The Immobilized Porphyrin-Mediator System Mn(TMePyP)/clay/HBT (clay-PMS): A Lignin Peroxidase Biomimetic Catalyst in the Oxidation of Lignin and Lignin Model Compounds

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A biomimetic system for lignin peroxidase (LiP) was designed by using a cationic porphyrin, [Mn(TMePyP)OAc_5], supported on the smectitic clay montmorillonite [Mn(TMePyP)/clay]. The natural role of the polypeptidic pocket of LiP was mimicked by the clay. The possibility to use low-molecular-weight redox mediators as active readily diffusible oxidizing species has been investigated. This assembly — a sort of "synthetic enzyme" — can be defined as an immobilized porphyrin-mediator system (clay-PMS). The clay-PMS was found to be a stable, recyclable, and efficient catalyst for the environmentally friendly H_2O_2-catalyzed oxidation of different lignins and representative lignin model compounds. The clay-PMS showed a higher reactivity than Mn(TMePyP)/clay alone due to an effective role of the redox mediator on the oxidation.

Introduction

Lignin peroxidase (ligninase, LiP) is an enzyme isolated from ligninolytic cultures of *Phanerochaete chrysosporium*[1] that performs a one-electron oxidation of the lignin aromatic moieties in the presence of hydrogen peroxide.[2] The catalytic cycle consists of a two-electron oxidation of Fe^{III} protoporphyrin IX (high spin) to give a highly reactive (oxo)iron(IV)−protoporphyrin IX π-cation radical, the LiP compound I.[3] LiP compound I is then reduced to the initial state by two different one-electron reductions by the substrate (Figure 1).[4]

In nature, the ligninolitic activity of LiP is enhanced by the presence of veratryl alcohol (VA), which acts as a diffusible redox mediator in lignin oxidation.[5] Thus, the oxidative potential can be transferred from the enzyme to the bulk of the polymer avoiding a kinetic barrier for the heterogeneous reaction.

Unfortunately, the use of LiP for delignification processes is not economically convenient with respect to inorganic catalysts, mainly due to the costs of enzyme purification. Moreover, LiP is inactivated when exposed to an excess of hydrogen peroxide (more than 20 equiv.), to give the inactive form LiP III (Figure 1).[6] Novel LiP biomimetic catalysts resistant to peroxide inactivation might be of pivotal importance for the design of environmentally friendly and economically feasible lignin degradation processes.

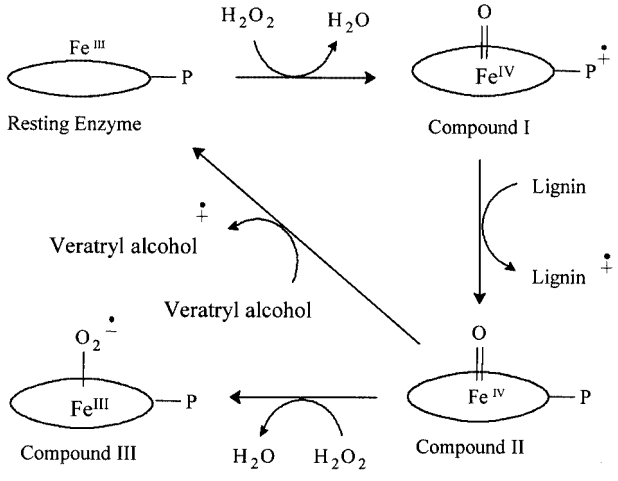

Figure 1. Catalytic cycle of lignin peroxidase

Metalloporphyrins are well-known biomimetic model compounds for protoporphyrin IX and have been extensively studied in the oxidative degradation of monomeric, dimeric, and oligomeric lignin model compounds.[7] The design of heterogeneous porphyrin systems that might be used in several cycles of transformations is a crucial point for their industrial application. In this context, a heterogenation technique based on the anchorage of the active catalyst onto appropriate inert and low-cost supports appears to be highly promising.[8] Smectite clay minerals, such as montmorillonite, have been used as support for the cationic manganese *meso*-tetrakis(tetramethylpyridinio)porphyrin pentaacetate [Mn(TMePyP)$(OAc)_5$] by use of ion-exchange methods.[9] This heterogeneous catalyst [named Mn(TMe-

