Biodegradation **8:** 321–328, 1998.

Transformation of 2-hydroxydibenzofuran by laccases of the white rot fungi *Trametes versicolor* and *Pycnoporus cinnabarinus* and characterization of oligomerization products

Ulrike Jonas[1], Elke Hammer[1], Frieder Schauer[1] & Jean-Marc Bollag[2]
[1] *Institut für Mikrobiologie und Molekularbiologie, Ernst-Moritz-Arndt-Universität, Greifswald D-17487, Germany;* [2] *Laboratory of Soil Biochemistry, Center for Bioremediation and Detoxification, The Pennsylvania State University, University Park, Pennsylvania 16802, USA*

Accepted 27 September 1997

Key words: dibenzofuran, laccase, polymerization, *Pycnoporus cinnabarinus*, *Trametes versicolor*, white rot fungi

Abstract

Laccase, a ligninolytic enzyme, was secreted by each of the white rot fungi *Trametes versicolor* and *Pycnoporus cinnabarinus* during growth in a nitrogen-rich medium under agitated conditions. After addition of 2-hydroxydibenzofuran to cell-free supernatants of the cultures, yellow precipitates were formed. These precipitates were poorly soluble in water and therefore readily separated from the supernatant. The products formed were more hydrophobic than the substrate, as indicated by their longer retention times on a reverse phase high-performance liquid chromatography column. Mass spectrometric analysis of the purified products indicated the formation of oligomers. Analysis of the mixture of products by gas chromatography and mass spectrometry after derivatization with diazomethane suggested the formation of at least three dimeric and nine trimeric products. Carbon-carbon and carbon-oxygen bonds were identified in the dimers and trimers, respectively. The nuclear magnetic resonance spectrum of the main dimer suggested coupling of the two monomers at the carbon one position.

Introduction

Halogenated dioxins and dibenzofurans reach the environment as by-products during the formation of polychlorinated phenols and incineration of refuse. Because of the highly toxic potential of these compounds, considerable effort is being directed toward development of biological techniques for their removal from the environment. Although very desirable, the total mineralization of biarylic dioxin-like xenobiotics such as dibenzofuran and diphenylether can be achieved by only a few special bacterial strains (Fortnagel et al. 1989; Pfeifer et al. 1989). Furthermore, a degradation of chlorinated dioxins and dibenzofurans was described for the white rot fungi *Phanerochaete chrysosporium* and *Phanerochaete sordida* (Valli et al. 1992; Takada et al. 1996). Although full mineralization is rare, the ability to oxidize such compounds seems to be widespread among bacteria, yeasts, and filamentous fungi (Smith et al. 1980; Cerniglia et al. 1992; Hammer & Schauer 1997). This oxidation results mainly in the formation of more water soluble hydroxylated derivatives that often exceed the parent compounds in toxicity and accumulate as unreactive end products (Henning 1993). In contrast to this, the ligninolytic enzymes laccase and peroxidase secreted by various white rot fungi show high affinity for hydroxyl-group-containing aromatic molecules (Thurston 1994). Many phenols and chlorophenols are transformed by these enzymes to radicals which subsequently form stable polymers spontaneously (Yu et al. 1994; Dec & Bollag 1994).

To date, experiments have focused on the transformation of monoaromatic compounds (Minard et al. 1981; Chang & Bumpus 1993; Tatsumi et al. 1994) rather than on the oxidation of biarylic substances. The purpose of the present study was to show that hydroxylated biarylic compounds such as 2-hydroxydibenzofuran (2-OH-DBF) can be transformed through oxidative coupling by the laccase of

the white rot fungi *Trametes versicolor* and *Pycnoporus cinnabarinus*. The major concern was to investigate the nature and fate of the reaction products.

Materials and methods

Organisms and culture conditions

Trametes versicolor SBUG-M 1050 was isolated from the wood of a deciduous tree, and *Pycnoporus cinnabarinus SBUG-M 1044* from an oak tree in the north of Germany. They are deposited at the strain collection of the Department of Biology of the University of Greifswald (SBUG).

