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Coupling of Manganese Peroxidase-Mediated Lipid Peroxidation with Destruction of Nonphenolic Lignin Model Compounds and ^{14}C-Labeled Lignins

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Linoleic acid, the predominant unsaturated fatty acid (UFA) in the lipids of wood-rotting fungi, was oxidized by manganese peroxidase (MnP) from the white-rot fungus *Phlebia radiata* through a peroxidation mechanism. The peroxidation was markedly stimulated by hydrogen peroxide. UFAs that are substrates for lipid peroxidation and surfactants that emulsify water-insoluble components were essential for the MnP-catalyzed destruction of a nonphenolic *β*-O-4-linked lignin model compound (LMC). Moreover, both components stimulated the MnP-catalyzed mineralization of ^{14}C-labeled synthetic lignin and ^{14}C-labeled wheat straw. A high level of destruction was obtained in reaction systems with Tween 80 acting both as surfactant and source of UFAs. The presence of the linoleic acid in reaction systems with MnP and Tween 80 additionally enhanced rate and level of LMC destruction and lignin mineralization. The results indicate that lipid peroxidation may play an important role in lignin biodegradation by wood-rotting basidiomycetes and support the hypothesis of coupling between the processes.

Lignin is one of the most recalcitrant biopolymers and an essential part of terrestrial biomass. Wood-rotting basidiomycetous fungi that cause white-rot are the most efficient lignin degraders in nature (1). Therefore, these fungi play a crucial role in maintaining the carbon cycle in terrestrial ecosystems.

It is generally thought that the major enzymes involved in lignin biodegradation by fungi are two extracellular heme-containing peroxidases: lignin peroxidase (LiP, EC 1.11.1.14) and manganese peroxidase (MnP, EC 1.11.1.13) (2–4). The main difference between LiP and MnP is the nature of the substrate that is oxidized. LiP is capable of oxidizing non-phenolic or phenolic lignin structures directly to yield aryl cation radicals and phenoxy radicals, respectively. Subsequently, these radicals undergo various non-enzymatic reactions that lead to many final products (2). For MnP, the primary reducing substrate is divalent manganese ion (Mn^{2+}). The catalytic cycle of MnP in the presence of appropriate chelators generates highly reactive Mn^{3+}-chelate complexes that are able to oxidize various phenols and carboxylic acids to yield phenoxy and carbon-centered radicals, respectively (5, 6). Usually, MnP is not able to oxidize or depolymerize the more recalcitrant non-phenolic lignin structures that make up about 90% of the lignin in wood. However, there are MnP-producing white-rot fungi that evidently lack LiP (for example *Dichomitus squalens* and *Ceriporiopsis subvermispora*), which nevertheless degrade lignin and non-phenolic lignin structures efficiently (7–9). Moreover, it seems that primary attack on lignin requires low molecular weight agents, because LiP and other enzymes are too large to penetrate lignoncellulose (10). Because of these discrepancies, it has been proposed that there are mechanisms that enable MnP to cleave non-phenolic lignin structures via the action of small mediators such as thiyl or lipid radicals (11, 12).

Linoleic acid is the predominant unsaturated fatty acid (UFA) in the lipids of wood-rotting fungi (13, 14). The content of the dienoic acid can reach more than 50% of total fatty acids and even more than 80% in fungal phospholipids (15). Therefore, lipids of wood-rotting fungi are easily oxidized and possess prooxidative properties. White-rot fungi produce reactive lipoxygenases that oxidize linoleic acid and initiate lipid peroxidation enzymatically in the fungal mycelium (16). Based on these findings, it was postulated in 1990

