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ORGANOPOLLUTANT DEGRADATION BY LIGNINOLYTIC FUNGI

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1. INTRODUCTION

The ligninolytic fungi that cause white rot of wood degrade an impressive array of organopollutants (for earlier reviews, see Bumpus and Aust, 1987b; Hammel, 1989, 1992). As this chapter shows, the xenobiotic metabolism of white-rot fungi is at least in part a consequence of the mechanisms these organisms have to degrade lignin. Although observations of aromatic pollutant breakdown by white-rot fungi date back 30 years (Lyr. 1963), the connection of this process with ligninolytic metabolism was noted more recently, when several groups observed that the basidiomycete *Phanerochaete chrysosporium* degrades benzo[*a*]pyrene, DDT, chlorinated anilines, various alkylhalide insecticides, and a polychlorinated biphenyl mixture under culture conditions that promote the expression of ligninolytic metabolism (Arjmand and Sandermann, 1985; Bumpus et al., 1985; Eaton, 1985; Huynh et al., 1985; Bumpus and Aust, 1987a; Kennedy et al., 1990). In some cases, significant mineralization of the pollutants was obtained.

The ability of *P. chrysosporium* to degrade most organopollutants correlates closely with ligninolytic activity in culture, both processes depending on secondary (idiophasic) metabolism, which is triggered by nutrient starvation and the cessation

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of cell growth. There are some, as yet unexplained, exceptions to this observation (Köhler et al., 1988), as well as many instances in which idiophasic metabolism is required but no direct involvement of ligninolytic enzymes has been shown (Bumpus et al., 1985; Eaton, 1985; Bumpus and Aust, 1987a; Kennedy et al., 1990). Here I focus only on those xenobiotic oxidations that show clear mechanistic connections with ligninolytic metabolism.

2. BIOCHEMICAL FEATURES OF FUNGAL LIGNINOLYSIS

Lignins are complex phenylpropane wood polymers, formed in plant cell walls via the coupling of phydroxycinnamyl alcohols, that make up a significant proportion (10%–30%) of the biomass in terrestrial higher plants. The function of lignin is to give these plants structural rigidity and to protect their cellulose and hemicelluloses from microbial attack. Lignins are amorphous, stereoirregular, water insoluble, nonhydrolyzable, and highly resistant to degradation by most organisms (Adler, 1977; Kirk and Farrell, 1987).

Rapid lignin degradation appears to be limited to white-rot basidiomycetes, which are inhabitants of dead wood and soil litter, and depend for their growth on the polysaccharides in these substrates (Kirk and Farrell, 1987; Gold et al., 1989). Ligninolysis *per se* does not support growth, but is rather intended to open up the structure of woody materials so that fungal polysaccharide-degrading agents can penetrate. The large size, irregular structure, and nonhydrolyzable nature of lignin dictate key features of the ligninolytic process: It is extracellular, highly non-specific, and oxidative. One review refers to fungal ligninolysis as "enzymatic combustion" to reflect these properties (Kirk and Farrell, 1987). and it is this low specificity that has led many investigators to suggest that organopollutants might be fortuitous targets of fungal ligninolytic metabolism (Bumpus et al., 1985; Haemmerli et al., 1986; Hammel et al., 1986a; Sanglard et al., 1986; Bumpus and Aust, 1987b).

Considerable progress has been made in our understanding of fungal ligninolysis. One of the principal reactions in this process is oxidative $C_\alpha - C_\beta$ cleavage of the lignin propyl side chain. For example, the principal arylglycerol-β-aryl ether structures of lignin, which comprise 50%–60% of the total polymer, are cleaved *in vivo* to give C_α-benzylic aldehydes (Fig. 9.1) (Kirk and Farrell, 1987; Gold et al., 1989). This reaction is catalyzed, both in simple lignin model compounds and in lignin, by extracellular, highly oxidizing peroxidases known as lignin peroxidases (LiPs) (Glenn et al., 1983; Tien and Kirk, 1983; Gold et al., 1984; Tien and Kirk, 1984; Kirk and Farrell, 1987; Gold et al., 1089; Hammel and Moen, 1991; Hammel et al., 1993). LiPs ionize, their aromatic substrates to give aryl cation radicals, acting in effect as minute mass spectrometers (Kersten et al., 1985; Dolphin et al., 1987). These cation radicals then undergo a variety of nonenzymatic reactions. including nucleophilic attack by water in the case of some simple aromatics (Kersten et al., 1985, 1990) or carbon-carbon bond fission in the case of phenylpropane models that imitate natural lignin substructures (Hammel et al., 1985, 1986b; Kirk

