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Journal of Biotechnology 77 (2000) 235–245

JOURNAL OF Biotechnology

www.elsevier.com/locate/jbiotec

Different fungal manganese-oxidizing peroxidases: a comparison between *Bjerkandera* sp. and *Phanerochaete chrysosporium*

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Received 17 June 1999; received in revised form 11 October 1999; accepted 20 October 1999

Abstract

Two manganese-oxidizing peroxidases differing in glycosylation degree were purified from fermenter cultures of *Bjerkandera* sp. They were characterized and compared with the three manganese-oxidizing peroxidase isoenzymes obtained from the well-known ligninolytic fungus *Phanerochaete chrysosporium*. All the enzymes showed similar molecular masses but those from *P. chrysosporium* had less acidic isoelectric point. Moreover, the latter strictly required Mn^{2+} to oxidize phenolic substrates whereas the *Bjerkandera* peroxidases had both Mn-mediated and Mn-independent activity on phenolic and non-phenolic aromatic substrates. Taking into account these results, and those reported for *Bjerkandera adusta* and different *Pleurotus* species, we concluded that two different types of Mn^{2+}-oxidizing peroxidases are secreted by ligninolytic fungi.

Keywords: Mn-oxidizing peroxidases; Catalytic properties; *Bjerkandera* sp.; *Phanerochaete chrysosporium*

1. Introduction

In order to depolymerize lignin some fungi have developed an unspecific system including the ligninolytic enzymes laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP), together with oxidases that provide H_2O_2 for peroxidase activity (Shimada and Higuchi, 1991). This enzymatic system is also able to degrade aromatic pollutants causing environmental problems (Barr and Aust, 1994). The exact role of the different ligninolytic enzymes in these degradation processes remains uncertain, mainly due to the possibility to catalyze similar reactions under specific conditions.

Phanerochaete chrysosporium, a model organism for lignin and xenobiotic biodegradation studies, produces a family of LiP and MnP isoenzymes (Holzbaur et al., 1991), glyoxal-oxidase (Kersten et al., 1995), and multiple laccase isoforms (Dittmer et al., 1997). *Bjerkandera* species are able to bleach Kraft pulp (Moreira et al., 1997a) and degrade aromatic hydrocarbons (Field

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0168-1656/00/$ - see front matter
PII: S0168-1656(99)00218-7

et al., 1992), synthetic dyes (Heinfling et al., 1997) and different wood components (Gutiérrez et al., 1999). The enzymatic system involved in these reactions includes multiple LiP isoenzymes (Kimura et al., 1991; Heinfling et al., 1998; ten Have et al., 1998), a manganese-independent peroxidase (de Jong et al., 1992), an aryl-alcohol oxidase (AAO; Muheim et al., 1990), and several Mn^{2+}-oxidizing peroxidases, which also present Mn-independent activity (Heinfling et al., 1998; Mester and Field, 1998) no laccase has been detected.

MnP was first described in *P. chrysosporium* (Kuwahara et al., 1984). This peroxidase was considered less important than LiP in lignin degradation due to its incapacity to directly oxidize non-phenolic compounds. However, it has been shown that MnP can depolymerize synthetic lignin (Wariishi et al., 1991) and degrade recalcitrant aromatic compounds via lipid peroxidation (Bao et al., 1994). Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. and *P. chrysosporium* are being investigated to bleach paper pulp (Moreira et al., 1997a). An advantage of the *Bjerkandera* peroxidases for this type of treatments could be related to their higher tolerance to hydrogen peroxide (Palma, 1998). This could be due to comparatively high extracellular H_2O_2 levels from aromatic aldehyde redox cycling (Guillén et al., 1994) involving Bjerkandera AAO (Muheim et al., 1990). The aim of the present paper is to compare the Mn^{2+}-oxidizing peroxidase produced by *Bjerkandera* sp. (strain BOS55) with *P. chrysosporium* MnP isoenzymes in order to verify the differences in catalytic properties.

