Enzyme and Microbial Technology 46 (2010) 136–140

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Oxidizability of unsaturated fatty acids and of a non-phenolic lignin structure in the manganese peroxidase-dependent lipid peroxidation system

Alexander N. Kapich[a,b,c,*], Tatyana V. Korneichik[c], Annele Hatakka[b], Kenneth E. Hammel[a]

[a] Institute for Microbial and Biochemical Sciences, USDA Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53726, USA
[b] Department of Applied Chemistry and Microbiology, University of Helsinki, Viikinkaari 9, Viikki Biocenter 1, P.O.Box 56, 00014 Helsinki, Finland
[c] International Sakharov Environmental University, 23 Dolgobrodskaya Str, 220009 Minsk, Belarus

ARTICLE INFO

Article history:
Received 25 June 2009
Received in revised form
25 September 2009
Accepted 29 September 2009

Keywords:
Lignin degradation
Lipid peroxidation
Manganese peroxidase
Unsaturated fatty acids
White rot

ABSTRACT

Unsaturated fatty acids have been proposed to mediate the oxidation of recalcitrant, non-phenolic lignin structures by fungal manganese peroxidases (MnP), but their precise role remains unknown. We investigated the oxidizability of three fatty acids with varying degrees of polyunsaturation (linoleic, linolenic, and arachidonic acids) by measuring conjugated dienes formation when lipid peroxidation was initiated either by MnP in the presence of Mn(II) or by chelated Mn(III). An inverse relationship between the degree of fatty acid unsaturation and the rate of peroxidation was found in both cases, but we also noted some differences between the two types of reaction. With MnP/Mn(II), the reaction developed slowly and resulted in sustained lipid peroxidation as determined by the formation of late-stage fatty acid degradation products. By contrast, the reaction with chelated Mn(III) was very rapid and did not result in the formation of these late-stage products, which suggests that this system failed to propagate the sustained radical chain reaction that is characteristic of complete lipid peroxidation. All three polyunsaturated fatty acids supported the co-oxidation of a non-phenolic lignin model compound by MnP, again showing an inverse relationship between the degree of unsaturation and reactivity, but chelated Mn(III) by itself supported only very low levels of fatty acid-mediated lignin model oxidation. These parallels in fatty acid reactivity are consistent with a reaction scheme in which Mn(III) acts as the proximal oxidant that initiates lipid peroxidation by MnP, thus generating fatty acid-derived radicals which in turn oxidize lignin structures. However, the results also suggest that the initial peroxyl radicals formed may not be the ligninolytic oxidants in this system. Instead, other radical oxidants produced during late-stage reactions of lipid peroxidation may be required.

1. Introduction

Manganese peroxidase (MnP) is a ligninolytic enzyme produced by almost all basidiomycetous fungi that cause white rot of wood [1–3]. This extracellular heme peroxidase requires Mn(II) as its reducing substrate, oxidizing it to Mn(III), which is then chelated by various organic acids. The Mn(III)–chelator complexes in turn act as low molecular weight, diffusible oxidants of the relatively labile phenolic substructures in lignin. However, non-phenolic units predominate in natural lignin and are recalcitrant to degradation by this mechanism.

It has been proposed that radical mediator mechanisms may fill this gap in the reactivity of MnP [4,5]. For example, MnP initiates the peroxidation of unsaturated fatty acids [6], generating in the process species that are capable of oxidizing and cleaving non-phenolic structures in synthetic lignins [7,8]. Unsaturated fatty acids have also been shown to enhance the ability of MnP to bleach (i.e., delignify) wood pulps [9]. The nature of the responsible oxidants in these systems is not clearly understood, but lipid peroxyl radicals are possible candidates because non-phenolic lignin structures are similarly co-oxidized during the thermolysis of azo radical initiators, which are well-known precursors of peroxyl radicals [7].

Although the involvement of lipid peroxidation in fungal ligninolysis remains hypothetical, observations that MnP-producing white rot fungi produce extracellular lipids are consistent with such a role [10,11]. Since linoleic acid (18:2) is the predominant unsaturated fatty acid in lipids of wood-decaying basidiomycetous fungi [12–16], and is also the major naturally occurring fatty acid in wood [17–19], it might serve as a natural co-oxidant to enable MnP-mediated ligninolysis. On the other hand, polyunsaturated fatty acids such as linolenic acid (18:3) or arachidonic acid (20:4) might be better candidates, given that previous work has shown the sus-

* Corresponding author at: Institute for Microbial and Biochemical Sciences, USDA Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53726, USA. Tel.: +1 608 231 9456; fax: +1 608 231 9262.
E-mail address: kapich@wisc.edu (A.N. Kapich).