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PyP)/clay] is highly efficient in the oxidation of monomeric and dimeric lignin model compounds. Nevertheless, it is not suitable for lignin oxidation mainly due the presence of a kinetic barrier to the approach of the polymeric substrate.[10] With the aim of solving this problem, we report here our results on the oxidation of lignins and lignin model compounds with the Mn(TMePyP)/clay system in the presence of veratryl alcohol (VA) and 1-hydroxybenzotriazole (HBT) as low-molecular-weight redox mediators. This novel biomimetic system of LiP — a clay/porphyrin mediator system (clay-PMS) — was able to efficiently degrade lignin model compounds resembling the fundamental bonding pattern in lignin. The degradation of milled wood and technical softwood lignins, carefully studied by ^{31}P NMR spectroscopy after phosphitylation of the oxidized samples, was also effective.[11]

Results and Discussion

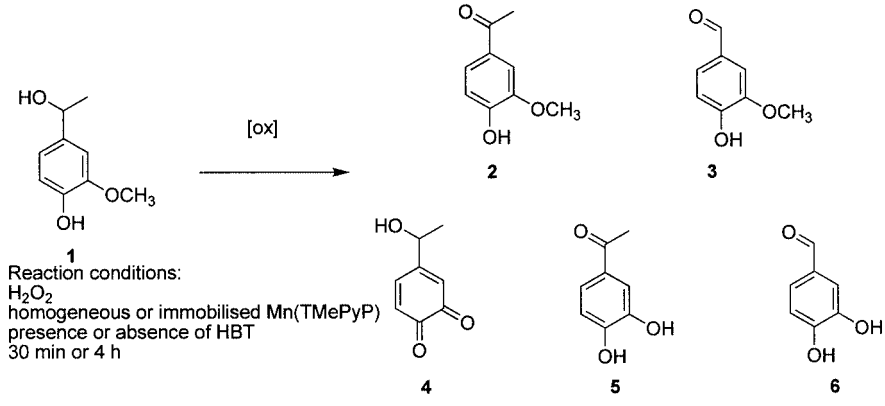
Oxidation of Lignin Model Compounds

The reactivity of some aromatic model compounds resembling the most representative lignin bonding patterns is of pivotal interest in order to rationalize the oxidative behavior of the polymer. Thus, an array of monomeric and dimeric, phenolic and nonphenolic lignin model compounds was selected in order to clarify the reactivity of clay-PMS with lignin subunits.

4-(1-Hydroxyethyl)-2-methoxyphenol (apocinol; **1**), the β-*O*-4-phenolic model 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (**7**), the non-phenolic models 1-(3,4-dimethoxyphenyl)ethanol (**14**), and 1-(4-ethoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (**16**) were oxidized as representative lignin model compounds. The reactions were carried out by treating the appropriate substrate (50 μmol) in the presence of a catalytic amount (2% mol/mol) of clay-PMS (0.27 mmol of active catalyst/g of support) using H_2O_2 (10% aqueous solution) as primary oxidant and HBT as redox mediator. HBT is known to generate long-lived radical intermediates with an electrostatic potential (1.04 V vs. NHE) high enough for the oxidation of either phenolic or non-phenolic lignin model compounds.[12] Oxidations with homogeneous $Mn(TMePyP)(OAc)_5$ in the presence or absence of HBT and with Mn(TMePyP)/clay were also performed as references. Irrespective of the experimental conditions used for the oxidation a low mass balance with respect to isolated products was observed. In accord with results previously reported in the literature, the loss of material from the reaction mixture might be due to formation of highly polar hydrophilic overoxidation products not recovered by usual workup procedures.[7] HBT, present as an oxidation mediator, was quantitatively recovered at the end of the reaction in all the experiments performed, as shown by GC quantitative measurements.

The reactivity of the catalysts toward lignin model compound degradation was studied by measuring the conversion of substrate after 30 min and 4 h for the oxidation of phenolic model compounds, and after 4 h for the oxidation of the more stable nonphenolic lignin model compounds (vide infra). All reaction products were characterized by means of GC-MS analysis after silylation with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and by ^1H and ^{13}C NMR spectroscopy. Control experiments in the absence of the catalysts were performed as previously reported in a study dealing with the activity of montmorillonite-immobilised porphyrins.[10] In this specific case, in order to clarify the role of the oxidation mediator, it is significant to consider the reactions performed in the absence of HBT (and in the presence of catalyst) as reference blank experiments.

