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Extracellular free radical biochemistry of ligninolytic fungi

Kenneth E. HAMMEL

USDA Forest Products Laboratory Madison, WI 53705, USA.

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Abstract. – Higher filamentous fungi are the primary degraders of lignin in nature. Research has shown that fungi employ several types of free radical chemistry to break down this abundant and recalcitrant wood polymer. Fungal ligninolytic mechanisms play an essential role in global carbon cycling, and may also serve as inspiration for new, environmentally friendly biomimetic catalysts.

Lignin is the most abundant aromatic chemical on earth, comprising roughly 15% of all terrestrial biomass. Formed through the free radical polymerization of several *p*-hydroxycinnamyl alcohols, it is a heterogeneous, highly crosslinked polymer that encases the structural polysaccharides of higher plant cell walls. The intermonomer linkages of lignin consist of alkyl aryl ether, arylpropane, biphenyl, and other bonds that resist cleavage by most biochemical agents[1,2]. These properties are consonant with the polymer's functions, which are to give vascular plants the rigidity they need to stand upright and to protect their structural polysaccharides (cellulose and hemicelluloses) from microbial degradation.

Despite the chemical recalcitrance of lignin, it and the polysaccharides it protects are degraded in nature. The overall mass balance of this process remains unknown, but it is clear that lignocellulose is biologically converted to partially degraded polymers such as humic and fulvic acids, to low molecular weight organics, and to CO_2[3]. Were this not the case, photosynthetically fixed carbon would accumulate irreversibly into lignified biomass, eventually causing the terrestrial carbon cycle to halt. Lignocellulose degradation is thus an essential ecological process.

The microorganisms believed chiefly responsible for lignocellulose degradation are higher fungi, i.e. basidiomycetes, that inhabit soil litter and dead wood. Certain lower fungi (ascomycetes) and perhaps bacteria also degrade lignified biomass[4], but little is known about the mechanisms or ecological significance of these much slower processes. Lignocellulose-degrading basidiomycetes fall into two distinct groups that employ different strategies to gain access to wood cellulose, their principal food. One group, referred to as brown-rot fungi, removes cellulose from wood selectively, leaving behind a brown, chemically modified, but still polymeric lignin. The mechanisms that brown-rot fungi use to circumvent the lignin barrier and degrade the cellulose are unknown and remain one of the greatest unsolved problems in microbial ecology [4].

Much more is known about the second group of lignocellulose degraders, the white-rot fungi. These basidiomycetes degrade the lignin in wood, giving it a bleached appearance and exposing the cellulose to enzymatic attack. Some white-rot fungi remove lignin and cellulose from wood at comparable rates, but in others lignin is degraded preferentially, leaving behind nearly intact cellulose fibers. An extreme example of selective white-rot is found in the temperate rain forests of Chile, where entire logs, referred to as *palo podrido,* are found so extensively delignified that they can be used directly as cattle fodder [4]. White-rot fungi have potential applications in two industrial delignification processes: wood pulping and pulp bleaching [5]. They may also be useful in the bioremediation of contaminated soils and wastewaters- since ligninolysis is necessarily a process of low specificity, fungal ligninolytic pathways also oxidize a wide variety of aromatic organopollutants [5,6].

Research has characterized several white-rot delignification mechanisms in some detail, and has shown that they all display one fundamental similarity: they depend on the generation of lignin free radicals which, because of their chemical instability, subsequently undergo a variety of spontaneous degradative reactions. The ligninolytic agents produced by white-rot fungi include lignin

