

## NOTE

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## Polyethylene degradation by manganese peroxidase in the absence of hydrogen peroxide

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**Abstract** A possible role of Tween 80 in the polyethylene degradation by manganese peroxidase (MnP) and the basis of the MnP action in the absence of hydrogen peroxide were investigated. The MnP activity in the system was retained its maximum level for 6 days in the presence of Tween 80. Tween 20 and CHAPSO stabilized MnP in the system similarly to Tween 80, and these surfactants also promote the polyethylene degradation. The system containing malonate buffer, Mn(II), and MnP produced Mn(III) in the absence of hydrogen peroxide, but the effect of Tween 80 addition on Mn(III) production in the absence of hydrogen peroxide was small. The results show that Mn(III) is generated by the MnP action initiated and amplified by the decomposition of malonate by Mn(III) and that a surfactant such as Tween 80 is required to stabilize MnP in the system.

**Key words** Polyethylene · Biodegradation · Manganese peroxidase · Surfactant

### Introduction

In a previous paper we reported that polyethylene membranes were degraded by the manganese peroxidase (MnP) system containing malonate buffer,  $\text{MnSO}_4$  as Mn(II), and Tween 80.<sup>1</sup> We also indicated that the Mn(III)-chelate generated by the action of MnP was the basis of the oxidative degradation of polyethylene. The system required Tween

80 but not exogenous hydrogen peroxide, which is essential for the initiation of MnP action. Hammel's group suggested MnP action in the absence of hydrogen peroxide.<sup>2,3</sup> In these studies, involvement of Tween 80 and unsaturated fatty acid in the degradation of nonphenolic lignin and phenanthrene was pointed out, but the detailed mechanisms for the initiation of the MnP action and for the degradation are still unclear. More recently, generation of Mn(III) by MnP action in the system containing malonic acid, MnP, and Mn(II) but not hydrogen peroxide was studied, and the oxidative decomposition of malonic acid and subsequent formation of radical species were found to be the basis of the MnP action.<sup>4</sup> This system was similar to our polyethylene degradation system except that Tween 80 was not employed. Therefore, the question arises whether Tween 80 is involved in the generation of Mn(III) by MnP and the polyethylene degradation in the system.

### Materials and methods

#### Enzyme preparation and enzyme assay

The preparation of partially purified MnP and the assay of its activity were performed by previously described methods.<sup>1</sup> The partially purified MnP fraction did not contain lignin peroxidase or laccase activity.

#### Treatment of polyethylene in the MnP system and evaluation of polyethylene degradation

Polyethylene membranes were treated in the MnP system at 30°C according to our previous paper.<sup>1</sup> The system contained 50mM malonate buffer (pH 4.5), 0.2mM  $\text{MnSO}_4$ , 0.1% Tween 80 (Pierce Chemical Co.), and partially purified MnP (30U/ml)<sup>1</sup> unless otherwise noted. In this study Tween 20 (Pierce Chemical) or CHAPSO (Wako Chemical Co.) was used instead of Tween 80 in the system. The polyethylene degradation was followed by measurement of the reductions in relative elongation and relative tensile strength according to the previous paper.<sup>1</sup>

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## Determination of the oxidation of 2,6-dimethoxyphenol and production of Mn(III) in the system

The effect of the surfactant on the oxidation of 2,6-dimethoxyphenol in the hydrogen peroxide-free reaction mixture was investigated. The MnP system was incubated at 30°C with polyethylene membranes. An aliquot (2750 µl) of the mixture from the system was added to 250 µl of 19.5 mM 2,6-dimethoxyphenol dissolved in 50 mM malonate buffer (pH 4.5). Oxidation of 2,6-dimethoxyphenol was carried out without adding hydrogen peroxide at 37°C for 2 min, and the oxidation of 2,6-dimethoxyphenol was measured at 470 nm.

Production of Mn(III) was determined by measuring the absorbance of the Mn(III) complex in the system. The system was incubated at 30°C without polyethylene membranes. The Mn(III)-malonate complex was determined at its maximal absorption (270 nm).<sup>5</sup> Various concentrations of Mn(III) were added to the system without Tween 80 to investigate the effect of Mn(III) on the production of Mn(III). The effects of oxalate (50 mM) and pyrophosphate (50 mM) as buffering agents on the production of Mn(III) were also investigated with and without Tween 80. In these cases the Mn(III)-oxalate complex and the Mn(III)-pyrophosphate complex were determined at 260 nm.<sup>6</sup>

## Results and discussion

Effects of surfactants on the degradation of polyethylene and MnP activity

Tween 80 is a surfactant composed of oleate (unsaturated fatty acid) and has often been used in biobleaching of kraft

pulps with MnP.<sup>7-10</sup> Hammel's group<sup>2,3</sup> applied this surfactant to the MnP-mediated degradation of nonphenolic aromatic compounds and nonphenolic lignin model compounds; they proposed that Mn(III)-dependent and hydrogen peroxide-independent lipid peroxidation may play an important role in the degradation. Therefore, we examined the effects of three surfactants – Tween 80, Tween 20, CHAPSO – on the degradation of polyethylenes by MnP in the absence of hydrogen peroxide (Fig. 1).