Acetophenone **2** was the only recovered product after 30 min reaction time with all the catalysts used for the oxidation of **1**, with a substrate conversion that ranged from 24.5 to 44.3% (Scheme 1, Table 1). It is noteworthy that the clay-PMS system was found to be more reactive than Mn(TMePyP)/clay alone, showing the important role of HBT in the oxidation. This effect was not revealed when the reaction was performed under homogeneous conditions. In fact, the reactivity of $[Mn(TMePyP)OAc_5]$ under identical experimental conditions decreased in the presence of the redox mediator (Table 1). A similar behavior was observed after 4 h reaction time. In this latter case, products of side-chain fragmentation to aldehyde (compounds **3** and **6**), demethylation (**6**) and formation of a benzoquinone de-



Scheme 1

Table 1. Substrate conversion in the [Mn(TMePyP)OAc_5] and Mn(TMePyP)/clay, Mn(TMePyP)OAc_5/HBT and clay-PMS catalysed H_2O_2 oxidation of phenolic lignin models **1** and **7**

Catalyst	Monomeric model **1**		β-*O*-4 model **7**	
	Reaction time	Conv. (%)	Reaction time	Conv. (%)
[Mn(TMeTPyP)OAc_5]	30 min	35.4	30 min	44.7
[Mn(TMeTPyP)OAc_5]	4 h	59.0	4 h	59.6
Mn(TMeTPyP)-clay	30 min	39.5	30 min	41.1
Mn(TMeTPyP)-clay	4 h	51.5	4 h	59.2
[Mn(TMePyP)OAc_5]/HBT	30 min	24.5	30 min	34.4
[Mn(TMePyP)OAc_5]/HBT	4 h	53.7	4 h	72.1
Clay-PMS[a]	30 min	44.3	30 min	57.1
Clay-PMS[a]	4 h	56.4	4 h	73.9

[a] Mn(TMePyP) immobilized on clay + HBT.

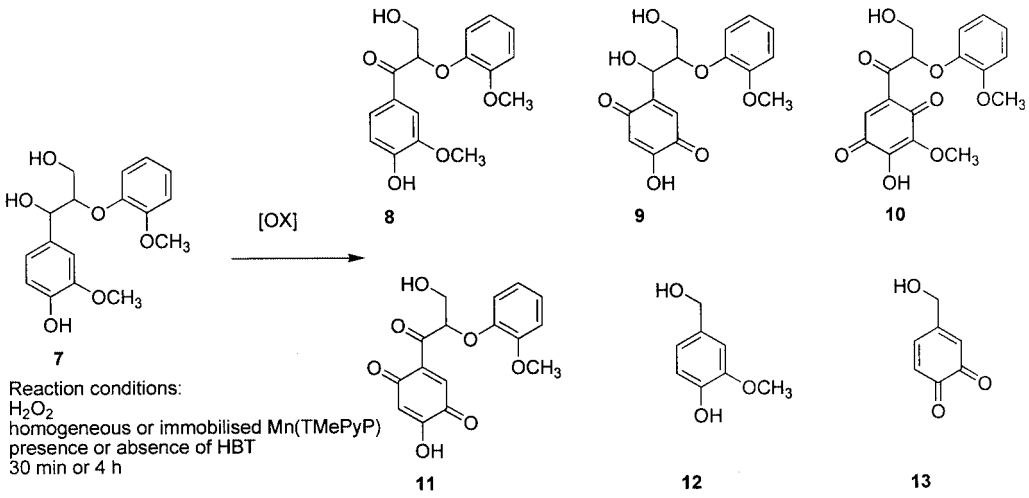
rivative (compound **4**; Scheme 1) were characterized in the reaction mixture. In a test for checking the leaching of catalyst, the oxidation of **1** with clay-PMS was stopped at about 50% conversion. After centrifugation, the colourless solution had lost its catalytic activity. Table 2 shows that the clay-PMS system is stable enough to perform at least five recycling experiments with similar conversion and selectivity.