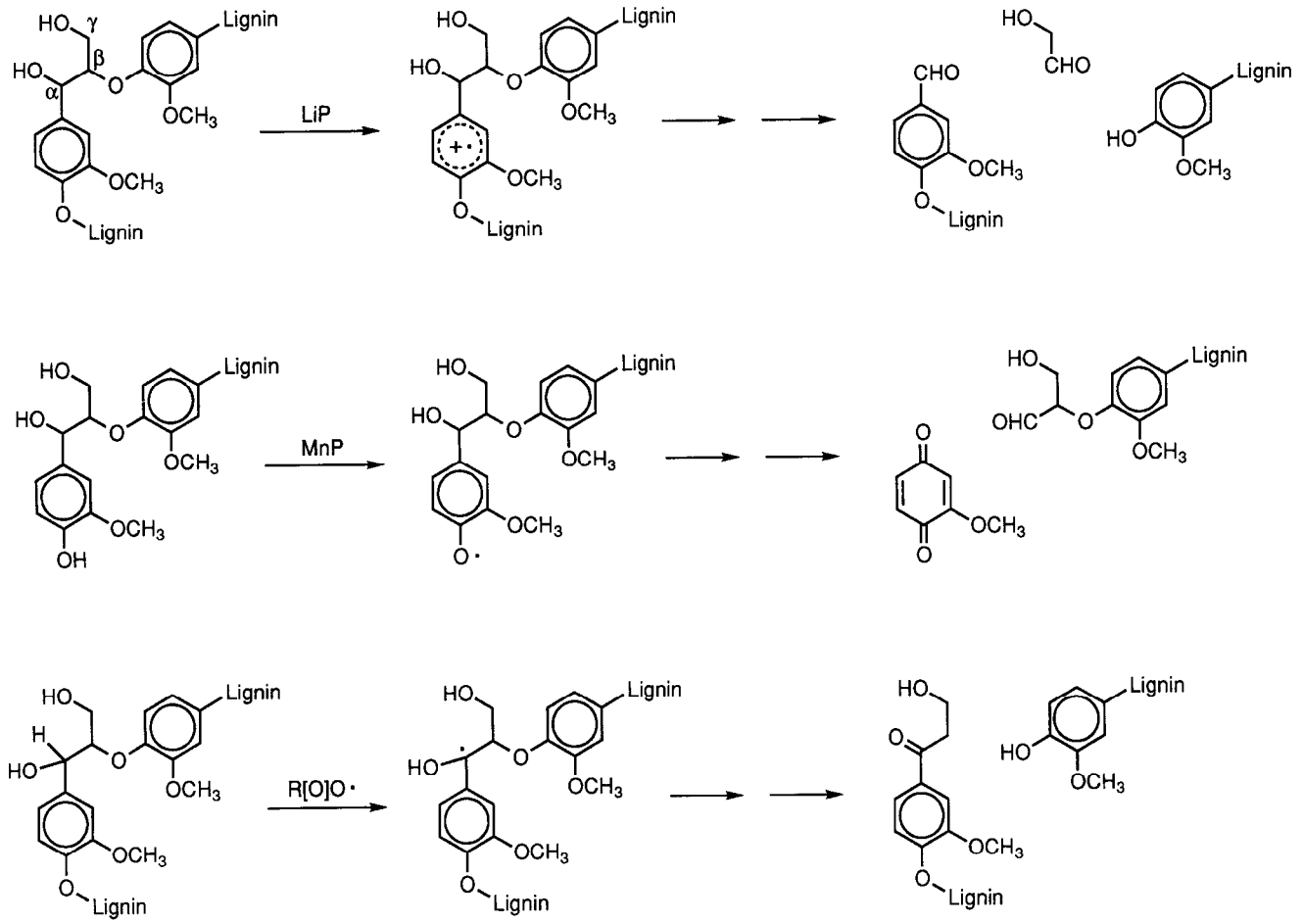

Figure 1. – Several ways that LiP, MnP, and intermediates of lipid peroxidation can cleave the major arylglycerol-*b*-aryl ether lignin structure. Many other products, not shown here, are also obtained in these oxidations [15,21,22].

peroxidases (LiPs), manganese peroxidases (MnPs), and perhaps low molecular weight oxygen-centered radicals (see Fig. 1).