The fungi were initially cultivated on malt agar plates that were incubated for 7 days at 30 °C and then maintained at 4 °C. Broth cultures were prepared by inoculating a nitrogen-rich medium (Hüttermann & Vogler 1973) with two or three 1-cm^2 agar culture fragments; incubation was performed under static conditions at 30 °C for 7 days. A uniform inoculum was obtained by homogenization of this culture with an Ultra-Turrax homogenizer T25 (IKA Labortechnik, Staufen, FRG) at 17000 rpm. For the production of laccase and peroxidase, 40 ml medium was incubated with 2 ml of the homogenized pre-culture in rubber stoppered 100-ml Erlenmeyer flasks for 9 days. Cultures were agitated in a water bath (GFL model 1092, Burgwedel, FRG) at 30 °C and 160 rpm under air for 3 days, after which they were purged with pure oxygen every 3 days.

Emzyme assays

At selected time intervals after inoculation, 1-ml samples of fungal culture medium were removed for spectrophotometric determination of enzyme activities using a double beam photometer (UVIKON 930, Kontron Instruments, Eching, FRG).

Laccase activity was measured by monitoring the oxidation of ABTS (2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonate); ε_{420} = 3.6 × 10^4 $M^{-1}\cdot cm^{-1}$; Bourbonnais & Paice 1990) for 1 min at 420 nm. Reaction mixtures (final volume 1 ml) contained 57 μl sample, 110 μl 5 mM ABTS, and 833 μl 100 mM sodium acetate buffer, pH 5 (Roy-Arcand & Archibald 1991).

Peroxidase activity was determined using phenol red according to Pick & Keisari (1980). In the assay 100 μl sample was incubated with 10 μl 8.2 mM H_2O_2 and 1 ml 0.01% phenol red (ε_{610} = 4.46 × 10^3 $M^{-1}\cdot cm^{-1}$; Michel et al. 1991) in 20 mM sodium succinate, pH 4.5. After 15 min at 37 °C the reaction was stopped by addition of 40 μl 2 M NaOH, and the absorbance was measured at 610 nm. In the case of manganese peroxidase activity determination, 10 μl 10 mM manganese sulfate was also added. To correct for the influence of biogenic H_2O_2, controls with catalase were used.

Lignin peroxidase activity was assayed by monitoring the change in absorbance at 310 nm corresponding to the oxidation of veratryl alcohol, as described by Kirk et al. (1990), but using 500 μl of sample, 50 μl of 8.2 mM H_2O_2, and 450 μl of 3.8 mM veratryl alcohol in 100 mM sodium tartrate buffer, pH 3.

Enzyme purification

To investigate the substrate specificity of individual enzymes in the culture supernatant, the laccase was separated from the manganese peroxidase. The culture medium was filtered through a glass fiber filter in a Buchner funnel, and the enzymes were eluted on a 2.5 × 3 cm DEAE-Sephacel (Sigma, Deisenhofen, FRG) column using 20 mM sodium acetate buffer, pH 5, and a linear gradient of 0 to 700 mM NaCl (Maltseva et al. 1991). The fractions containing enzyme activity were concentrated by ultrafiltration (Centriprep, 10 KDa, Amicon GmbH, Witten, FRG) and then desalted with PM 10 columns (Pharmacia, Freiburg, FRG).

Incubation with biaryl compounds and extraction of products

Experiments were performed with cell-free culture medium (laccase activity 200 $nmol\cdot ml^{-1}\cdot min^{-1}$) diluted with 20 mM sodium acetate buffer, pH 5, in a ratio of 1:4. To 10 ml of this culture filtrate 48 μl of 215 mM 2-OH-DBF dissolved in dimethylformamide was added, and the assay was incubated for 3 h at 30 °C with agitation at 100 rpm. Reactions were stopped by addition of 2 M HCl to achieve a final pH of 2, and the samples were extracted three times with an equal volume of ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and then evaporated in a vacuum rotator at 40 °C. The samples were evaporated to dryness under a stream of nitrogen. All samples obtained were redissolved in 1 ml ethyl acetate.

High-performance liquid chromatography and mass spectrometry

For the detection of metabolites, a high-performance liquid chromatography (HPLC) system (Hewlett-Packard GmbH, Bad Homburg, FRG) consisting of an HP 1050 Series Pump, and HP 1040 M Series I Diode-Array-Detector, and an HP HPLC Chem Station was used. The purification of metabolites was performed on a HPLC module system (Merck, Darmstadt, FRG) equipped with a Model L 6200A Intelligent Pump, a Rheodyne 7161 injection valve with a 20-μl loop and a Model L-4250 absorbance detector operating at 254 nm. Separation of products was achieved on an endcapped, 5-μm, LiChroCart 125-4 RP 18 column (Merck, Darmstadt, FRG) at a flow rate of 1.5 ml/min. An initial elution was carried out for 6 min with methanol (eluent A) and 0.01% acetic acid (eluent B) in a ratio of 65% A:35% B. The ratio was then changed within 30 sec to 75%A:25% B and held for the next 8.5 min. In the linear gradient that followed, the final solvent, 100% methanol, was achieved after 10 min and held for an additional 5 min. As metabolites from the column, samples were collected, evaporated to dryness, dissolved in methanol and analyzed by mass spectrometry (MS).