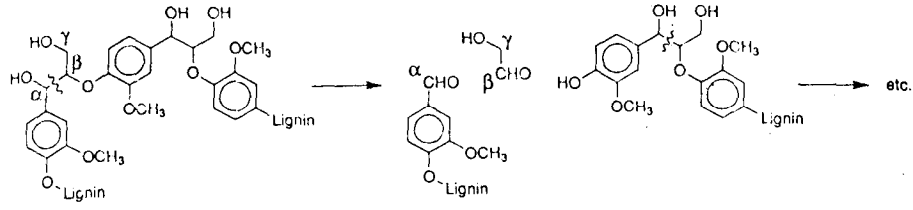

Figure 9.1. A linear region of softwood lignin, showing the major arylglycerol-β-aryl ether structure of the polymer and principal sites of fungal side-chain cleavage.

et al., 1986). The initial steps of fungal ligninolysis thus result from enzymatic one-electron oxidations of lignin.

White-rot fungi also produce other extracellular one-electron oxidants, in the form of laccases and manganese-dependent peroxidases (MnPs) (Glenn and Gold, 1985; Glenn et al., 1986; Kirk and Farrell, 1987; Gold et al., 1989). Laccases oxidize substrates directly, whereas MnPs operate by oxidizing manganese(II) in the presence of various organic chelators to give complexes of manganese(III), which act as diffusible oxidants at a distance from the enzyme active site (Glenn and Gold, 1985; Glenn et al., 1986). The low molecular weight and diffusibility of Mn(III) make MnPs particularly attractive candidates for ligninolytic catalysis in wood. However, chelated Mn(III) is a relatively weak oxidant that is limited to attack on easily oxidized substrates such as phenols. MnPs have been shown to depolymerize lignins *in vitro*, presumably via attack on phenolic structures in the polymer (Wariishi et al., 1991), and are now thought to constitute an important part of the fungal ligninolytic machinery. Both LiPs and MnPs, because of their low specificity, stand out as obvious candidates for the catalysis of xenobiotic oxidations by white-rot fungi.

3. FUNGAL ORGANOPOLLUTANT DEGRADATION

3.1. Polycyclic Aromatic Hydrocarbons

3.1.1. *Fungal Metabolism*

Eukaryotes in general do not degrade polycyclic aromatic hydrocarbons (PAHs). Instead, they metabolize these compounds, using well-described monooxygenase systems, to hydroxylated metabolites that can be excreted directly or as conjugates with more polar molecules (Gibson and Subramanian, 1984). Hydroxylation and excretion are standard eukaryotic strategies for xenobiotic detoxification, and most fungi fit this pattern. For example, members of the genus *Cunninghamella* utilize membrane-bound cytochrome P_{450} systems to oxidize naphthalene, phenanthrene, anthracene, benz[*a*]anthracene, 3-methylcholanthrene, and benzo[*a*]pyrene (Cerniglia and Gibson, 1979; Cerniglia and Yang, 1984; Gibson and Subramanian, 1984). Recently, Sutherland et al. (1991) showed that the white-rot basidiomycete

P. chrysosporium metabolizes phenanthrene to *trans*-dihydrodiols and phenanthrol conjugates when grown under nonligninolytic conditions in a rich nutrient medium, PAH metabolism by white-rot fungi under nonligninolytic conditions is accordingly unexceptional, resembling the process in nonligninolytic fungi and other eukaryotes.

