used (**C**, **B**, or **A**, respectively). No pH increase was detected in the control sample. After about 1 h of reaction, a black precipitate began to form and to accumulate. Addition of fresh oxalate buffer at this point will cause the black solids to redissolve with the return of the characteristic red color.

Sample Weight Increase during the Reaction. Weight variation in each oxidized sample due to incomplete removal of precipitated low valent Mn species was determined relative to the control. No variations were detected when wheat straw was oxidized in medium **B** and **C**. In medium **A** (high manganese and oxalate concentrations), a weight increase up to 11% of the control sample was sometimes observed. The proportion of Mn precipitate in the lignocellulosic material was then taken into account for the quantitative and accurate determination of the content of cell wall components.

Analysis of Extractibles. Analysis by HP−SEC and reverse phase HPLC of ethyl acetate extracts of the reaction medium did not reveal any significant release of substances absorbing at 280 nm from the oxidized sample. Similarly, only traces of soluble mono- or oligosaccharides could be detected in the reaction medium of either oxidized or control wheat straw.

Chemical Modifications Introduced by MnO_2/Oxalate in Wheat Straw. *Lignin Content.* The con-

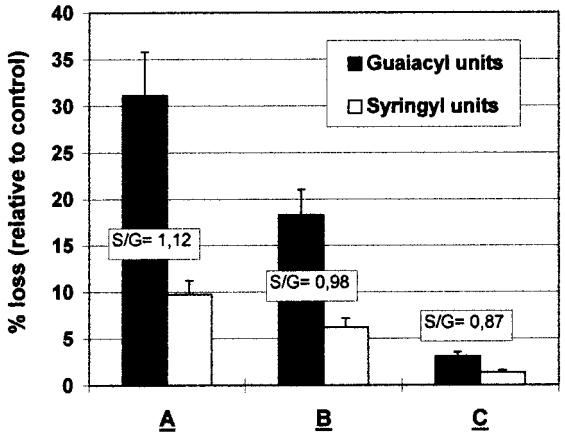

Figure 2. Disappearance of β-O-4 linked units in lignins oxidized in situ. Reaction conditions: **A**: 50 mM MnO_2, 100 mM oxalate buffer pH 2.5; **B**: 10 mM MnO_2, 20 mM oxalate buffer pH 2.5; **C**: 1 mM MnO_2, 2 mM oxalate buffer pH 2.5; lignin content of β-O-4 linked units in the control sample (μmol/g sample): $G = 136.4 \pm 3.8$, $S = 116.5 \pm 3.1$; S/G ratio = 0.85.

Table 1. Yields of Degradation Products Issued from Thioacidolysis of Lignins Permethylated within Wheat Straw before Oxidation by MnO_2/Oxalate

	G^a	MeG^a	S^a	MeS^a
control - oxalate 100 mM, pH 2.5	85.9 ± 1.4	47.3 ± 0.5	121.9 ± 2.8	3.7 ± 0.1
oxidized by MnO_2 50 mM, oxalate 100 mM pH 2.5 (**A**)	84.8 ± 0.5	46.3 ± 0.1	114.5 ± 0.2	3.8 ± 0.1

[a] In μmol/g of sample; G, S = released Guaiacyl and Syringyl structures, respectively, originally engaged in 4-O ether bonds; MeG, MeS = released methylated Guaiacyl and Syringyl monomers, respectively, with originally free 4-OH group—see also Figure 1.

tent of wheat straw in Klason lignin (21.0 ± 1.3%) was not affected by any of the oxidative treatments **A**, **B**, **or C**.

Analysis of the β-O-4 Linked Fraction of Lignin. The effect of MnO_2/oxalate on the β-O-4 bonding pattern of lignin is shown in Figure 2. The decrease in the lignin content in ether-linked guaiacyl (G) and syringyl (S) units and the corresponding increase of the S/G ratio were more pronounced when higher manganese dioxide/oxalate concentrations were employed. When permethylated wheat straw was used as the MnO_2/oxalate substrate, no loss of β-O-4 linked structures occurred (Table 1).