2. Materials and methods

2.1. Fungal strains and culture conditions

P. chrysosporium VKM F-1767 (= ATCC 24725) was grown on 2% malt agar at 37°C. Conidia were harvested, filtered through glass-wool, and kept at −20°C. The fungus was cultivated in N-limited BIII medium (Tien and Kirk, 1988), with 10 g l^{-1} glucose, 0.36 mM 3,4 dimethoxy-benzyl (veratryl) alcohol, 235 μM Mn^{2+}, 0.5% Tween 80, and 20 mM sodium acetate buffer (pH 4.5). Stationary cultures were inoculated with conidia, incubated for 2 days, homogenized and used to inoculate 250-ml flasks containing 90 ml of medium (10% v v^{-1}). The flasks were incubated at 150 rpm and flushed with 100% water-saturated O_2 (0.5 bar) for 3 min at the time of inoculation and then once a day for 5 days.

Bjerkandera sp. strain BOS55 was grown in a fermenter (Biostat E controlled by a computer) containing 10 l of Basal BIII medium, with 2 g l^{-1} glucose and 2 g l^{-1} peptone, at 30°C, 150 rpm and 6 l min^{-1} aeration. Homogenized mycelium from 5- to 7-day-old cultures grown at 30°C and 150 rpm in twenty 250-ml flasks containing 90 ml of medium was used as inoculum (10%).

2.2. Enzyme assays and analytical procedures

MnP activity was estimated by the formation of Mn^{3+}-tartrate complex ($\varepsilon_{238} = 6500\ M^{-1}\ cm^{-1}$) or by the Mn^{2+}-dependent oxidation of 0.1 mM 2,6-dimethoxyphenol (DMP) to coerulignone ($\varepsilon_{469} = 27500\ M^{-1}\ cm^{-1}$ referred to substrate concentration) in the presence of 0.1 mM H_2O_2. Peroxidase kinetic constants were calculated at the pH optimum for oxidation of each substrate as described by Martínez et al. (1996a). Mean values and 95% confidence limits were obtained from triplicate determination.

The total carbohydrate content of purified peroxidases from *Bjerkandera* sp. was determined by the anthrone procedure (Trevelyand and Harrison, 1952) and N-linked carbohydrates were estimated by SDS-PAGE before and after deglycosylation with *endo*-β-*N*-acetylglucosaminidase (Endo-H from Boehringer). Protein concentration was determined using the Bradford reactive (BioRad).

2.3. Purification of Mn^{2+}-oxidizing peroxidases of P. chrysosporium and Bjerkandera sp.

During purification hemeproteins were detected by absorbance at 410 nm and MnP activity by formation of Mn^{3+}-tartrate. Extracellular fluid

from 5-day-old *P. chrysosporium* cultures was filtrated, concentrated 50-fold by ultrafiltration (10 kDa-cut-off membrane), and dialyzed against 10 mM sodium tartrate buffer, pH 5.7. It was stored at −20°C without loss of MnP activity. Samples of 1 ml were loaded on Mono-Q column (Pharmacia HR 5/5) equilibrated with 10 mM sodium tartrate buffer, pH 5.7, and proteins eluted with a linear gradient of 0–0.25 M NaCl in the same buffer (30 min at 0.8 ml min^{-1}). The fractions containing MnP activity were pooled, concentrated by ultrafiltration (Microsep-Filtron 3 kDa) and applied to a Superdex-75 column (Pharmacia HR 10/30), equilibrated and eluted with the above buffer and 150 mM NaCl (45 min at 0.4 ml min^{-1}). The peak containing MnP was collected, concentrated, dialyzed, and stored at −20°C.

In the case of *Bjerkandera* sp. the fermentation was stopped after 8 days, and the culture liquid was 50-fold concentrated (10 kDa-cut-off membrane) and dialyzed against 10 mM sodium tartrate buffer, pH 4.5. The crude enzyme was loaded onto a Biorad Q-cartridge equilibrated with 10 mM sodium tartrate buffer, pH 4.5, (1 ml min^{-1}) and retained protein was eluted with a gradient of 0–0.25 M NaCl in the same buffer (20 min). Retained and non-retained fractions with MnP activity were pooled separately, concentrated and 1 ml-samples applied onto a Sephacryl S-200 HR (Pharmacia K16/100) column equilibrated with the same buffer (0.8 ml min^{-1}). Fractions containing MnP activity were pooled, concentrated (Microsep-Filtron 3 kDa) and dialyzed against 10 mM sodium tartrate, pH 5. The enzyme was applied to a Mono-Q column and eluted in 10 mM sodium tartrate buffer pH 5, using a linear gradient of 0–0.25 M NaCl (20 min at 0.8 ml min^{-1}). A final chromatographic step in Superdex-75, under the same conditions described for *P. chrysosporium*, was used to estimate the relative molecular mass of these proteins and confirm their homogeneity.

