Factors Controlling the Reactivity of a Ligninase Model Based on the Association of Potassium Monopersulfate to Manganese and Iron Porphyrin Complexes

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Received February 7, 1989

An efficient ligninase model based on the association of potassium monopersulfate to iron and manganese porphyrin in solution or immobilized onto an ion-exchange resin is reported. High catalytic conversion of veratryl alcohol or 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol, a useful model molecule for checking the ability of cleaving C_α-C_β bonds of arylglycerol-β-aryl ether linkages existing in lignin itself, is obtained at room temperature in a single-phase solution (buffered water/acetonitrile, 75/25, v/v) at pH 2-3 for iron porphyrin or at pH 4.5-6.0 for manganese porphyrin. The porphyrin ligand used in the present study is the *meso*-tetrakis(*p*-sulfonatophenyl)porphyrin (TPPS). Catalytic activities can be as high as eight cycles per second.

Since the recent purification of lignin peroxidase (LiP), a heme-containing H_2O_2-requiring enzyme purified from the extracellular medium of the fungus *Phanerochaete chrysosporium*,[1-4] some chemical models of this peroxidase have been reported. Most of these ligninase models are based on iron porphyrin complexes associated with alkyl hydroperoxide, sodium hypochlorite, or molecular oxygen and an electron source.[5-8] There are several reasons for current intensive studies on biomimetic models of ligninase: (i) the possibility of developing robust chemical catalysts for the industrial delignification of wood chips, (ii) the contribution to the study of the ligninase mechanism, and (iii) the design of new catalysts for the detoxification of polychlorinated phenols, a major class of environmental pollutants. Because our own interest in peroxidase-catalyzed reactions[9] and cytochrome P-450 modeling,[10-12] we have recently developed an efficient ligninase model resulting from the association of potassium monopersulfate, $KHSO_5$, with manganese or iron porphyrin either free or immobilized on an ion-exchange resin.[13]

Here we report data on the key factors (nature of the oxidant, hydrophobicity of the reaction medium, and pH) involved in the catalytic activity of $KHSO_5$/manganese or iron porphyrin as models of ligninase. Studies have been performed by using veratryl alcohol (1) and 1-(3,4-dimethoxphenoxy)-2-(2-methoxyphenoxy)propane-1,3-diol (2) (see Figure 1 for molecular structures), two classical molecules generally used in lignin degradation modeling.[14,15]

Experimental Section

Chemicals. 3,4-Dimethoxybenzyl alcohol (veratryl alcohol, 1) and other usual chemicals were obtained from Aldrich. 1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol (2) was a gift from J.-L. Seris (Elf-Aquitaine, Lacq). Hydrogen peroxide, ~30 wt % in water (8.8 M), and potassium monopersulfate, available as the triple salt of $2KHSO_5$, $KHSO_4$, and K_2SO_4 (Oxone), were purchased from Janssen and Alfa Ventron, respectively.

Two categories of catalysts were used: (i) free water-soluble iron and manganese derivatives of tetrasodium *meso*-tetrakis(*p*-sulfonatophenyl)porphyrin (TPPS) and (ii) the same two metalloporphyrins immobilized onto an ion-exchange resin, Amberlite IRA-900 EGA, by strong physical adsorption. The porphyrin ligand, $TPPSH_2$, was prepared according to ref 16, and its metalation was performed either in refluxing water for 1-2 h, at pH values ranging from 5 to 8, by manganese(II) acetate (5 equiv), or by an air-stable iron(II) salt (e.g., ammonium iron(II) sulfate hexahydrate, 10 equiv). In both cases, the yield of isolated pure metalloporphyrin ranges between 70 and 80%. MnTPPS and FeTPPS immobilized onto Amberlite IRA 900 (MnTPPS-Ad and FeTPPS-Ad) was prepared according to ref 17 (5 mg of MTPPS are immobilized onto 100 mg of resin).