Analysis of the C−C Linked Fraction of Lignin. The thioacidolysis reaction depolymerizes specifically lignins at the level of the alkyl−aryl ether bonds, thus releasing a mixture of monomeric compounds and C−C linked oligomeric structures, as exemplified in Figure 1. Analysis of the molecular size distribution of the thioacidolysis products recovered from sample oxidized in condition **A** revealed a slightly higher amount of new C−C linked oligomeric structures (marked **N**, Figure 3) with higher

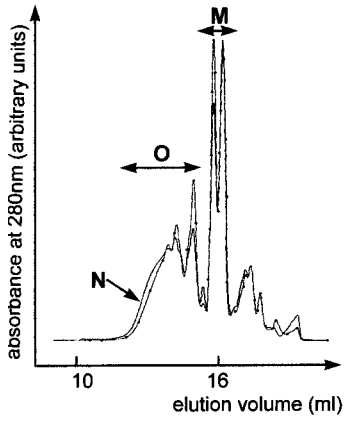

Figure 3. HP−SEC chromatograms of the thioacidolysis products recovered from control wheat straw (•—•) and wheat straw oxidized in condition **A** (—); M = elution volume of thioethylated monomers (MW ~ 350); O = elution volume of dimers to oligomers; N = new oligomeric products. See also Figure 1.

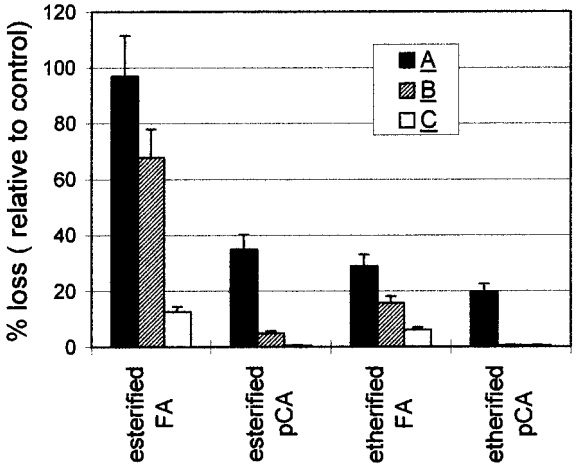

Figure 4. Effect of MnO_2/oxalate on wheat straw content in ferulic (FA) and *p*-coumaric (pCA) acids. **A**, **B**, and **C**: reaction conditions described in Figure 2; Cinnamic acids content in the control sample (μmol/g) esterified FA = 14.6 ± 1.9; esterified pCA = 21.9 ± 0.5; etherified FA = 14.6 ± 1.9; etherified pCA = 4.0 ± 0.5.

hydrodynamic volumes, as compared to the control sample. In contrast, elution profiles of thioacidolysis products recovered from samples oxidized in condition **B** and **C** were identical to those of the nonoxidized control samples (data not shown).

Cinnamic Acids. Table 2 shows the extent of total cinnamic acids depletion after MnO_2/oxalate catalysis in condition **A**. Irrespective of the protocol used for analysis (sequential or strong alkaline hydrolysis), the decrease in ferulic acid content was greater than that of the coumaric acid.

The results in Figure 4 not only confirm this feature but also show that depletion of ester bonded cinnamic acids was higher than that of ester analogues in MnO_2/

Table 2. Determination of Total Cinnamic Acid Content of Wheat Straw Cell Wall by Sequential Hydrolysis and Alkaline Hydrolysis at 170 °C

	sequential hydrolysis		alkaline hydrolysis at 170 °C	
	control^a	oxidized^b	control^a	oxidized^b
pCA (μmol/g of sample)	48.2 ± 3^c	28.1 ± 7.2^c (41.6)^d	41.8 ± 0.8	37.6 ± 4.4 (10.0)
FA (μmol/g of sample)	29.4 ± 1.9^c	11.2 ± 0.9^c (62.0)	36.9 ± 0.5	22.1 ± 3.9 (40.1)

[a] Sample incubated in 100 mM oxalate pH = 2.5. [b] Oxidized in condition **A**. [c] Sum of ethers + esters. [d] Percent loss relative to control in brackets.