2.4. Gel electrophoresis and isoelectric focusing

SDS-PAGE was performed in 12% polyacrylamide gels. Isoelectric focusing (IEF) was performed in 5% polyacrylamide gels with a thickness of 1 mm and a pH range of 2.5–5 (prepared with LKB-Pharmacia Ampholines) with 0.5 M H_3PO_4 and 0.5 M NaOH in anode and cathode, respectively. The pH gradient was measured on the gel, using a contact electrode. Protein bands after SDS/PAGE were stained with $AgNO_3$ (Silver Stain Plus of Biorad), and IEF gels were stained with Coomassie blue R-250.

2.5. Amino acid composition and sequencing

The amino acid composition was determined with a Biochrom 20 autoanalyzer (Pharmacia) after hydrolysis of 20 μg of protein with 6 M HCl at 110°C for 24 h. The N-terminal sequences were obtained by automated Edman degradation of 5 μg of protein in an Applied Biosystems (Perkin Elmer-model Procise™ 494).

3. Results

3.1. Purification of *P. chrysosporium* MnP

MnP purification was carried out after 5 incubation days, when the fungus showed the maximal activity (900 mU ml^{-1}), just before the maximal LiP activity. Table 1A summarizes the chromatographic steps used to purify the MnP isoenzymes: (i) during Mono-Q chromatography five hemeprotein peaks were obtained, two exhibiting LiP activity (labeled PCH3 and PCH7) and three exhibiting MnP activity (labeled PCH4, PCH5 and PCH6); and (ii) during Superdex-75 minor proteins were removed and the purification process was completed. PCH4 was the major MnP isoenzyme with a yield of 65% whereas PCH5 and PCH6 showed much lower yield.

3.2. Purification of Mn^{2+}-oxidizing peroxidases from *Bjerkandera sp.*

Bjerkandera sp. BOS55 was cultivated in a fermentor using a medium supplemented with peptone which increases the production of ligninolytic peroxidases by this fungus (Kaal et al., 1995). Mn^{2+}-oxidizing peroxidases were

purified after 8 days of growth, when maximal MnP activity (1400 mU ml^{-1}) was attained. Fig. 1 show two of the three chromatographic steps used for peroxidase purification. During low-performance anion exchange chromatography (Fig. 1(A)), two fractions with MnP activity were obtained, one of them (labeled BOS1) eluted at pH 4.5, whereas the second one (labeled BOS2) was retained and released by the NaCl gradient. As shown in Fig. 1(B), the purification process was completed using a Mono-Q column. The purification process is summarized in Table 1B, which includes yield, specific activity and purification factor for the two Mn^{2+}-oxidizing peroxidases produced by *Bjerkandera* sp. BOS55 under the growth conditions used in the present study.

3.3. Properties of Mn^{2+}-oxidizing peroxidases

Based on gel filtration on Superdex-75, the molecular mass of native Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. and *P. chrysosporium* was estimated to be around 40 kDa. The molecular mass of the denatured peroxidases was estimated by SDS-PAGE (Fig. 2A, C) to be 45 kDa for BOS1, PCH4 and PCH6, 44 kDa for BOS2, and 43 kDa for PCH5. Anthrone analysis revealed that both *Bjerkandera* sp. peroxidases contained around 5–7% total carbohydrate. SDS-PAGE after Endo-H treatment showed 4.5% N-linked carbohydrate content in BOS1 and 2.3% in BOS2, being the molecular masses of both deglycosylated proteins 43 kDa. Analytical IEF of

Table 1
Purification of MnP isoenzymes from *P. chrysosporium* (A) and *Bjerkandera* sp. (B)[a]