Tween 20 accelerated the polyethylene degradation similarly to Tween 80, as determined by reductions in elongation and tensile strength. CHAPSO also facilitated the polyethylene degradation, whereas its effect on the degradation of polyethylene was less than that of the other two (Fig. 1). Tween 20 is composed of laurate (saturated fatty acid), and CHAPSO does not contain fatty acid. Thus, it is not likely that the radicals generated by the lipid peroxidation in the system can oxidatively degrade polyethylene.

In our previous study,<sup>1</sup> no degradation was observed in the system containing acetate buffer instead of malonate buffer, and we concluded that the Mn(III)-chelate was closely related to the polyethylene degradation. Therefore, the oxidation of 2,6-dimethoxyphenol by hydrogen peroxide-free reaction mixture in the presence of Tween 80, Tween 20, or CHAPSO was determined (Fig. 2). In the case of the mixture containing no surfactant, oxidation of 2,6-dimethoxyphenol was observed at the first day of incubation but was not seen after the second day. The oxidation of 2,6-dimethoxyphenol by the mixture containing Tween 80 or Tween 20 was higher and retained its high level until the sixth day of incubation. Oxidation by the mixture containing CHAPSO gradually decreased, but more than 60% of its maximal oxidation remained until the sixth day of incu-

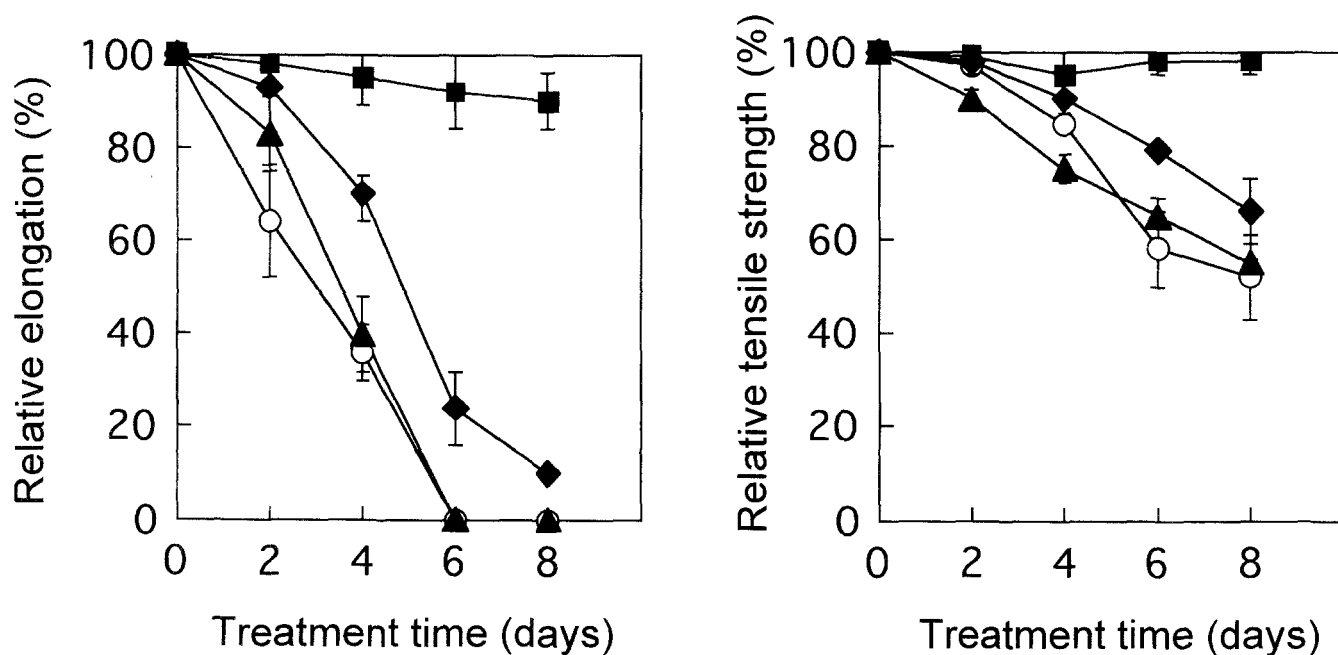
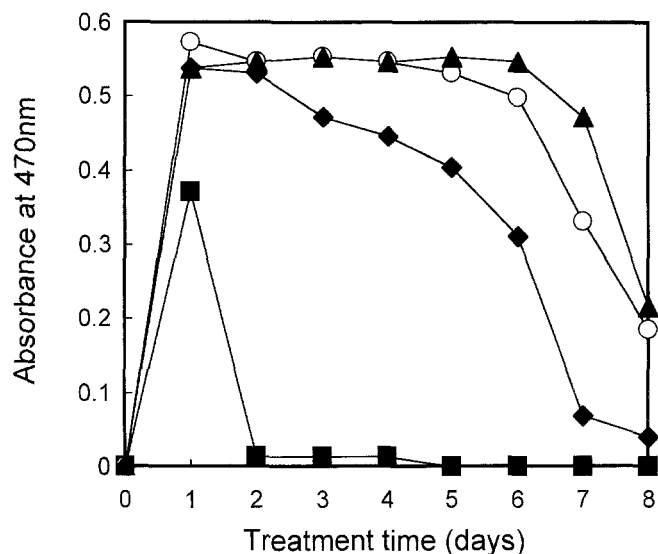