Table 2. Apocinol (**1**) conversion after recycling of the clay-PMS system[a]

Cycle[a]	**1** (% conv.)
1	59.0
2	58.2
3	58.8
4	57.3
5	54.0

[a]Reaction conditions: substrate 50 μmol, active catalyst 1 μmol, hydrogen peroxide 10% in 0.5 mL of dioxane/buffer citrate phosphate pH = 6 (100 mM) (1:4, v/v), 60 °C, reaction time 4 h.

The oxidation of compound **7**, an aromatic phenolic β-*O*-4 model that represents the most diffuse bonding network in lignin, showed a reactivity pattern close to that observed for apocinol (**1**). The clay-PMS system was again the most active catalyst at both 30 min and 4 h reaction time (Table 1). On the other hand, HBT decreased the reactivity of the homogeneous catalyst [Mn(TMePyP)OAc_5]. Compounds of side-chain oxidation (**8**, **10**), demethylation (**9**), benzoquinone formation (**11** and **13**), and side-chain fragmentation (**12**; Scheme 2) were obtained. On the basis of these data some further considerations are needed. The novel porphyrin-mediator system, clay-PMS, was the most active catalyst in the oxidative degradation of phenolic monomeric and dimeric lignin model compounds. The reaction proceeds mainly by side-chain oxidation and/or fragmentation, demethylation of alkyl aryl ether moieties, and aromatic ring oxidation to *ortho*- and *para*-benzoquinones. The minor reactivity observed for Mn(TMePyP)/clay in the absence of a redox mediator is probably due to the presence of a kinetic barrier for the approach of the substrate to the catalyst when it is embedded and surrounded by the polymeric environment. Thus, the presence of HBT in the



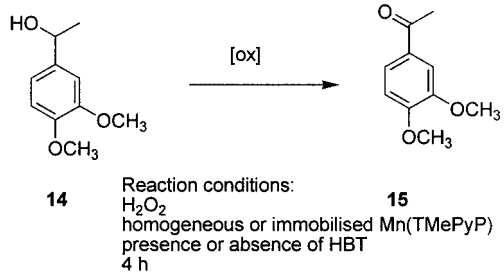
Scheme 2

reaction mixture increases the reactivity of the metalloporphyrin towards the phenolic lignin model compounds by acting as a diffusible oxidation carrier. This effect might be switchable depending on the homogeneous or heterogeneous character of the catalyst. In fact, the presence of HBT decreased the reactivity of [Mn(TMePyP)OAc_5] under homogeneous conditions. Although we have not studied the mechanism of the reaction in detail, it is reasonable to suggest that under homogeneous conditions HBT might compete with the substrate in the H_2O_2-catalysed oxidation.

Oxidation of Non-Phenolic Lignin Model Compounds

Next we turned our attention to the oxidation of non-phenolic lignin model compounds. They represent the inner lignin chain bonding network rather than the terminal units of the polymer. The redox potential of non-phenolic lignin model compounds is higher than that of the corresponding phenolic ones, therefore their oxidation is more difficult, and they are usually resistant to biological treatments.

First we considered the monomeric model, 1-(3,4-dimethoxyphenyl)ethanol (**14**; Scheme 3). Treatment of compound **14** under previously reported experimental conditions gave, after 4 h of reaction time, the corresponding acetophenone derivative **15** as the only recovered product with a substrate conversion that ranged from 24.9 to 50.5% (Table 3). Again, the porphyrin-mediator system clay-PMS was found to be more reactive than heterogeneous Mn(TMePyP)/clay and homogeneous [Mn(TMePyP)OAc_5] systems (Table 3). In this case, HBT also increased the reactivity of the catalysts under homogeneous conditions, and [Mn(TMePyP)OAc_5] showed a higher conversion of substrate in the presence of the redox mediator than in its absence (Table 3).



Scheme 3

Table 3. Substrate conversion in the [Mn(TMePyP)OAc_5], Mn(TMePyP)/clay, Mn(TMePyP) immobilized on clay + HBT, and clay-PMS catalysed H_2O_2 oxidation of nonphenolic lignin models **14** and **16**

Treatment	Substrate **14** (conv. %)	Substrate **16** (conv. %)
[Mn(TMePyP)OAc_5]	24.9	81.0
Mn(TMePyP)-clay	47.8	80.4
[Mn(TMePyP)OAc_5]/HBT	33.1	85.2
Clay-PMS[a]	50.5	86.3

[a] Mn(TMePyP) immobilized on clay + HBT.