0141-0229/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2009.09.014

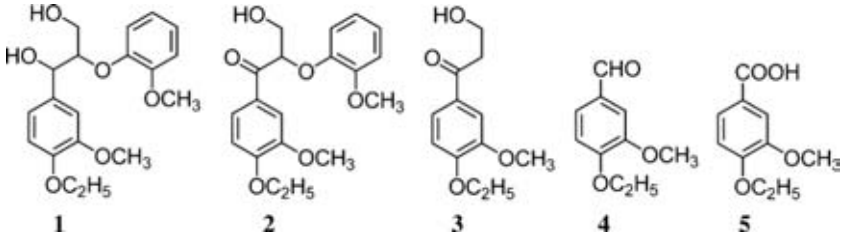

Fig. 1. Chemical structures of lignin model **1** and products derived from it.

ceptibility of fatty acids to peroxidation in homogeneous solution to increase with the number of double bonds they contain [20,21].

The goal of the present work was to compare the efficacy of linoleic acid with that of other common unsaturated fatty acids in the MnP-mediated lipid peroxidation system. Specifically, we have determined whether there is any correlation between the degree of unsaturation in fatty acids, their susceptibility to peroxidation by MnP, and their efficacy in promoting the co-oxidation of a non-phenolic lignin model compound.

2. Materials and methods

2.1. Chemicals and solutions

Tween 20, Mn(III) acetate, oleic, linoleic, linolenic and arachidonic acids were purchased from Sigma–Aldrich. Mn(III) tartrate solutions were prepared by dissolving manganese(III) acetate in 100 mM sodium tartrate (pH 4.5) in darkness immediately prior to use [22]. The non-phenolic β-*O*-4 lignin model compound (**1**, Fig. 1), 1-(4-ethoxy-3-methoxy-*ring*-[^{14}C]phenyl)-2-(2-methoxyphenoxy)-propane-1,3-diol, was prepared by reducing 1-(4-ethoxy-3-methoxy-*ring*-[^{14}C]phenyl)-1-oxo-2-(2-methoxyphenoxy)-propan-3-ol (**2**) with $NaBH_4$ in 95% ethanol at room temperature. The lignin model **1** was purified by preparative high performance liquid chromatography (HPLC) to greater than 99% chemical and radiochemical purity as previously described [7]. Compound **2** was prepared from *ring*-[^{14}C]-acetovanillone by the method of Landucci et al. [23]. *ring*-[^{14}C]-Acetovanillone was custom synthesized by New England Nuclear. A standard of 1-(4-ethoxy-3-methoxyphenyl)-1-oxo-propane-2,3-diol (compound **3**) was prepared as described [7]. 4-Ethoxy-3-methoxybenzaldehyde (compound **4**) was purchased from Pfaltz and Bauer, and 4-ethoxy-3-methoxybenzoic acid (compound **5**) was obtained by alkylating vanillic acid with ethyl iodide [24].

2.2. Enzyme preparation and assays

Recombinant *Phanerochaete chrysosporium* MnP (isoenzyme H4) was expressed in cultures of *Aspergillus oryzae* and purified by anion-exchange chromatography on DEAE-Biogel A as described [25]. MnP activity was quantified spectrophotometrically as described [22]. The reaction mixture (1 ml) contained 50 mM sodium tartrate (pH 4.5), 1 mM $MnSO_4$, 0.5 mM 2,6-dimethoxyphenol, and MnP. The reaction was started by adding 0.1 mM hydrogen peroxide. One unit (U) represents 1 μmol of 2,6-dimethoxyphenol dimer (diphenoquinone) formed in 1 min at room temperature. The MnP isoenzyme preparation was also tested for oxidation of veratryl alcohol to veratraldehyde at 310 nm to confirm that lignin peroxidase activity was undetectable [26].