It is therefore singular that idiophasic *P. chrysosporium,* grown under nutrient-limiting conditions that induce the ligninolytic system, is able to mineralize benzo[*a*]pyrene. The rate and extent of degradation initially reported were low–about 10% of initially added (0.125 μM) benzo[*a*]pyrene was oxidized to CO_2 in 30 days–but the finding was nonetheless unprecedented (Bumpus et al., 1985). Later work (Sanglard et al., 1986) demonstrated greater degradation of this PAH by *P. chrysosporium*. Low-nitrogen fungal cultures with biomass levels of about 2 g dry weight L^{-1} were able to metabolize 3 μM benzo[*a*]pyrene completely in 7 days. About 15% of the starting material was mineralized, about 60% was oxidized to water-soluble products, and about 20% was oxidized to organic-soluble metabolites, with the remainder (approx. 5%) present as mycelium-bound material. The maximum rate of benzo[*a*]pyrene mineralization observed with this PAH load was about 2 pmol h^{-1} mg^{-1} mycelial dry weight. When the initial benzo[*a*]pyrene concentration was increased to 30 μM, the maximal mineralization rate was about 6 pmol h^{-1} mg^{-1} dry weight.

Recent studies by Field et al. (1992) have shown that relatively high initial loads (10 mg L^{-1}) of anthracene or benzo[*a*]pyrene can be removed by a variety of white-rot fungi growing in liquid media. Most of the strains tested, including various species of *Trametes* and *Bjerkandera,* were more effective than the standard laboratory organism *P. chrysosporium.* In soils, the results are similar, but less clear-cut. Fluorene, added at 75 ppm to sterile soil, was effectively removed by *P. chrysosporium* in laboratory studies (George and Neufeld, 1989). 9-Fluorenone was reported to be an intermediary metabolite and was also depleted from soil by the fungus. In a study done on creosote-contaminated soil, fluorene and a variety of other PAHs with fewer than five rings were depleted by the related fungus *P. sordida,* but high variability in the data made a reliable assessment of the rates and extents of degradation difficult. PAHs with more than five rings, *e.g.*, benzo[*a*]pyrene, were not degraded by the fungus in soil, presumably because their greater hydrophobicity promoted tight binding to organic constituents in the soil, thereby preventing biological attack (Davis et al., 1993).

it is pertinent to compare fungal rates of PAH degradation with those exhibited by bacteria. To take one example, a *Mycobacterium* sp. has been described that co-oxidizes pyrene during growth on a complex organic medium (Heitkamp et al., 1988). This organism mineralized approximately 50% of initially added (2.5 μM) pyrene in 2 days, during which time it grew to a biomass level of about 0.1 g dry weight L^{-1}. Stationary-phase *P. chrysosporium,* by contrast, mineralized 16% of initially added (0.2 μM) pyrene in 2 weeks, at a biomass level of about 2 g dry weight L^{-1} (Hammel et al., 1991). We can conclude from this large difference that bacteria appear more promising for practical application in PAH bioremediation than wrhite-rot fungi do. However, from an ecological standpoint, ligninolytic fungi

probably play a significant role in the natural breakdown of PAH contaminants: Although these organisms are slow biodegraders, they are highly nonspecific, and they are ubiquitous inhabitants of woodland soils (Frankland, 1982).

3.1.2. Oxidation by Ligninolytic Enzymes

The possible involvement of the ligninolytic system in PAH degradation by white-rot fungi was shown some years ago in work with purified LiP. Benzo[*a*]pyrene was found to be a substrate for the enzyme, and it was proposed that LiPs might participate in the degradation of this PAH *in vivo* (Haemmerli et al., 1986). The products of enzymatic benzo[*a*]pyrene oxidation were shown to be the benzo[*a*]pyrene 1,6-, 3,6-, and 6.12-quinones. which is consistent with a reaction mechanism in which LiP oxidizes the PAH to its cation radical. However, the quinones were not identified as metabolic intermediates in fungal cultures, and it remained unclear whether LiP is actually involved in the degradative pathway.

Concurrent work showed that PAHs in general are LiP substrates, provided that they have ionization potentials lower than about 7.6 eV (Hammel et al., 1986a). Perylene (7.1 eL), benzo[*a*]pyrene (7.2 cV), unthracene (7.4 eV), pyrene (7.5 eV), and benz[*a*]anthracene are oxidized, whereas benzo[*e*]pyrene (7.7 eV), chrysene (7.8 eV), benzo[*c*]phenanthrene (7.9 eV), phenanthrene (8.1 eV), and naphthalene (8.1 eV) give no reaction. Classical peroxidases such as the horseradish enzyme have also been reported to oxidize PAHs, but only when the ionization potential is lower than about 7.35 eV (Cavalieri et al., 1983). These results suggest that the oxidized states (compounds I and II) of LiP are more oxidizing than those of typical peroxidases. Experiments on the relative ability of LiP and horseradish peroxidase to oxidize methoxybenzenes with known ionization potentials have confirmed this conclusion (Kersten et al., 1990), as have measurements of the Fe(II)-Fe(III) redox potentials of the two enzymes (Millis et al., 1989).

