Preliminary

# Reaction of Manganese-dependent Peroxidase from *Bjerkandera adusta* in Organic Solvents

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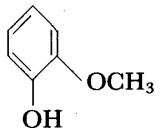
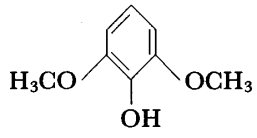
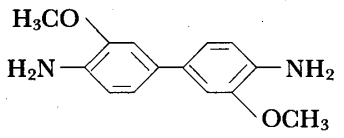
White-rot fungi have been reported to secrete lignin-degrading peroxidases, lignin peroxidase (LiP) and manganese-dependent peroxidase (manganese (II)-peroxidase, MnP). LiP catalyzes hydrogen peroxide-dependent one-electron oxidation of various non-phenolic compound to generate aryl cation radicals. These radicals undergo subsequent non-enzymatic reactions to yield a variety of final products. On the other hand, MnP oxidizes Mn (II) to Mn (III), a strong oxidant, in the presence of hydrogen peroxide. Mn (III), in tern, oxidizes phenolic substrates to phenoxy radicals which are degraded to low molecular metabolites in a similar manner to LiP.

Usually, characterization of enzymes are carried out in aqueous media. This is also a case for ligninolytic peroxidases. Reaction in organic solvents are expected to enhance the abilities of peroxidases by solubilizing hydrophobic substrates and products. We reported the reaction of LiP retains its activity in ethylene glycol, acetone and other organic solvents[1-3]. This research aims to characterize the activity of MnP in water-miscible as well as in water-immiscible organic solvents.

## 1. Isolation and purification of MnP from the culture of *Bjerkandera adusta*

*B. adusta* (K-2679) was cultivated in a glucose-peptone medium[4] stationary at 30°C for 13 days. The culture filtrate was concentrated by a rotary evaporator and dialyzed against 20 mM Na-succinate buffer (pH 4.5). The dialyzate was concentrated by ultrafiltration using Amicon PM and then applied to a Sepharose CL-6B column. Fractions which showed MnP activity were pooled and cencentrated by ultrafiltration. The concentrate was subjected to ion-exchage chromatography on a Pharmacia Mono-Q columun (10/10). The elution was carried out with Na-acetate buffer (pH 6.0) using a linear gradient of 10 mM to 1.5 M at a flow rate of 1.0 ml/min. Each of two main fractions, MnP I and MnP II,

Table 1. Activity of MnP in organic solvents

| Solvents | Relative activity (%) | | |
|---|---|---|---|
| | Guaiacol  | 2, 6-DMP  | 3, 3′-DMB  |
| Water | 100 | 100 | 100 |
| 70% Acetone | 44 | 82 | 168 |
| 70% Acetonitile | 60 | 50 | 118 |
| 70% DEGDE | 17 | 94 | 53 |
| 70% DEGME | 18 | 36 | 0 |
| 70% Diethyleneglycol | 18 | 18 | 219 |
| 70% 1, 2-Dimethoxyethane | 23 | 90 | 51 |
| 70% Dioxane | 7 | 11 | 1 |
| 70% DMF | 0 | 0 | 0 |
| 70% DMSO | 0 | 0 | 0 |
| 70% Ethanol | 9 | 0 | 0 |
| 70% Ethyleneglycol | 15 | 12 | 15 |
| 70% Methanol | 0 | 0 | 0 |
| 70% Methylcellosolve | 15 | 12 | 33 |
| 70% 1-Propanol | 16 | 5 | 36 |
| 70% 2-Propanol | 29 | 13 | 7 |
| 70% Pyridine | 0 | 0 | 0 |
| 70% THF | 1 | 0 | 0 |
| Benzene | 0 | | 0 |
| Chloroform | 0 | | 0 |
| Ethylacetate | 0 | | 0 |
| Toluene | 0 | | 0 |

Note) 2, 6-DMP, 2, 6-dimethoxyphenol; 3, 3′-Dimethoxybenzine; DEGDE, diethylene glycol diethyl ether; DEGME, diethylene glycol monomethyl ether; DMF, *N*, *N*-dimethylfomamide; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran.
Specific activity in water) Guaiacol, 404 U/mg; 2, 6-DMP, 1987 U/mg; 3. 3′-DMB, 518 U/mg.

were pooled and concentrated to 1 ml through a Centriprep-10 microconcentrator (Amicon). Specific activities and yields of activitiy were 478 and 442 U/mg protein and 27.7% and 17.9% for MnP I and MnP II, respectively. MnP I was used in the following experiments.

