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NOTE

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Change in oxidation state of manganese atoms in kraft pulp during biological bleaching with white-rot fungus *Phanerochaete sordida* YK-624

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Abstract Change in the oxidation state of manganese atoms in unbleached hardwood kraft pulp (UKP) during the biological bleaching of UKP with *Phanerochaete sordida* YK-624 was determined by the electron spin resonance (ESR) method. The spectrum of Mn(II), which reveals hyperfine splitting, was not observed in the ESR analysis of UKP, but the spectrum for manganese dioxide was observed. After fungal treatment of UKP with *P. sordida* YK-624, the spectrum of Mn(II) was detected. The reduction of manganese dioxide was triggered by the increase in NADPH-dependent ferrireductase activity. It is concluded that the manganese dioxide dominant in UKP was reduced by *P. sordida* YK-624 to Mn(II), which stimulates the production and function of manganese peroxidase.

Key words Electron spin resonance · Biological bleaching · *Phanerochaete sordida* YK-624 · Manganese · NADPH-dependent ferrireductase

Introduction

In response to environmental concerns and increasingly stringent emissions standards, the pulp and paper industry is looking for ways to decrease the level of chlorinated lignin residues in its effluents through both production process changes and improved treatment technologies. Murata et al.[1] and Tsuchikawa et al.[2] reported a chlorine-free biobleaching process of kraft pulp with white-rot fungi. During the biological bleaching of kraft pulp with white-rot fungi, manganese peroxidase (MnP) plays an important role,[3–6] and the bleaching of UKP was conducted successfully with MnP secreted by *P. sordida* YK-624[7,8] and IZU-154[9].

Kondo et al. reported that the addition of Mn(II) ions was necessary to bleach UKP with MnP,[7] and Harazono et al. found that the bleaching of UKP with MnP was successful without the addition of Mn(II), using oxalate as an effective Mn(III)-chelating agent and manganese dioxide-reductive agent.[8] These results suggest that most of the manganese in UKP is manganese dioxide, and that the manganese dioxide is reduced during the biological bleaching of UKP with white-rot fungi.

Herein, the oxidation state of manganese atoms present in UKP was analyzed with an electron spin resonance (ESR) method, and the change in the oxidation state during the biological bleaching of UKP with *P. sordida* YK-624 was determined.

Materials and methods

Fungus strain *P. sordida* YK-624 (ATCC 90872) was used in this study. Fungal treatment of UKP was performed by the method described in our previous report.[4] ESR samples were vacuum-dried with P_2O_5. ESR spectra were measured using a JEOL JES-FE1XG spectrometer operating at 9.5 GHz with 100 kHz field modulation, a microwave power of 5 mW, and a modulation amplitude of 20 G.

Manganese dioxide dispersed in cellulose powder was synthesized by the oxidation of Mn(II) with hydrogen peroxide and ammonia in a suspension of cellulose. The reaction mixture (10 ml) consisted of $MnSO_4$ (26 μmol), hydrogen peroxide (260 μmol), cellulose powder (1 g), and ammonia (260 μmol). The reaction mixture was stirred for 10 min at room temperature and then was freeze-dried. Acid-treated UKP (A-UKP) was produced by soaking UKP in 0.05 N HCl aqueous for 12 h, and washing it with distilled water to remove the metal ions in the UKP.

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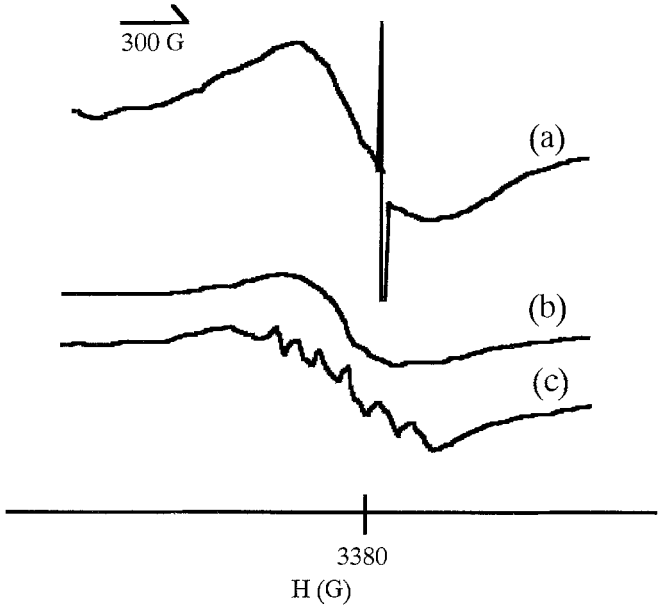