Fig. 1. Effect of surfactant on the degradation of polyethylene in the manganese peroxidase (MnP) system. Filled squares, without surfactant; open circles, with Tween 80; filled triangles, with Tween 20; filled diamonds, with CHAPSO

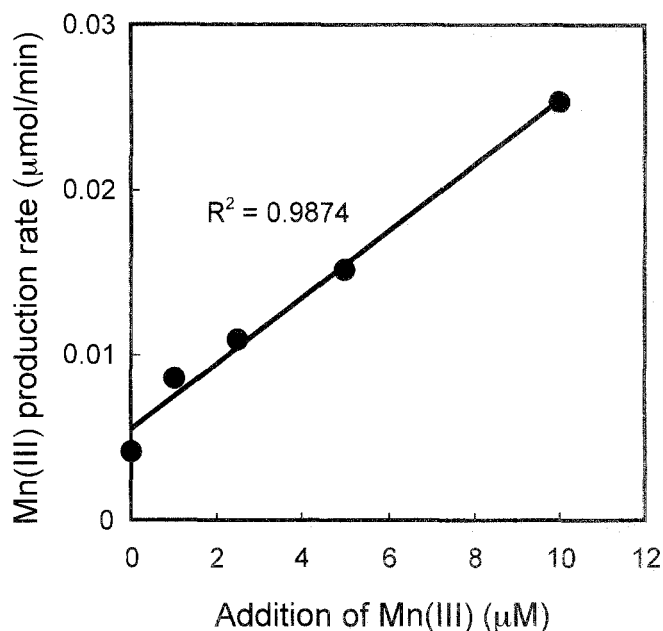


**Fig. 2.** Effect of surfactant on the oxidation of 2,6-dimethoxyphenol by the hydrogen peroxide-free reaction mixture from the MnP system. See Fig. 1 for explanation of symbols

bation. Venkatadri et al. reported that Tween 80 protects lignin peroxidase against mechanical inactivation due to agitation.<sup>11</sup> Thus, we assume that the surfactants used here stabilized MnP in the system, and this effect is mostly independent of the constituents of the surfactants. Judging from the results in Figs. 1 and 2, the extensive polyethylene degradation in the presence of surfactants may be attributed to the ability of surfactants to stabilize MnP.