The hypothesis that LiP-catalyzed PAH oxidations proceed through a PAH cation radical intermediate received strong support in $H_2{}^{18}O$–labeling studies of pyrene oxidation (Hammel et al., 1986a). Pyrene was oxidized by the enzyme to its 1.6- and 1.8-quinones, and the newly introduced oxygens were derived from water. These results indicate that a cationic reaction intermediate undergoes nucleophilic attack by water at its positions of minimum charge density. Deprotonation and further oxidation then presumably lead to the formation of hydroxylated PAHs, which undergo further, similar reactions to yield the quinones (Fig. 9.2). It was also observed in this study that intact cultures of *P. chrysosporium* product low levels of pyrene quinones as transient products of pyrene metabolism, but it was not possible to conclude whether these products are precursors for ring fission and mineralization.

Recent work has shown that LiPs are actually involved in the oxidation by *P. chrysosporium* of one PAH, anthracene, that is a LiP substrate (Hammel et al., 1991). Both purified LiP and idiophasic (nitrogen-limited) cultures of *P. chrysosporium* oxidized anthracene to 9.10-anthraquinone. Chromatographic (Fig. 9.3) and isotope dilution experiments with [^{14}C]anthracene showed that the fungal oxi-

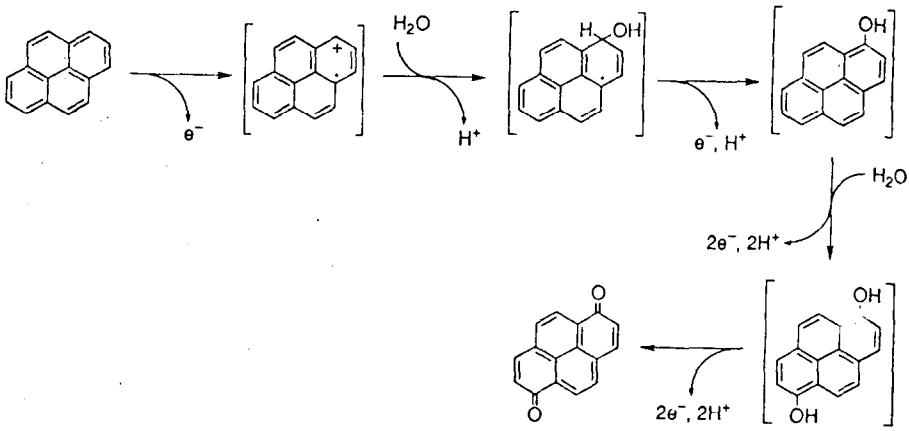

Figure 9.2. Mechanism proposed for the LiP-catalyzed oxidation of pyrene to pyrene-1,6-quinone. (Reproduced from Hammel, 1992, with permission of the publisher.)

