APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1996, p. 1788–1792 Vol. 62, No. 5
0099-2240/96/$04.00+0

Fluorene Oxidation In Vivo by *Phanerochaete chrysosporium* and In Vitro during Manganese Peroxidase-Dependent Lipid Peroxidation

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The oxidation of fluorene, a polycyclic hydrocarbon which is not a substrate for fungal lignin peroxidase, was studied in liquid cultures of *Phanerochaete chrysosporium* and in vitro with *P. chrysosporium* extracellular enzymes. Intact fungal cultures metabolized fluorene to 9-hydroxyfluorene via 9-fluorenone. Some conversion to more-polar products was also observed. Oxidation of fluorene to 9-fluorenone was also obtained in vitro in a system that contained manganese(II), unsaturated fatty acid, and either crude *P. chrysosporium* peroxidases or purified recombinant manganese peroxidase. The oxidation of fluorene in vitro was inhibited by the free-radical scavenger butylated hydroxytoluene but not by the lignin peroxidase inhibitor $NaVO_3$. Manganese(III)-malonic acid complexes could not oxidize fluorene. These results indicate that fluorene oxidation in vitro was a consequence of lipid peroxidation mediated by *P. chrysosporium* manganese peroxidase. The rates of fluorene and diphenylmethane disappearance in vitro were significantly faster than those of true polycyclic aromatic hydrocarbons or fluoranthenes, whose rates of disappearance were ionization potential dependent. This result indicates that the initial oxidation of fluorene proceeds by mechanisms other than electron abstraction and that benzylic hydrogen abstraction is probably the route for oxidation.

White rot basidiomycetes such as *Phanerochaete chrysosporium* have long been known to possess a highly nonspecific battery of extracellular enzymes that allows them to degrade the plant polymer lignin. This same enzymatic system is believed to underlie the ability of these fungi to degrade many organopollutants. In the past decade, extensive work has accumulated to implicate two *P. chrysosporium* peroxidases, lignin peroxidase (LiP) (31, 38), and manganese peroxidase (MnP) (14, 28), in pollutant degradation.

LiP directly oxidizes polycyclic aromatic hydrocarbons (PAHs) with ionization potentials (IPs) of ≤7.55 eV, including perylene, benzo[*a*]pyrene, anthracene, pyrene, and benz[*a*]anthracene (17). Recent work with a LiP-tetrahydrofuran system argued that the cutoff value was slightly higher (approximately 8.0 eV) in the case of certain alkylaromatic and heteroaromatic polycyclic compounds, identifying carbazole, acenaphthene, fluoranthene, and dibenzothiophene as LiP substrates (42). Purified LiP also oxidizes dibenzo[*p*]dioxin (17) and 2,7-dichlorodibenzo[*p*]dioxin (41), as well as numerous chlorinated and polychlorinated phenols (18, 40), methoxybenzenes (19), and chloro- and nitromethoxybenzenes (39–41).

Similarly, purified MnP of *P. chrysosporium* has been observed to oxidize many phenolic organopollutants (39–41). On the basis of the ability of manganic acetate to cause the one-electron oxidations of benzo- and dibenzopyrenes (9, 10) and of certain $Mn(III)$-H_2SO_4 systems to oxidize many PAHs (30), it has been suggested that compounds of this nature may also be substrates for MnP via the action of Mn^{3+} (12).

Some compounds which are degraded by *P. chrysosporium*, however, are apparently not substrates for LiP or the MnP-Mn^{2+} system. The insecticide DDT [1,1,1,-trichoro-2,2-bis(4-chlorophenyl)ethane] is extensively degraded by whole fungal cultures (6), yet the involvement of extracellular peroxidases in initial oxidative steps could not be shown (21). Polycyclic aromatic compounds with ionization potentials of greater than 7.55 eV, which are not substrates for LiP (e.g., phenanthrene), are nonetheless known to be degraded by the intact fungus (5, 16). Recent research in which an MnP-based lipid peroxidation system degraded phenanthrene may help to reconcile some of these observations (27). Further, the products observed with this cell-free system were the same as those obtained with whole fungal cultures (16).

Fluorene, a polycyclic hydrocarbon with a benzylic methylene functional group, is not a LiP substrate (42). It is, nonetheless, oxidized by *P. chrysosporium* in liquid culture (this study) and in soil (13) and is depleted from soil slurries in bioreactors (37). Here we sought to determine whether transformation of fluorene occurs during the lipid peroxidation reactions mediated by *P. chrysosporium* peroxidases. Our results provide further evidence of the function of the MnP-based lipid peroxidation system in liquid culture and indicate that it may participate in xenobiotic degradation in contaminated soils inoculated with *P. chrysosporium.*