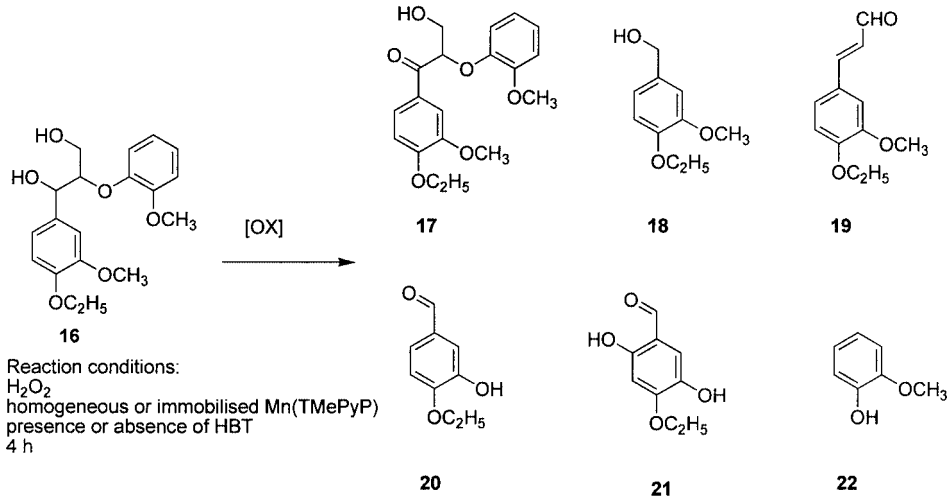
A similar trend was observed upon oxidation of the dimeric lignin model compound 1-(4-ethoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (**16**; Scheme 4). In this case, both Mn(TMePyP)/clay and [Mn(TMePyP)OAc_5] were more reactive in the presence of HBT than alone (Table 3), with clay-PMS being the best catalyst. As reported in Table 3 the conversion of substrate ranged from 80.4% [Mn(TMePyP)/clay] to 86.3% (clay-PMS). A complex mixture of reaction products was recovered after GC-MS analysis and chromatographic purification. Among them, products of side-chain oxidation (ketone derivative **17**), side-chain fragmentation (compounds **18**, **19** and **22**), and side-chain fragmentation/demethylation (compounds **20** and **21**) were unambiguously identified by GC-MS analysis; their low yields showed the high efficiency of the oxidative degradation. All of these transformations are potentially useful for delignifying processes. It is noteworthy that even though HBT is always efficient in the reactions catalysed by the porphyrin mediator system clay-PMS, it shows a different effect in the oxidation of non-phenolic lignin model compounds with respect to phenolic ones when the reactions are performed in the presence of homogeneous catalyst. In fact, the reactivity of [Mn(TMePyP)$(OAc)_5$] was increased by HBT only in the case of non-phenolic compounds.

Both [Mn(TMePyP)OAc_5] and Mn(TMePyP)-clay are able to oxidize HBT to benzotriazol *N*-oxide radical (HBT$^{\cdot}$). In turn, HBT$^{\cdot}$ can oxidize the model compound presumably by a side-chain hydrogen atom abstraction (HAT) process.[13]

The reaction of metalloporphyrins with lignin model compounds is thought to occur by electron transfer.[14] One might hypothesize that, in the presence of [Mn(TMePyP)(OAc_5)] the direct reaction of porphyrin with phenolic model compounds could be faster than the hydrogen atom abstraction process by HBT$^{\cdot}$. When non-phenolic substrates are used, the oxidation of HBT to HBT$^{\cdot}$ and further side-chain oxidation of the model by HAT could be faster than the direct oxidation both in homogeneous and heterogeneous phase.