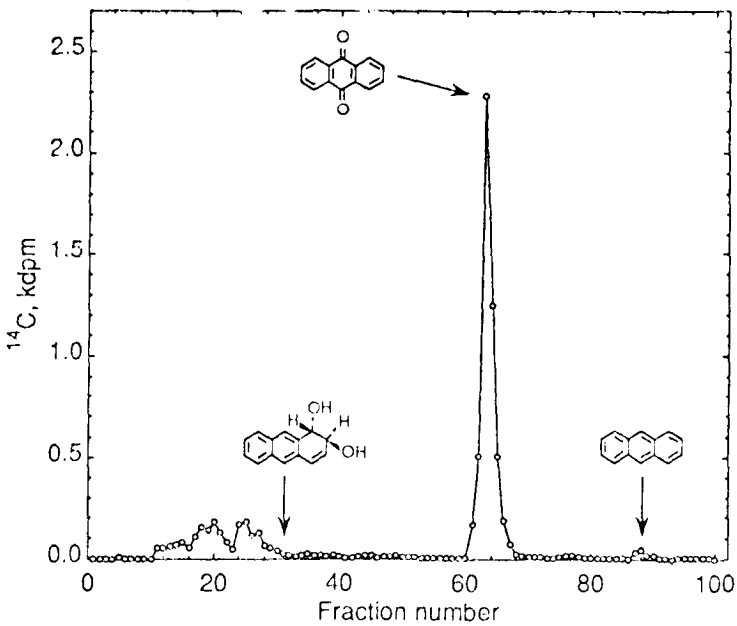

Figure 9.3. High-performance liquid chromatography of neutral metabolites formed from [^{14}C]anthracene by nitrogen-limited *P. chrysosporium* in 2 days. the major anthracene metabolite formed is 9,10-anthraquinone. Anthracene *trans*-1,2-dihydrodiol, the major thracene metabolite found in non ligninolytic fungi (Cerniglaia and Yang, 1984), was not found. (Reproduced from Hammel et al., 1991, with permission of the publisher)

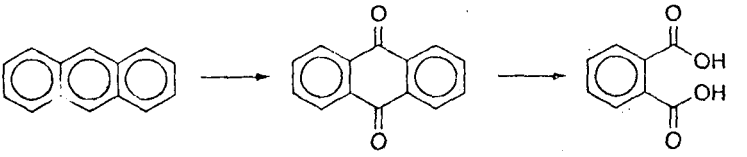

Figure 9.4. Known reactions of anthracene metabolism in idiophasic *P. chrysosporium.*

dation of anthracene to anthraquinone was rapid, preceding the onset of anthracene mineralization, and accounted after 48 hours for about 40% of the ^{14}C initially added. Both anthracene and anthraquinone were mineralized to the same extent in culture (approx. 13% of 0.2 µM in 2 weeks), and both compounds were cleaved by the fungus to give equivalent yields (12%–13%) of the same diagnostic ring-fission metabolite, phthalic acid. Phthalate production from the quinone was demonstrated to occur only under ligninolytic culture conditions. These results show that the major pathway for anthracene degradation in *P. chrysosporium* proceeds via the 9,10-quinone to phthalate (Fig. 9.4) and that it is probably mediated by LiPs and other enzymes of ligninolytic metabolism. The mechanism that the fungus employs for the cleavage of anthraquinone to phthalate remains unknown.

The degradation of PAHs by ligninolytic fungi is not limited to those compounds that are LiP substrates. Phenanthrene, which repeated investigations have shown is not oxidized by the enzyme, is nevertheless rapidly metabolized by ligninolytic *P. chrysosporium.* The major metabolite found in fungal cultures is 2,2′-diphenic acid, which results from cleavage of the phenanthrene 9,10-bond (Fig. 9.5) (Hammel et al., 1992). Recent work suggests that the MnPs of *P. chrysosporium* are instrumental in this process (Moen and Hammel, 1994).

3.2. Chlorinated Phenols

3.2.1. *Fungal Metabolism*

Early work by Lyr (1963) in Germany showed that the white-rot fungus *Trametes versicolor* was able to dechlorinate polychlorinated phenols to unspecified products. Subsequent research has confirmed this finding and showed that white-rot fungi

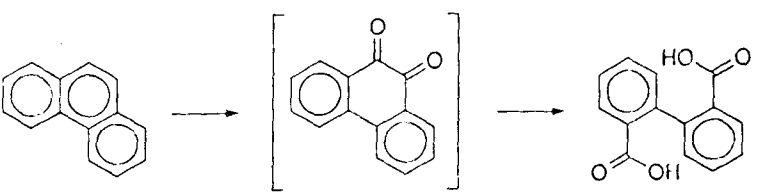

Figure 9.5. Oxidation of phenanthrene by idophasie *P. chrysosporium.* The intermediacy of the quinone is likely, but not firmly established.

degrade a wide variety of chlorinated phenols, some of which are significant pollutants formed during the chlorine bleaching of wood pulp. For example, *P. chrysosporium* degrades 2,4,6-trichlorophenol, various polychlorinated guaiacols, and several polychlorinated vanillins, all of which are toxic constituents of pulp bleachery effluents (Haynh et al., 1985). Products in these studies were identified by gas chromatography/mass spectrometry, and although direct precursor/product relationships were not determined, it could be concluded that the mechanisms of removal include oxidation, dechlorination, and methylation.