MATERIALS AND METHODS

Chemicals. [9-^{14}C]fluorene (11.3 mCi mmol^{-1}, >98% radiochemical purity), unlabeled fluorene, benzo[*a*]pyrene, *n*-dodecyl-β-D-maltoside, and sodium *meta*-vanadate were obtained from Sigma (St. Louis, Mo.), as was oleic acid (approximately 99% pure), which was stored under argon at −20°C. [9-^{14}C]9-fluorenone (11.3 mCi mmol^{-1}, radiochemical purity, >99%) was prepared from [^{14}C]fluorene by the ceric ammonium sulfate method of Periasamy and Bhatt (29) and purified by the high-performance liquid chromatography (HPLC) system described below. The purity of the product thus isolated was confirmed by gas chromatography (GC)-electron impact mass spectrometry. Unlabeled 9-fluorenone, 9-hydroxyfluorene, anthracene, fluoranthene, benzo[*b*]fluoranthene, benzo[*e*]pyrene, chrysene, phenanthrene, and diphenylmethane were purchased from Aldrich (Milwaukee, Wis.). Benz[*a*]anthracene was from Supelco (Bellefonte, Pa.). Benzo[*c*]phenan-

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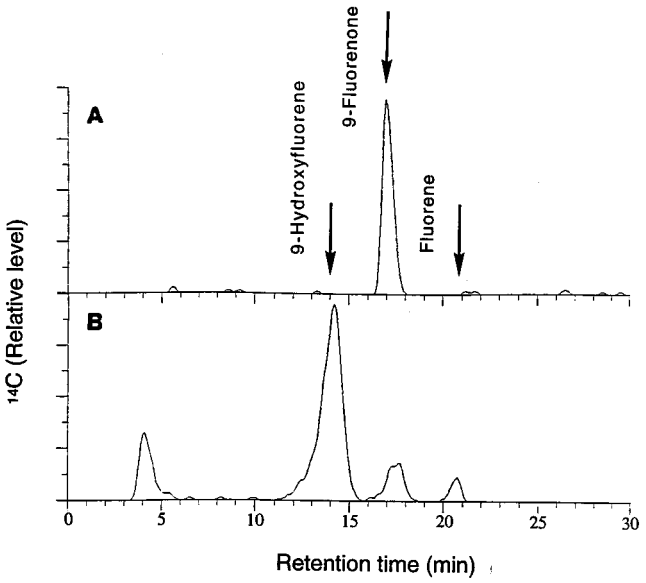

FIG. 1. HPLC profiles of the product formed during lipid peroxidation-mediated [^{14}C]fluorene oxidation (A) and the organic-soluble products of *P. chrysosporium* liquid cultures, supplemented with [^{14}C]fluorene, after 3 weeks of incubation (B). Arrows denote retention times of standards of 9-hydroxyfluorene, 9-fluorenone, and fluorene.

threne and dibenz[*a,c*]anthracene were generously donated by J. A. Miller (McArdle Cancer Research Laboratory, University of Wisconsin-Madison). Silanizing reagent (Aqua-Sil) was from Pierce (Rockford, Ill.). All of the solvents used were of HPLC grade.

Fungus. *P. chrysosporium* Burds. BKM-F-1767 (ATCC 24725) was obtained from the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wis. Master cultures were subcultured onto yeast extract-malt extract-peptone-glucose agar slants (22) and maintained at 4°C until use.

Enzyme preparation. Cultures of *P. chrysosporium* for peroxidase production contained 750 ml of BIII medium (20), with 10 mM *trans*-aconitic acid substituted for dimethylsuccinic acid. Following a 6-day incubation (with agitation at 200 rpm and daily flushing with O_2), the resultant fungal pellets were removed by filtration. The culture filtrate was then concentrated 100-fold. High-molecular-weight polysaccharide was precipitated by freezing-thawing and removed by centrifugation. Following extensive dialysis against 5 mM sodium acetate (pH 6.0), the crude peroxidase preparation was further concentrated (final volume, ≈20 ml) and sterilized by filtration through low-protein binding filters (Acrodisc 13; Gelman Sciences; Ann Arbor, Mich.). The enzyme activities in this final preparation were 2×10^4 nmol min^{-1} ml^{-1} for LiP by veratryl alcohol oxidation (38) and 3×10^4 nmol min^{-1} ml^{-1} for MnP on the basis of the vanillyl acetone assay (28).

Recombinant *P. chrysosporium* MnP isozyme H4, purified from *Aspergillus oryzae* cultures (36), was used in some experiments. This preparation had an activity of 2×10^5 nmol min^{-1} ml^{-1}.