2.3. Conjugated dienes formation

The kinetics of MnP and Mn(III)-mediated formation of conjugated dienes in unsaturated fatty acids was assayed spectrophotometrically by monitoring the increase in absorption at 233 nm. The reactions were carried out in 1-cm path length quartz cuvettes. The reaction mixtures for MnP-initiated reactions (1 ml) contained 50 mM sodium acetate buffer (pH 4.5), 1 mM $MnSO_4$, and 1 mM of an unsaturated fatty acid that had been emulsified immediately beforehand in argon-saturated Tween 20 (0.1% wt/vol final concentration). The reactions were initiated by adding 0.015 U of MnP. H_2O_2 was not included because previous work has shown that it is not required [5–7]. The reaction mixtures for Mn(III)-initiated lipid peroxidation contained the same buffer and fatty acid emulsions as above without MnP and $MnSO_4$. Instead, these reactions were initiated by adding 0.04 mM Mn(III) tartrate.

2.4. Determination of thiobarbituric acid-reactive substances (TBARS)

The accumulation of TBARS was followed during peroxidation of linoleic acid initiated by MnP/Mn(II) or Mn(III) tartrate. The reaction mixtures for MnP-initiated reactions (1 ml) contained 20 mM sodium acetate buffer (pH 4.5), 1 mM $MnSO_4$, and 5 mM of linoleic acid that had been emulsified in argon-saturated Tween 20 (1% wt/vol final concentration). The reactions were initiated by adding 0.3 U of MnP. The reaction mixtures for Mn(III)-initiated lipid peroxidation contained the same buffer and linoleic acid emulsion as above without MnP and $MnSO_4$. Instead, these reactions were initiated by adding 0.2 mM Mn(III) tartrate. Controls without MnP and Mn(III) tartrate were set up for each variant. The assay mixtures for TBARS contained 150 μl of the reaction mixtures, 200 μl of 6% sodium lauryl sulfate, 1.5 ml of 2% phosphoric acid, 0.5 ml of thiobarbituric acid solution (0.8% wt/vol) and 50 μl of 10 mM butylated hydroxytoluene in ethanol. The thiobarbituric acid solution was prepared as described [8]. The mixtures were capped to prevent evaporation and incubated at 100 °C for 15 min. The absorbance of the cooled assay mixtures was measured at 532 nm.

2.5. Oxidation of ^{14}C-labeled non-phenolic lignin model *1*

Reactions with lignin model **1** were carried out in loosely capped 6-ml scintillation vials that were incubated on a rotary shaker (100 rpm) at 40 °C in the dark. The reaction mixtures for MnP-initiated reactions with different unsaturated fatty acids contained 20 mM sodium acetate buffer (pH 4.5), 10 mM of an emulsified unsaturated fatty acid, 3% (wt/vol) Tween 20, 1 mM $MnSO_4$, 0.34 mM ^{14}C-labeled **1** (1.1×10^6 dpm) and 0.6 U of MnP in a total volume of 2 ml. The reaction mixtures for Mn(III)-initiated reactions contained the same compounds but without MnP and $MnSO_4$. Instead, these reactions were initiated by adding 0.2 mM Mn(III) tartrate. The reaction mixture used to assess the detailed time course of lignin model **1** oxidation during MnP-initiated linoleic acid peroxidation contained the same compounds as for the other reactions with MnP but 5 mM of linoleic acid and 1% (wt/vol) Tween 20. Samples (130 μl) of the reaction mixtures were collected at indicated intervals, filtered through a 0.45-μm pore size nylon membrane, and analyzed by high performance liquid chromatography (HPLC).

2.6. HPLC analyses

A 100-μl portion of each filtered reaction mixture was fractionated by reverse phase HPLC on a C18 column (Vydac 201TP104, 10 μm particle size, 250 mm × 4.6 mm). The column was eluted at 1.0 ml/min and ambient temperature with the following program: 0–5 min: $H_2O/CH_3OH/HCOOH$, 75:25:0.1. 44 min: $H_2O/CH_3OH/HCOOH$, 50:50:0.1. 46 min: $CH_3OH/HCOOH$ 100:0.1. Fractions (0.5 ml) were collected and assayed for ^{14}C by scintillation counting.

3. Results and discussion

3.1. Peroxidation of fatty acids initiated by MnP or Mn(III)

To assess the oxidation of unsaturated fatty acids by MnP in the presence of its substrate Mn(II), we monitored the formation of conjugated dienes spectrophotometrically at 233 nm. This assay is diagnostic for the first propagation reaction in lipid peroxidation, which consists of hydrogen abstraction from the methylene unit situated between two fatty acid double bonds, followed by rapid isomerization and addition of O_2 to give a peroxyl radical. It should be noted that monounsaturated fatty acids give a negative result in this assay, even though they are peroxidizable [27], because they cannot yield the necessary products with two conjugated double bonds.