Mass spectral determinations were made by electron impact analysis at 70 eV with sample introduction via a direct insertion probe. Molecular weights were confirmed by fast atom bombardment (+FAB) analysis on a KRATOS MS-50 (Manchester, UK) using glycerol as a matrix.

Gas chromatography and mass spectrometry

After conversion of metabolites to their methyl derivatives by diazomethane (De Boer & Backer 1956) in a mini-apparatus (Aldrich, Steinheim, FRG), the separation of products for MS was performed on a gas chromatograph (Carlo Erba Instruments, Mainz-Kastel, FRG) equippped with a fused silica capillary column (DB-5 HT, 30 m $\times$ 0.25 mm, J&W Scientific, Folson, CA, USA). The following conditions were used: temperature program, 80–380 °C at 6 °C min^{-1}; injection mode, on column; carrier gas, H_2. For MS analysis a CH7A mass spectrometer (Varian, Darmstadt, FRG) was coupled with the gas chromatograph (GC) and the sample was analyzed over a scan range of 35 to 600 Da.

Proton nuclear magnetic resonance (NMR) spectroscopy

Proton NMR spectra were recorded on a 300 MHz spectrometer (Bruker GmbH, Karlsruge, FRG) using deuterated methanol as a reference.

Chemicals

2-OH-DBF was obtained from Aldrich (Steinheim, FRG). All other chemicals were of the highest purity available.

Results

Incubations with 2-OH-DBF

In agitated cultures with nitrogen-rich medium, the white rot fungi *Trametes versicolor* and *Pycnoporus cinnabarinus* secreted extracellular laccase after 9 days of incubation with activity at a level of 500 $nmol \cdot ml^{-1} \cdot min^{-1}$. This represents the maximum value, which remained stable at least for the next three days. Under these conditions *T. versicolor* also produced manganese peroxidase, while *P. cinnabarinus* did not, and no lignin peroxidase was detected in either culture.

Addition of 2-OH-BDF (250 μM final concentration) to the fungal cultures three days after inoculation enhanced laccase activity continuously threefold for 9 days in comparison to that of control cultures without the compound. The medium became yellow in color, and precipitates were formed. The products formed were poorly soluble in water, 2 N acetic acid, 2 N NaOH, chloroform, methylene chloride, acetonitrile, and n-hexane, and only partially soluble in methanol, ethylacetate, dimethylformamide and acetone. Precipitates similar in appearance were obtained when a cell-free culture medium with laccase activity was used. In order to distinguish between laccase- and manganese peroxidase-catalyzed reactions, enzymes were purified by ion exchange chromatography. The purified laccase of the two fungi was also able to transform 2-OH-DBF. On the other hand, after incubation with manganese peroxidase (activity 170 $nmol^{-1} \cdot min^{-1}$), no decrease of substrate and no products were found by HPLC analysis.

HPLC analysis of the organic extract of the *P.*

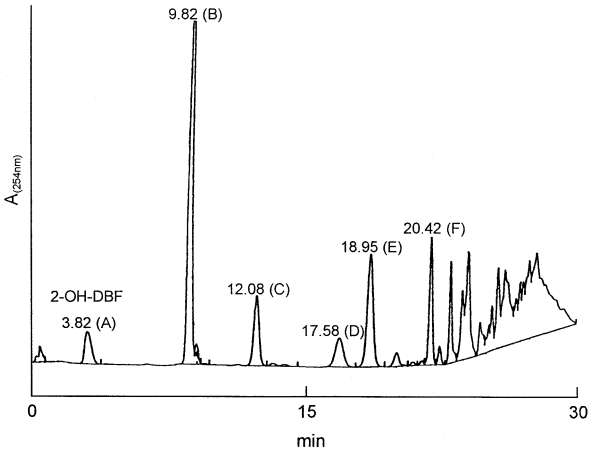

Figure 1. HPLC elution profile of an extract from an incubation assay of 2-OH-DBF (1 mM) and *Pycnoporus cinnabarinus* laccase (50 nmol·ml^{-1}·min^{-1}).