Recent work with individual chlorophenols has confirmed the ability of *P. chrysosporium* to degrade this class of compound. 2-Chlorophenol, added at initial concentrations as high as 0.5 g L^{-1}, was removed from the extracellular medium in packed-bed bioreactors (Lewandowski et al., 1990). Although no organic degradation products were identified, dehalogenation of the aromatic ring must have occurred, because inorganic chloride was produced. 2,4,6-Trichlorophenol and pentachlorophenol are also degraded in *P. chrysosporium* liquid cultures (Guo et al., 1990). In this study, both phenols were rapidly depleted from the extracellular medium, in part because they were methylated to give the corresponding anisoles, which were also removed by unspecified mechanisms.

Some polychlorinated phenols are mineralized by *P. chrysosporium* when the fungus is grown in nitrogen-limited liquid culture. It was recently reported that *ring*-^{14}C-labeled 2,4-dichlorophenol and 2,4,5-trichlorophenol, added at 180 µM initial concentration, were mineralized over 30 days in 50%–60% yield (Valli and Gold, 1991; Joshi and Gold, 1993). Pentachlorophenol is also mineralized by *P. chrysosporium.* In nitrogen-limited liquid cultures to which 0.5-36 µM [*ring*$^{-14}$C]pentachlorophenol was added, variable proportions (20%–50%) of the total were reportedly trapped as $^{14}CO_2$. The times required to produce this much CO_2 varied considerably, from 4 to 30 days (Mileski et al., 1988). Subsequent studies have suggested that some of the volatile ^{14}C produced from pentachlorophenol by *P. chrysosporium* actually consists of organic products, thus leading to overestimates of mineralization (Lamar et al., 1990b). The ability of *P. chrysosporium* to degrade pentachlorophenol is markedly better in pregrown cultures with appreciable biomass levels than it is when the pollutant is added to freshly inoculated cultures (Mileski et al., 1988), a result attributable to the not unexpected finding that pentachlorophenol, a fungicidal wood preservative, inhibits the growth of *P. chrysosporium* and other white-rot fungi.

Experiments with liquid cultures of ligninolytic fungi provide useful information on what these organisms might do to chlorophenols during wastewater biotreatment. However, when ligninolytic fungi are grown in soil, the picture is evidently different. The mineralization of pentachlorophenol by *P. chrysosporium* or the related white-rot fungus *P. sordida* in this environment was found to be negligible. Nevertheless, both fungi did deplete pentachlorophenol from soil (Lamar and Dietrich, 1990; Lamar et al., 1990a). Some of the depletion was attributable to the formation of soil-bound metabolites, which have not thus far been characterized, but might have resulted from oxidative radical coupling between phenolic pentachlorophenol metabolites and phenolic constituents in the soil. The amount of soil-bound versus

organic-extractable pentachlorophenol-derived material was influenced by soil type, with high clay content favoring binding. Another prominent fate of pentachlorophenol in soil was methylation to give pentachloroanisole, which was in turn slowly converted to unknown products by both of the fungi examined.

3.2.2. Oxidation by Ligninolytic Enzymes

Two frequently made observations suggest that the fungal degradation of polychlorinated phenols might involve ligninolytic metabolism: Degradation is highly nonspecific, and it is stimulated under nutrient limitation. Moreover, polychlorinated phenols have been shown to be substrates for *P. chrysosporium* lignin peroxidases (Hammel and Tardone, 1988). The results showed that these enzymes catalyze the oxidative 4-dechlorination of polychlorinated phenols to give 1,4-benzoquinones. For example, 2,4,6-trichlorophenol is quantitatively oxidized to 2,6-dichloro- 1,4-benzoquinone and inorganic chloride by LiP *in vitro.* Similarly, pentachlorophenol is oxidized to tetrachloro- 1,4-benzoquinone. Reactions of this type presumably involve two sequential one-electron oxidations of the phenol to give a cyclohexadienone cation, which then eliminates halide upon nucleophilic attack by water (Fig. 9.6). Other studies on the LiP-catalyzed oxidation of pentachlorolphenol by LiP have given results in agreement with this scheme (Mileski et al., 1988; Lin et al., 1990). Varying degrees of enzyme inactivation are observed during LiP-catalyzed phenol oxidations (Aitken et al., 1989), and MnP and laccase are now known to catalyze these oxidative dechlorinations more efficiently than does LiP (Roy-Arcand and Archibald, 1991).