Oxidation of Lignins

Lignin is a complex tridimensional phenylpropanoic polymer whose units are connected by an array of interunit bondings that makes its chemical characterization extremely difficult. An efficient method to study the modifications induced by the chemical treatment of lignin polymers is ^{31}P NMR spectroscopy; ^{31}P-based magnetic resonance analysis of lignin is capable of detecting and quantitatively determining all functional groups in lignin that possess reactive hydroxy groups — aliphatic OH groups, the various forms of phenolic OH groups, and carboxylic acid groups. More specifically, it allows the unambiguous quantification of the amount of condensed phenolic structures of lignin. Oxidative processes with lignin depolymerization and/or oxidative coupling typically induce a decrease in the number of aliphatic OH groups and noncondensed phenolic subunits and an increase in the number of carboxylic acid



Scheme 4

groups.[15] The number of condensed phenolic OH groups is usually greater after oxidative coupling treatment. Furthermore, condensed lignin subunits are resistant to most of the oxidation treatments. Thus, an increase in these groups indicates the occurrence of oxidative coupling together with side-chain oxidation, while their decrease is identificative of efficient delignification processes.

In order to verify the activity of the clay-PMS on bulky polymeric substrates we selected Black Spruce (BS) milled wood (MWL) and residual kraft lignins (RKL). The oxidations were carried out with an excess of hydrogen peroxide (10% aqueous solution) in buffer citrate phosphate (pH = 6) in the presence of a catalytic amount of the appropriate porphyrin catalyst and, where necessary, HBT. In this case, with the aim of mimicking LiP, VA was also evaluated as a redox mediator for the reaction. Lignin samples recovered from the reaction mixture after usual workup procedures were phosphitylated and then subjected to quantitative ^{31}P NMR analysis in the presence of a suitable amount of cholesterol as an internal standard. The assignment of the different signals was carried out on the basis of earlier work.[11] Table 4 shows the quantitative distribution of the OH groups as revealed by ^{31}P NMR spectroscopy. In agreement with data previously recovered on the side-chain oxidation of phenolic and non-phenolic lignin model compounds, the treatment of MWL with the porphyrin-mediator systems clay-PMS-HBT and clay-PMS-VA yielded an appreciable decrease in the number of aliphatic OH groups (Table 4). The reference systems [Mn(TMePyP)OAc_5] and Mn(TMePyP)/clay were not efficient catalysts under these experimental conditions. Moreover, the reaction performed with HBT was more efficient than that with VA, probably because of the higher stability of the first redox mediator intermediate (Table 4).

BS MWL contains a small amount of condensed OH groups. Irrespective of the experimental conditions used in the oxidation, this did not vary significantly during the treatment of MWL. Thus, oxidative coupling reactions are not operative side-processes. In agreement with the data reported above for the oxidation of lignin model compounds, the number of guaiacyl OH groups decreased after oxidation showing a depolymerization process. It is noteworthy that the number of carboxylic acid groups, which arise from lignin oxidation of both side-chains and aromatic rings, increased after treatment of MWL with clay-PMS-HBT more than with clay-PMS-VA. This different behavior could be due to the longer lifetime of HBT radical with respect to veratryl alcohol radical cation.

Table 4. Distribution of aliphatic, phenolic and carboxylic hydroxy groups in MWL and RKL (mmol/g of lignin) after metalloporphyrin- and PMS-catalyzed oxidations as obtained by quantitative ^{31}P NMR spectroscopy

Sample/catalyst	Aliphatic OH	Condensed OH [mmol/g]	Guaiacyl OH	COOH
Black spruce MWL	4.59	0.05	0.75	0.15
MWL/[Mn(TMePyP)OAc_5]	4.57	0.05	0.63	0.16
MWL/Mn(TMePyP)-Clay	4.55	0.05	0.65	0.12
MWL/clay-PMS-HBT[a]	3.93	0.04	0.68	0.17
MWL/clay-PMS-VA[b]	4.14	0.04	0.73	0.19
Black spruce RKL	1.94	0.92	1.27	0.27
RKL/[Mn(TMePyP)OAc_5]	1.64	0.91	1.09	0.65
RKL/Mn(TMePyP)-Clay	1.39	0.80	0.78	0.27
RKL/clay-PMS-HBT[a]	1.19	0.63	0.52	0.17

[a] Mn(TMePyP) immobilized on clay + HBT. [b] Mn(TMePyP) immobilized on clay + veratryl alcohol.