Fig. 1. Electron spin resonance (ESR) spectra of unbleached hardwood kraft pulp (UKP) (*a*), cellulose powder containing manganese dioxide 26 μmol/g (*b*), and cellulose powder containing 1 μmol Mn(II)/g (*c*)

Crude enzyme solution was prepared from fungus-treated UKP by the method of our previous report.[4] Extraction buffer was 20 mM sodium phosphate (pH 7.0) containing 0.05% Tween 80 and 0.004% phenylmethylsulfonyl fluoride. MnP activity was measured by the oxidation of 2,6-dimethoxyphenol at 470 nm,[4] and intracellular NADPH-dependent ferrireductase activity was determined by the formation of the Fe(II)–1,10-phenanthroline complex at 510 nm.[10]

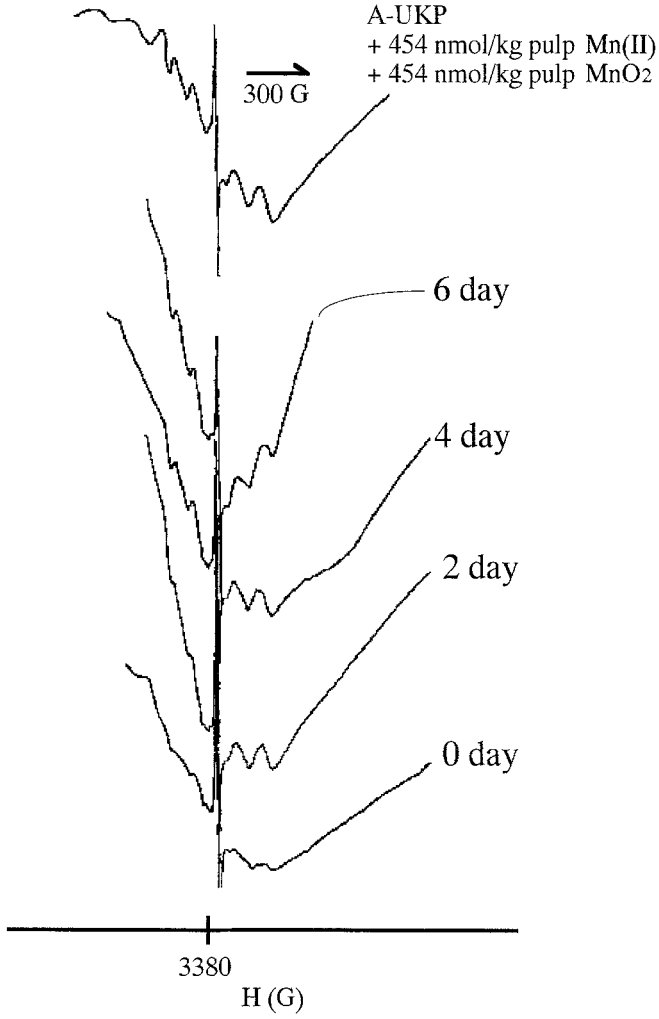

Fig. 2. ESR spectra of fungus-treated UKP. Acid-treated UKP (A-UKP) was made by soaking UKP in 0.05% HCl aqueous to remove metal ions in UKP

Results and discussion

ESR spectrum of UKP

The ESR spectrum of UKP showed a broad signal, as shown in Fig. 1. Similarly, a broad signal was observed in the ESR spectrum of the cellulose powder containing manganese dioxide 26 μmol/g, although hyperfine splitting was observed only in the cellulose powder containing Mn(II) 1 μmol/g. These results suggest that the manganese in UKP may be manganese dioxide. Then, it is thought that Mn(II) in wood chips could be oxidized to manganese dioxide during kraft cooking (under alkaline conditions).