The observations that LiP, MnP, and laccase can dehalogenate polycholrinated phenols were, in retrospect, not surprising: Peroxidases and laccases have long been known to oxidize a wide variety of phenols. Lyr demonstrated in 1963 that a crude laccase from *T. versicolor* oxidized chlorophenols to unknown products with the release of chloride, and subsequent studies with plant peroxidases showed that chlorophenols were enzymatically dechlorinated to give uncharacterized products (Saunders and Stark, 1967). However, it was not immediately evident how these (per)oxidative reactions might be integrated into metabolic pathways that lead to the extensive degradation of chlorinated phenols.

Gold and coworkers have recently provided the first evidence that fungal peroxidases are directly involved in polychlorophenol degradation by P. chrysosporium (Valli and Gold, 1991; Joshi and Gold, 1993). These biodegradations evidently result from the simultaneous operaiton of several extracellular and intracellular

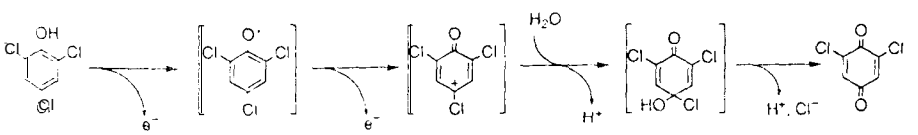

Figure 9.6. Mechanism proposed for the LiP-catalyzed oxidation of 2,4,6-trichlorophenol to 2,6-dichloro-1,4-benzoquinone. (Reproduced from Hammel, 1992, with permission of the publisher.)

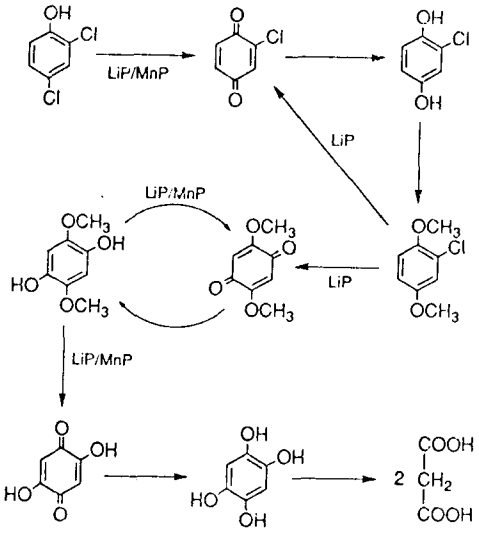

Figure 9. . . Route proposed by Valli and Gold (1991) for 2,4-dichlorophenol degradation in cultures of *P. chrysosporium.* (Reproduced from Hammel, 1992, with permission of the publisher.)

fungal processes: (1) peroxidative dechlorination of the phenols (by LiPs and MnPs) to give quinones; (2) reduction of these quinones to hydroquinones, possibly by a previously described intracellular oxidoreductase (Buswell and Eriksson, 1988); (3) methylation, in some cases, of the hydroquinones to give dimethoxybenzenes; and (4) oxidative dechlorination of these hydroquinones (by MnP and LiP) and dimethoxybenzeness (by LiP) to give hydroxyquinones (Fig. 9.7).

All of these dechlorination pathways contain futile cycles. In particular, the oxidation of chlorinated hydroquinones and methoxybenzenes by peroxidases leads predominantly to the formtion of quinones without dechlorination. These chloroquinones can then be reduced to hydroquinones, which can be attacked again by peroxidases, and so on. Oxidative dechlorination occurs as a low-efficiency side reaction during these cycles, but iterations of the process evidently result in the complete dechlorination of some polychlorinated phenols.