Residual kraft lignin (RKL) is the lignin fraction that can be isolated by acidolysis of kraft pulps. This lignin is severely altered with respect to native and milled wood lignins and contains higher amounts of condensed subunits.[16] In order to clarify the ability of the PMS to oxidize these units, we performed experiments of oxidation of a sample of black spruce RKL with [Mn(TMePyP)(OAc_5)], Mn(TMePyP)-clay and the HBT-clay-PMS (Table 4). While [Mn(TMePyP)(OAc_5)] was not able to degrade condensed OH groups and Mn(TMePyP)-clay induced a modest decrease, HBT-clay-PMS reduced their amount by 32%. The guaiacyl units were reduced to 79% and the aliphatic OH groups were reduced to 61% of their initial content (Table 4). These data are a clear indication of the extensive degradation induced by the clay-PMS on a resistant polymer such as RKL.

Conclusion

An advanced biomimetic system for lignin peroxidase has been designed and realized. When using such systems, the application of the "mediator" concept avoids kinetic problems due to the solid nature of the substrate and of the heterogeneous catalyst, and opens the way to advanced biomimetic ligninase mediator systems (porphyrin mediator systems). Phenolic and non-phenolic, monomeric and β-*O*-4-lignin model compounds were efficiently oxidized with the occurrence of side-chain and aromatic ring oxidation reactions. Oxidation of BS MWL and RKL revealed the occurrence of efficient delignification processes and absence of significant coupling reactions. The porphyrin mediator systems represent the last step in the development of a "synthetic enzyme" for the pulp and paper transformations.

Experimental Section

General: ^1H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker AM 400 spectrometer. Mass spectrometry (MS) was performed with a GC Shimadzu GC-17A and a mass-selective detector QP 6000. All solvents were ACS reagent grade and were redistilled and dried according to standard procedures. Chromatographic purifications were performed on columns packed with Merck silica gel 60, 230−400 mesh for flash technique. Thin-layer chromatography was carried out using Merck platten Kieselgel 60 F254. Lignin model compounds **1**, **7**, **14** and **16** were synthesized according to literature procedures.[17]

Catalyst Preparation: The manganese porphyrin [Mn(TMePyP)(OAc_5)] was synthesized according to a literature procedure.[18] [Mn(TMePyP)(OAc_5)} was immobilized on montmorillonite as previously reported by refluxing in the presence of Na^+-saturated montmorillonite.[9] The determination of the content of metalloporphyrin supported on the clay was performed spectrophotometrically, as reported previously.[9]

Lignin Isolation: Milled lignin was prepared from ultraground extractive free powder according to Bjorkman's procedure. Extractive free powders were ultraground for three weeks in a rotatory ball mill. The ML fraction was then extracted with dioxane/water (96:4, v/v). The residue was concentrated under reduced pressure and freeze-dried. Purification was performed by dissolving the lignin in 90% acetic acid. The solution was then added dropwise to stirred water. The precipitated lignin was centrifuged and freeze-dried. It was then dissolved again in a mixture of 1,2-dichloroethane/ethanol (2:1, v/v) and precipitated by addition of diethyl ether.[19] Residual kraft lignin was isolated from kraft pulp using a slightly modified acidolysis procedure. The yield was 38%, and the purity was confirmed by UV and klason lignin content measurements.[16,20]

General Procedure for the Oxidation of Lignin Model Compounds: Hydrogen peroxide (10%) was added with stirring at 60 °C as 20-μL aliquots every hour to a solution of the porphyrin catalyst (1 μmol) in 0.5 mL of dioxane/buffer citrate phosphate pH = 6 (100 mM) (1:4, v/v) containing 50 μmol of substrate and 5 μmol of HBT. As an example, the amount of active catalyst corresponds to 12% (w/w) in the case of the monomeric model compound **1** and to 6% (w/w) for the dimeric model **7**. After 4 h, the reaction mixtures were extracted with CH_2Cl_2 in the presence of saturated aqueous NaCl solution. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The residues were dissolved in 20 μL of pyridine in the presence of 3,4-dimethoxytoluene as an internal standard for GC-MS analysis. The mixture was than silylated by addition of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA).

Oxidation of Lignin. General Procedure: Oxidation of black spruce and cypress MWL, and black spruce residual kraft lignin were carried out in buffer citrate phosphate pH = 6 (100 mM) (10 mL) at 90 °C in the presence of 100 mg of lignin, 10 mg of active catalyst, 50 μmol of HBT or veratryl alcohol, and 200 μL of 10% H_2O_2. Aliquots of H_2O_2 (200 μL) were added every hour for 6 h. The reaction mixture was washed with water several times, centrifuged and freeze-dried. The samples obtained were submitted to quantitative ^{31}P NMR analysis (see below).