Table 3. Content in Monomeric Sugars of Wheat Straw Cell Walls Oxidized by MnO_2 and Oxalate[a]

	glucose	xylose	arabinose	mannose
control - oxalate 100 mM, pH 2.5	396.1 ± 0.2	242.9 ± 1.8	20.8 ± 0.5	tr[b]
oxidized by MnO_2 50 mM, oxalate 100 mM pH 2.5 (**A**)	402. ± 4.7	243.9 ± 7.3	21.1 ± 0.2	tr
oxidized by MnO_2 10 mM, oxalate 20 mM pH 2.5 (**B**)	421.3 ± 2.4	257.2 ± 0.8	21.7 ± 0.2	tr
oxidized by MnO_2 1 mM, oxalate 2 mM pH 2.5 (**C**)	409.8 ± 2.7	253.0 ± 0.5	20.7 ± 0.4	tr

[a] In mg/g of sample. [b] Traces (<5 mg/g).

oxalate treated samples. This effect was even greater for the highest concentration of catalyst used.

Hemicellulosic and Cellulosic Components. The composition of wheat straw cellulose and hemicellulose in glucose, xylose, arabinose, and mannose released by acid hydrolysis did not reveal any significative variation between control and oxidized samples (Table 3).

DISCUSSION

Reaction between MnO_2 and Oxalate. Interaction between solid MnO_2 and oxalate may generate soluble Mn chelates at low pH (Chrétien et al., 1960; Xyla et al., 1992). Considering the complexity of Mn chemistry, it is likely that many different high valencies Mn/oxalate chelates as well as binuclear Mn(III)/Mn(IV) complexes transiently coexist in solution (Donne et al., 1997; Fatiadi, 1976).

The pH increase, the concomitant bubbling, and the colors changes of the reaction media observed during oxidation of wheat straw is consistent with the progressive reductive dissolution of Mn(IV) to Mn(II) by oxalic acid, which is concomitantly decarboxylated (Xyla et al., 1992; Perezbenito et al., 1996). The pH increase during reaction could thus be related to the formation of a mixed oxalate/carbonate buffer, due to CO_2 solubilization in the medium.

As the reaction proceeded, black solid particles precipitated in the medium. These are probably composed of Mn(IV) species formed by the dismutation of Mn(III). However, the black material may also contain insoluble Mn(III) intermediates (MnOOH) as well as MnO and MnOH species (Chrétien et al., 1960). Nevertheless, it was possible to recycle this material for catalysis simply by adding fresh oxalate solution, suggesting a pH and chelate control of the solubilization/precipitation process of Mn species. With this respect, the weight increase sometimes evidenced in oxidized samples may be a result of this process. Mn species which have first permeated the straw could then reprecipitate inside the cell wall. The formation of calcium oxalate precipitates is also likely to occur in situ.

Chemical Modification of Wheat Straw by MnO_2/Oxalate. *Specificity for Aromatics.* The oxidants generated by the interaction between MnO_2 and oxalate were shown to specifically oxidize the aromatic components in wheat straw. Indeed, a strong modification of the lignin bonding pattern as well as an extensive depletion of etherified and esterified cinnamic acids occurred. In contrast, hemicellulose and cellulose remained apparently unmodified.

However, partial degradation without release of free sugars cannot be ruled out since oxalic acid at low pH is able to hydrolyze cellulose and hemicellulose, albeit over a long period time (Shimada et al., 1991, 1994). Since the pH of the oxalate buffer increases as a function of time, polysaccharide hydrolysis, if any, should only take place at the beginning of the reaction.

Modification of Cell Wall Aromatics. The MnO_2/oxalate system studied here cannot be described as a true delignifying system since no soluble aromatics were released during oxidation and the content in Klason lignin remained unchanged. However major modifications of aromatic components in cell wall have been introduced.

Lignin. The decrease of the lignin content in β-O-4 linked monomers can be explained by two main types of structural modifications catalyzed by MnO_2/oxalate: (i) alteration of the lignin bonding pattern by various reactions such as Cα−Cβ-, alkyl−aryl-, ether-bond-cleavages as well as by coupling reactions forming new C−C linked structures and (ii) oxidation of the G and S lignin monomers into new products such as aldehydes, acids, α-ketones, and muconates. All these reactions were already shown to be catalyzed by ligninolytic enzymes and related biomimetic systems on model compounds (reviewed in Higuchi, 1989; Kirk and Farrell, 1987; Gold et al., 1989; Cui et al., 1990). In our case, the determination of the degradation pathways is more complex as macromolecular lignin is oxidized in situ (Kurek et al., 1998). Several important points could however be stressed for MnO_2/oxalate catalysis.