All of the polyunsaturated fatty acids that we tested gave positive results, as shown in Fig. 2 for linoleic acid. We observed an inverse relationship between the degree of fatty acid unsaturation and the rate of peroxidation, i.e., linoleic acid (18:2) > linolenic acid (18:3) > arachidonic acid (20:4) (Fig. 3). Although the peroxidizability of fatty acids is commonly thought to increase with their degree of unsaturation, our results agree with studies that concluded more unsaturated fatty acids are less peroxidizable in micellar systems

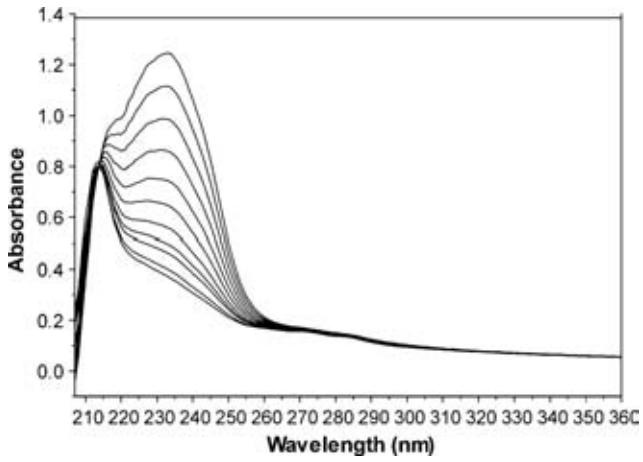

Fig. 2. UV absorption spectral changes during MnP-initiated peroxidation of linoleic acid. Spectra were taken at 30 s intervals.

because the resulting peroxyl radicals are more polar and consequently migrate to the periphery of the micelles, where they cannot participate efficiently in radical chain propagation [28,29]. Oleic acid (18:1) gave the expected negative result (Fig. 3), but we were able to confirm that it was peroxidized by MnP plus Mn(II) by showing that oxygen uptake occurred under our reaction conditions (data not shown).

Since Mn(III) chelates are the immediate products of Mn(II) oxidation by MnP, we next assessed the ability of Mn(III) tartrate to produce diene conjugation in unsaturated fatty acids. The results confirmed that this species was able to initiate lipid peroxidation by itself, in agreement with past work [30], and showed in addition that the order of oxidizability of the fatty acids was the same as that shown by MnP in the presence of Mn(II) (Fig. 4). Thus, it is apparently not necessary for MnP to react directly with components in the lipids to initiate peroxidation. Instead, it suffices for the enzyme to produce Mn(III), which itself acts as the proximal oxidant of the lipids. Mn(III) probably does this by oxidizing pre-existing hydroperoxides, which are universal contaminants in unsaturated lipids, and also by oxidizing the enolic form of free fatty acids to give chain-propagating acyl radicals [30].

However, further investigation showed that the course of lipid peroxidation initiated by Mn(III) was not identical to that initiated by MnP plus Mn(II). We observed that the Mn(III)-mediated reaction with linoleic acid failed to generate thiobarbituric acid-reactive substances (TBARS), whereas the enzyme-mediated reaction gave

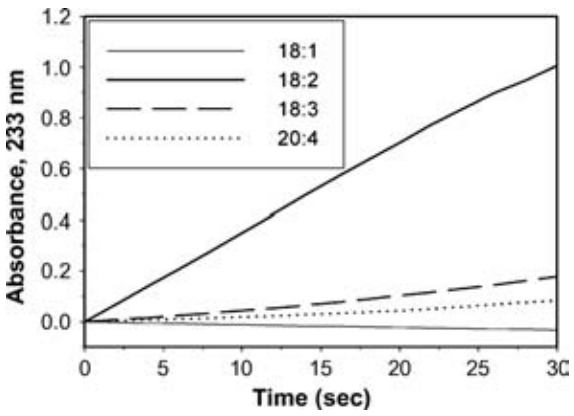

Fig. 4. Time course of conjugated dienes formation during Mn(III)-initiated peroxidation of unsaturated fatty acids.

a positive reaction (Fig. 5). TBARS are mixtures of late-stage degradation products, including malondialdehyde, that are formed from peroxidizing polyunsaturated fatty acids and give a colorimetric reaction with thiobarbituric acid [27]. That is, although Mn(III) alone clearly sufficed to generate peroxyl radicals in linoleic acid, as shown by our diene conjugation experiment (Fig. 4), under our reaction conditions it was unable to support the subsequent chain reactions that lead to extensive oxidative fragmentation of this fatty acid.