Catalytic Oxidation of Lignin Model Compounds 1 and 2 by FeTPPS, MnTPPS, FeTPPS-Ad, and MnTPPS-Ad. Metalloporphyrin-catalyzed oxidations of 1 and 2 were performed in a single-phase solution constituted of acetonitrile and a buffer solution, at room temperature, under aerobic conditions. Each experiment was carried out under the same general conditions: to 500 μL of a 0.04 M acetonitrile solution of the substrate (20 μmol of 1 or 2) was added the appropriate molar ratio of catalyst with respect to the substrate (e.g., 2 μmol in the case of 10% catalyst versus substrate; for MTPPS-Ad, M = Fe or Mn, this amount corresponds to 100 mg of loaded Amberlite IRA 900) solubilized in 1 mL of a 0.1 M citrate-phosphate buffer for reactions performed at pH 3.0 (or a 0.5 M acetate buffer for pH 4.5, or a 0.5 M phosphate buffer for pH 6.0 and 7.0). For some reactions with a proximal nitrogen ligand, 200 μmol of 4-*tert*-butylpyridine was added by syringe at this stage. Catalytic oxidations were started by addition of 500 μL of a buffered solution of the oxygen donor and 100 μmol of hydrogen peroxide or potassium monopersulfate. In the latter case, 100 μmol of $KHSO_5$ corresponds to 30.7 mg of Oxone. Final reaction volume: 2 mL (CH_3CN/buffered H_2O: 25/75, v/v).

Conversion of the substrate was determined after 1 min of reaction by HPLC (μ-Bondapak C_{18} column). Isocratic elution was performed with methanol/water (1/1, v/v) at a flow rate of 1 mL/min (UV detection at 280 nm). After total conversion of the substrate, the reaction product was extracted with di-

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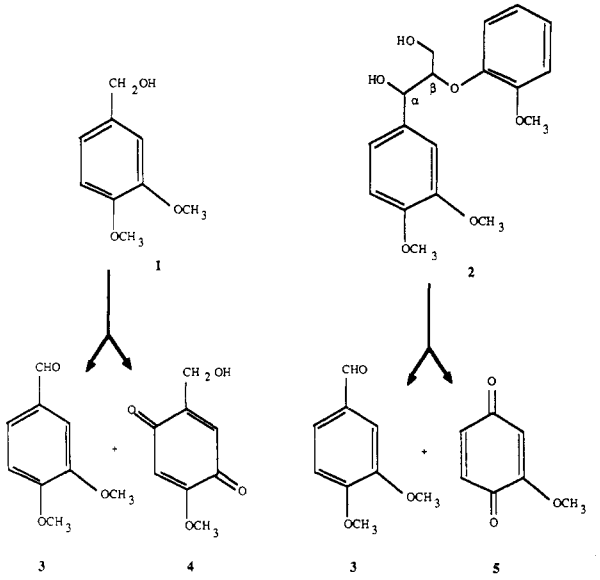

Figure 1. Oxidative degradation of lignin model compounds 1 and 2 by MTPPS (free or adsorbed on Amberlite) and $KHSO_5$.

chloromethane and separated on Merck PLC plates RP-18 F 254S (eluent: methanol/water, 50/50).

The major products from 1 (3 and 4) or from 2 (3 and 5) were identified by ^1H and ^{13}C NMR and also by mass spectrometry. They are identical with authentic samples (4 and 5 were prepared by HRP/$KHSO_5$ oxidation of 3-hydroxy-4-methoxybenzyl alcohol and by HRP/H_2O_2 oxidation of guaiacol, respectively).

Results and Discussion

Three different factors of this biomimetic oxidation of lignin models were investigated: (i) the comparative efficiency of $KHSO_5$ and H_2O_2 as primary oxidants, (ii) the influence of the pH of the buffer solution associated with acetonitrile in a single-phase system, and (iii) the influence of the hydrophobicity of the reaction medium.

Most of the studies were performed by using veratryl alcohol (1) as the lignin model since this material is readily available, but data are also reported in the oxidation of the dimer molecule 2, a useful lignin model to evidence the C_α–C_β bond cleavage, a key step in lignin degradation.