Table 1. Molecular weights and proposed structures of the products formed during transformation of 2-hydroxydibenzofuran (MW 184) by *Pycnoporus cinnabarinus* laccase

Product	Molecular weight	Proposed oligomer
B	366	Dimer
C	366	Dimer
D	549	Trimer
E	366	Dimer
F	549	Trimer

cinnabarinus incubation assay revealed that at least five products easy to separate (Figure 1, peaks B-F) and a pool of products with retention times higher than 21 min resulted from the transformation of 2-OH-DBF. All products were more hydrophobic than the parent compound (peak A), as revealed by the elution pattern. The same products resulted from incubations with *T. versicolor*, suggesting that product formation was independent of fungal species. Therefore, all further experiments were performed only with the cell-free culture medium of *P. cinnabarinus*.

Kinetic studies

For kinetic investigations, the disappearance of 2-OH-DBF and formation of the five major products were determined by HPLC. After 30 min, 97% of 2-OH-DBF (1 mM) was transformed (Figure 2), and the reaction mixture became dark yellow in color. The concentrations of the investigated products reached maxima

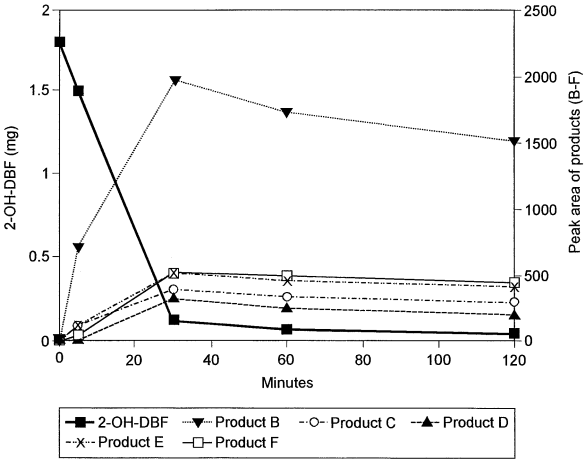

Figure 2. Kinetics of the transformation of 2-OH-DBF (1 mM) by *Pycnoporus cinnabarinus* laccase (50 nmol·ml^{-1}·min^{-1}) as determined by HPLC. Peaks B-F: products of the transformation, as indicated in Figure 1.

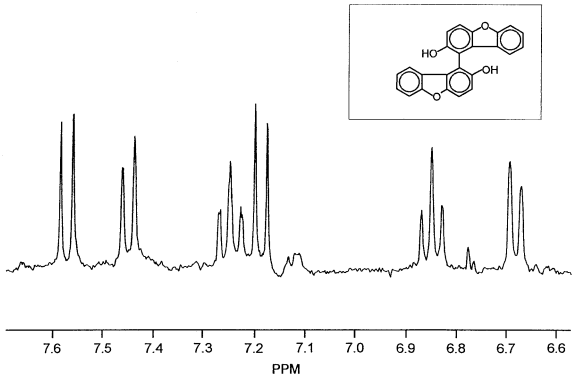

Figure 3. ^{1}H-NMR spectrum of dimer B, 1,1-di(2-OH-DBF), formed during transformation of 2-OH-DBF by *Pycnoporus cinnabarinus* laccase (300 MHz; CD_3OD). The dimer structure is shown inset.

at this time and decreased thereafter, that of product B most noticeably.

Separation of products by HPLC and analysis by MS and ^{1}H-NMR

The five products (B-F) separated by HPLC (Figure 1) were collected and further analyzed on a +FAB mass spectrometer. The molecular weights (MW) of the products (Table 1) were two- to threefold greater than that of the substrate, 2-OH-DBF (MW 184). Products B, C and E, with a molecular weight of 366, appear to be dimers, while products D and F with a molecular weight of 548 seem to be trimers. The ^{1}H-NMR spectrum of compound B is shown in Figure 3. The low

Table 2. Molecular weights after methylation, number of free hydroxyl groups and bond types of oligomer products from the transformation of 2-hydroxydibenzofuran by *Pycnoporus cinnabarinus* laccase

Molecular weight after methylation	Number of free hydroxyl groups	Type of bond	Product
380	1	C-O	E
394	2	C-C	B, C
562	1	2 C-O	*
576	2	1 C-C; 1 C-O	*
590	3	2 C-C	*

* These bonds were not assigned to products.