Reaction conditions. Lipid peroxidation-based reactions were based on those of Moen and Hammel (27), with some modifications. Reactions contained 20 mM sodium malonate buffer (pH 4.5), 200 μM Mn^{2+} (as $MnSO_4$), 0.5% *n*-dodecyl-β-D-maltoside, and 300 μM oleic acid (dispersed in *n*-dodecyl-β-D-maltoside prior to addition). Reactions (total volume, 1.0 ml) were conducted in silanized, sterilized 7-ml borosilicate scintillation vials at room temperature as previously described (4, 27). Fluorene (20 μM final concentration, 1.0×10^5 dpm of ^{14}C) was added in acetonitrile (10 μl). Butylated hydroxytoluene, when used, was emulsified in *n*-dodecyl-β-D-maltoside prior to addition. Crude enzyme (see above) was added daily in 5-μl aliquots. Enzyme reactions to be analyzed by HPLC were terminated by addition of 1 ml of acetonitrile and filtered prior to injection.

The kinetics of fluorene oxidation were monitored by using reactions identical to those described above. Samples were taken as indicated (see Fig. 2) and analyzed by HPLC. The disappearance kinetics of 11 other compounds (benzo[*a*]pyrene, anthracene, benz[*a*]anthracene, dibenz[*a,c*]anthracene, benzo[*e*]pyrene, benzo[*c*]phenanthrene, benzo[*b*]fluoranthene, fluoranthene, chrysene, phenanthrene, and diphenylmethane) were assessed by adding them (20 μM) individually to reaction mixtures as previously described (4). These reactions were sampled periodically, and percent disappearance of each PAH was determined by HPLC. Previous experiments (4) indicated that loss of PAHs from no-enzyme controls of this type was minimal (≤10% of the input PAH in 7 days).

Mn(III)-malonate complex preparation and reaction. Mn(III) acetate (20 μM) was dissolved in 200 mM sodium malonate (pH 4.5) immediately prior to use. Reaction mixtures for assessment of fluorene oxidation (1.0 ml) contained 20 μM [^{14}C]fluorene (1.0×10^5 dpm), 0.5% *n*-dodecyl-β-D-maltoside, 20 mM sodium malonate (pH 4.5), and 400 μM Mn(III)-malonate complex.

Liquid culture studies. *P. chrysosporium* was inoculated as a spore suspension into 20 ml of *trans*-aconitic acid-buffered BIII medium in 125-ml Erlenmeyer flasks fitted with gas exchange ports. Cultures were maintained without agitation at 39°C. Metabolite profiles for [^{14}C]fluorene and [^{14}C]9-fluorenone were generated by adding the compound (30 μM final concentration; 2.0×10^5 dpm) in 50 μl of *N,N*-dimethylformamide after 3 days of mycelial growth. Following 3 weeks of incubation (with O_2 flushing every 3 to 5 days), cultures were individually homogenized and extracted three times with 20-ml volumes of ethyl acetate. The resultant extracts were dehydrated with anhydrous Na_2SO_4, evaporated to dryness under N_2, and redissolved in acetonitrile for HPLC analysis as described below.

HPLC and GC-mass spectrometry. All HPLC analyses were performed with a Vydac 201TP54 (25 by 0.46 cm) C_{18} reverse-phase column (Nest Group, Southboro, Mass.). The HPLC gradient consisted of water-acetonitrile (each with 1% glacial acetic acid) as follows: 0 to 2 min, 80:20; 2 to 23 min, ramp to 0:100. The flow rate was 1 ml min^{-1}. For quantitation of ^{14}C, column eluent was passed through a Flo-One radiochromatography detector (Packard Instrument Co., Downers Grove, Ill.) operated in the time-resolved liquid scintillation counting mode with 5 ml of Flo-Scint V (Packard) min^{-1} as the scintillation cocktail.

GC-mass spectrometry analyses were done on a Hewlett-Packard 5890 Series II gas chromatograph (injector temperature, 220°C; detector temperature, 300°C) with a DB-5 fused silica capillary column (30 m by 0.321 mm; J & W Scientific, Folsom, Calif.) connected to a Hewlett-Packard 5972 Series mass-selective detector. The GC temperature gradient was as follows: 0 to 1 min, 60°C; 1 to 10 min, ramp to 150°C (10° min^{-1}); 10 to 30 min, ramp to 180°C (1.5° min^{-1}).

Statistical analysis. Rates of disappearance of each compound were calculated from linear regressions of datum points taken during the linear phase of in vitro reactions (see Table 1). Nonlinear regression analyses of the relationship between disappearance rate and IP, including calculation of 95% confidence bands for the regression, were performed as described by Bates and Watts (3).

RESULTS

In vitro product identification. The product of the in vitro fluorene oxidation reaction had an HPLC retention time identical to that of a 9-fluorenone standard (Fig. 1). The in vitro product, when isolated by HPLC, also had a GC retention time (15.3 min) identical to that of the authentic 9-fluorenone, and the mass spectrometry spectra of the two compounds were the same: *m/z* 180 (M+; relative intensity, 100), 152 (-CO; relative intensity, 44), 151 (relative intensity, 23), and 150 (relative intensity, 15). Positive identification of the in vitro product as 9-fluorenone can therefore be made.