#### Action of MnP in the absence of hydrogen peroxide

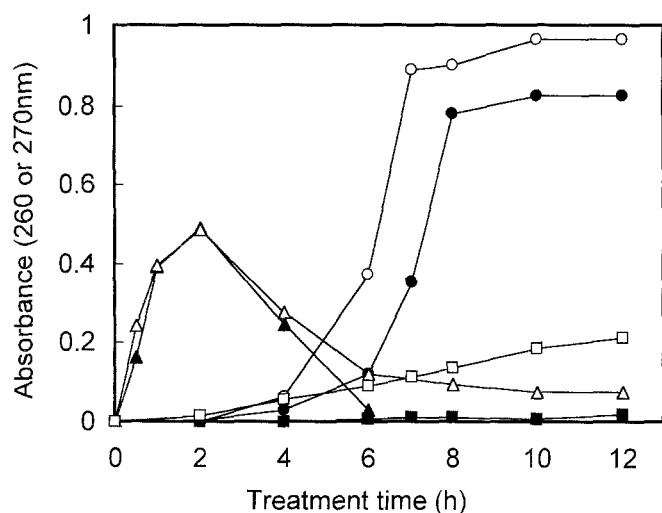
More recently, it was reported that MnP oxidatively decomposed malonate in the absence of hydrogen peroxide in a reaction system.<sup>4</sup> The explained mechanisms involved the oxidative decomposition of malonate mediated by Mn(III); the peroxide formed by the decomposition of malonate can be used for the MnP catalytic cycle, and the superoxide anion radical is converted to hydrogen peroxide by the dismutation with Mn(II). They also postulated that a trace amount of Mn(III) formed by autoxidation of Mn(II) initiates the reaction cascade and can be amplified by the action of MnP. This explanation fits our polyethylene degradation system because the MnP catalyzes the oxidation of Mn(II) to Mn(III) in the absence of hydrogen peroxide. Previously, we reported that incubation of our system under nitrogen atmosphere did not afford production of Mn(III), but incubation under an oxygen atmosphere prompted extensive production.<sup>12</sup> The other experiment in which Mn(II) was preincubated in the malonate buffer for 7 h and then MnP was added to the solution showed that preincubation of Mn(II) in malonate buffer clearly shortens the lag period of Mn(III) production for about 4 h.<sup>13</sup> These results indicate that manganese oxides initiate the MnP action. When Mn(III) was added in the system, the rate of the production of Mn(III) in the system was plotted against the initially



**Fig. 3.** Effect of the initial concentration of Mn(III) on the production of Mn(III) in the MnP system containing no Tween 80

added Mn(III) concentration (Fig. 3). Here, the rate of the Mn(III) production was computed from the data of produced Mn(III) and reaction time at the point of the half-time of the Mn(III) production to reach its maximum. The obvious linear line indicates that Mn(III) production mediated by MnP was dependent on the initial Mn(III) concentration. Although several oxidation states of manganese are known, it is established that manganese oxides with an oxidation state above three do not form readily in the laboratory except under extreme experimental conditions.<sup>14</sup> Our previous and present results strongly support that the Mn(III) formed by autoxidation of Mn(II) initiates the MnP action.

We should point out that surfactant was required for polyethylene degradation in our system. The question that arises here is whether the surfactant as well as malonate initiates the MnP action. Mn(III) production in the system with and without Tween 80 was determined (Fig. 4). In the system containing malonate buffer and no Tween 80, Mn(III) production appeared after a lag of 6 h of incubation and reached its maximum at 8 h. The addition of Tween 80 to the system shortened this lag period about 2 h. This finding indicates that the lipid peroxidation derived from the oxidation of Tween 80 can be used for initiating the MnP, but the Mn(III) production originating from the oxidation of Tween 80 was smaller than that from the malonate. In a further experiment, production of Mn(III) in the system containing pyrophosphate buffer or oxalate buffer instead of malonate buffer was investigated (Fig. 4) because pyrophosphate is not being decomposed by Mn(III) and can form a chelating complex with Mn(III), and oxalate initiates the MnP action with its decomposition process.<sup>15</sup> In the system containing pyrophosphate buffer, no Mn(III) production was observed. The addition of Tween 80 to the



**Fig. 4.** Effect of oxalate or pyrophosphate on the production of Mn(III) in the MnP system with or without Tween 80. Filled circles, malonate; open circles, malonate plus Tween 80; filled triangles, oxalate; open triangles, oxalate plus Tween 80; filled squares, pyrophosphate; open squares, pyrophosphate plus Tween 80

pyrophosphate buffer confirmed that Mn(III) production via oxidation of Tween 80 was slow. When oxalate buffer was used in the system, markedly quick production of Mn(III), within 30 min, was observed; and the production disappeared after 6 h of incubation (Fig. 4). The oxidative decomposition of oxalate can generate hydrogen peroxide and Mn(III) through dismutation of the superoxide anion radical by Mn(II).<sup>15</sup> The results in Fig. 4 indicate that oxalate is decomposed by Mn(III) faster than malonate on the basis for the initiation and amplification of the MnP action in the absence of hydrogen peroxide.

The polyethylene degradation in the MnP system containing malonate buffer, Mn(II), Tween 80, and no hydrogen peroxide can be summarized as follows. Mn(III) formed by autoxidation of Mn(II) followed by decomposition of malonate initiates and amplifies MnP. This hypothesis does not conflict with the previous observations by Hofrichter et al.<sup>4</sup> and is strongly supported by the experiment that used pyrophosphate buffer in the system. In this system Tween 80 is also oxidized by Mn(III), but participation of its lipid peroxidation in amplifying MnP action is slight. The active agent responsible for polyethylene degradation is Mn(III) produced by the MnP action, but the radicals derived from the oxidation of Tween 80 do not participate in the polyethylene degradation. The surfactants such as Tween 80 may retain the MnP activity during the degradation reaction.

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