$KHSO_5$: A Better Primary Oxidant than H_2O_2 in This Metalloporphyrin-Catalyzed Oxidation of Veratryl Alcohol (1). Comparative data on the respective efficiency of $KHSO_5$ and H_2O_2, the natural cofactor of peroxidases including lignin peroxidase, are reported in Table I. Both soluble catalysts, FeTPPS and MnTPPS, with H_2O_2 always gave very low conversion of 1 at a reaction time of 1 min, arbitrarily chosen short: 5% with FeTPPS (run 1), 2% with MnTPPS (run 7). The conversion is not improved by addition of imidazole, 100 equiv with respect to the catalyst (runs 2 and 8). The same low activity is observed for both catalysts supported on Amberlite, FeTPPS-Ad and MnTPPS-Ad: 6% conversion with FeTPPS-Ad (run 5) and 8% conversion with MnTPPS-Ad (run 10). On the other hand, $KHSO_5$ gives high conversions of veratryl alcohol when the same molar ratios of substrate, catalyst, and oxidant are used.

With FeTPPS and MnTPPS, conversion of 1 reaches 67% within 1 min in both cases (runs 3 and 9). Similar conversions are obtained with the supported catalysts, FeTPPS-Ad and MnTPPS-Ad: 50% and 61%, respectively (runs 6 and 11). In addition, it must be noted that MnTPPS-Ad can be recycled, maintaining 95% of the initial activity measured in run 11.

This higher activity of $KHSO_5$ compared to H_2O_2 has also been recently observed in DNA breaks generated by

Table I. Hydrogen Peroxide and Potassium Monopersulfate Oxidation of Veratryl Alcohol (1), Catalyzed by FeTPPS, MnTPPS, FeTPPS-Ad, or MnTPPS-Ad[a]

run	catalyst	% of catalyst vs 1	oxygen donor	ratio of imidazole to catalyst	substr convsn in % for a reactn time of 1 min
1	FeTPPS	0.2	H_2O_2		5
2	FeTPPS	0.2	H_2O_2	100	7[b]
3	FeTPPS	0.2	$KHSO_5$		67
4	FeTPPS	0.2	$KHSO_5$	100	65
5	FeTPPS-Ad	10	H_2O_2		6
6	FeTPPS-Ad	10	$KHSO_5$		50
7	MnTPPS	3	H_2O_2		2
8	MnTPPS	3	H_2O_2	100	7
9	MnTPPS	3	$KHSO_5$	100	67
10	MnTPPS-Ad	10	H_2O_2	100	8
11	MnTPPS-Ad	10	$KHSO_5$	100	61

[a] See Experimental Section for conditions used. [b] Reaction performed in the presence of 100 equiv of imidazole/catalyst (4 μmol of imidazole).

(bleomycin)iron(III) and cationic metalloporphyrins.[18,19] Since the peroxidic O–O bond is more unsymmetrical in $KHSO_5$ compared to H_2O_2, the heterolytic cleavage (the pathway leading to a high-valent metal–oxo) is highly favored (SO_4^{2-} being a better leaving group than HO^-).

Influence of the Buffer pH Value on the $KHSO_5$ Oxidation of Veratryl Alcohol Catalyzed by MTPPS or MTPPS-Ad (M = Fe and Mn) in Single-Phase Buffer/Acetonitrile (75/25, v/v). During preliminary investigations, we found that the pH of the single phase constituted by a mixture of buffered water and acetonitrile has an outstanding effect on the efficiency of the $KHSO_5$/MTPPS system in solution or adsorbed on Amberlite.

Studies have been performed on the oxidation of veratryl alcohol by the two soluble catalysts, FeTPPS and MnTPPS, and the corresponding insoluble ones, FeTPPS-Ad and MnTPPS-Ad. In each case, conversion of 1 was determined for a reaction time of 1 min at six different pH values (2, 3, 4.5, 6, 7 and 8) and are reported in Figure 2.