Requirements of the in vitro system/Mn(III) oxidation of fluorene. Table 1 shows the yield of 9-fluorenone in enzyme-mediated lipid peroxidation reactions. Full 7-day reactions contained most (88% ± 4%) of the input radioactivity, all as 9-fluorenone. Maximal conversion of fluorene to 9-fluorenone required unsaturated fatty acid, Mn^{2+}, and crude enzyme (Table 1). A small amount of 9-fluorenone was produced in the

TABLE 1. Formation of 9-fluorenone in vitro after 7 days under various reaction conditions[a]

Reaction condition	Concn of 9-fluorenone formed (μM)
Complete with crude enzyme	17.6 (0.8)
Without oleate	0.8 (0.3)
Without $MnSO_4$	1.6 (0.4)
Without enzyme	0.2 (0.2)
With 200 μM BHT[b]	0.2 (0.2)
With 100 μM $NaVO_3$	15.5 (1.4)
Complete with rMnP[c]	20.3 (1.4)

[a] The data shown are means of multiple experiments ($n = 3$) with standard deviations in parentheses. The initial fluorene concentration was 20 μM.
[b] BHT, butylated hydroxytoluene.
[c] rMnP, recombinant *P. chrysosporium* MnP isozyme H4.

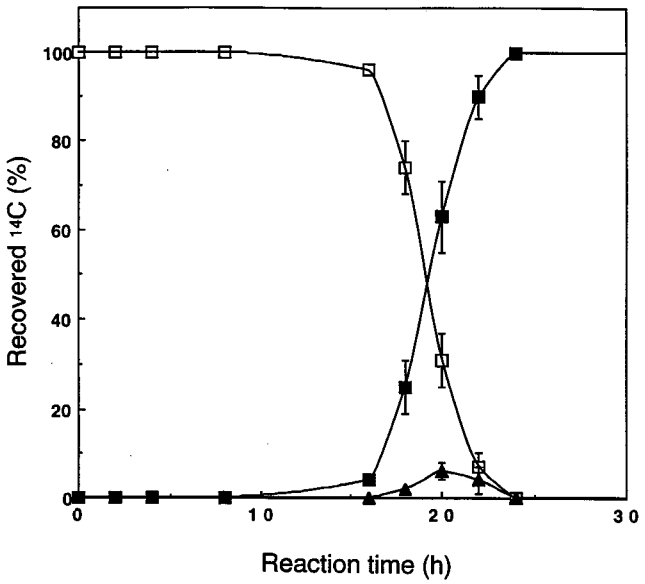

FIG. 2. Time course of [^{14}C]fluorene disappearance (□) and accumulation of [9-^{14}C]fluorene (■) during lipid peroxidation reactions. Note the transient accumulation of small amounts of 9-hydroxyfluorene (▲). The final product distribution (100% 9-fluorenone) remained constant throughout the 7-day duration of the experiment (data not shown). The initial concentration of fluorene was 20 μM.

absence of oleate or Mn^{2+}. The free-radical scavenger butylated hydroxytoluene (200 μM) blocked the formation of 9-fluorenone, whereas the selective LiP inhibitor sodium *meta*-vanadate (100 μM) (1) did not. Purified recombinant MnP was as efficient at fluorene oxidation as was the crude peroxidase preparation.

HPLC analysis of Mn(III)-malonate reactions with fluorene showed no transformation of fluorene by chelated Mn^{3+}. All of the ^{14}C recovered after incubation for either 2 or 24 h was present as unchanged fluorene (data not shown).

In vitro kinetics and intermediates. Analysis of reactions containing [^{14}C]fluorene showed a lag time of 12 to 15 h, followed by rapid, almost quantitative conversion of fluorene to 9-fluorenone between 16 and 24 h (Fig. 2). The reaction was linear during this time (r = 0.99), and the rate of fluorene oxidation was 3.1 ± 0.2 nmol h^{-1}. Small amounts of 9-hydroxyfluorene (≤10% of the input ^{14}C; therefore, ≤2 μM) were transiently observed. No evidence of further oxidation of 9-fluorenone was detected in the in vitro system.

The kinetics of fluorene oxidation in vitro were compared to the disappearance kinetics of eight PAHs (benzo[*a*]pyrene, anthracene, benz[*a*]anthracene, dibenz[*a,c*]anthracene, benzo[*e*]pyrene, benzo[*c*]phenanthrene, chrysene, and phenanthrene), two fluoranthenes (fluoranthene and benzo[*b*]fluoranthene), and diphenylmethane. Diphenylmethane resembles fluorene in that it also contains a benzylic hydrogen. Data for these compounds are shown in Table 2. Following a lag phase, linear disappearance kinetics were observed for all compounds; r values ranged from 0.82 (phenanthrene) to 0.99 (benz[*a*]anthracene) and averaged 0.95. Previous studies (4) demonstrated that essentially all input PAH was recovered from no-enzyme control reactions after 7 days, with no transformation observed.