Characterization of Products: Gas chromatography and gas chromatography/mass spectrometry of the reaction products were performed using a DB1 column (30 m × 0.25 mm and 0.25 mm film thickness), and an isothermal temperature profile of 100 °C for the first 2 min, followed by a 20 °C/min temperature gradient to 300 °C and finally an isothermal period at 300 °C for 10 min. The injector temperature was 280 °C. Chromatography-grade helium was used as the carrier gas. The identification of **2**, **3**, **5**, **6**, **8**, **12**, **15**, **17**, **18**, **19**, **20** and **22** was carried out by comparison with samples of authentic products. Compounds **5**, **13** and **21** were assigned by comparison with the fragmentation spectra of original compounds. Products **9**, **10** and **11** were tentatively identified on the basis of the fragmentation spectra. The fragmentation patterns are shown in Table 5. Quantitative ^{31}P NMR spectra were obtained using methods identical to those described by Argyropoulos et al.[11] The chemical shifts were referenced to phosphoric acid. Derivatization of the lignin samples with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane were performed as described previously.[11] The ^{31}P NMR spectroscopic data reported in this effort are averages of three phosphitylation experiments followed by quantitative ^{31}P NMR acquisitions. The maximum standard deviation of the reported data was 2×10^{-2} mmol/g, while the maximum standard error was 1×10^{-2} mmol/g.

Acknowledgments

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Table 5. Mass spectrometric data

Product	Derivative[a]	*m/z* (%)
2	$Si(CH_3)_3$	238 (41) [M^+], 223 (35), 209 (9), 193 (23), 91 (10), 73 (100)
3	$Si(CH_3)_3$	224 (45) [M^+], 209 (15), 193 (22), 177 (5), 137 (8), 73 (100)
4	$Si(CH_3)_3$	224 (18) [M^+], 209 (10), 193 (15), 179 (31), 151 (9), 73 (100)
5	$Si(CH_3)_3$	296 (8) [M^+], 224 (30), 209 (10), 193 (23), 151(84), 73 (100)
6	$Si(CH_3)_3$	282 (8) [M^+], 223 (40), 207 (6), 192 (35), 177 (6), 73 (100)
8	$Si(CH_3)_3$	224 (15), 209 (10), 194 (5), 166 (12), 150 (76), 73 (100)
9	$Si(CH_3)_3$	297 (12), 223 (5), 181 (5), 166 (6), 109 (8), 73 (100)
10	$Si(CH_3)_3$	252 (9), 209 (5), 166 (8), 124 (7), 103 (12), 73 (100)
11	$Si(CH_3)_3$	224 (9), 209 (6), 194 (8), 166 (10), 150 (45), 73 (100)
12	$Si(CH_3)_3$	298 (13) [M^+], 283 (6), 268 (9), 209 (9), 179 (6), 73 (100)
13	$Si(CH_3)_3$	210 (12) [M^+], 179 (18), 136 (20), 147 (10), 105 (8), 73 (100)
14	–	182 (42) [M^+], 167 (64), 139 (100), 124 (30), 108 (21), 77 (45)
15	–	180 (45) [M^+], 165 (100), 137 (17), 122 (10), 107 (8), 77 (26)
16	–	330 (16) [$M^+ - 18$], 206 (17), 179 (26), 151 (33), 109 (47), 77 (100)
17	–	328 (17) [$M^+ - 18$], 207 (35), 179 (100), 151 (63), 137 (7), 123 (21)
18	–	180 (50) [M^+], 154 (38), 137 (40), 122 (48), 109 (21), 65 (100)
19	–	206 (33) [M^+], 179 (8), 151 (100), 123 (18), 77 (24), 55 (90)
20	–	180 (37) [M^+], 151 (100), 109 (17), 95 (10), 81 (20), 65 (25)
21	–	196 (22) [M^+], 181 (37), 153 (30), 135 (33), 125 (51), 93 (100)
22	–	124 (65) [M^+], 109 (100), 81 (89), 65 (15), 77 (21), 53 (55)

[a] – = underivatized; $Si(CH_3)_3$ = trimethylsilylated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide.

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