3.2. Lignin model compound oxidation as a result of lipid peroxidation by MnP

Next, we investigated the ability of MnP/Mn(II) to oxidize a non-phenolic β-*O*-4-linked lignin model compound **1** in the presence of fatty acids with various degrees of unsaturation (Fig. 6, Table 1). The principal products were the ketones **2** and **3**, the benzaldehyde **4**, and the benzoic acid **5**. The routes for formation of these compounds, already discussed in our previous work [7], probably involve benzylic hydrogen abstraction to give intermediate carbon-centered radicals in the case of products **2** and **3**, and electron abstraction to give intermediate cation radicals in the case of products **4** and **5**. The results showed that the abilities of linoleic, linolenic, and arachidonic acids to promote lignin model oxidation were inversely related to their degrees of unsaturation, in agreement with the relative rates at which MnP/Mn(II) and Mn(III) tartrate caused them to undergo diene conjugation

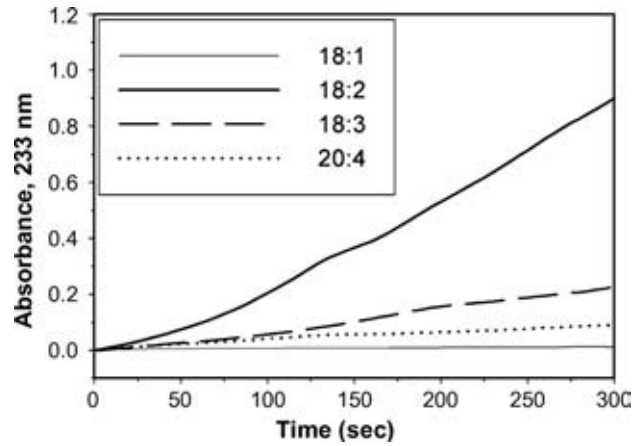

Fig. 3. Time course of conjugated dienes formation during MnP-initiated peroxidation of unsaturated fatty acids.

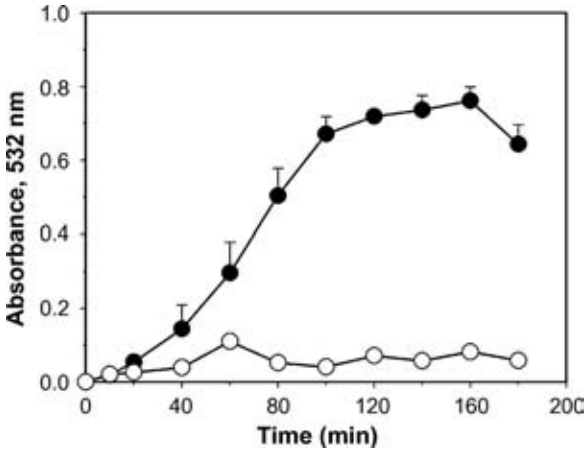

Fig. 5. Time course of TBARS formation during peroxidation of linoleic acid initiated by MnP/Mn(II) (●) or Mn(III) tartrate (○).

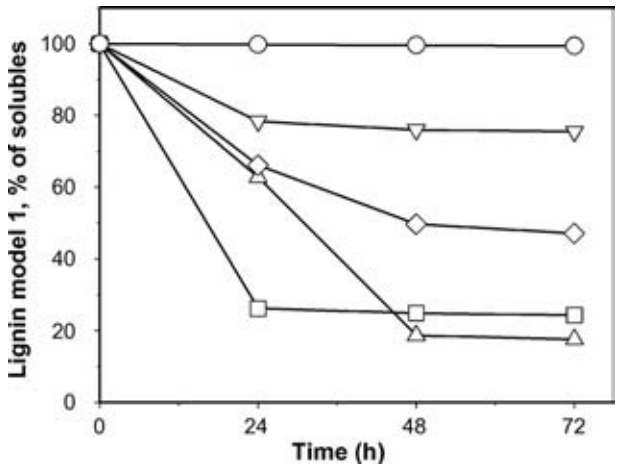

Fig. 6. Time course of lignin model **1** removal by MnP in the presence of Mn(II) and various unsaturated fatty acids as follows: 18:1 (△); 18:2 (□); 18:3 (◊); 20:4 (▽); control without fatty acids (○).