In vivo metabolite profiles. An HPLC profile of organic-soluble radioactivity recovered from a typical 3-week culture containing [^{14}C]fluorene is shown in Fig. 1B. As expected (13), one peak coeluted with 9-fluorenone. The major peak, however, was slightly more polar, with a retention time equal to that of a standard of 9-hydroxyfluorene. Addition of [9-^{14}C]-fluorenone to cultures resulted in a profile similar to that seen with labeled fluorene (Table 3). Again, most of the recovered radioactivity coeluted with 9-hydroxyfluorene. Thus, it can be concluded that at least some of the hydroxyfluorene peak seen in whole cultures is produced by reduction of 9-fluorenone and that the polar metabolites generated in vivo are the result of further transformation of one or both of these intermediates.

DISCUSSION

The same in vitro system which has been used to oxidize phenanthrene and other high-IP PAHs (4, 27) with crude *P. chrysosporium* peroxidases was found to oxidize fluorene efficiently, yielding the ketone 9-fluorenone. Maximal formation of 9-fluorenone in this system required an unsaturated fatty acid, Mn^{2+}, and crude enzyme and was almost totally blocked by inclusion of the general free-radical scavenger butylated hydroxytoluene (Table 1). However, although manganese is required for optimal fluorene conversion, Mn^{3+} chelates, the product of normal MnP turnover, cannot oxidize fluorene, which has an IP of 7.78 to 8.52 eV (11, 34, 35, 42). This is not unexpected; Mn(III) chelates do not oxidize phenanthrene (IP, 8.03 to 8.19 eV [8]) (27), and Cavalieri and Rogan (7) have shown a cutoff value of ca. 7.75 eV for one-electron PAH ionization by Mn^{3+} in manganic acetate systems. Fluorene oxidation in vitro is therefore connected in some way to the MnP-dependent lipid peroxidation known to occur in this system. It is clear that LiP is not an essential component of the system because crude enzyme could be replaced by recombinant MnP. Moreover, fluorene oxidation to 9-fluorenone by crude enzyme was not inhibited by 100 μM $NaVO_3$. This concentration is sufficient to inhibit veratryl alcohol oxidation by *P. chrysosporium* LiP almost totally but still allows approximately 50% of MnP activity (as measured by vanillyl acetone oxidation) under the range of enzyme levels used in this experiment (data not shown). It should also be noted that the IP of fluorene is well outside the LiP substrate range defined by Hammel et al. (17) and Vazquez-Duhalt et al. (42).

TABLE 2. Kinetic data for disappearance of eight PAHs, two fluoranthenes, and fluorene and diphenylmethane from in vitro lipid peroxidation reactions[a]

Compound	Rate of disappearance (nmol h^{-1})	Lag time (h)	Linear phase of reaction (h)
PAHs			
Anthracene	0.93 (0.07)	10	10–30
Benzo[*a*]pyrene	0.96 (0.08)	20	20–36
Benz[*a*]anthracene	1.08 (0.05)	20	20–36
Dibenz[*a,c*]anthracene	0.60 (0.05)	20	20–48
Benzo[*e*]pyrene	0.31 (0.02)	20	20–48
Chrysene	0.07 (0.01)	20	20–48
Benzo[*c*]phenanthrene	0.21 (0.04)	20	20–48
Phenanthrene	0.06 (0.02)	20	20–48
Fluoranthenes			
Benzo[*b*]fluoranthene	0.19 (0.02)	20	20–48
Fluoranthene	0.14 (0.02)	20	20–48
Fluorene	3.10 (0.20)	12–15	16–22
Diphenylmethane	0.30 (0.02)	20	20–48

[a] Calculated rates of disappearance during the linear portion of the reaction are given as mean values (n = 3) with 95% confidence intervals in parentheses.

TABLE 3. Distribution of products from *P. chrysosporium* liquid cultures supplemented with [^{14}C]fluorene or [9-^{14}C]fluorenone[a]

Compound	% of product[b]			
	Fluorene	9-Fluorenone	9-Hydroxyfluorene	More-polar compounds
Fluorene	4 (2)	13 (2)	64 (5)	17 (5)
9-Fluorenone		12 (3)	70 (3)	14 (1)

[a] Initial concentration, 30 μM.
[b] The data are mean percentages of three replicate cultures with standard deviations in parentheses.

Recent work with high-IP PAHs (4) found a strong correlation between IP and disappearance from both lipid peroxidation reactions and intact fungal cultures, arguing for a one-electron oxidative mechanism for these compounds. These conclusions were based on analyses of in vitro reactions and whole cultures sampled at single time points. The in vitro kinetic data presented here clearly show a relationship between IP and the rates of disappearance of individual PAHs, thus strongly supporting the centrality of one-electron oxidative processes in this system.