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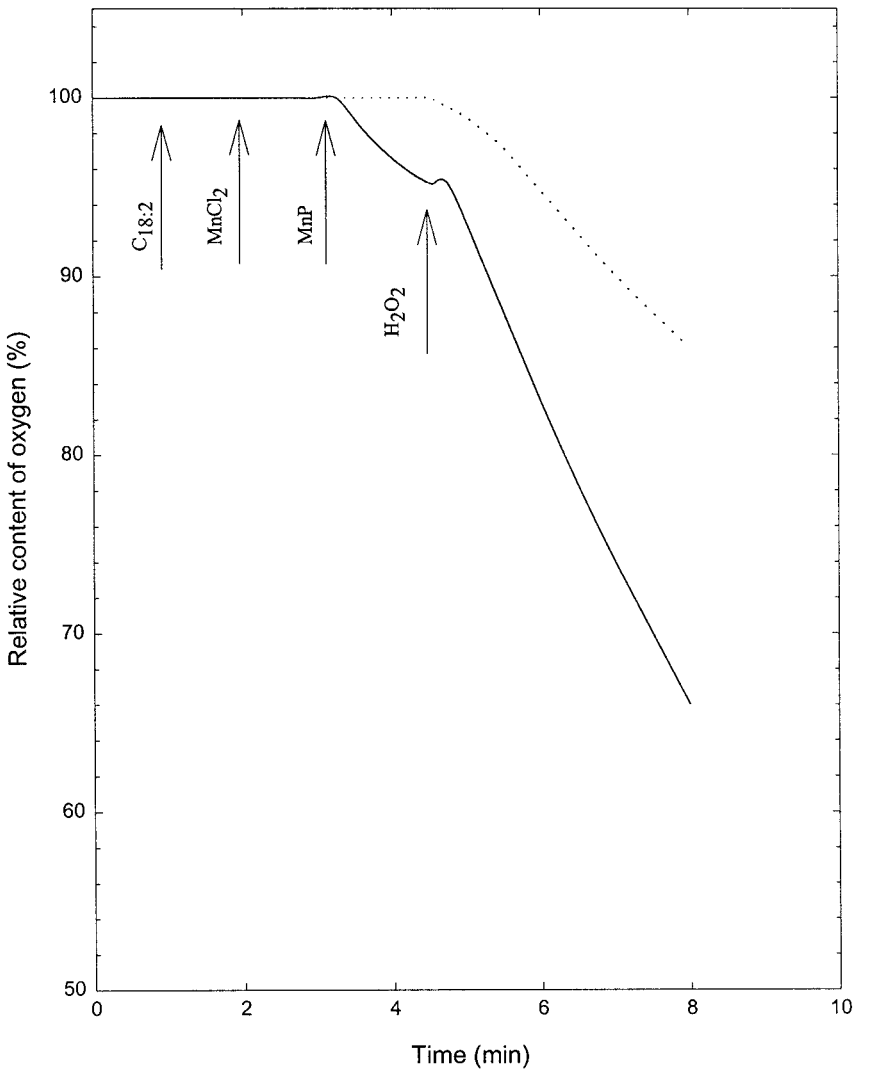

FIG. 1. Oxygen consumption in reaction systems with MnP in the presence of linoleic acid (—) and without (···). Reactions were carried out in 50 mM sodium malonate buffer (pH 4.5) as described in Materials and Methods.

that the degradation of lignin by white-rot fungi may be coupled with peroxidation of their highly unsaturated membrane lipids (13, 17).

Later, it was shown that purified MnP from the white-rot fungus *Phanerochaete chrysosporium* stimulates peroxidation of emulsified UFAs and that this system cleaves non-phenolic lignin model compounds (LMC) oxidatively (12). MnP from another white-rot fungus, *Ceriporiopsis subvermispora*, which does not produce LiP, also degrades non-phenolic lignin structures in the presence of Tween 80 as the source of UFAs (18). Based on these findings, it has been proposed that the oxidation of non-phenolic lignin model compounds may be caused by lipid peroxyl or alkoxyl radicals which appear during MnP-initiated peroxidation of unsaturated lipids/UFAs.

Phlebia radiata is a basidiomycetous white-rot fungus producing LiP, MnP and laccase (4, 19, 20). It degrades non-phenolic lignin model compounds (21). Here we report that purified MnP from the fungus catalyzes peroxidation of linoleic acid and other unsaturated lipids and that the reaction system cleaves a non-phenolic dimeric β-O-4-linked lignin model compound (LMC). Moreover, it also promotes the MnP-catalyzed mineralization of ^{14}C-labeled synthetic lignin and ^{14}C-labeled wheat straw in a cell-free system.