First, MnO_2 is known to specifically oxidize benzyl alcohols into the corresponding aldehydes (Endo et al., 1996; Fatiadi, 1976; Adler and Becker, 1961). This implies that lignin structures bearing benzylic hydroxyl group would be oxidized into the corresponding ketones, provided that MnO_2 is active during the reaction. However, analysis by GC/MS of the thioacidolysis products recovered from oxidized samples did not reveal any significant increase in Ar−CSEt=CHSEt monomers typically released during thioacidolysis from α-carbonylated G and S structures (Lapierre et al., 1995; Rolando et al., 1992; data not shown). This indicates (i) that α-ketone formation on lignin is not the main reaction catalyzed by these hitherto unidentified Mn(III) and/or Mn(IV) species and (ii) that solid MnO_2 may probably not participate directly to lignin oxidation.

Second, the MnO_2/oxalate system was inactive on lignin which has been extensively permethylated in situ. The presence of free phenols on lignin monomers is thus required for efficient MnO_2/oxalate catalysis. This also confirms that lignin attack may proceed through oxidation of the aromatic moiety, as described for Mn(III)-chelates generated enzymatically by Mn peroxidase but probably not through side-chain oxidation, as for solid MnO_2 catalysis (Fatiadi, 1976).

Third, HP−SEC analysis of the thioacidolysis products indicated a slight enrichment of lignin in C−C linked oligomers of higher hydrodynamic volume (Figure 3). This suggests that some condensation reactions and/or rearrangement occurred in situ, which may also explain to some extent the decrease in the recovery of β-O-4 linked monomers. A similar phenomenon is reported for isolated alkali lignin and spruce milled wood lignin (MWL) treated by lignin peroxidases and a synthetic porphyrin (Martinez-Inigo and Kurek, 1997; Kurek et al., 1996) but not when porphyrins or MnO_2/oxalate are oxidizing lignin within spruce or poplar wood sawdust (Hames and Kurek, 1997; Kurek et al., 1996).

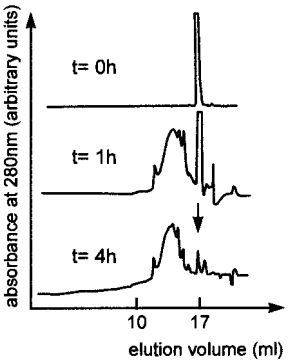

Figure 5. HP−SEC of product recovered from ferulic acid incubated with MnO_2/oxalate in conditions **A** (refer to Figure 2) during 0, 1, and 4 h; arrow: elution volume of ferulic acid (MW = 208).

Such differences between wood and wheat straw could be related (i) to the higher relative content of phenolic monomeric units in their lignins, compared to poplar or spruce (Lapierre et al., 1988; Lapierre and Rolando, 1988) and (ii) to the presence of phenolic acids in their cell wall. These latter structures were shown to be oxidized by MnO_2/oxalate and may therefore be involved in coupling reactions with lignin, as discussed below.

Cinnamic Acids. One peculiarity of graminaceous cell walls is the presence of cinnamic acids linked to lignin and/or polysaccharides. We have evidenced here for the first time different levels in the reactivity of cinnamic acids toward abiotic lignin oxidizing agents, considering their disappearance rate in oxidized wheat straw (Figure 4).

Indeed, ester linked phenolic acids were more reactive than ether linked ones, and ferulates were always prone to be oxidized, compared to coumarates. As no free phenolic acids have been detected in the reaction media, it is likely that oxidized cinnamates may be retained within the cell wall. Another hypothesis is that they may overreact with lignin, forming new structures which escape to classical chemical analyses. With this respect, ferulic acid was shown to be readily polymerized by MnO_2/oxalate in condition **A** (Figure 5), methyl ester of ferulate was dimerized (Wallace and Fry, 1995), and incorporation of ferulic or coumaric acid in synthetic lignin was proven to be effective during peroxidasic reaction (Jacquet et al., 1995; Ralph et al., 1994). It is therefore highly probable that new cinnamic acids—ferulate and/or coumarate-based polymeric material would be formed within the cell walls in the presence of MnO_2/oxalate, thus explaining the unique in situ lignin repolymerization observed here—see also discussion above.

