

Degradation of polymeric substances by free radicals and multifunctional peroxidase produced by white rot basidiomycetes

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Degradation of lignin by white rot basidiomycetes proceeds by oxidative depolymerization either directly by fungal ligninolytic enzymes, or indirectly *via* mediator molecules oxidized by the enzymes or transition metal complexes. The extracellular oxidative systems have been extensively studied for pulp and paper production and bioremediation of polluted environments containing various hazardous organic compounds. Lignin peroxidase (LiP) and manganese peroxidase (MnP) have been isolated and characterized from numerous white rot fungi. LiP from *Phanerochaete chrysosporium* is unique in its ability to oxidize polymeric substances such as a synthetic azo dye, Poly R-478 and ribonuclease (RNase) *via* its long-range electron transfer pathway, but the oxidation of these molecules by LiP is dependent on the presence of mediator molecules such as veratryl alcohol. In *Pleurotus* species, hybrid-type peroxidases sharing the functions of LiP and MnP have been isolated. The multifunctional peroxidase (versatile peroxidase, VP) possess a manganese binding site, heme access channel and long-range electron transfer pathway. Recently we first demonstrated that the multifunctional peroxidase (VP, MnP2) from *Pleurotus ostreatus* directly oxidized the polymeric substances, Poly R-478 and RNase without the aid of redox mediators, and that a Trp residue exposed on the enzyme surface is involved in the polymer oxidation [1]. We also demonstrated that expression of VP (MnP2) was down-regulated at the transcription level by nutrient nitrogen, e.g., NH_4^+ , arginine or urea [2]. When the fungus was cultivated in chemically-defined media containing Poly R-478, the azo dye did not act as a transcriptional inducer but suppressed inactivation of VP (MnP2) from excess of H_2O_2 [2].

MnP abstracts one electron from phenolic compounds to generate a phenoxy radical, although it also oxidizes recalcitrant compounds with high ionization potential exceeding 7.5 eV in the presence of lipids. The MnP-dependent lipid peroxidation is involved in lignin degradation by a selective white rot basidiomycete, *Ceriporiopsis subvermispora*. The MnP-catalyzed lipid peroxidation is initiated by hydrogen abstraction from enolic form of fatty acids and proceeds by acyl radical chain reactions to produce aldehydes, accompanied by emission of chemiluminescence [3,4]. We found that vulcanized rubbers were degraded by the lipid peroxidation initiated by MnP and laccase [5]. In collaboration with SRI R & D Ltd., research on the recycling system of waste rubber products by the use of lipid peroxidation is in progress. We also found that *C. subvermispora*, degraded vulcanized natural rubber sheets in wood cultures, accompanied by cleavage of sulfide bonds between the polyisoprene chains [6]. To our knowledge, this is the first report of basidiomycetes capable of degrading vulcanized rubber. The enzymatic lipid peroxidation were also applied to degradation of ether-type polyurethane in collaboration with Toyota Motor Corporation. Studies on the radical reactions catalyzed by ligninolytic enzymes would allow us to decompose various polymers which are hardly recognized by a substrate binding site buried in enzymes.

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