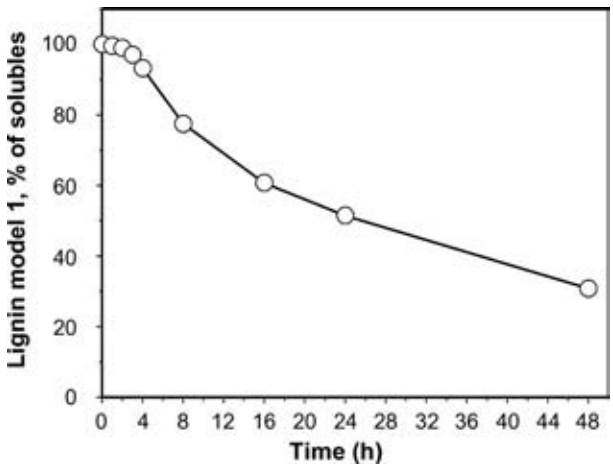

Fig. 7. Detailed time course of lignin model **1** removal by MnP in the presence of Mn(II) and linoleic acid.

(Figs. 3 and 4). That is, the extent of lignin model oxidation we found was correlated with the extent of initial peroxyl radical generation that occurred in the polyunsaturated fatty acids we used as co-oxidants. However, an additional experiment with linoleic acid as the co-oxidant showed that the detailed time courses for these two processes differed: although linoleate-derived peroxyl radicals were generated immediately by MnP in the presence of Mn(II) (Fig. 3), the maximal rate of lignin model oxidation by this system occurred only after a lag of several hours (Fig. 7).

Our experiments also showed that the monounsaturated lipid oleic acid was effective in promoting model **1** oxidation (Fig. 6, Table 1), which is not surprising because this fatty acid is peroxidizable [27]. We could not compare the extent of lipid peroxidation that occurred in this case with the extents that occurred with the polyunsaturated fatty acids, because the peroxidation of oleic acid does not result in the formation of diene conjugates, as discussed above. Moreover, although late-stage products of fatty acid scission are formed from oleic acid, they do not react with thiobarbituric acid [27]. The high efficacy of oleic acid as a mediator for model **1** oxidation by MnP-initiated lipid peroxidation may be related to the relatively lower polarity of its radicals as compared with the radicals of polyunsaturated fatty acids, which is expected to increase the efficiency of peroxidation, as mentioned above [28,29].

In further work, we attempted to oxidize lignin model **1** with the Mn(III) tartrate system in the presence of unsaturated fatty acids. This experiment gave only 6% lignin model conversion when linoleic acid was used as the co-oxidant, and even lower yields with the other fatty acids (Table 1). The result with linoleic acid, Mn(III), and lignin model **I** contrasts with the high extent to which Mn(III) alone was able to produce initial peroxyl radicals in linoleic acid (i.e., diene conjugates, Fig. 4), but appears in line with the inability of Mn(III) alone to produce late-stage products of linoleic acid peroxidation (i.e., TBARS, Fig. 5).

3.3. Conclusions

In summary, in our experiments a peroxidative system that generated high levels of initial fatty acid peroxyl radicals was required for optimal lignin model oxidation, but was not by itself sufficient for this reaction to occur. One possible explanation is that the peroxyl radicals generated by Mn(III) alone may have reacted with each other before they could react with the lignin model compound. As the rate of lipid peroxidation rises, the bimolecular reaction of lipid peroxyl radicals by the Russell mechanism [27] to give non-radical products plus singlet oxygen becomes an increasingly dominant process because its rate increases as the square of the peroxyl radical concentration. Thus, since the rate of diene conjugate formation was roughly ten times faster with Mn(III) tartrate than it was with MnP/Mn(II) in our experiments (Figs. 3 and 4), the proportion of peroxyl radicals removed via bimolecular self-reactions was probably about tenfold greater in the Mn(III) tartrate reaction. If the initially formed fatty acid peroxyl radicals are the ligninolytic oxidants in this system, a competing self-reaction between them is expected to reduce the efficiency of lignin structure oxidation because singlet oxygen does not cleave lignin [31].