## 2. Reaction of MnP in organic solvents

Activities of MnP in 70% water-miscible organic solvents and water-immiscible organic solvents were measured using guaiacol, 2, 6-dimethoxyphenol and 3, 3′-dimethoxybenzidine as the substrates. As shown in Table 1, in the reaction using guaiacol as a substrate, MnP

Table 2. Effect of organic solvents on the oxidation of Mn (II) by MnP

| Solvents | Specific activty (U/mg) | | $E_T$ (30) (kcal/mol) |
|---|---|---|---|
| | 240 nm | 340 nm | |
| Water | 4,248 | 1,168 | 63.1 |
| 70% Acetone | — | 960 | 37.0 |
| 70% Acetonitile | 6,960 | 696 | 46.0 |
| 70% DEGDE | 9,304 | 1,280 | 53.8 |
| 70% Diethyleneglycol | 2,712 | 208 | 53.8 |
| 70% 1, 2-Dimethoxyethane | 9,096 | 720 | 38.2 |
| 70% Dioxane | — | 0 | 36.0 |
| 70% DMF | — | 0 | 43.8 |
| 70% DMSO | — | 0 | n. l. |
| 70% Ethanol | 0 | 0 | 51.9 |
| 70% Ethyleneglycol | 1,280 | 112 | 56.3 |
| 70% Methanol | 0 | 0 | 55.5 |
| 70% Methylcellosolve | — | 16 | 52.3 |
| 70% Pyridine | — | 0 | 40.2 |

Note) DEGDE, diethylene glycol diethyl ether; *N, N*-dimethylformamide; DMSO, dimethyl sulfoxide; n. l., not listed.

activitieis in 70% aqueous acetone and acetonitril were 44 and 60% of those observed in water, respectively. 2, 6-Dimethoxyphenol found to be oxidized also in diethylene glycol. However, reactions in dimethylformamide, dimethylsulfoxide, methanol, pyridine, tetrahydrofuran and water-immiscible solvents such as chloroform and ethyl acetate resulted in loss of activity for three substrates used in this experiments. MnP activity with 3, 3′-dimethoxybenzidine reached maximam in acetone concentration between 40–50%.

Since MnP oxidizes directly Mn (II) to Mn (III), oxidation of Mn (II) by MnP was also determined in several organic solvents by measuring the increase in absorbance at 240 and/or 340 nm. As shown in Table 2, MnP oxidized Mn (II) in water-miscible organic solvents including ethylene glycol, diethylene glycol, acetone, acetonitrile, diethylene glycol dietyl ether and 1, 2-dimethoxyethane. Interestingly, in the last three solvents, the activity was higher than that in water. The values of $E_T$ (30) of organic solvents were measured by solvatochromism methods[5] and these values are also listed in Table 2. This value has been used for evaluating conformational changes of enzymes in organic solvents[6]. The result suggests that the active center of heme in MnP is emerged to outside of MnP molecule by the conformational change of protein molecule and Mn (II) can approach easily to the heme.

Absorption spectra of MnP in various organic solvents were measured to investigate the effects of organic solvents on the structure of active site. MnP showed strong absorption at 407 nm with weak absorption maxima at 503 and 642 nm in water. Similar spectra were obtained in 70% acetone, ethylen glycol and diethylene glycole, indicating that the heme of

MnP was little affected by the addition of these solvents. Solvents causing decrease in the reaction resulted in extensive changes of the spectra. Interestingly, in 70% acetonitrile, despite the change of the spectrum, MnP activity was retained in this solvent.

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