As can be seen in Fig. 3, however, the rates of disappearance of fluorene (3.1 ± 0.2 nmol h^{-1}) and diphenylmethane (0.30 ± 0.05 nmol h^{-1}) were found to be anomalously high compared with those of either true PAHs or fluoranthenes, which were clearly correlated with substrate ionization potential (Fig. 3). Peroxyl and/or alkoxyl radicals, generated from lipid hydroperoxides by any of several agents (Mn^{2+}, Mn^{3+}, and MnP heme iron), could react with xenobiotic substrates via several mechanisms: hydrogen abstraction, electron abstraction, and/or direct addition of the radical to an olefinic bond (26, 33). Oxidation of fluorene would most likely be dominated by electron or hydrogen abstraction pathways, as the rate for the gas phase reaction of fluorene with ·OH, primarily an addition reaction (2), is one of the lowest among PAHs (26). However, because the rate of fluorene transformation in vitro is far greater than that predicted by ionization potential (Fig. 3), we consider electron abstraction an unlikely route for fluorene oxidation. By contrast, H atom abstraction from fluorene is known to be caused by a variety of oxyradicals, including superoxide (23) and *t*-butyl perbenzoate-derived peroxyl radicals (32), in a relatively facile reaction. The ratio of the rate of fluorene disappearance to that of diphenylmethane disappearance observed in our system was approximately 10. This result agrees reasonably well with those obtained by Russell (32), who observed an approximately 2.5-fold higher rate of peroxyl radical-mediated oxidation of fluorene than of diphenylmethane. In summary, abstraction of fluorene's benzylic hydrogen, probably by an oxyradical species, appears to be the most likely pathway for formation of a 9-hydroxyfluorene intermediate, which is subsequently rapidly oxidized to 9-fluorenone.

The only product of fluorene metabolism by *P. chrysosporium* identified to date is the ketone 9-fluorenone, which has previously been isolated from soil cultures (13) and has now been isolated from liquid culture. In the present study, 9-hydroxyfluorene accumulated in liquid cultures to a greater extent than did 9-fluorenone. In vitro experiments produced the ketone with transient accumulation of 9-hydroxyfluorene. The 9-hydroxyfluorene observed in cultures containing fluorene may be produced either as a transient intermediate during fluorene oxidation or via the later reduction of 9-fluorenone.

Previous evidence of the relevance of the in vitro lipid peroxidation system to fungal bioremediation processes has been confined to the commonality of its products and those observed in whole liquid cultures of *P. chrysosporium* (16, 27). Now it is evident that this process may account for at least some xenobiotic transformation by ligninolytic fungi in soil. The hyphae of *Phanerochaete* and similar species are rich in unsaturated lipids (15, 24, 25), and soils frequently contain high levels of manganese and lipid material; thus, lipid peroxidation-mediated co-oxidation of xenobiotics by white rot fungi in soil can be easily envisioned.

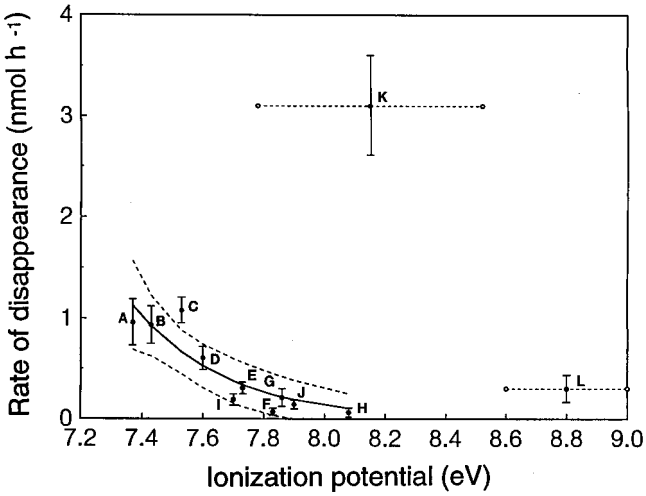

FIG. 3. Rates of disappearance from lipid peroxidation reactions of PAHs, fluoranthenes, and benzylic hydrogen-containing polyaromatic compounds as a function of ionization potential. Best-fit nonlinear regression (solid line) and 95% confidence limits (dashed lines) are presented. Ionization potentials for PAHs are the electron absorption values cited by Cavalieri et al. (8); those for fluoranthenes were calculated on the basis of the report of Simonsick and Hites (34) as previously described (4). The full range of IP values reported in the literature is shown for fluorene and diphenylmethane (○----○). Compounds: A, benzo[*a*]pyrene; B, anthracene; C, benz[*a*]anthracene; D, dibenz[*a,c*]anthracene; E, benzo[*e*]pyrene; F, chrysene; G, benzo[*c*]phenanthrene; H, phenanthrene; I, benzo[*b*]fluoranthene; J, fluoranthene; K, fluorene; L, diphenylmethane.