It is noteworthy that bimolecular reactions between lipid peroxyl radicals are also expected to compete with the intramolecular

Table 1
Oxidation products derived from ^{14}C-labeled lignin model **1** after MnP- and Mn(III)-initiated peroxidation of unsaturated fatty acids for 72 h.

Initiator of lipid peroxidation	Co-oxidized fatty acid	Starting material remaining and products formed (% of soluble ^{14}C)[a]			
		1	**2**	**3**	**4+5**
MnP	Control (Tween 20)	99.6	<0.1	ND[b]	ND
	18:1	17.7	26.4	6.3	6.3
	18:2	24.4	16.2	5.2	6.0
	18:3	47.1	15.1	3.5	4.0
	20:4	75.5	6.5	3.0	1.7
Mn(III) tartrate	Control (Tween 20)	99.7	<0.1	ND	ND
	18:1	99.4	0.4	ND	ND
	18:2	94.2	3.0	0.1	0.4
	18:3	98.3	1.0	<0.1	0.1
	20:4	98.9	0.6	<0.1	<0.1

[a] See Fig. 1 for structures of compounds **1**–**5**. Additional structure verification and discussion of the oxidative reactions is given in ref. [7].
[b] ND, not detected.

scission reactions that generate TBARS [27], in accord with our observation that few TBARS were formed in the rapid reaction between linoleic acid and Mn(III). We also observed that oxygen consumption ceased within several minutes during polyunsaturated fatty acid oxidation by Mn(III) tartrate, which suggests that this system failed to propagate the sustained radical chain reaction that is characteristic of complete lipid peroxidation (data not shown). These results point to the possibility that the ligninolytic oxidants in the MnP/Mn(II) system may not be the initially formed fatty acid peroxyl radicals, but may rather be some other oxidant associated with late reactions of lipid peroxidation. For oleic acid, our methods could not have detected these products [27], but in the case of linoleic acid these late reactions resulted in TBARS formation (Fig. 5). TBARS themselves are unlikely to be ligninolytic oxidants, but TBARS formation is an indirect reporter of complex reactions that involve the formation of diverse peroxyl and alkoxyl lipid-derived radicals [27], and some of these radicals may be ligninolytic. Our finding that a lag phase occurred before linoleic acid-mediated lignin model oxidation is consistent with this conclusion (Fig. 7). However, the nature and importance of these putative downstream oxidants remains to be determined.

Acknowledgments

This work was supported by Ministry of Education of the Republic of Belarus grant (09/06) 20065179 (A.N.K.) and grant DE-AI02-07ER64480 from the Office of Biological and Environmental Research, U.S. Department of Energy (K.E.H.).

