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Mn(III)-dependent Breakdown of 13(S)-Hydroperoxy-9Z, 11E-octadecadienoic Acid: A Key Free Radical Reaction in Lipid Peroxidation of Linoleic Acid by Manganese Peroxidase

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A heme-containing Mn(II)-oxidizing enzyme, manganese-dependent peroxidase (MnP) has been implicated to be involved in lignin biodegradation by white rot fungi. In the catalytic cycle of MnP, ferric enzyme is oxidized by H₂O₂ to generate a ferryl- π -porphyrin cation radical, compound I, followed by a one-electron reduction to compound II and then back to ferric enzyme^{1,2}. In the catalysis of MnP, Mn(II) is oxidized to Mn(III), which in turn oxidizes phenolic compounds when suitable chelating agents are present. The resulting Mn(III) chelates are diffusible oxidants of low molecular weight that can act at a distance from the enzyme. They are not strong enough to be able to attack the recalcitrant nonphenolic structures that predominate in lignin. In the presence of unsaturated lipids, however, MnP promotes peroxidation of the lipids, and the operation of this system co-oxidizes a variety of aromatic molecules³⁻⁶, including nonphenolic lignin model compounds. Although it has been assumed that free radicals from unsaturated lipids are involved in this oxidation system, free radicals in the MnP/Mn(II)/lipid reactions have not been detected. In addition, it is not understood why the oxidation proceeds in the presence of strong antioxidant, Mn(II)^{7,8} and what is the primary agent that initiates lipid peroxidation. In this study, free radicals produced by the MnP/Mn(II)/lipid were analyzed by ESR to understand the unique peroxidation processes that occur in the presence of the antioxidant Mn(II).

A MnP isozyme (pl 3.40) was purified from culture filtrates of *Ceriporiopsis subvermispora* FP-90031 grown on birch wood/wheat bran/peptone cultures for 7 days at 28°C. When the MnP was reacted with 13(S)-hydroperoxy-9Z, 11E-octadecadienoic acid (13(S)-HPODE) in the presence of Mn(II), H₂O₂ and *tert*-nitrosobutane (*t*-NB), ESR spectrum composed of a sharp triplet of acyl radical was observed. Formation of acyl radical was also observed in the reactions of Mn(III)-tartrate with 13(S)-HPODE and with 9Z, 12Z-octadecadienoic acid (linoleic acid), but the latter reaction occurred explosively after an induction period of around 30 min. Reactions of MnP with linoleic acid in the presence of Mn(II), H₂O₂ and *t*-

NB gave no spin adducts while addition of *t*-NB after preincubation of linoleic acid with MnP/Mn(II)/H₂O₂ for 2 h gave spin adducts of carbon-centered and acyl radicals. In contrast to linoleic acid, methyl 9Z, 12Z-octadecadienoate (methyl linoleate) and 9Z-octadecenoic acid (oleic acid) were not peroxidized by MnP within a few hours, indicating that structures containing both the 1, 4-pentadienyl moiety and a free carboxyl group are necessary for inducing the peroxidation in a short reaction time. The same reactivity was observed also with a Mn(III)-tartrate complex. When the MnP was reacted with linoleic acid in the presence of α -4-pyridyl-1-oxide-*N*-*tert*-butylnitron (4-POBN), weak signals from O₂⁻ adduct were observed independently of the presence of the fatty acid⁹, but no other spin adduct was detected. This is in sharp contrast to lipid peroxidation by soybean lipoxygenase in which bis-allylic hydrogen is abstracted during turnover to produce a 4-POBN spin adduct of pentyl radical. These results indicate that MnP-dependent lipid peroxidation is not initiated by direct abstraction of hydrogen from the bis-allylic position during turnover but proceeds by a Mn(III)-dependent hydrogen abstraction from enols and subsequent propagation reactions involving the formation of acyl radical from lipid hydroperoxide. This expands the role of chelated Mn(III) from a phenol oxidant to a strong generator of free radicals from lipids and lipid hydroperoxides.

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