Exclusive overexpression and structure-function analysis of a versatile peroxidase from white-rot fungus, *Pleurotus ostreatus*

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White-rot fungi play a critical role in the carbon cycle in arborsphere by degrading plant cell wall substances produced in photosynthesis. Especially, the unique ability to decompose a recalcitrant polymer lignin in a mild condition has advantages as a strong tool for conversion of biomass resources to useful materials including energy compounds and biodegradable plastics, by which the naturally-occurring system would function more extensively in carbon cycling within humanosphere. Toward the elucidation and application of the mechanism for lignin biodegradation, we are operating manifold approaches including biochemical, enzymological and molecular genetical methodologies.

Versatile peroxidases (VPs) are a family of peroxidases secreted by several white-rot basidiomycetes belonging to the *Pleurotus* and *Bjerkandera* genera and are considered to be the key enzyme in the lignin biodegradation system by these fungi. VPs are characterized for their extraordinary wide substrate specificity and retain features of the other two fungal peroxidase families, manganese peroxidases (MnPs) and lignin peroxidases (LiPs). Therefore, a highly efficient VP-overproduction system is desired for biotechnological applications in industrial processes and bioremediation of recalcitrant pollutants, and also detailed analysis of the structure-function relationship of the enzyme.

Although *Pleurotus ostreatus* MnP2 was earlier mentioned as a typical MnP isozyme, we reported that MnP2 can oxidize veratryl alcohol [1] and even high molecular-weight compounds such as RNase A and a polymeric azo dye Poly R-478 [2]. 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 [2]. Recently, genetically modified *P. ostreatus* strains with elevated MnP2 productivity were isolated by transforming an expression construct containing the coding region of *mnp2* under the control of a homologous *sdi1* promoter sequence [3]. *sdi1* encodes for Iron-sulphur protein subunit of succinate dehydrogenase, one of the components of the mitochondrial respiratory chain and the recombinant *mnp2* gene is expected to be transcribed constitutively. The recombinant strains exhibited enhanced Poly R-478-decolorizing and benzo[*a*]pyrene-removing activities [3], demonstrating their high potential as biocatalysts in biological processes. However, the production yield of the extracellular enzymes with Mn^{2+}-dependent peroxidase activity by the recombinants was at most 230 U/l in a stationary culture using a synthetic medium. Through optimization of the culture conditions, an exclusive expression of the recombinant MnP2, upto 7300 U/l, without background expression of endogenous MnP isozymes was achieved [4]. Using this system, MnP2 variants constructed with site-directed mutagenesis 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.

REFERENCES

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