# PRODUCTION OF DIOXINS-TRAPPING COMPOUND FROM UNSATURATED FATTY ACID DURING REACTION OF FUNGAL MANGANESE PEROXIDASE

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## Introduction

Various methods for treatment of dioxins released to the environment were proposed. It may be suitable to apply a pollutant-degradable microorganism to treatment of wide-range-contaminated soil or water containing lower concentration of dioxins.

White rot fungi (basidiomycetes) such as *Phanerochaete chrysosporium* have been known to possess a highly nonspecific battery of extracellular enzymes that allows them to degrade the plant polymer lignin [1]. The random nature of the structure of lignin requires lignin degradation to function in a nonspecific manner. It is generally thought that the major enzymes involved in lignin biodegradation by fungi are two extracellular heme-containing peroxidases: lignin peroxidase (LiP) [2] and manganese peroxidase (MnP) [3]. These enzymatic systems can degrade other compounds that have an aromatic structure, such as many xenobiotic compounds. LiP directly oxidizes polycyclic aromatic hydrocarbons [4], dibenzo-*p*-dioxin [5] and 2,7-dichlorodibenzo-*p*-dioxin [6]. MnP is unique enzyme that oxidizes Mn(II) to Mn(III), which attack to phenolic lignin and organopollutants. The MnP system can not degrade nonphenolic compounds. However, there are MnP-producing white rot fungi that evidently lack LiP, which nevertheless degrade non-phenolic lignin structures efficiently. Recently, it is reported that the MnP system is capable to mineralize nonphenolic compounds in the presence of an unsaturated lipid [7,8].

In previous study, we reported that the MnP system in the presence of unsaturated fatty acid could degrade dibenzo-*p*-dioxin [9]. When we tried the degradation of 2,7-dichlorodibenzo-*p*-dioxin by this system successively, we found a phenomenon that almost amount of added 2,7-dichlorodibenzo-*p*-dioxin is trapped in water layer during MnP reaction in the presence of higher unsaturated fatty acid. In this study, we report the phenomenon in detail.

## Methods and Materials

*Phanerochaete sordida* YK-624 (ATCC 90872) were used for MnP preparation. MnP was purified as described [10, 11] and used to below experiments.

The volume of reaction mixture was 5 ml. Reactions contained 25 U MnP, 50 mM sodium malonate buffer (pH 4.5), 0.1 mM Mn(II) (as $MnSO_4$), 2 mM unsaturated fatty acid, 0.5 mM glucose and 1 U glucose oxidase. 50 μl (final concentration; 50 μM) of 5 mM 2,

7-dichlorodibenzo-*p*-dioxin (DCDD) dissolved in dimethylformamide (DMF) was added to the reaction mixture. Each tube (30 mm X 200 mm) was sealed with a glass stopper and sealing tape. The tubes were shaken at 37°C and 150 rpm for 24 h.
After incubation, 50 µl of 4 M $H_2SO_4$ or 50 µl of 10 M NaOH and 500 µl of biphenyl in DMF solution (internal standard, 2 mM) were added to the reaction mixture. Mixtures were extracted with n-hexane (two 10-ml portions). The organic phases were evaporated under reduced pressure. The extracts were analyzed by GC-MS (TurboMass GC Mass Spectrometer, Perkin Elmer).
Reaction with lipoxidase (EC 1.13.11.12, from soybean, SERVA) contained 50 µM DCDD, 6000 U lipoxidase, 2 mM unsaturated fatty acid in 50 mM sodium malonate buffer (pH 4.5) and was done at 30°C for 24 h.

**Results and Discussion**
MnP reactions were done in the presence of 4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA, 2 mM). Although Reaction with addition of inactive MnP by heating did not show decrease in DCDD, complete MnP system decreased DCDD by 95% (Table 1). To ensure the decrease in DCDD by MnP system, Several extract methods were carried out. When the MnP reaction mixture was extracted with n-hexane after alkalification of the mixture to pH 10 with NaOH, 70% of DCDD was yielded. It shows that a DCDD-trapping compound, which is opened by alkalification, is produced during MnP reaction in the presence of DHA This phenomenon, DCDD-trapping in water layer, was not happened in the case that DCDD was added to the mixture after MnP reaction in the absence of DCDD.

Treatments of DCDD by the MnP system in the presence of seven unsaturated fatty acids (2 mM) were tested (Table 2). After MnP reactions for 24h in the presence of oleic acid, linoleic acid and linolenic acid, all amount of DCDD was yielded to n-hexane from water layer. On the other hand, after MnP reactions for 24 h in the presence of arachidonic acid, 5,8,11,14,17-icosapentaenoic acid (EPA) and DHA, DCDD was trapped in water layer. The addition of fatty acids containing higher unsaturated linkage (from four to six) increased in the extent of DCDD-trapping.

It has been reported that MnP supports the Mn-dependent peroxidation of unsaturated fatty acids [7,8]. Reaction with lipoxidase, which does peroxidation of unsaturated fatty acid, was carried out in the presence of DCDD and DHA in the 50 mM malonate buffer (pH 4.5) (Table 3). It occurred to trap DCDD by lipoxidase reaction as same as MnP reaction. It is seemed that DCDD-trapping compound may be produced from unsaturated fatty acid via peroxidation.

To ensure effect of carboxylic acid, MnP reaction in the presence of ethyl ester of DHA was done (table 4). After the reaction for 24 h, DCDD was not yielded by extraction with n-hexane after alkalification as same as by extraction after acidification. This result suggests that calboxylic functional group affect to the formation of DCDD-trapping compound.

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Table 1 Treatment of DCDD by MnP system in the presence of DHA.

| Reaction conditions | pH adjusted [a] | Yield of DCDD (%) |
|---|---|---|
| Complete | 2 | 3.86 |
| With boiled MnP | 2 | 100 |
| Minus MnP | 2 | 100 |
| Complete | 10 | 72.6 |

[a] pHs of reaction mixtures were adjusted to 2 or 10 before extraction with *n*-hexane.

Table 2 Treatment of DCDD by MnP system in the presence of various fatty acids.

| Fatty acids (C No.: C=C No.) [a] | $Yield^{acidic}$ / $Yield^{alkali}$ [b] |
|---|---|
| Oleic acid (18:1) | 1.09 |
| Linoleic acid (18:2) | 0.953 |
| Linolenic acid (18:3) | 1.11 |
| Arachidonic acid (20:4) | 0.389 |
| EPA (20:5) | 0.398 |
| DHA (22:6) | 0.0532 |

[a] EPA, 5,8,11,14,17-icosapentaenoic acid;
DHA, all *cis*-4,7,10,13,16,19-docosahexaenoic acid.
[b] Ratio of yields of DCDD by extraction with n-hexane after acidification and alkalification.

Table 3 Treatment of DCDD by MnP and lipoxidase [a].

| Reaction conditions | | Yield of DCDD (%) |
|---|---|---|
| MnP complete | | 100 |
| Minus MnP | | 49.0 |
| Lipoxidase | 0 U | 100 |
| | 3000 U | 59.3 |
| | 6000 U | 39.4 |

[a] Reactions were done at 30°C for 24 h.

Table 4 Treatment of DCDD by MnP system in the presence of DHA methyl ester.

| Reaction conditions | pH adjusted[a] | Yield of DCDD (%) |
|---|---|---|
| Control (minus MnP) | 2 | 100 |
| Complete | 2 | 41.3 |
| | 10 | 25.4 |

[a] pHs of reaction mixtures were adjusted to 2 or 10 before extraction with *n*-hexane.