MATERIALS AND METHODS

Organisms, enzyme preparations, and reagents. The basidiomycetous white-rot fungus *Phlebia radiata* 79 (ATCC 64658) was used for MnP production (22). The fungus was cultivated in a low-nitrogen medium (2 mM N) containing 168 μM Mn^{2+} in a 2 liter bioreactor (23) and the isoenzyme MnP2 was purified by FPLC as reported previously (20). MnP activity was determined by monitoring the formation of Mn^{3+}-malonate complexes according to Wariishi et al. (5).

Linoleic acid [*cis*-9,*cis*-12-octadecadienoic acid]; linoleic acid hydroperoxide [13(S)-(9Z,11E)-hydroperoxyoctadecadienoic acid], phosphatidylcholine (Type IV-S, from soybean); 2-thiobarbituric acid, Tween 80 and Tween 20 were obtained from Sigma (Steinheim, Germany). Non-phenolic β-O-4-linked LMC [1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol] was synthesized as described earlier (21). Synthetic ^{14}C-ring-labeled lignin (^{14}C-DHP) was synthesized by Odier and Heckman as described by Eriksson et al. (1). Uniformly ^{14}C-labeled wheat straw was obtained from wheat plants that had been grown with a continuous supply of $^{14}CO_2$ as reported earlier (24) and was milled in a rotating jar ball mill to give ^{14}C-labeled milled wheat straw (^{14}C-MWS) (25).

Oxygen consumption. Oxygen consumption experiments were performed with a YSI 5300 Biological Oxygen Monitor Standard System (Yellow Spring Instrument Co, Ohio, USA). Reaction mixtures were incubated in 5-ml-reaction chambers at 25°C and oxygen uptake was measured with a Clark type YSI 5300 oxygen electrode fitted into the reaction chamber. 3 ml of a freshly prepared, oxygen-saturated solution of 50 mM sodium malonate buffer (pH 4.5) was initially placed into the chamber, then the oxygen electrode was inserted. All other components were added at the indicated final concentrations in the following order: an emulsion of linoleic acid (1 mM), $MnCl_2$ (1 mM), MnP (0.86 U/ml), Mn(III)acetate (0.2 mM) and H_2O_2 (0.2 mM). The linoleic acid emulsion was prepared by vortexing of 31 μl of linoleic acid with 100 μl of ethanol, 100 μl of 1 M Tris and 769 μl of water. Oxygen consumption rates were calculated on the assumption that the initial oxygen concentration in buffer at 25°C was 260 μM (26).

Determination of the thiobarbituric acid-reactive substances (TBA-RS). The complete assay mixture (4.7 ml) contained 0.3 ml of the TBA-RS sample, 0.4 ml of 6% sodium lauryl sulfate, 3 ml of 2% phosphoric acid and 1 ml of thiobarbituric acid solution (0.8% wt/vol) that had been adjusted with 10% NaOH to pH 7.4. The mixtures were capped to prevent evaporation and incubated at 100°C for 15 min. The absorbances of the cooled mixtures were measured at 532 nm against a blank that contained all the reagents but 50 mM malonate buffer in place of the TBA-RS sample. The concentrations of TBA-RS in the samples were estimated using the extinction coefficient of the malonic dialdehyde (MDA) complex with TBA ($\varepsilon_{532\,nm} = 1.54 \times 10^5\ M^{-1}\ cm^{-1}$) (27).

Oxidation of LMC. Reactions were carried out in 10-ml reaction tubes that were covered with parafilm and incubated on a rotary shaker (180 rpm) at 37°C in the dark. The basal reaction mixture contained 50 mM sodium malonate buffer (pH 4.5), 1 mM $MnCl_2$, 0.2 mM H_2O_2, 0.1 mM LMC and 0.86 U MnP in a total of 1 ml. The same reaction mixture without MnP was used as control. Other components were added to give the following final concentrations: linoleic acid as a water emulsion (3 mM), Tween 20 and Tween 80 in water (1% wt/vol), phosphatidylcholine in ethanol (0.2% wt/vol) and linoleic acid hydroperoxide in ethanol (16 μM). Samples (100 μl) of the reaction mixtures were collected daily over a period of three days to determine the degree of LMC degradation.