With both iron porphyrin complexes (Figure 2A), either soluble or immobilized, high conversions are obtained at low pH values. For pH below 3, all conversions of 1 are above 50%, whereas very low conversions (<10%) are observed for pH 6–8. The acidic medium presumably favors the cleavage of inactive μ-oxo iron porphyrin dimers known to be formed in the oxidation of water-soluble porphyrin complexes[20] and that represent a dead-end in the catalytic cycle of this biomimetic oxidation of veratryl alcohol.

An opposite pH effect is observed for both manganese porphyrins, MnTPPS and MnTPPS-Ad (Figure 2B). An optimum is reached between pH 4.5 and 6.0, whereas low pH values considerably slow down the conversion of 1. This effect is mainly due to the protonation of the 4-*tert*-butylpyridine, thus preventing the activation of the high-valent manganese species by a proximal effect. Only a small decrease in the conversion of 1 is observed for pH values above 6.

From these data, it is clear that the activity maxima of the iron and manganese porphyrins are not reached in the

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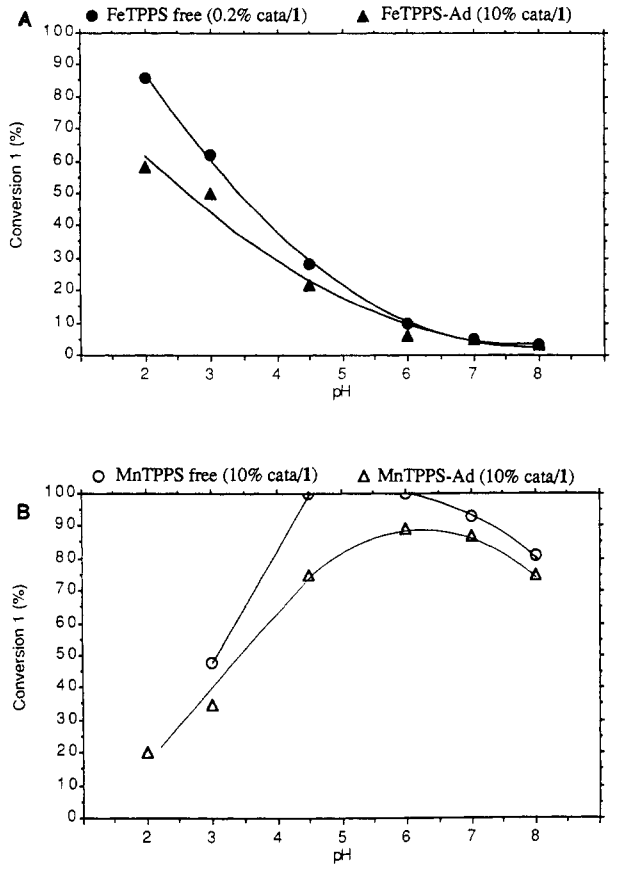

Figure 2. Influence of pH buffer solution on oxidation of veratryl alcohol (1) catalyzed by MTPPS (free or adsorbed)/$KHSO_5$ (single-phase solution constituted of 75% buffer solution and 25% acetonitrile, v/v). A: (●) FeTPPS free (0.2% cat./subs.); (▲) FeTPPS-Ad (10% cat./subs.). B: (△) MnTPPS-Ad (10% cat./subs.); (○) MnTPPS free (10% cat./subs.). All assays with manganese porphyrins were performed with 100 equiv of 4-*tert*-butylpyridine as axial ligand/catalyst.

same pH range making less obvious a direct comparison. However, the soluble FeTPPS is far more active at pH 2 than MnTPPS at pH 6: for 1, a 87% conversion is obtained with 0.2% of catalyst with respect to the substrate at pH 2 for FeTPPS. This high conversion of 1 with FeTPPS as catalyst corresponds to eight catalytic cycles per second, i.e., the highest catalytic activity reported so far for a biomimetic model of ligninase (Figure 2A). The same conversion requires 5% of MnTPPS at pH 6 (data not shown on Figure 2). For immobilized catalysts, the manganese porphyrin is more active than the corresponding iron complex: for the same catalyst/substrate ratio of 0.1, 1 is completely oxidized by MnTPPS-Ad in 1 min at pH 4.5, whereas the maximum activity for FeTPPS-Ad is only 60% at pH 2.

