Mechanisms of Degradation by White Rot Fungi

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White rot fungi use a variety of mechanisms to accomplish the complete degradation of lignin and a wide variety of environmental pollutants. Both oxidative and reductive reactions are required for the metabolism of both lignin and environmental pollutants. The fungi secrete a family of peroxidases to catalyze both direct and indirect oxidation of chemicals. The peroxidases can also catalyze reductions using electron donors to generate reductive radicals. A cell-surface membrane potential can also be used to reduce chemicals such as TNT. — Environ Health Perspect 103(Suppl 5):59-61 (1995)

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White rot fungi, the fungi responsible for the biodegradation of lignin in wood, are remarkable in their ability to degrade a wide variety of environmental pollutants (1-3). Insoluble, generally very recalcitrant chemicals are mineralized by the fungi. The fungi also mineralized (oxidized to CO2) some chemicals that are already highly oxidized. In general it is thought that this biodegradative ability is related to the ability of these fungi to degrade lignin. This ability, which is unique to white rot fungi, is thought to be dependent on a family of peroxidases secreted by the fungi (4,5); however, a purely oxidative, peroxidase-based system cannot be used to completely degrade lignin. Lignin is highly oxidized so it is difficult to oxidize further. Lignin is a complex heteropolymer with no stereochemical regularity, due at least in part to the free radical mechanism of synthesis (6). Lignin biodegradation must therefore involve a nonspecific and nonstereoselective mechanism.

The mechanism of oxidation generally applicable to peroxidases is shown in Figure 1. The ferric form of the enzyme, usually referred to as resting enzyme, is oxidized by two electrons by hydrogen peroxide to a form of peroxidases referred to as compound I. Compound I can be reduced by one electron by chemicals having a suitable reduction potential. The enzyme is reduced to a form called compound II whereas the chemical is oxidized by one electron. This one-electron oxidized chemical, or the original chemical, can then reduce compound II back to resting enzyme. In order to be oxidized by two electrons (compound I), one electron is removed from ferric iron to form ferryl while the second electron is withdrawn from the porphyrin ring. The latter is reduced first to form compound II. The reduction potential for compound I is thus higher than for compound II.

The lignin peroxidases are somewhat unique in that they have higher oxidation potentials (~1.35 V) than do most peroxidases (~0.8 V) (7). In this way these enzymes have a somewhat greater range of chemicals that they can oxidize; however this does not explain why so many chemicals are oxidized by these fungi. There obviously must be additional oxidative mechanisms.

In a somewhat analogous way, some chemicals must first be reduced before they can be oxidized by the lignin peroxidases. It would appear that there are several mechanisms by which this can occur but most significantly, this must be the manner in which the fungi mineralize a number of highly oxidized chemicals such as TNT (2,4,6-trinitrotoluene) and DDT (1,1, trichloro-2,2-bis(4-chlorophenyl))ethane. One method is the indirect oxidation via the cation radical of veratryl alcohol. For compounds that may have sufficiently low oxidation potential but are apparently without access to the heme of lignin peroxidases, indirect oxidation occurs upon the inclusion of veratryl alcohol. This was demonstrated by our laboratory for aminotriazole (8) and for lignin by Kurek et al. (9).

The mechanisms discussed above involve oxidation; however, many of the chemicals that are oxidized by white rot fungi are already highly oxidized. For example, TNT is oxidized by white rot fungi (10) but TNT is an oxidant, not a reductant. Its metabolism by all other organisms involves reduction, primarily by nitro reductases (11). Although a search for nitro reductases produced by Phanerochaete chrysosporium was unsuccessful, P. chrysosporium mycelium was found to reduce TNT (12). Reduction seemed to occur by a membrane dependent reduction potential that the fungus apparently uses to maintain an acidic external environment.

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Figure 1. Catalytic cycle for lignin peroxidases. The iron is in heme (protoporphyrin IX).
The fact that TNT is reduced externally by a membrane potential has significance for the detoxification of TNT and for the design of TNT bioremediation systems based on these fungi. Subsequent to reduction, TNT can be oxidized by Mn-dependent peroxidases and is then mineralized when lignin peroxidases are produced (13).

The fungal membrane potential may be involved in other reductions catalyzed by the fungi. A number of redox dyes are reduced by this system, and the system can also be used for reductive dechlorination. The disappearance of DDT, for example, occurs long before any peroxidases are produced or any DDT is mineralized and before reduced metabolites (DDD and DDE) are observed (14).

A second mechanism for reduction was clarified when it was discovered that the fungi also produce chemicals that inhibit veratryl alcohol oxidation. This mechanism was actually discovered while investigating the mechanism by which EDTA inhibits not only lignin peroxidases but all peroxidases (15). The mechanism became clear when it was realized that EDTA is an excellent reductant. It is used as a reductant for photoreductions (16). We demonstrated that this reaction involves the decarboxylation of EDTA by the veratryl alcohol cation radical (15). There was also a report in the literature that the fungus produces oxalate and that it is decarboxylated by lignin peroxidase in the presence of veratryl alcohol (17). The mechanism by which veratryl alcohol and oxalate can catalyze reduction is shown in Figure 2.

Lignin peroxidases oxidize veratryl alcohol when one electron produces the veratryl alcohol cation radical. Oxalate (or EDTA) will rapidly reduce this cation radical back to veratryl alcohol, giving an apparent inhibition of veratryl alcohol oxidase activity. This is a one-electron oxidation of oxalate (or EDTA). The remaining electron is thus available to reduce other chemicals. The resulting product of oxalate (and EDTA) is carbon dioxide, thus the reaction equilibrium lies towards reduction and is irreversible. This mechanism can also catalyze reductive dechlorination, of carbon tetrachloride, for example (18).

The oxalate radical can also reduce molecular oxygen (19). Superoxide can be used as either a reductant or an oxidant, depending on the pH. Superoxide is also useful for generating another powerful oxidant, the hydroxyl radical (OH) by the Haber-Weiss reaction sequence:

\[
\begin{align*}
O_2^+ + Fe^{3+} & \rightarrow O_2 + Fe^{2+} \\
2O_2^+ + 2H^+ & \rightarrow H_2O_2 + O_2 \\
Fe^{2+} + H_2O_2 & \rightarrow \cdot OH + HO^- + Fe^{3+}
\end{align*}
\]

Since the fungus already produces hydrogen peroxide, superoxide may only be needed to reduce iron. It is possible that the hydroxyl radical is involved in the oxidation of chemicals that are not oxidized directly by lignin peroxidases but which are mineralized by the fungus.

In summary, the biodegradative abilities of white rot fungi are remarkable, both in terms of the number of different chemicals that can be oxidized, and in the type of chemicals that can be oxidized. Thus, complex mixtures can be addressed, and chemicals that are usually regarded as very recalcitrant to biodegradation can in fact be degraded. The biodegradation system of white rot fungi is complex. Its application must therefore be preceded by an understanding of the mechanisms involved.

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