Products of linoleic acid peroxidation were prepared by incubation of linoleic acid emulsion with MnP. The complete reaction mixture (final volume 3 ml) consisted of linoleic acid (5 mM), $MnCl_2$ (1 mM), MnP (0.72 U/ml), Mn(III)acetate (0.17 mM) and H_2O_2 (0.33 mM). The mixture was incubated on a rotary shaker at 37°C for 24 h in 50 ml flasks covered with foil. The final concentration of TBA-RS in the reaction mixture was 17.5 μM (calculated with the extinction coefficient $\varepsilon_{532\,nm} = 1.54 \times 10^5\ M^{-1}\ cm^{-1}$). In some experiments, the products of linoleic acid peroxidation were added to the reaction mixtures that contained LMC to a final concentration of 5 μM.

HPLC analyses. Oxidation of LMC and formation of aromatic metabolites was analyzed using an HPLC system (HP 1090 Liquid Chromatograph, Hewlett Packard) fitted with a C_{18} reversed phase column (125 × 4 mm, Merck, Darmstadt, Germany). A mixture of acetonitrile and water (20/80, vol/vol) was used as the solvent at a flow rate of 1 ml/min. Routinely, 20 μl of the reaction mixture was injected into the HPLC system and chromatograms were recorded at 275 nm. Authentic standards of LMC and veratraldehyde were used for calibration.

TABLE 1

The Influence of Linoleic Acid on the Maximum Rate of Oxygen Consumption in the Reaction Systems with MnP

Mn^{3+}	H_2O_2	Linoleic acid	Rate of O_2 consumption (nmol/ml · min)
−	−	−	0
−	−	+	4.1
+	−	−	4.0
+	−	+	4.6
−	+	−	5.6
−	+	+	11.6
+	+	−	7.7
+	+	+	11.6

Note. + and −, presence and absence of ingredients. Reactions were carried out in 50 mM sodium malonate buffer (pH 4.5) in the presence of 1 mM $MnCl_2$ and 0.86 U/ml MnP.

Mineralization experiments. Mineralization of ^{14}C-DHP and ^{14}C-MWS by MnP was performed as described recently (25, 28, 29). The reaction mixtures (1 ml) contained 50 mM Na malonate (pH 4.5), 1 mM $MnCl_2$, 0.2 mM H_2O_2, 1% Tween 20 or 80, 2 U MnP and 100 μg ^{14}C-DHP (2.2×10^4 dpm) or 5 mg solid ^{14}C-MWS (1×10^5 dpm). In addition, certain samples contained 3 mM linoleic acid. Controls did not contain MnP. Released $^{14}CO_2$ was trapped every 24–48 h and determined by liquid scintillation counting (LSC; Model 1411 counter, Wallac Oy, Turku, Finland).

RESULTS

Linoleic acid peroxidation initiated by MnP. As shown in Fig. 1, *P. radiata* MnP initiated oxygen consumption in a reaction mixture that contained Na malonate buffer, Mn^{2+} and linoleic acid. The reaction started immediately after addition of MnP, but slowed down quickly. Oxygen consumption was not observed when linoleic acid was omitted. The oxygen uptake was probably caused by MnP-initiated peroxidation of linoleic acid. Thus, it can be concluded that MnP possesses a lipoxygenase-like activity. Addition of H_2O_2 (0.2 mM) stimulated oxygen consumption in the presence of linoleic acid and initiated its in the absence. In the presence of linoleic acid, however, the rate of oxygen consumption was twice as high.

Oxygen consumption did occur in reaction mixtures without linoleic acid when Mn^{3+} and/or H_2O_2 were added to the system containing malonate, Mn^{2+} and MnP (Table 1). However, the presence of linoleic acid in all these reaction mixtures increased the oxygen uptake.