Influence of the Hydrophobicity of the Reaction Medium on the $KHSO_5$ Oxidation of 1 Catalyzed by MTPPS or MTPPS-Ad. The second important factor in the catalytic oxidation by $KHSO_5$/MTPPS and MTPPS-Ad is the ratio of acetonitrile/buffer, namely, the hydrophobicity of the reaction mixture. Such effect has seldom been mentioned in reactions catalyzed by metalloporphyrin complexes. The only precedent is the catalytic dismutation of hydrogen peroxide by catalase models.[21]

The data obtained for FeTPPS and FeTPPS-Ad catalyzed oxidation of veratryl alcohol at the same pH value

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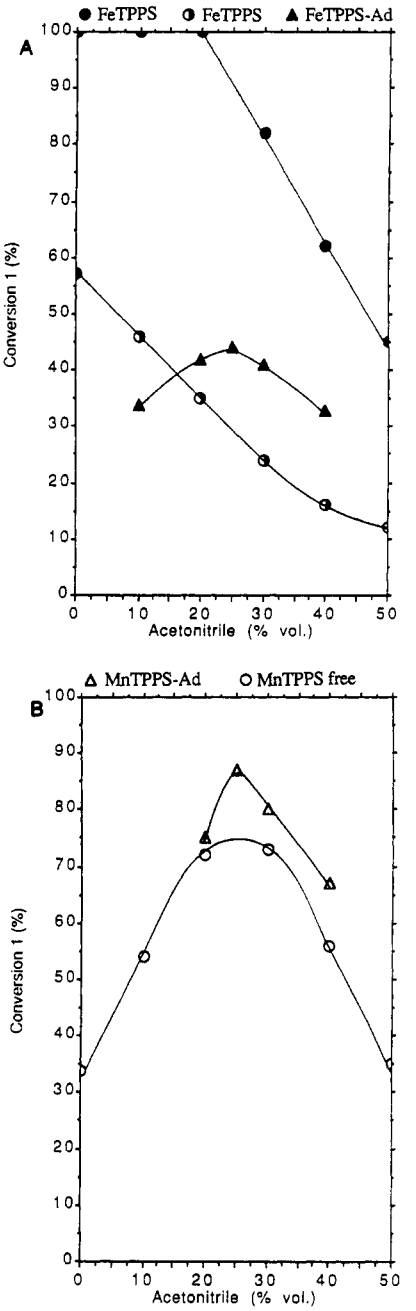

Figure 3. Influence of the percentage of acetonitrile in the single-phase solution constituted by acetonitrile and a buffer solution at pH = 3.0 on the oxidation of veratryl alcohol catalyzed by MTPPS (free or adsorbed)/$KHSO_5$. A: (●) FeTPPS free (0.3% cat./subs.); (◑) FeTPPS free (0.1% cata./subs.); (▲) FeTPPS-Ad (10% cata./subs.). All three experiments were performed in citrate–phosphate buffer at pH = 3.0. B: (○) MnTPPS free (3% cat./subs.); (△) MnTPPS-Ad (10% cat./subs.). These two experiments were performed in 0.5 M phosphate buffer at pH = 6.0 with 100 equiv of an axial ligand, 4-*tert*-butylpyridine/catalyst. All assays were performed as indicated in Materials and Methods sections.

(3.0) are reported in Figure 3A. With 0.1% of FeTPPS with respect to the substrate, the conversion of 1 decreases when the percentage of acetonitrile in the reaction medium increases, i.e., when the hydrophobicity of the medium increases.