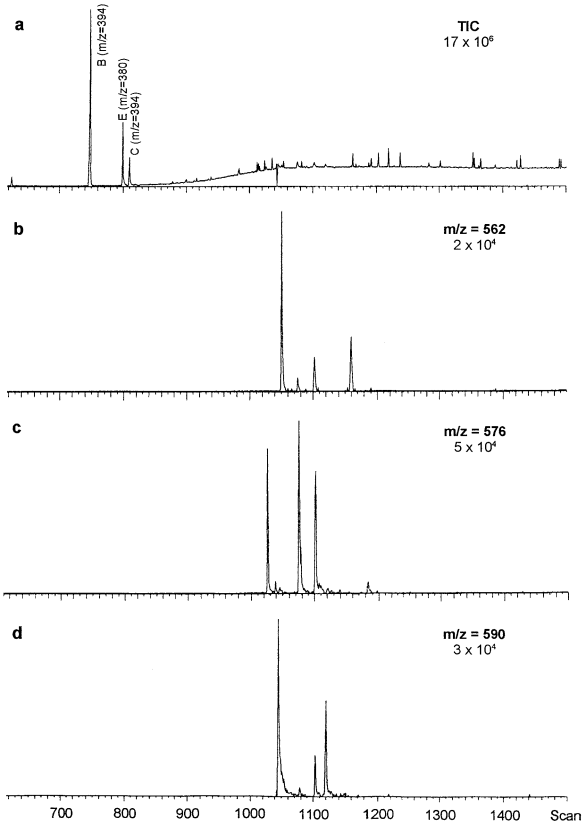

Figure 4. GC analysis of methylated extract from an incubation assay of 2-OH-DBF and *Pycnoporus cinnabarinus* laccase; a) total ion chromatogram; b) trimers with m/z = 562; c) trimers with m/z = 576; d) trimers with m/z = 590.

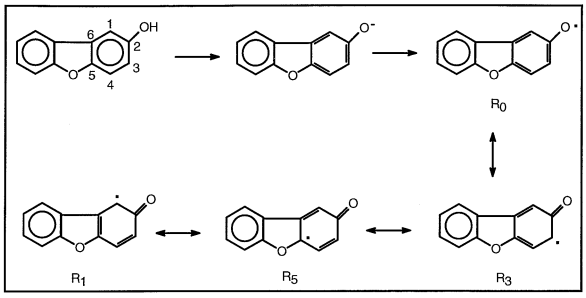

Figure 5. Proposed reaction mechanism for the formation of radicals of 2-OH-DBF by a laccase, R_0, R_1, R_3 and R_5 denote radicals formed at the free oxygen and carbons 1, 3 and 5, respectively.

number of proton-coupling signals indicates a very symmetrical chemical structure. The four doublets, two triplets, and the lack of the singlet at 7.35 ppm as indicated in the NMR of the 2-OH-DBF (data not shown) suggest the coupling of two radicals in position C1, from which we infer that 1,1-di(2-hydroxy dibenzofuran) was formed.

Analysis of methylated products by GC/MS

Further information regarding functional groups and bond types was obtained by GC and MS analysis of products. Methylation can confirm the presence of hydroxyl groups in a molecule, since the binding of one methyl group per substituent causes a mass increase of 14. From such increases in mass, inferences can be drawn concerning the number of free hydroxyl groups and the types of bonds involved.

Dimers. The purified compounds B and C (Figure 1) were identified as C-C-coupled dimers because of their molecular mass of 394 after methylation (Table 2). The mass spectrum of product B displayed 5 ion peaks: at m/z 394 (molecular mass, M^+, $C_{26}H_{18}O_4$), 379 ($M^+ - C_2H_6$), 348 ($M^+ - C_2H_6O$), 197 ($M^+ - C_{13}H_9O_2$ or the fragment ion $C_{13}H_9O_2$); 183 ($C_{12}H_7O_2$ or 197 – CH_3), and 125 ($C_7H_9O_2$), respectively (data not shown). This mass spectrum differed from that of product C inasmuch as product B produced stable ion peaks of 183 and 197 which were not detectable after fragmentation of product C. These peaks indicate the breakdown of a symmetric dimer into two monomers, confirming the structure suggested by NMR analysis above.