Oxygen consumption in reaction mixtures that contained linoleic acid was accompanied by the development of the fatty acid peroxidation, as shown by the appearance of substances that reacted with thiobarbituric acid (TBA-RS). Figure 2 shows that TBA-RS were actively produced only in complete system when both

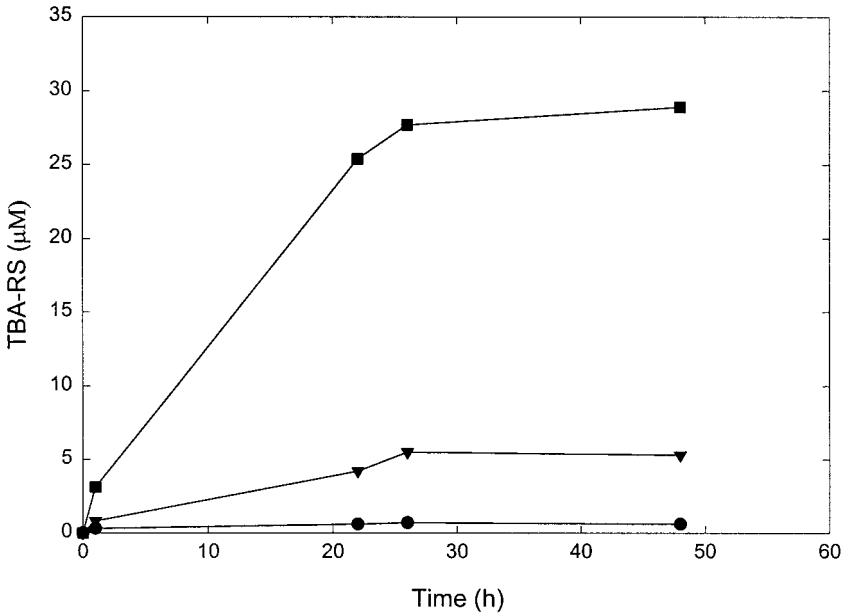

FIG. 2. Accumulation of TBA-RS in the complete reaction mixture containing MnP, Mn^{2+}, malonate, linoleic acid, H_2O_2 (■), and in reaction mixtures lacking MnP (▼) or linoleic acid (●).

components, linoleic acid and MnP, were added. There was no production of TBA-RS in the reaction system without linoleic acid. In the absence of MnP, the process of linoleic acid peroxidation developed autocatalytically, but very slowly.

Destruction of non-phenolic LMC by MnP. Addition of different sources of UFAs to reaction mixtures with MnP resulted in partial destruction of the non-phenolic LMC within 72 h (Table 2). MnP alone converted only 2% of the lignin model compound. In the presence of linoleic acid or phosphatidylcholine, LMC degradation increased a little, but was rather low (7.4% and 14%, respectively). The best results were obtained with Tween 80, which acts both as a surfactant for the system and as source of UFAs. Its addition to the reaction mixture enhanced LMC degradation considerably (51.3%). LMC destruction in the presence of linoleic acid was enhanced when the reactions contained linoleic acid that had already been oxidized by MnP. However, pre-oxidized linoleic acid did not enhance LMC degradation in the systems with Tween 80 and phosphatidylcholine.

When Tween 20, which contains only saturated fatty acids, was introduced to the reaction mixtures, LMC degradation was low (3.8%; Table 3). However, Tween 20 and linoleic acid together gave significant LMC degradation (27.9%) which was about three times higher than that in the system with linoleic acid alone (9.1%). The addition of linoleic acid enhanced the degree of LMC degradation even in the systems with Tween 80 (from 43.8% to 51.3%). HPLC analysis combined with UV spectrophotometry (diode array detection) demonstrated that veratraldehyde was one product of LMC destruction (chromatograms not shown). Its concentration, however, was relatively low (0.5–5 μM). Thus, the formation of other products has to be assumed, e.g. of the LMC ketone described by Jensen et al. (18).

Linoleic acid hydroperoxides are the major primary molecular products that are produced during linoleic acid peroxidation and facilitate the process of the peroxidation. However, addition of alcoholic solutions of 13(S)-[9Z,11E]- hydroperoxyoctadecadienoic acid to the MnP reaction mixtures slightly inhibited the LMC degradation in all reactions.