	Activity (U)	Protein (mg)	Yield (%)	Specific activity	Purification factor
A					
Culture liquid	1620.0	25.20	100.0	64.3	1.0
Ultrafiltration	1519.0	6.4	93.8	237.3	3.7
Mono Q					
PCH4	1057.1	3.00	65.3	352.3	5.5
PCH5	85.3	0.26	5.3	328.1	5.1
PCH6	87.8	0.34	5.4	58.2	4.0
Total	1230.2	3.60	76.0		
Superdex					
PCH4	1028.6	2.86	63.5	359.7	5.6
PCH5	40.8	0.09	2.5	453.3	7.0
PCH6	25.0	0.07	1.5	357.1	5.6
Total	1094.4	3.02	67.5		
B					
Culture liquid	14 000	1140.0	100.0	12.2	1.0
Ultrafiltration	11 750	371.0	83.9	31.7	2.6
Q cartridge					
BOS1	7600	75.0	54.3	101.3	8.2
BOS2	2500	16.5	18.9	151.5	12.3
Total	10 100	91.5	73.2		
Sephacryl					
BOS1	7000	28.0	50.0	250.0	20.5
BOS2	2100	11.0	15.0	190.9	15.6
Total	9100	39.0	65.0		
Mono Q					
BOS1	6550	24.0	46.8	272.9	22.4
BOS2	1600	6.5	11.4	246.1	20.1
Total	8150	30.5	58.2		

[a] The enzymatic activities in the culture liquid and after the different purification steps were estimated by the formation of Mn^{3+}-tartrate.

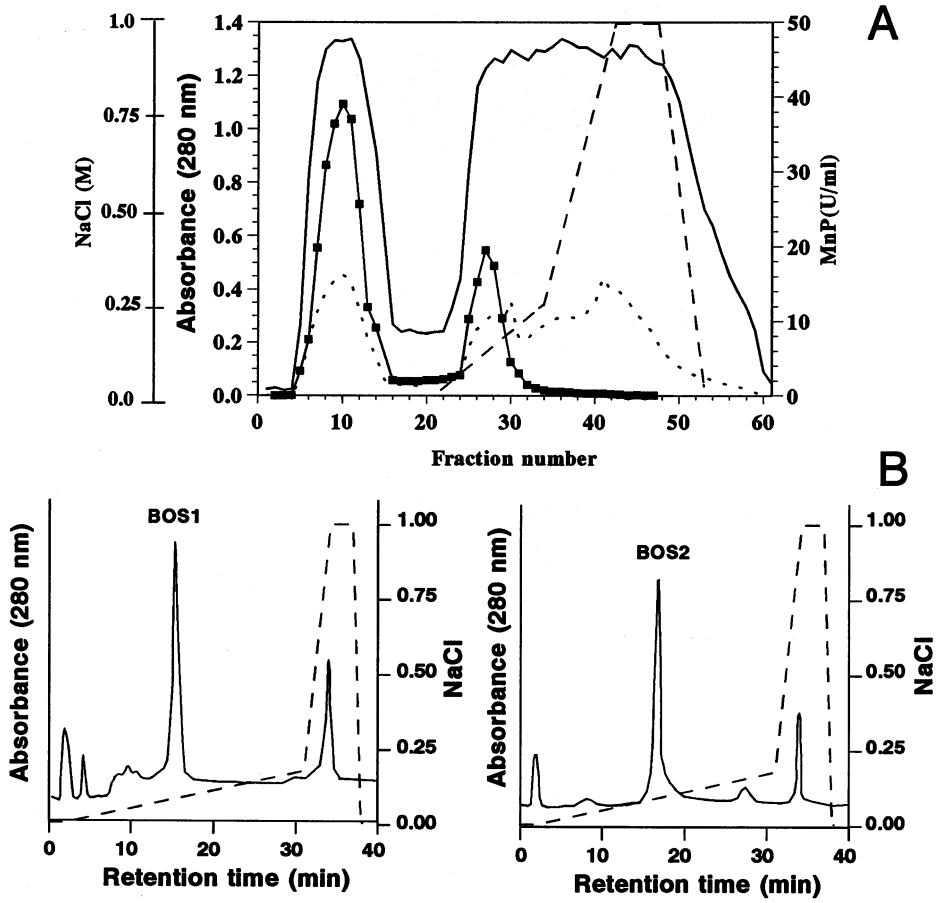

Fig. 1. Purification of Mn^{2+}-oxidizing peroxidase from *Bjerkandera* sp. (A) Profiles corresponding to Q-cartridge column: absorbances at 280 (——) and 410 nm (- - - -), NaCl gradient (— —) and MnP activity (■) are shown. (B) Profiles at 280 nm corresponding to Mono-Q column of BOS1 and BOS2, after previous Sephacryl S-200 chromatographies.

the two Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. showed different p*I* around 3.45 and 3.40 for BOS1 and BOS2, respectively (Fig. 2B).