After methylation, mass analysis of product E gave a molecular weight of 380, indicating only one free hydroxyl group and therefore a coupling of free oxygen and a carbon (Table 2). The mass spectrum of the methyl derivative of product E contained ion peaks at 380 (molecular mass, M^+, $C_{25}H_{18}O_4$), 337 ($M^+ - CH_3CO$) and 139 ($C_{11}H_7$, a typical fragment ion of dibenzofuran), respectively (data not shown).

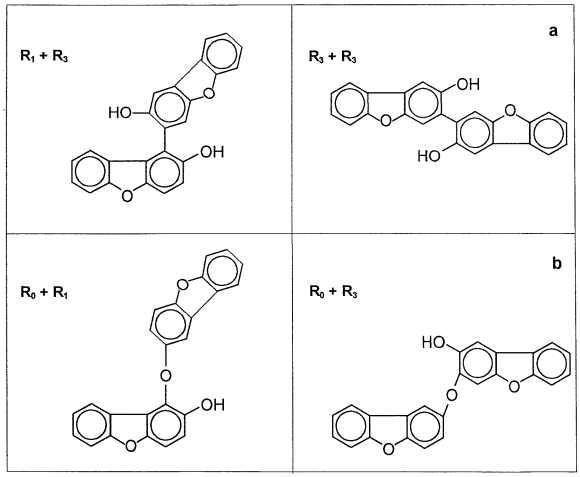

Figure 6. Proposed dimer formation from 2-OH-DBF with a m/z of (a) 394 and (b) 380.

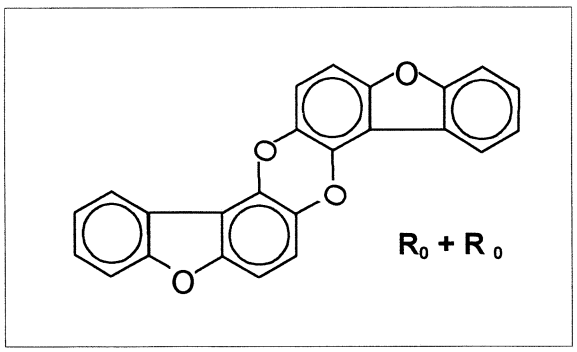

Figure 7. Proposed structure of a dimer with dioxin-like bonds.

As a result of GC and MS analysis of all products, a dimer pool (scan number 740–820) was observed containing only one methyl derivative with a m/z of 380 (scan 800) and two derivatives with a m/z of 394 (scans 723, 810) (Figure 4). No further products with C-C- or C-O-ether bonds for the coupling of two 2-OH-DBF radicals were found. Peroxide or dioxin-like linkages were also not detected.

Trimers. GC and MS analysis of the polymer mixture revealed at least nine trimers (Figure 4; scan 1020–1170), representing three groups differing in molecular weight after methylation (m/z 562, 576, 590) and thus differing in the number of free hydroxyl groups and types of polymerization bonds (Table 2). Despite the use of mass and NMR spectra, the trimer structures could not be characterized in further detail.

Discussion

The results demonstrated that 2-OH-DBF was oligomerized by laccase of the white rot fungi *T. versicolor* and *P. cinnabarinus* secreted into the culture medium within few minutes. In contrast to this fast reaction, other degradation processes of dioxin-like compounds by fungi take some days or weeks (Takada et al. 1996; Hammer & Schauer 1997). It is obvious that not only monoaromatic but also biarylic compounds with at least one hydroxyl group were polymerized. It was also shown that the manganese peroxidase produced by *T. versicolor* was not involved in an oligomerization and did not transform 2-OH-DBF at all. However, polymerization processes catalyzed by manganese peroxidase were described by Rüttimann-Johnson & Lamar (1996) for the coupling of polychlorinated phenols and ferulic acid.

In our experimental system, formation of oligomers with C-C and C-O bonds suggests a reaction mechanism similar to that described by Dec & Bolag (1994) for the polymerization of monoaromatic compounds. Four different radicals can be formed at the reactive hydroxylated ring (Figure 5). The preferred entry of a new substituent is at positions *ortho* or *para* to the hydroxyl group; the *meta* position is non-reactive (Walter 1991). A radical can also be formed on the oxygen atom.