The mechanism by which cinnamate esters and ethers are removed and/or recondensed remains to be determined. However, the cross-linking between polysaccharides and lignin should be significantly altered by MnO_2 oxalate treatment and should therefore change drastically the physical and mechanical properties of cell wall.

Reactivity of Aromatics toward MnO_2/Oxalate. The different studies on lignin structural modification catalyzed by ligninolytic enzymes and porphyrins have always revealed a preferential degradation of β-O-4 linked G monomers (Martinez-Inigo and Kurek, 1997; Kurek et al., 1998). This was also the case for wood and wheat straw samples treated by MnO_2/oxalate (this study and Hames and Kurek, 1997). This general phenomenon seems to be related to the phenolic nature of G domain in lignins, compared to the S domain (Kurek et al., 1998).

Our data also demonstrated the different reactivity of phenolic acids toward Mn oxidants. The regiochemistry of the cinnamic acids would be important in determining their oxidation rate by Mn chelates. Indeed, the higher reactivity of esterified FA could be related to its phenolic nature, whereas esterifed pCA could be less reactive because of its preferential localization in S domains, only slightly oxidized by Mn/oxalate (Grabber et al., 1996; Chabbert et al., 1994). Finally, the etherified fraction of cinnamic acids is nonphenolic and therefore would be more difficult to oxidize by Mn. Their degradation could then only reflect a secondary event related to the modification of lignin and/or polysaccharides by MnO_2/oxalate. Complementary studies are required to delineate the complex process that takes place during Mn/oxalate catalysis, and model compounds such as cinnamates-containing synthetic lignins (DHP) would be useful tools for such purpose (Jacquet et al., 1995; Ralph et al., 1994; Terashima et al., 1995).

On the Significance of Abiotic Oxidations in Lignocellulose Degradation. The action of low molecular weight oxidants generated by MnO_2 and oxalate on in situ wheat straw lignin exhibited similar features to those encountered with ligninolytic enzymes acting on the isolated macromolecule (Martinez-Inigo and Kurek, 1997). The catalysis was however different. Indeed, as demonstrated here for the first time, the (unknown) Mn(III) and/or -(IV) species were able to diffuse within cell wall and rearrange strongly the lignin and the cinnamic acids components, a result that was never obtained with enzymes in situ (C. Lequart and B. Kurek, unpublished). This observation precludes the involvement of enzymes in the early stage of lignin degradation process confirming that their catalysis occurs only where accessibility of wood is sufficient for protein diffusion (Blanchette, 1991).

With this respect, one can propose that abiotic oxidation of lignin through molecules already present in wood or accumulated by microorganisms such as MnO_2 and oxalate could be an efficient pretreatment step of the lignified cell wall. Indeed, either the chemical structure of lignin as well as the cross-linking between the cell wall macromolecular contituents have been altered. How far these modifications affect lignin reactivity and/or the physicochemical properties of cell wall for further microbial oxidation and degradation is currently not known and has to be established.

Finally, considering that MnO_2 as well as oxalate are commonly found in soil at pH values compatible with the reaction described in this study (Pedler et al., 1996; Bromfield and Skerman, 1949; Ghiorse, 1988), the involvement of abiotic Mn complexes in ligninolysis would perhaps not be restricted to wood decay. Indeed, the lignins activated into radical species by MnO_2/oxalate could be also involved in coupling reactions with other nucleophilic compounds formed during the degradation of organic material in soils, participating therefore directly in the more general process of humus formation (Blanchette, 1984; Daniel and Bergman, 1997; Shindo and Huang, 1982; Lovley et al., 1996).

CONCLUSION

The action of the oxidants generated by MnO_2 and oxalate on in situ wheat straw lignin were shown here

for the first time to specifically and strongly rearrange the lignin and the cinnamic acids components. These modifications at the level of the lignin cement and of the cross-linking agents in cell wall would have a strong impact on its physico chemical properties, suggesting that abiotic oxidation could be a significant step in the overall process of lignocellulose degradation. How microorganisms (bacteria and/or fungi) participate in the formation of Mn(IV) species in wood and soil and coordinate the action of such abiotic complexes and enzymes for efficient lignin degradation remains to be determined.

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