Fig. 3 shows a multiple alignment of the N-terminal sequences of the Mn^{2+}-oxidizing peroxidases studied here from *Bjerkandera* sp. and *P. chrysosporium*. Both Mn^{2+}-oxidizing from *Bjerkandera* sp. appeared identical (although the residue 17 was non-identified in BOS1). However, they differed from the N-terminal sequences of the three *P. chrysosporium* isoenzymes (PCH4, PCH5 and PCH6).

The amino acid composition of the two *Bjerkandera* sp. Mn^{2+}-oxidizing peroxidases was similar, although some small differences were found (e.g. in Met, Ser and Pro content).

3.4. Catalytic properties

Table 2 shows the apparent kinetic constants of the different *Bjerkandera* sp. and *P. chrysosporium* peroxidases studied. The five Mn^{2+}-oxidizing peroxidases purified showed high peroxidase activity on Mn^{2+} and Mn-mediated peroxidase activity on DMP in the presence of adequate chelators. Their kinetic constants showed high affinity for H_2O_2 and Mn^{2+}, and similar V_{max} during Mn^{2+} oxidation. Mn^{2+}-independent activity on phenolic (such as DMP) and non-phenolic compounds

Table 2
Steady-state kinetic constants of Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. and *P. chrysosporium*: apparent K_m and V_{max}[a]

	Bjerkandera sp.				*P. chrysosporium*					
	BOS1		BOS2		PCH4		PCH5		PCH6	
	K_m (μM)	V_{max} (U mg^{-1})	K_m (μM)	V_{max} (U mg^{-1})	K_m (μM)	V_{max} (U mg^{-1})	K_m (μM)	V_{max} (U mg^{-1})	K_m (μM)	V_{max} (U mg^{-1})
H_2O_2[b]	3.1 ± 0.4	247 ± 10	4.7 ± 0.2	131 ± 6.7	9.2 ± 3.5	513 ± 20	6.0 ± 0.8	357 ± 7.0	5.0 ± 0.05	139 ± 3.0
Mn^{2+}	16.5 ± 1.7	253 ± 10	25.4 ± 1.9	150 ± 4.9	15.6 ± 2.3	469 ± 12	17.5 ± 2.4	344 ± 5.5	45.0 ± 1.1	285 ± 2.5
DMP+Mn^{2+}	19.5 ± 1.1	50 ± 1.1	11.0 ± 0.8	51 ± 0.8	18.0 ± 2.5	58 ± 2.5	11.0 ± 2.1	41 ± 2.0	6.6 ± 0.3	42 ± 0.9
DMP	121.0 ± 9.9	22 ± 1.2	106.0 ± 6.7	14 ± 0.7	nd[c]	nd	nd	nd	nd	nd
Veratryl alcohol	3200.0 ± 412	20 ± 0.8	5330.0 ± 763	22 ± 0.7	ns[d]	ns	ns	ns	ns	ns

[a] All reactions were in 0.1 M sodium tartrate (pH 5 when carried out in presence of Mn^{2+}, and pH 3 in the absence of Mn^{2+}) containing 0.1 mM H_2O_2. Mean values and 95% confidence limits are shown.

[b] Mn^{2+} (0.1 mM) was used to determine kinetic constants for H_2O_2.

[c] nd, not determined.

[d] ns, no substrate.

(such as veratryl alcohol) was investigated in 0.1 M tartrate buffer, pH 3 (Table 2). No oxidation of veratryl alcohol by *P. chrysosporium* MnP isoenzymes was found, whereas the *Bjerkandera* sp. Mn^{2+}-oxidizing peroxidases oxidized this aromatic alcohol with K_m values of 3–6 mM. In the same way, these *Bjerkandera* sp. peroxidases showed Mn^{2+}-independent activity on DMP with a K_m value around 0.1 mM. Very slow oxidation of DMP by *P. chrysosporium* MnP was observed only at very high substrate concentrations (Fig. 4), compared with low concentrations oxidized by *Bjerkandera* sp. Mn^{2+}-oxidizing peroxidase in the absence of Mn^{2+} (the enzyme from *P. chrysosporium* being not saturated even at the highest DMP concentration assayed). Specific activities of less than 2.5 U mg^{-1} were obtained for oxidation of 5 mM DMP by *P. chrysosporium* MnP, compared with 22 and 15 U mg^{-1} for *Bjerkandera* BOS1 and BOS2 isoenzymes using ten-fold lower substrate concentration. In order to explain the results obtained with *P. chrysosporium* MnP it is necessary to take into account that some chemical oxidation of DMP by hemin was observed (1 U mg^{-1} of hemin) at high substrate concentrations (30 mM).