After transformation of 2-OH-DBF, three dimers and at least nine trimers with free hydroxyl groups were detected. Derived from the reaction mechanism of radicals (Figure 5), three configurations of dimers with C-C bonds are possible, as shown in Figures 3 and 6a. One of these, the 1,1-di(2-OH-DBF) formed by the joining of two R_1 radicals (Figure 3), was always the predominant product. A similar result was obtained by Yu et al. (1994), who found the ortho-ortho dimer to be the most prevalent coupling form for phenol in the presence of horseradish peroxidase. This dimer proved to be a poor substrate for further oligomerizations, whereas in the present study dimer B (1,1-di(2-OH-DBF) appears to be a good substrate for this purpose, as evidenced by its decrease in concentration following the peak at 30 min (Figure 2). In contrast to the C-C-linked dimers (m/z 394) the methyl derivatives of C-O-linked oligomers would have masses of 380. From the radicals shown in Figure 5, two dimer structures with C-O bonds are possible (Figure 6b), but only one with this molecular weight (product E; scan 800, Figure 4) was observed

upon GC and MS analysis. The exact structure was not assignable until now.

Theoretically, 2-OH-DBF radicals of the structure R_0 (Figure 5) can couple as well, resulting in a dioxin-like structure with m/z 364 (Figure 7). However, this compound was not observed upon GC or +FAB-MS analysis of the dimers, suggesting that it was either absent or present at a very low concentration. From an ecological point of view, the formation of a dioxin bond would be unfavorable, e.g., in the case of subsequent chlorination. The possibility of biogenous dioxin production from chlorinated phenols has been described recently (De Jong et al. 1994; Öberg et al. 1990), but Dec & Bollag (1994) did not find a dioxin-like structure formed from monoaromatic substances.

In complex systems like soil, the processes described here can be of considerable importance. Many microorganisms in soil are able to hydroxylate unsubstituted aromatic compounds during mineralization. After oligomerization of these intermediates by laccase, complete degradation of xenobiotics would be prevented. Often up to 50% of the original substance seems to disappear during biodegradation in soil without an increase of products (Richnow et al., 1995).

However, polymerization processes and the binding of phenols to the soil humic fraction may also be classified as 'detoxification' (Dec & Bollag 1994; Bollag & Myers 1992). Polymers are less bioavailable and are subject to very slow degradation to carbon dioxide and water by soil microorganisms (Dec & Bollag 1988). Moreover, Dec & Bollag (1994) observed a release of chloride ions during the oxidative coupling of chlorinated phenols with humic monomers or humic acid.

The first step in the elucidation of such polymerization processes is the structural characterization of products. This paper sought to describe some possible structures of dimers and trimers of 2-OH-DBF, and from these results it is possible to speculate concerning probable tetramers and further oligomers. The investigation suggests the potential of laccase to assist in transformation and detoxification of a variety of biarylic compounds such as diphenylethers, biphenyls and dioxins.

Acknowledgements

We are grateful to Dr. R. Minard, Pennsylvania State University, for support in structure analysis, Dr. A. Benesi, Pennsylvania State University, for providing NMR facilities and Dr. H.-H. Richnow, University Hamburg, for providing GC/MS facilities. We thank also Errol Fernandes for critical reading of the manuscript and help in NMR analysis. This work was supported by a grant of the Kultusministerium des Landes Mecklenburg-Vorpommern.

References

Bollag JM (1992) Decontaminating soil with enzymes. Environ. Sci. Technol. 26: 1876–1881

Bollag JM & Meyers CJ (1992) Detoxification of aquatic and terrestrial sites through binding of pollutants to humic substances. Sci. Total Environ. 117/118: 357–366

Bourbonnais R & Paice MG (1990) Oxidation of non-phenolic substrates: An expanded role for laccase in lignin biodegradation. FEBS Lett. 267: 99–102

Cerniglia CE, Sutherland JB & Crow SA (1992) Fungal metabolism of aromatic hydrocarbons. In: Winkelmann G (Ed) Microbial degradation of natural products (pp 193–217). VHC Press, Weinheim, Germany

Chang C & Bumpus JA (1993) Oligomers of 4-chloroaniline are intermediates formed during its biodegradation by *Phanerochaete chrysosporium*. FEMS Microbiol. Lett. 107: 337–342

De Boer TD & Backer HJ (1956) Diazomethane. In: Leonard NJ (Ed) Organic synthesis, Vol. 36 (pp 14–16). John Wiley and Sons, New York

