Molecular mechanism of direct oxidation of polymeric substrate by a versatile peroxidase from white-rot fungus, *Pleurotus ostreatus*

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Conversion of biomass resources to useful materials such as energy compounds or biodegradable plastics are getting received more and more interests in the light of world-wide demands to prevent global warming and to substitute fossil resources. White-rot fungi play a critical role in the carbon cycle in biosphere by degrading plant cell wall substances produced by photosynthesis. Especially, the unique ability to decompose a recalcitrant polymer, lignin, in a mild condition has advantages as a strong tool for pretreatment of wood biomass subjected to saccharification. Toward the elucidation and application of the mechanism for lignin biodegradation, we are operating intensive approaches using biochemical, enzymological and molecular genetical methodologies.

A white-rot basidiomycete,*Pleurotus ostreatus* MnP2 was earlier mentioned as a typical manganese peroxidases (MnP) isozyme, MnP2 oxidizes veratryl alcohol, a typical substrate for lignin peroxidases (LiPs), and even high molecular-weight compounds such as RNase A and a polymeric azo dye Poly R-478. From these results MnP2 is called as a versatile peroxidase (VP) and are considered to be the key enzyme in the lignin biodegradation system by the fungus. The deduced amino acid sequence from the cloned *mnp2* gene demonstrated that this isozyme contains the tryptophane residue (W170) conserved among LiPs and VPs. Chemical modification of purified MnP2 by *N*-bromsuccimide blocked the oxidizing activity for veratryl alcohol, RNase A and Poly R-478, suggesting that W170 plays an essential role in oxidizing these compounds. Using a genetic transformation system, *P. ostreatus* strains with elevated MnP2 productivity were isolated [1]. In these strains, the recombinant *mnp2* was expressed under the control of *P. ostreatus* promoter sequence from *sdi1* which encodes for Iron-sulphur protein subunit of succinate dehydrogenase, one of the components of the mitochondrial respiratory chain. The recombinant strains exhibited enhanced Poly R-478-decolorizing and benzo[*a*]pyrene-removing activities [1], demonstrating their high potential as biocatalysts in biological processes such as pretreatment of wood biomass and bioremediation of polluted soil or water. Under a certain culture condition, one of the transformant showed about 30-fold production of MnP2 compared to wild type strain.

Furthermore, through optimization of the culture conditions, an exclusive expression of the recombinant MnP2 without background expression of endogenous MnP isozymes was achieved [2]. We used this exclusive expression system for recombinant MnP2, to elucidate the unique mechanism of direct polymer oxidation by this enzyme. Four variants with site-directed mutation at W170 and its surrounding amino acid residues were successfully expressed and their catalytic activities for low and high molecular-weight substrates are being analyzed [3]. It was demonstrated that W170 is essential for oxidation of aromatic and polymeric substrates and that relatively open circumstances of its proximal microenvironment make the polymeric substrates accessible to the oxidation site, which is not possible in case of other fungal ligninolytic peroxidases including LiP from *Phanerocheate chrysosporium*.

REFERENCES

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