TABLE 2

The Influence of Products of Linoleic Acid Peroxidation and Different Sources of UFAs on the Degree of LMC Degradation in Reaction Systems with MnP

Sources of UFAs	Products of linoleic acid peroxidation	Degree of LMC degradation (%)
Without UFAs	−	2
Linoleic acid	−	7.4
Linoleic acid	+	15.5
Tween 80	−	51.3
Tween 80	+	50.1
Phosphatidylcholine	−	14.0
Phosphatidylcholine	+	10.4

Note. + and −, presence and absence of ingredient. Reactions were carried out in 50 mM sodium malonate buffer (pH 4.5) in the presence of 1 mM $MnCl_2$, 0.2 mM H_2O_2, 0.1 mM LMC, and 0.86 U/ml MnP. Time of incubation: 72 h.

TABLE 3

The Influence of Linoleic Acid Hydroperoxide, Linoleic Acid, and Different Surfactants on the Degree of LMC Degradation in Reaction Systems with MnP

Linoleic acid	Surfactants	Linoleic acid hydroperoxide	Degree of LMC degradation (%)
−	−	−	2
+	−	−	9.1
+	−	+	7.1
−	Tween 20	−	3.8
−	Tween 20	+	2
+	Tween 20	−	27.9
+	Tween 20	+	20.6
−	Tween 80	−	43.8
−	Tween 80	+	27.9
+	Tween 80	−	51.3
+	Tween 80	+	40.1

Note. + and −, presence and absence of ingredients. The basal reaction mixture contained the same components as described in the footnote to Table 2. Time of incubation: 24 h.

Mineralization of ^{14}C-DHP and ^{14}C-MWS. The MnP-catalyzed mineralization of ^{14}C-DHP was stimulated by linoleic acid and Tween 80. Figure 3 shows the time courses of $^{14}CO_2$ evolution from ^{14}C-DHP during incubation with MnP in different reaction mixtures. In all cases, most $^{14}CO_2$ was evolved during the first 72 hours, after which mineralization decreased rapidly. The highest mineralization rate was achieved in the complete reaction system that contained Tween 80 and linoleic acid (approximately 6% $^{14}CO_2$/d). $^{14}CO_2$ evolution began immediately after starting the reaction with H_2O_2 in this sample, whereas a lag-period of 12 h was observed for all other samples. Evidently, the addition of H_2O_2 initiated the action of MnP on UFAs and lignin, whereas this process had to develop autocatalitically in the absence of H_2O_2 (25). In all, about 16% of the synthetic lignin was converted to CO_2 within seven days in the complete reaction. Samples that contained Tween 80 without linoleic acid mineralized 11% of ^{14}C-DHP while attaining a maximum mineralization rate of 4% $^{14}CO_2$/d. MnP in the presence of Tween 20 mineralized only 4.5% of lignin—a percentage which is comparable with that which had been measured in earlier studies with MnP (29). The addition of linoleic acid to the MnP-Tween-20-samples increased $^{14}CO_2$ release considerably, which indicates that this UFA is in fact somehow involved in lignin mineralization.

Similar results were obtained with solid ^{14}C-MWS, although rates and extent of mineralization were lower and the effects of Tween 80 and linoleic acid were less marked (Table 4). In part, these results probably reflect the fact that the substrate was solid lignocellulose rather than colloidal lignin. Nevertheless, it is clear that more straw was mineralized by MnP in the presence of both Tween 80 and linoleic acid than without these additions and also that linoleic acid increased $^{14}CO_2$-formation from ^{14}C-MWS in MnP-Tween-20-samples.