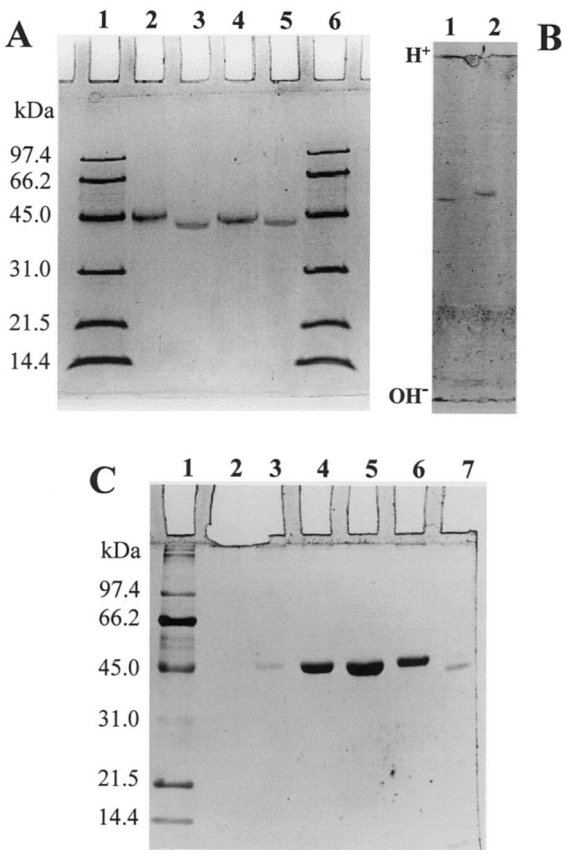

Fig. 2. Estimation of relative molecular masses and p*I* of Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. and *P. chrysosporium*. A. SDS-PAGE of *Bjerkandera* sp. BOS55 peroxidases BOS1 (lanes 2 and 3) and BOS2 (lanes 4 and 5), glycosylated (lanes 2 and 4) and deglycosylated (lanes 3 and 5). BioRad low molecular-mass standards are shown in lanes 1 and 6. B. IEF of glycosylated peroxidases BOS1 (lane 1) and BOS2 (lane 2). C. SDS-PAGE of *P. chrysosporium* isoenzymes PCH3 (lane 3), PCH4 (lane 4), PCH5 (lane 5), PCH6 (lane 6) and PCH7 (lane 7).

4. Discussion

MnP is a ligninolytic enzyme produced by most white-rot fungi (Hatakka, 1994; Peláez et al., 1995). The different MnP isoenzymes from *P. chrysosporium* are well documented, and three genes encoding MnP1, MnP2 and MnP3 have been isolated (Godfrey et al., 1990; Mayfield et al., 1994; Alic et al., 1997). In the present study we purified these three MnP isoenzymes, as confirmed by N-terminal sequencing, in order to compare their physicochemical and catalytic properties with those of Mn^{2+}-oxidizing peroxidases produced by *Bjerkandera* sp. BOS55 (using the same assay conditions). The molecular mass of Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. and *P. chrysosporium* are included in the 43–45-kDa range, similar to that described in other ligninolytic fungi. By contrast, the isoelectric point of the *Bjerkandera* sp. peroxidases was more acidic than those of MnP isoenzymes from *P. chrysosporium* (Pease and Tien, 1992) and *Poria* (synonym: *Ceriporiopsis*) *subvermispora* (Lobos et al., 1994), and more similar to those described for *Pleurotus* peroxidases (Becker and Sinitsyn, 1993; Martínez et al., 1996b; Sarkar et al., 1997).

The N-terminal sequence obtained here for the two Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. was coincident with those reported for similar enzymes of *Bjerkandera adusta* (Heinfling et al., 1998) and *Bjerkandera* sp. (Mester and

N-terminal sequence	
VAXPDGVNTATNAAXXXLFAVRDDI	*Bjerkandera* sp. BOS1
VAXPDGVNTATNAAXXALFAVRDDI	*Bjerkandera* sp. BOS2
AVCPDGTRV-SHAACCAFIPLAQDL	*P. chrysosporium* PCH4
AVCPDGTRV-TNAACC	*P. chrysosporium* PCH5
ATCPDGT	*P. chrysosporium* PCH6

Fig. 3. Multiple alignment of N-terminal sequences of Mn-oxidizing peroxidases from *Bjerkandera* sp. BOS55 and *P. chrysosporium*.