References

[1] Hatakka A. Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. FEMS Microbiol Rev 1994;13:125–35.
[2] Gold MH, Youngs HL, Sollewijin Gelpke MD. Manganese peroxidase. In: Sigel A, Sigel H, editors. Metal Ions in Biological Systems, vol. 37. New York-Basel: Marcel Dekker, Inc.; 2000. p. 559–86.
[3] Martinez AT, Speranza M, Ruiz-Dueñas FJ, Ferreira P, Camarero S, Guillén F, et al. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int Microbiol 2005;8:195–204.
[4] Wariishi H, Valli K, Renganathan V, Gold MH. Thiol-mediated oxidation of non-phenolic lignin model compounds by manganese peroxidase of *Phanerochaete chrysosporium*. J Biol Chem 1989;264:14185–91.
[5] Bao W, Fukushima Y, Jensen KA, Moen MA, Hammel KE. Oxidative degradation of non-phenolic lignin during lipid peroxidation by fungal manganese peroxidase. FEBS Lett 1994;354:297–300.
[6] Moen MA, Hammel KE. Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Appl Environ Microbiol 1994;60:1956–61.
[7] Kapich AN, Jensen KA, Hammel KE. Peroxyl radicals are potential agents of lignin biodegradation. FEBS Lett 1999;461:115–9.
[8] Kapich A, Hofrichter M, Vares T, Hatakka A. Coupling of manganese peroxidase mediated lipid peroxidation with destruction of nonphenolic lignin model compounds and ^{14}C-labeled lignins. Biochem Biophys Res Commun 1999;259:212–9.
[9] Bermek H, Li K, Eriksson K-E. Studies on mediators of manganese peroxidase for bleaching of wood pulps. Biores Technol 2002;85:249–52.
[10] Foisner R. Isolation and characterization of extracellular 3-lamellar structures of *Sporotrichum pulverulentum*. J Ultrastruct Res 1985;92:36–46.
[11] Foisner R. Wood decay by basidiomycetes: extracellular tripartite membranous structures. Trans Brit Mycol Soc 1985;85:257–66.
[12] Kapich AN, Romanovetz ES, Voit SP. Content and composition of lipids in mycelium of wood-rotting Basidiomycetes. Mikol Fitopatol 1990;24:51–6 [in Russian].
[13] Kapich AN, Shishkina LN. Changes in composition and content of the lipids in wood-rotting Basidiomycetes during growth. Nauchnye Dokl Vyss Shkoly Biol Nauki 1992;6:89–95 [in Russian].
[14] Enoki M, Watanabe T, Nakagame S, Koller K, Messner K, Honda Y, et al. Extracellular lipid peroxidation of selective white-rot fungus, *Ceriporiopsis subvermispora*. FEMS Microbiol Lett 1999;180:205–11.
[15] Gutierrez A, del Rio JC, Martinez-Inigo MJ, Martinez MJ, Martinez AT. Production of new unsaturated lipids during wood decay by ligninolytic basidiomycetes. Appl Environ Microbiol 2002;68:1344–50.
[16] Elissetche JP, Ferraz A, Freer J, Mendonça R, Rodríguez J. Thiobarbituric acid reactive substances, Fe^{3+} reduction and enzymatic activities in cultures of *Ganoderma australe* growing on *Drimys winteri* wood. FEMS Microbiol Lett 2006;260:112–8.
[17] Fengel D, Wegener G. Wood chemistry, ultrastructure, reactions. Berlin: Walter de Gruyter; 1984.
[18] Demirbas A. Analysis of beech wood fatty-acids by supercritical acetone extraction. Wood Sci Technol 1991;25:365–70.
[19] Piispanen R, Saranpää P. Neutral lipids and phospholipids in Scots pine (*Pinus sylvestris*) sapwood and heartwood. Tree Physiol 2002;22:661–6.
[20] Cosgrove JP, Church DF, Pryor WA. The kinetics of the autooxidation of polyunsaturated fatty acids. Lipids 1987;22:299–304.
[21] Gardner HW. Oxygen radical chemistry of polyunsaturated fatty acids. Free Radical Biol Med 1989;7:65–86.
[22] Wariishi H, Valli K, Gold MH. Manganese (II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. Kinetic mechanism and role of chelators. J Biol Chem 1992;267:23688–95.
[23] Landucci LL, Geddes SA, Kirk TK. Synthesis of ^{14}C-labeled 3-methoxy-4-hydroxy-α-(2-methoxyphenoxy)-β-hydroxyoropiophenone, a lignin model compound. Holzforschung 1981;35:66–9.
[24] Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR, editors. Vogel's textbook of practical organic chemistry. New York: John Wiley & Sons; 1989.
[25] Stewart P, Whitwam RE, Kersten PJ, Cullen D, Tien M. Efficient expression of a *Phanerochaete chrysosporium* manganese peroxidase gene in *Aspergillus oryzae*. Appl Environ Microbiol 1996;62:860–4.
[26] Tien M, Kirk TK. Lignin peroxidase of *Phanerochaete chrysosporium*. Methods Enzymol 1988;161:238–49.
[27] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: University Press; 1999.
[28] Yazu K, Yamamoto Y, Niki E, Miki K, Ukegawa K. Mechanism of lower oxidizability of eicosapontaenoate than linoleate in aqueous micelles. II. Effect of antioxidants. Lipids 1998;33:597–600.
[29] Nagler LG, Lankin VZ, Kozachenko AI, Gurevich SM. Rate of free-radical oxidation of C18 diene and triene fatty acids in aqueous micellar solutions and effectiveness of beta-carotene as an inhibitor of their oxidation. Biochemistry (Moscow) 2003;68:203–8.
[30] Watanabe T, Katayama S, Enoki M, Honda Y, Kuwahara M. Formation of acyl radical in lipid peroxidation of linoleic acid by manganese-dependent peroxidase from *Ceriporiopsis subvermispora* and *Bjerkandera adusta*. Eur J Biochem 2000;267:4222–31.
[31] Kirk TK, Nakatsubo F, Reid ID. Further study discounts role for singlet oxygen in fungal degradation of lignin model compounds. Biochem Biophys Res Commun 1983;111:200–4.