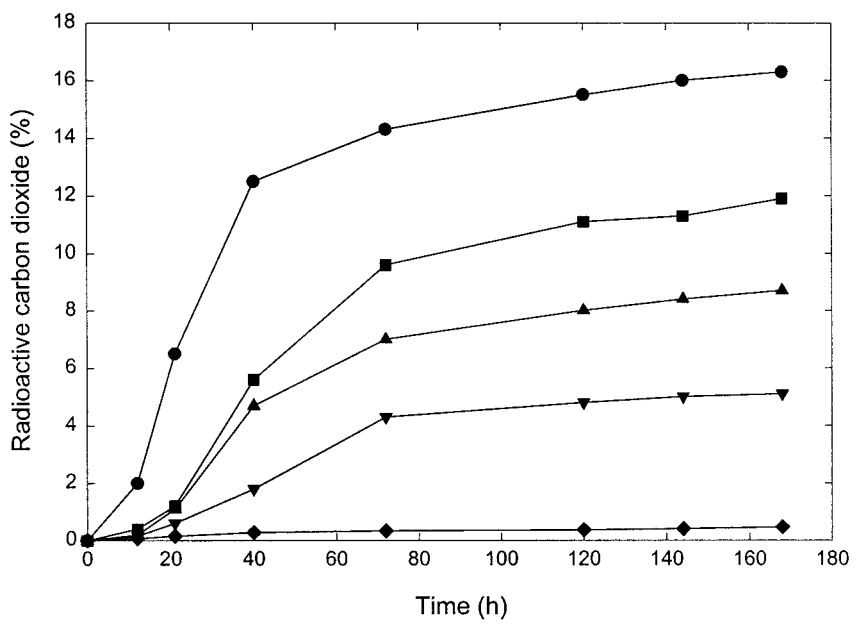

FIG. 3. Mineralization of ^{14}C-ring-labeled lignin (^{14}C-DHP) by MnP from *P. radiata* in the presence of different sources of unsaturated fatty acids in a cell-free system. MnP + Tween 80 + linoleic acid (●), MnP + Tween 80 (■), MnP + Tween 20 + linoleic acid (▲), MnP + Tween 20 (▼), control without MnP (◆).

TABLE 4

The Influence of Linoleic Acid and Different Surfactants on Mineralization of ^{14}C-Labeled MWS in Reaction Systems with MnP

Sample	$^{14}CO_2$ (%) after 24 h	$^{14}CO_2$ (%) after 168 h
control without MnP	<0.1	0.1
MnP + Tween 20	0.1	1.8
MnP + Tween 20 + linoleic acid	0.4	2.2
MnP + Tween 80	0.7	2.6
MnP + Tween 80 + linoleic acid	0.4	3.6

Note. Reactions were carried out in 50 mM sodium malonate buffer (pH 4.5) in the presence of 1 mM $MnCl_2$, 0.2 mM H_2O_2, 2 U/ml MnP, and 5 mg/ml solid ^{14}C-MWS (milled wheat straw).

DISCUSSION

Linoleic acid and the UFA containing surfactant Tween 80 enabled MnP from *P. radiata* to destroy the non-phenolic dimeric β-O-4-linked LMC substantially and enhanced the MnP-catalyzed mineralization of synthetic lignin (^{14}C-DHP) as well as of solid milled wheat straw (^{14}C-MWS).

The best results were obtained in reaction systems with Tween 80, which acts both as a surfactant and a source of UFAs. Recently, it has been shown that, in the presence of Tween 80, MnPs of other white-rot fungi (*Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora*) destroy similar dimeric non-phenolic β-O-4 LMC oxidatively (12, 18). However, the system worked slowly: no more than half of the LMC was oxidized in 112 h.

Tween 80 contains oleic acid, which is monounsaturated, as its predominant fatty acid. Thus, it is not an optimal substrate for peroxidation, because the readiness of fatty acids to be peroxidized increases with their degree of unsaturation (30). We observed that the addition of diunsaturated fatty acid, linoleic acid, to the reaction system with MnP and Tween 80 enhanced the rate and extent of LMC destruction. The degree of destruction approached 50% within 24 h. The higher efficiency of our system is also probably related to the higher incubation temperature (37°C) that we employed.

Tween 20, another surfactant that does not contain any UFAs, was found to be ineffective in supporting MnP-catalyzed oxidation of LMC, in agreement with the results of Bao et al. (12). However, substantial degradation of LMC was observed when linoleic acid was added to the MnP-Tween-20-system. By contrast, linoleic acid alone was less effective. Thus, two properties of the reaction system are important for the destruction of LMC by MnP: the presence of UFAs as substrates for lipid peroxidation and the presence of surfactants that provide maximum homogeneity of the water-insoluble components.