ESR spectra of fungus-treated UKP

As shown in Fig. 1, signals of Mn(II) were not observed in UKP. However, Mn(II) is necessary for production and function of MnP from *P. chrysosporium*[11,12] and *P. sordida* YK-624.[5,13] Hence reduction of manganese dioxide to Mn(II) in UKP during biological bleaching with *P. sordida* YK-624 should be necessary. In this context, we analyzed the ESR spectra of UKP treated with *P. sordida* YK-624 (Fig. 2). Hyperfine splitting that originated from Mn(II) was observed in the ESR spectra of UKP treated with *P. sordida* YK-624 for 2, 4, and 6 days. By comparison with acid-treated UKP containing both 454 nmol Mn(II) and 454 nmol manganese dioxide, it was estimated that much or the same amount of Mn(II) was probably present in fungus-treated UKP (2, 4, and 6 days).

Enzyme activities during biological bleaching of UKP with *P. sordida* YK-624

Figure 3 shows enzyme activities during biological bleaching of UKP with *P. sordida* YK-624. The activity of intracellular NADPH-dependent ferrireductase reached a maximum on the first day of incubation and then decreased. After 3 days of incubation, intracellular NADPH-dependent ferrireductase increased again and showed a second maximum on day 4. Because Mn(II) was detected in

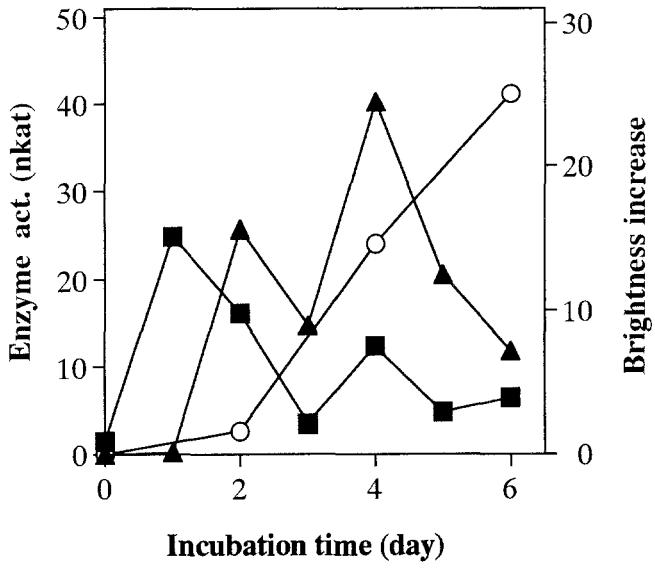

Fig. 3. Enzyme activities during biological bleaching of UKP with *P. sordida* YK-624. *Squares*, NADPH-dependent ferrireductase; *triangles*, MnP; *circles*, brightness increase

fungus-treated UKP after 2 days of incubation, as shown in Fig. 2, it is reasonable that MnP activity was detected after 2 days in Fig. 3, and the maximum activity of MnP was observed on day 4. These results suggest that the production (or presence) of NADPH-dependent ferrireductase activity on the first day triggered the reduction of manganese dioxide to the Mn(II) state. The produced Mn(II) ions triggered the production of MnP on the second day. We reported that NADH and NADPH-dependent ferrireductase[14] were present in the cells of *P. sordida* YK-624, which reduces manganese dioxide with NAD(P)H.[10] It was also observed that various wood-rot fungi showed NAD(P)H-dependent ferrireductase activity, and that there is a linear correlation between extracellular MnP activity and intracellular NADPH-dependent ferrireductase in white-rot fungi.[15] In the present study, the second peak of maximum activity of NADPH-dependent ferrireductase was observed on day 4, and the maximum activity of MnP was detected on day 4. These results suggest that the production of MnP corresponds to the production of NADPH-dependent ferrireductase.

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