ACKNOWLEDGMENTS

We thank M. A. Moen for technical assistance, P. Stewart for recombinant MnP isozyme H4, P. Lebow and C. A. Hatfield for statistical analysis, and T. K. Kirk for helpful criticism of the manuscript.

B.W.B. is supported by a predoctoral fellowship from the Howard Hughes Medical Institute.

REFERENCES

1. **Archibald, F. S.** 1992. Lignin peroxidase activity is not important in biological bleaching and delignification of unbleached Kraft pulp by *Trametes versicolor*. Appl. Environ. Microbiol. **58:**3101–3109.
2. **Atkinson, R.** 1985. Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions. Chem. Rev. **85:**69–201.
3. **Bates, D. M., and D. G. Watts.** 1988. Nonlinear regression analysis and its applications. John Wiley & Sons, Inc., New York.
4. **Bogan, B. W., and R. T. Lamar.** 1995. One-electron oxidation in the degradation of creosote polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **61:**2631–2635.
5. **Bumpus, J. A.** 1989. Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **55:**154–158.
6. **Bumpus, J. A., and S. D. Aust.** 1987. Biodegradation of DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **53:**2001–2008.
7. **Cavalieri, E. L., and E. G. Rogan.** 1985. Role of radical cations in aromatic hydrocarbon carcinogenesis. Environ. Health Perspect. **64:**69–84.
8. **Cavalieri, E. L., E. G. Rogan, R. W. Roth, R. K. Saugier, and A. Hakam.** 1983. The relationship between ionization potential and horseradish peroxidase/hydrogen peroxide-catalyzed binding of polycyclic aromatic hydrocarbons to DNA. Chem.-Biol. Interact. **47:**87–109.