De Jong E, Field JA, Spinnler HE, Wijnberg JBPA & de Bont JAM (1994) Significant biogenesis of chlorinated aromatics by fungi in natural environments. Appl. Environ. Microbiol. 60: 264–270

Dec J & Bollag JM (1988) Microbial release and degradation of catechol and chlorophenols bound to synthetic humic acid. Soil Sci. Soc. Am. J. 52: 1366–1371

— (1994) Dehalogenation of chlorinated phenols during oxidative coupling. Environ. Sci. Technol. 28: 484–490

Fortnagel P, Wittich R, Harms H & Schmidt S (1989) New bacterial degradation of the biaryl ether structure. Naturwissenschaften 76: 523–524

Hammer E & Schauer F (1997) Fungal hydroxylation of dibenzofuran. Mycol. Res. 101: 433–436

Henning K (1993) Oxidation von Diphenylether durch die Hefe *Trichosporon beigelii*. PhD Thesis. Universität Grefiswald. Institut für Mikrobiologie und Molekularbiologie

Hüttermann A & Vogler C (1973) Induction of arylglucosidase in *Fomes annosus* by cellobiose. Arch. Microbiol. 93: 195–204

Kirk TK, Tien M, Kersten PJ, Kalyanaraman B, Hammel KE & Farrell RL (1990) Lignin peroxidase from fungi: *Phanerochaete chrysosporium*. In: Lidstrom ME (Ed) Methods in Enzymology: Hydrocarbons and Methylotrophy, Vol. 188 (pp 159–169). Academic Press, San Diego

Maltseva OV, Niku-Paavola ML, Leontievsky AA, Myasoedova NM & Govleva LA (1991) Ligninolytic enzymes of the white-rot fungus *Panus tigrinus*. Biotechnol. Appl. Biochem. 13: 291–302

Michel FC, Das SB, Grulke FA & Reddy CA (1991) Role of manganese peroxidase and lignin peroxidase of *Phanerochaete chrysosporium* in the decolorization of bleach plant effluent. Appl. Environ. Microbiol. 57: 2368–2375

Minard RD, Bollag JM & Liu SY (1981) Oligomers and quinones from 2,4-dichlorophenol. J. Agr. Food Chem. 29: 250–253

Öberg LG, Glas B, Swanson SE, Rappe CP & Paul KG (1990) Peroxidase-catalyzed oxidation of chlorophenols to polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Arch. Environ. Contam. Toxicol. 19: 930–938

Pick E & Keisari Y (1980) A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J. Immunol. Meth. 38: 161–170

Pfeifer F, Schacht S, Klein J, Trüper HG (1989) Degradation of diphenylether by *Pseudomonas cepacia*. Arch. Microbiol. 152: 512–519

Richnow HH, Seifert R, Kästner M, Mahro B, Horsfield B, Tiedgen U, Böhm S & Michaelis W (1995) Rapid screening of PAH-residues in bioremediated soils. Chemosphere 31: 3991–3999

Roy-Arcand L & Archibald FSJ (1991) Direct dechlorination of chlorophenolic compounds by laccases from *Trametes (Coriolus) versicolor*. Enzyme Microb. Technol. 13: 194–203

Smith RV, Davis PJ, Clark AM & Glover-Milton S (1980) Hydroxylations of biphenyl by fungi. J. Appl. Bacteriol. 49: 65–73

Tatsumi K, Freyer A, Minard RD & Bollag JM (1994) Enzyme-mediated coupling of 3,4-dichloroaniline and ferulic acid: A model for pollutant binding to humic materials. Environ. Sci. Technol. 28: 210–215

Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140: 19–26

Valli K, Wariishi H & Gold MH (1992) Degradation of 2,7-dichlorodibenzo-*p*-dioxin by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. J. Bacteriol. 174: 2131–2137

Walter W (1991) Mechanismen der mehrfachen elektrophilen Substitution am Benzolkern. In: Beyer H & Walter W (Eds) Lehrbuch der organischen Chemie (pp 485–487). S. Hirzel Verlag, Stuttgart

Yu J, Taylor KE, Zou H, Biswas N & Bewtra JK (1994) Phenol conversion and dimeric intermediates in horseradish peroxidase-catalyzed phenol removal from water. Environ. Sci. Technol. 28: 2154–2160