MnP of *P. radiata* oxidized linoleic acid in a reaction system that contained malonate and Mn^{2+}. This process was accompanied by the consumption of oxygen and the accumulation of TBA-RS. It is generally agreed that lipid peroxidation proceeds by a free radical mechanism (27). Oxygen consumption during this process results mainly from reactions between carbon-centered lipid radicals and molecular oxygen, which results in the formation of peroxyl radicals.

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \quad [1]$$

An additional uptake of oxygen takes place in subsequent decomposition reactions. Therefore, the measurement of oxygen uptake is considered a useful indication for the development of lipid peroxidation. Lipid peroxyl radicals are highly reactive and can react with other lipid molecules to propagate the oxidation (31).

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad [2]$$

Lipid peroxyl radicals are also able to abstract hydrogen from other organic compounds to give other radicals. They are relatively stable and can diffuse over comparatively long distances in biological systems. Thus, if peroxyl radicals are produced by white-rot fungi in wood, they may be involved in lignin degradation (12, 32). However, the mechanism that couples MnP-mediated lipid peroxidation with lignin degradation is probably more complex. We have shown that LMC is not destroyed when linoleic acid is peroxidized by fungal lipoxygenase, which does produce lipid peroxyl radicals. It may be that lipid-derived peroxyl radicals alone are not capable of attacking lignin in the absence of MnP and that the cooperation of Mn^{3+}-chelates and lipid radicals is required for efficient attack on lignin.

We also observed oxygen consumption in systems without linoleic acid when Mn^{3+} and/or H_2O_2 were added to reaction mixtures that contained malonate, Mn^{2+} and MnP. In this case, the uptake of oxygen was connected with the oxidation of malonic acid. Hofrichter et al. (6) have shown that MnP from *P. radiata* oxidizes malonate aerobically even in the absence of H_2O_2 via an autocatalytic process. These reactions have a long lag-period (about 3 h), but the addition of Mn^{3+} into the system promoted malonate oxidation. This oxygen uptake is caused by the reaction of O_2 with carbon-centered radicals that are generated from malonic acid. Thus, this reaction proceeds via a similar mechanism as that between lipid alkyl radicals and oxygen, Eq. [1]. As a result of malonate decomposition, peroxyl acetic acid radicals can appear in the reaction system (6). It can be assumed that these and other

radicals derived from malonate oxidation are able to attack unsaturated fatty acids and thus enhance the total rate of MnP-mediated lipid peroxidation and LMC destruction. Hydrogen peroxide, which may be produced through MnP-dependent oxidation of organic acids, may also stimulate the processes (33). Moreover, Mn^{3+} itself is very likely involved in this process.

It is well-known that lipid peroxidation is propagated by its own products. For example, iron-catalyzed lipid peroxidation is dependent on the presence of lipid hydroperoxides (34). However, we found that stimulation of lipid peroxidation by linoleic acid hydroperoxide did not stimulate LMC degradation in our experiments. A possible explanation for this could be the radical quenching effect of ethanol in which the hydroperoxide was dissolved.

Both linoleic acid and Tween 80 promoted the MnP-catalyzed direct mineralization of lignin ("enzymatic combustion"). This process probably enables white-rot fungi to convert a part of lignin outside their hyphae to CO_2. In case of ^{14}C-DHP, the stimulating effect of Tween 80 and linoleic acid was even greater than that of glutathione, which also enhances the mineralization of lignin by MnP (25, 28, 29). Moreover, white-rot fungi are more likely to secrete UFA-containing lipids than organic thiols like glutathione.

Lipid peroxidation is well-known first of all as undesirable process that damages cell membranes and thus has the potential to initiate different types of diseases in human beings and animals (27, 35). But it is not an entirely negative event, since some "normal" processes seem to involve lipid peroxides. Examples of such bioprocesses are phagocytosis and prostaglandin synthesis (27, 36). Much less is known about the possible positive functions of lipid peroxidation in microorganisms. It has been proposed that lipid peroxidation may play a substantial role in such processes as bioluminiscence, oxidative transformation of metals and organopollutant oxidation (37–39). The present work gives indications that lipid peroxidation may also be engaged in lignin biodegradation and confirms the hypothesis of coupling between the processes.

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