For a higher concentration of catalyst, viz. 0.3% of FeTPPS, this linear decrease is observed from 20 to 50% of acetonitrile: the conversion of 1 decreases from 100% to 45% for 1 min of reaction. However, the influence of the hydrophobicity is not the same for the immobilized

Table II. Potassium Monopersulfate Oxidation of Lignin Model 2, Catalyzed by FeTPPS, MnTPPS, FeTPPS-Ad, or MnTPPS-Ad[a]

catalyst[b]	% of catalyst vs substr	substr convsn in % for a reactn time of 1 min
FeTPPS	10	100
MnTPPS[c]	10	100
FeTPPS-Ad	10	63 (5)[d]
MnTPPS-Ad[c]	10	100

[a] See Experimental Section for used conditions. All data indicated in this table have been obtained in solutions at pH = 3.0 (citrate–phosphate buffer). [b] A molar ratio of 10% of catalyst versus substrate was used in all form cases. [c] In this case 100 equiv of 4-*tert*-butylpyridine were present in the reaction mixture. [d] The same reactions performed with 100 equiv of 4-*tert*-butylpyridine per equivalent of catalyst gives 5% of substrate conversion.

catalyst, FeTPPS-Ad. In this case, an optimum value for the substrate oxidation is reached for 25% of acetonitrile.

This bell-shaped curve is also observed in the case of manganese porphyrins, MnTPPS and MnTPPS-Ad, at pH 6.0 (Figure 3B). In both cases, with the Mn catalyst either in solution or immobilized, the maximum catalytic activity is obtained for 25% of acetonitrile. The data reported in Figure 3A and 3B show that, as observed during the study on the pH influence, the soluble iron catalyst is more active than the corresponding manganese one. For 25% of acetonitrile, only 0.3% of FeTPPS is necessary to oxidize 1 completely, whereas 3 of MnTPPS is required to reach 90% of conversion.

The same reverse situation is found for the immobilized catalyst: at 25% of acetonitrile and 10% of catalyst with respect to veratryl alcohol, the conversion is 87% with MnTPPS-Ad and only 45% with FeTPPS-Ad.

The influence of acetonitrile on these metalloporphyrin-catalyzed oxidations may be due to the fact that acetonitrile may act as axial ligand, limiting the formation of high-valent metal–oxo species or influencing the substrate–catalyst interaction during the electron-transfer step.

Identified Oxidation Products of Veratryl Alcohol Oxidation by These Ligninase Models. Two main products have been isolated: veratraldehyde (3) and 2-methoxy-5-(hydroxymethyl)-1,4-benzoquinone (4). 3 has been identified by comparison to an authentic sample and 4 by NMR and MS data.[22a] These two molecules are the two major products of the veratryl alcohol oxidation catalyzed by lignin peroxidase itself.[22b] Quinone 4 is obtained in higher yield in FeTPPS-catalyzed oxidations: 50–70% instead of 20–25% in the case of MnTPPS. Veratraldehyde (3) is favored in MnTPPS-catalyzed reactions: 40% instead of 20–30% for FeTPPS. These yields were determined when the conversion of 1 was complete (after 15 min for efficient reactions like runs 3, 6, 9, or 11).

Oxidation of the Lignin Model 2 by $KHSO_5$/MTPPS or MTPPS-Ad: C_α–C_β Bond Cleavage Catalyzed by Such Ligninase Models. Besides biphenyl and phenylcoumarin bond types, the major type of bond contributing to the tridimensional structure of lignin itself is the arylglycerol–β-aryl ether bond type. The latter class represents 40–50% of the bonds whose cleavage is a key step in the enzyme-catalyzed degradation of lignin. We report in Table II that the four metalloporphyrin complexes studied are able to catalyze the oxidation of 2, a molecule representative of arylglycerol–β-aryl ether linkages in lignin.[14,15] Full conversion of 2 is easily reached within 1 min for at least three catalysts, viz. FeTPPS, MnTPPS, and MnTPPS-Ad. Only FeTPPS-Ad gives a lower conversion (65%) under the same experimental conditions.