9. **Cremonesi, P., E. L. Cavalieri, and E. G. Rogan.** 1989. One-electron oxidation of 6-substituted benzo[*a*]pyrenes by manganic acetate. A model for metabolic activation. J. Org. Chem. **54:**3561–3570.
10. **Cremonesi, P., B. Hietbrink, E. G. Rogan, and E. L. Cavalieri.** 1992. One-electron oxidation of dibenzo[*a*]pyrenes by manganic acetate. J. Org. Chem. **57:**3309–3312.
11. **Dewar, M. J. S., E. Haselbach, and S. D. Worley.** 1970. Calculated and observed ionization potentials of unsaturated polycyclic hydrocarbons; calculated heats of formation by several semiempirical S.C.F. M.O. methods. Proc. R. Soc. Lond. A **315:**431–442.
12. **Field, J. A., E. de Jong, G. Feijoo-Costa, and J. A. M. de Bont.** 1993. Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. Trends Biotechnol. **11:**44–49.
13. **George, E. J., and R. D. Neufeld.** 1989. Degradation of fluorene in soil by fungus *Phanerochaete chrysosporium*. Biotechnol. Bioeng. **33:**1306–1310.
14. **Glenn, J. K., L. Akileswaran, and M. H. Gold.** 1986. Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. Arch. Biochem. Biophys. **251:**688–696.
15. **Hadar, Y., and E. Cohen-Arazi.** 1986. Chemical composition of the edible mushroom *Pleurotus ostreatus* produced by fermentation. Appl. Environ. Microbiol. **51:**1352–1354.
16. **Hammel, K. E., W. Z. Gai, B. Green, and M. A. Moen.** 1992. Oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **58:**1832–1838.
17. **Hammel, K. E., B. Kalyanaraman, and T. K. Kirk.** 1986. Oxidation of polycyclic aromatic hydrocarbons and dibenzo[*p*]dioxins by *Phanerochaete chrysosporium* ligninase. J. Biol. Chem. **261:**16948–16952.
18. **Hammel, K. E., and P. J. Tardone.** 1988. The oxidative 4-dechlorination of polychlorinated phenols is catalyzed by extracellular fungal lignin peroxidases. Biochemistry **27:**6563–6568.
19. **Kersten, P. J., M. Tien, B. Kalyanaraman, and T. K. Kirk.** 1985. The ligninase of *Phanerochaete chrysosporium* generates cation radicals from methoxybenzenes. J. Biol. Chem. **260:**2609–2612.
20. **Kirk, T. K., S. Croan, M. Tien, K. E. Murtaugh, and R. L. Farrell.** 1986. Production of multiple ligninases by *Phanerochaete chrysosporium*: effect of selected growth conditions and use of a mutant strain. Enzyme Microb. Technol. **8:**27–32.
21. **Kohler, A., A. Jäger, H. Willershausen, and H. Graf.** 1988. Extracellular ligninase of *Phanerochaete chrysosporium* Burdsall has no role in the degradation of DDT. Appl. Microbiol. Biotechnol. **29:**618–620.
22. **Lamar, R. T., M. J. Larsen, and T. K. Kirk.** 1990. Sensitivity to and degradation of pentachlorophenol by *Phanerochaete* spp. Appl. Environ. Microbiol. **56:**3519–3526.
23. **Lee-Ruff, E., and N. Timms.** 1980. Superoxide-mediated autoxidation of hydrocarbons. Can. J. Chem. **58:**2138–2141.
24. **Leštan, D., M. Cernilec, A. Strancar, and A. Perdih.** 1993. Influence of some surfactants and related compounds on ligninolytic activity of *Phanerochaete chrysosporium*. FEMS Microbiol. Lett. **106:**17–22.
25. **Leštan, D., A. Strancar, and A. Perdih.** 1990. Influence of some oils and surfactants on ligninolytic activity, growth, and lipid fatty acids of *Phanerochaete chrysosporium*. Appl. Microbiol. Biotechnol. **34:**426–428.
26. **Mabey, W. R., J. H. Smith, R. T. Podoll, H. L. Johnson, T. Mill, T.-W. Chou, J. Gates, I. W. Partridge, H. Jaber, and D. Vandenberg.** 1982. Aquatic fate processes for organic priority pollutants. U.S. Environmental Protection Agency, Washington, D.C.
27. **Moen, M. A., and K. E. Hammel.** 1994. Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Appl. Environ. Microbiol. **60:**1956–1961.
28. **Paszczyński, A., V.-B. Huynh, and R. Crawford.** 1985. Enzymatic activities of an extracellular, manganese-dependent peroxidase from *Phanerochaete chrysosporium*. FEMS Microbiol. Lett. **29:**37–41.
29. **Periasamy, M., and M. V. Bhatt.** 1977. A convenient method for the oxidation of polycyclic aromatic hydrocarbons to quinones. Synthesis **5:**330–332.
30. **Periasamy, M., and M. V. Bhatt.** 1978. Facile oxidation of aromatic rings by $Mn_2(SO_4)_3$. Tetrahedron Lett. **46:**4561–4562.
31. **Renganathan, V., K. Miki, and M. H. Gold.** 1985. Multiple molecular forms of diarylpropane oxygenase, an H_2O_2-requiring lignin-degrading enzyme from *Phanerochaete chrysosporium*. Arch. Biochem. Biophys. **241:**304–314.
32. **Russell, G. A.** 1956. The rates of oxidation of aralkyl hydrocarbons. Polar effects in free radical reactions. J. Am. Chem. Soc. **78:**1047–1054.
33. **Simic, M. G., E. P. L. Hunter, and S. V. Jovanovic.** 1987. Electron vs. H-atom transfer in chemical repair, p. 17–24. *In* P. A. Cerutti, O. F. Nygaard, and M. G. Simic (ed.), Anticarcinogenesis and radiation protection. Plenum Press, New York.
34. **Simonsick, W. J., and R. A. Hites.** 1986. Characterization of high molecular weight polycyclic aromatic hydrocarbons by charge exchange chemical ionization mass spectrometry. Anal. Chem. **58:**2114–2121.
35. **Slifkin, M. A.** 1967. Measurement of ionization potentials from contact charge transfer spectra. Nature (London) **215:**949–950.
36. **Stewart, P., R. Whitwam, P. J. Kersten, D. Cullen, and M. Tien.** 1996. Efficient expression of a *Phanerochaete chrysosporium* manganese peroxidase gene in *Aspergillus oryzae*. Appl. Environ. Microbiol. **62:**860–864.
37. **Stroo, H. F., M. A. Jurek, J. A. Bumpus, M. F. Torpy, and S. D. Aust.** 1989. Bioremediation of wood preserving wastes using the white rot fungus *Phanerochaete chrysosporium*. Proc. Am. Wood-Preserv. Assoc. **85:**236–243.
38. **Tien, M., and T. K. Kirk.** 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. Proc. Natl. Acad. Sci. USA **81:**2280–2284.
39. **Valli, K., B. J. Brock, D. K. Joshi, and M. H. Gold.** 1992. Degradation of 2,4-dinitrotoluene by the lignin-degrading fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **58:**221–228.
40. **Valli, K., and M. H. Gold.** 1991. Degradation of 2,4-dichlorophenol by the lignin-degrading fungus *Phanerochaete chrysosporium*. J. Bacteriol. **173:**345–352.
41. **Valli, K., H. Wariishi, and M. H. Gold.** 1992. Degradation of 2,7-dichlorodibenzo-*p*-dioxin by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. J. Bacteriol. **174:**2131–2137.
42. **Vazquez-Duhalt, R., D. W. S. Westlake, and P. M. Fedorak.** 1994. Lignin peroxidase oxidation of aromatic compounds in systems containing organic solvents. Appl. Environ. Microbiol. **60:**459–466.