A quantitative measure of these pathways is had to obtain, because the crucial dehalogenation reactions occur in low yield when chlorinated hydroquinones or dimethoxybenzenes are oxidized *in vitro* with LiP or MnP, which makes it difficult to assess the importance of specific chloride-releasing reactions *in vivo.* It is noteworthy in this connection that certain polychlorinated phenols, e.g., the 2,4-dichloro-, 2,4,6-trichloro-, and pentachloro-isomers are also methylated to give polychlorinated anisoles in *P. chysosporium* liquid cultures. These anisoles are not substrates for LiP, MnP, or laccase, yet they gradually disappear when added to fungal cultures (Guo et al., 1990), suggesting that mechanisms in addition to those proposed by Gold et al. play a role in polychlorophenol degradation.

3.3. Amino-, Nitro-, and Azoaromatics

The ability of *P. chrysosporium* to mineralize aminoaromatics was demonstrated by Arjmand and Sandermann (1935). A variety of chlorinated anilines and chloroaniline-lignin conjugates was shown to be mineralized by the fungus in idiophasic culture. Later, the aniline dye crystal violet (Bumpus and Brock, 1988) and the explosive 2,4,6-trinitrotoluene (Fernando et al., 1990) were shown to be mineralized by the fungus. However, high concentrations of trinitrotoluene inhibit fungal growth, raising doubts about the utility of the fungus for bioremediation of this pollutant (Spiker et al., 1992).

Work by Gold's group indicates that MnPs and Lips are likely participants in many of these reactions (Valli et al., 1992). These investigators have recently shown that *P. chrysosporium* reduces 2,4-dinitrotoluene to 2-amino-4-nitrotoluene, which is oxidatively demethylated and deaminated by MnP to give 4-nitro-1,2-benzoquinone, 4-Nitro-1,2-benzoquinone is then reduced and methylated by the fungus to give 4-nitro-1,2-dimethoxybenzene, which in turn is oxidized by Lip, with the elimination of methanol and nitrite, to give 2-methoxy-1,4-benzoquinone. 2-Methoxy-1,4-benzoquinone is a central intermediate in the intracellular ligninolytic metabolism of white-rot fungi. It is degraded by *P. chrysosporium,* via reactions shown by Valli et al. (1992) and others (Buswell et al., 1979; Buswell and Eriksson, 1979, 1988) to give ring-fission products and CO_2.

Work in several other laboratories has implicated LiPs in the degradation of azo dyes by white-rot fungi (Cripps et al., 1990; Paszczynski and Crawford, 1991). Research shows that dyes with ring substituents that make them better LiP substrates, e.g., methoxyl groups, are more easily degraded. These findings have resulted in innovative attempts to synthesize new LiP-susceptible azo dyes that might easily be degraded in waste treatment schemes (Paszczynski et al., 1991).

3.4. Polymerized Organopollutants

The demonstration by Arjmand and Sandermann (1985, 1986) that *P. chysosporium* degrades polymerized anilines is a particularly significant finding, because peroxidases readily catalyze free radical coupling reactions between aminoaromatics, hydroxyaromatics, or mixtures of the two. That is, the peroxidative metabolism of anilines or phenols by white-rot fungi leads not only to dechlorination, deamination, and other degradative reactions, but also to covalent cross-linking of the organopollutant with polymeric constituents of soil or wood (Bollag and Siu, 1985). It is therefore important that these basidiomycetes can degrade not only the original aromatic pollutants, but also polymers of them. Subsequent work has shown that idiophasic *P. chrysosporium* is able to degrade a variety of organopollutants when they are covalently linked to humic acids (Haider and Martin. 1989). Conjugates between chloroanilines, chlorophenols, and chlorocatechols were all significantly mineralized. Humic acids themselves are also degraded by the fungus (Bollag and Siu, 1985; Blondeau, 1989; Haider and Martin, 1989; Dehorter et al., 1992).

It remains to be seen whether the degradation of these xenobiotic polymers is catalyzed by ligninolytic enzymes, and much work obviously remains to be done. However, these results argue convincingly that the biodegradative abilities of ligninolytic fungi are not just curiosities of the microbiological laboratory, but rather play a significant ecological role in the natural breakdown of organopollutants.

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