Two oxidation products of 2 have been isolated and identified by NMR and MS data: veratraldehyde (3) and 2-methoxy-1,4-benzoquinone (5).[23] This latter compound is one of the oxidation products of guaiacol by peroxidases. The C_α–C_β bond cleavage of 2 leads to the formation of guaiacol[15] and we checked that the presently described ligninase models are also able to readily oxidize guaiacol to 4 under the same experimental conditions as the oxidation of 2. So the "potassium monopersulfate–metalloporphyrin" system is able to mimick efficiently under mild conditions the key step of lignin degradation, namely, the C_α–C_β bond cleavage. 3 and 5 are obtained in yields ranging from 15 to 30% (determined when the conversion 2 is complete), but other nonidentified products are also formed.

Conclusion

High conversions of lignin model molecules, veratryl alcohol and 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol, are obtained by associating potassium monopersulfate, a readily available single oxygen atom donor, with iron and manganese derivatives of the sulfonated derivative of tetraphenylporphyrin (TPPS). The catalytic activity is higher for soluble catalysts, FeTPPS and MnTPPS, compared to the corresponding resin-immobilized catalysts, FeTPPS-Ad and MnTPPS-Ad. However, the latter supported catalyst can be recycled, losing only 5% of the initial activity. Then the association "$KHSO_5$/MnTPPS-Ad" might have a real future as a chemical model of lignin peroxidase and probably also as a chemical catalyst for the oxidation of aromatic pollutants. This kind of work on ligninase models might also provide some informations on the factors that control the prosthetic site reactivity of ligninases and the future new ligninases that will be readily available in the near future since the recent characterization of cDNAs that encode for various ligninases.[24]

Acknowledgment. Gilles Labat is indebted to the Société ELF-Aquitaine for a Ph.D. fellowship and both authors thank J.-L. Seris for fruitful discussions.

(22) (a) NMR, MS, IR and UV–vis data of 4: ^1H NMR (250 MHz, D_2O) δ 3.92 (s, 3 H, OCH_3), 4.52 (d, 2 H, J = 2.1 Hz, CH_2), 6.09 (s, 1 H, H_3), 6.75 (t, 1 H, H_6); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 57.3 (q, OCH_3), 59.3 (t, CH_2OH), 108.7 (d), 129.8 (d), 151.2 (s), 160.8 (s), 179.0 (s), 189.1 (s); MS (DCI, NH_3), m/e 186 (MNH_4^+), 169 (MH^+), 153 (M – Me); IR (KBr, cm^{-1}) 1665 (s), 1644 (vs), 1619 (m), 1607 (s)8 1226 (m) and 1114 (m); UV–vis (MeOH, c = 143 μM) λ_{max} (ε, M^{-1} cm^{-1}) 261 (9.9×10^3) and 360 (6.8×10^2). (b) Haemmerli, S. D.; Schoemaker, H. E.; Schmidt, H. W. H.; Leisola, M. S. A. *FEBS Lett.* **1987**, *220*, 149–154.

(23) NMR, MS, IR, and UV–vis data of 5: ^1H NMR (200 MHz, $CDCl_3$) δ 3.93 (s, 3 H), 5.93 (d, 1 H, J = 1.6 Hz), 6.70 (s and d, 2 H, J = 1.6 Hz); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 57.2 (q, OCH_3), 107.7 (d), 134.4 (d), 137.2 (d), 158.6 (s), 181.7 (s)8 187.4 (s); MS (DCI, NH_3), m/e 156 (MNH_4^+), 141 (MH_3^+), 139 (MH^+); IR (KBr, cm^{-1}) 1674 (vs), 1640 (vs), 1616 (s), 1590 (vs), 1237 (s), 1112 (s); UV–vis (MeOH, c = 107 μM) λ_{max} (ε, M^{-1} cm^{-1}) 253 (1.3×10^4) and 355 (1.1×10^3).

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