LiPs occur in many but not all white-rot fungi, and were the first ligninolytic enzymes to be discovered [7,8]. They resemble other peroxidases such as the classical enzyme from horseradish, in that they contain ferric heme and operate through a typical peroxidase catalytic cycle. That is, LiP is oxidized by H_2O_2 to a two-electron deficient intermediate, which returns to its resting state by performing two one-electron oxidations of donor substrates [2,9]. However, LiPs are more powerful oxidants than typical peroxidases are, and consequently oxidize not only the usual peroxidase substrates such as phenols and anilines, but also a variety of non-phenolic lignin structures and other aromatic ethers that resemble the basic structural unit of lignin [2,10]. It is not yet clear what structural features make LiPs so oxidizing, but progress on this question is likely to be rapid now that the three-dimensional structure of the enzyme has been solved [11,12].

The LiP-catalyzed oxidation of aryl ethers begins with the abstraction of one electron from the donor substrate's aromatic ring, and the resulting species, an aryl cation radical, then undergoes a variety of postenzymatic reactions [13,14]. For example, dimeric model compounds that represent the major arylglycerol-*b*-aryl ether structure of lignin undergo C_a-C_b cleavage upon oxidation by LiP [15]. Synthetic polymeric lignins are also cleaved at this position by the enzyme in vitro, in a reaction that gives net depolymerization [16]. These results strongly support a ligninolytic role for LiP, because C_a-C_b cleavage is a major route for ligninolysis in many white-rot fungi [2]. However, it has been noted that LiPs, and other enzymes in general, are too large to penetrate the secondary plant cell wall. Some researchers therefore

believe that other, lower molecular weight agents must participate in conjunction with LiP to delignify solid wood [17].

MnPs occur in most white-rot fungi, and are similar to conventional peroxidases, except that Mn(II) is the obligatory electron donor for reduction of the one-electron deficient enzyme to its resting state, and Mn(III) is produced as a result [18,19]. This reaction requires the presence of bidentate organic acid chelators such as glycolate or oxalate, which stabilize Mn(III) and promote its release from the enzyme. The resulting Mn(III) chelates are small, diffusible oxidants that can act at a distance from the MnP active site. They are not strongly oxidizing and are consequently unable to attack the recalcitrant nonphenolic structures that predominate in lignin. However, Mn(III) chelates do oxidize the more reactive phenolic structures that make up approximately 10% of lignin. These reactions result in a limited degree of ligninolysis via C_a-aryl cleavage and other degradative reactions [16,20,21]. Mn(III) chelates, because of their small size, might be able to penetrate sound wood and initiate its delignification to facilitate later attack by the bulkier but more powerful oxidant LiP.

The production of diffusible oxyradicals by white-rot fungi may provide them with a third ligninolytic mechanism. For example, in the presence of Mn(II), MnP promotes the peroxidation of unsaturated lipids, and the operation of this system co-oxidizes a variety of aromatic molecules. The MnP/lipid peroxidation system, unlike MnP alone, oxidizes and cleaves nonphenolic lignin model compounds via benzylic hydrogen abstraction and perhaps other mechanisms. It also depolymerizes both nonphenolic and phenolic synthetic lignins [22]. The proximal oxidant in this system remains unknown, but the most likely candidates appear to be lipid peroxy or alkoxy radicals, which are known to be intermediates formed during lipid peroxidation. Several studies have provided evidence that white-rot fungi also produce extracellular hydroxyl radicals, which might play a role in ligninolysis [23,24]. However, ·OH, which is among the most reactive of chemical species, oxidizes not just lignin structures but also cellulose [25]. White rot mediated by ·OH would therefore have to be nonselective, unless the radical is generated in a highly site-specific manner within the lignin portion of the woody cell wall.

Despite recent progress, important components of fungal ligninolytic metabolism evidently remain to be discovered. Perhaps the major obstacle to further understanding is that although the enzymes isolated from white-rot fungi can degrade isolated lignins in vitro, they are not by themselves able to delignify solid wood. The search for fungal ligninolytic agents and for new ways to apply them therefore continues. There has also been much interest in the possibility that biomimetic catalysts based on fungal enzymes might provide a cheap and effective way to delignify wood products [26-28]. A better understanding of the fungal ligninolytic machinery may thus lead to a new generation of environmentally friendly oxidants for industrial application.

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