Field, 1998), and showed the highest similarity with the N-terminal sequences of *Trametes versicolor* (Johansson and Nyman, 1996) and *Pleurotus eryngii* peroxidases (Martínez et al., 1996a). Unidentified residues in positions 3, 15 and 16 should be cysteins, since Cys residues at these positions are involved in disulphide bridges conserved in fungal peroxidases (Banci, 1997).

The Mn^{2+}-oxidizing peroxidases from *Bjerkandera* sp. and *P. chrysosporium* showed similar steady-state kinetic constants during Mn^{2+} oxidation. However, an important difference between both enzymes concerns the Mn^{2+}-independent activity on phenolic and non-phenolic aromatic compounds of the *Bjerkandera* sp. peroxidases. No oxidation of veratryl alcohol, and only a very low Mn^{2+}-independent activity on DMP at very high substrate concentrations were observed with the MnP isoenzymes from *P. chrysosporium* (at 1 mM DMP concentration this was around 50–100-fold lower than found with the *Bjerkandera* sp. peroxidase). It is necessary to mention that some Mn-independent activity of *P. chrysosporium* MnP had been described on pinacyanol, being 1% of the activity on Mn^{2+} (Glenn and Gold, 1985).

Table 3 summarizes the K_m values of Mn^{2+}-oxidizing peroxidases from different *Bjerkandera* and *Pleurotus* species, exhibiting Mn^{2+}-independent activity on phenols and non-phenolic aromatic compound, compared with *P. chrysosporium* MnP (Martínez et al., 1996b; Sarkar et al., 1997; Heinfling et al., 1998). The three peroxidases reported from *Bjerkandera* sp. presented similar physico-chemical properties, and the same *N*-terminal sequences. However, the enzyme reported as an hybrid MnP-LiP enzyme showed lower affinity on Mn^{2+} and higher affinity on veratryl alcohol (Mester and Field, 1998). These studies suggest that *Bjerkandera* species produce several Mn^{2+}-oxidizing peroxidase isoenzymes, as occur for other ligninolytic enzymes. However, the small differences in BOS1 and BOS2 kinetics properties could be due to the carbohydrate moiety, since both show the same molecular mass after deglycosylation. In this sense, some LiP isoenzymes in *P. chrysosporium*

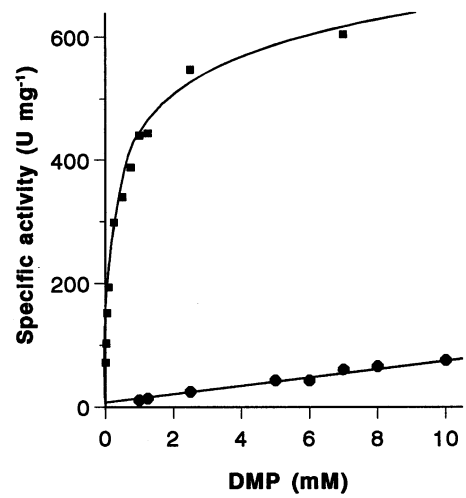

Fig. 4. Influence of substrate concentration on Mn-independent peroxidase activity. Comparison of DMP oxidation by peroxidase BOS1 from *Bjerkandera* sp. (■) and PCH4 from *P. chrysosporium* (●) in 0.1 M sodium tartrate, pH 3.

Table 3
Michaelis Menten constants of Mn^{2+} oxidizing peroxidases from *P. chrysosporium* and different *Bjerkandera* and *Pleurotus* species (DMP, 2,6-dimethoxyphenol; VA, veratryl alcohol; 0.1 mM H_2O_2 was used to determine kinetic constants for reducing substrates)

	K_m (μM)		
	Mn^{2+} (pH 5)	DMP (pH 3)	VA (pH 3)
P. chrysosporium PCH4	15.6	ns[a]	ns
P. chrysosporium PCH5	17.5	ns	ns
P. chrysosporium PCH6	45	ns	ns
Bjerkandera sp. BOS1	16.5	121	3200
Bjerkandera sp. BOS2	25.4	106	5330
Bjerkandera sp. Hybrid MnP-LiP (Mester and Field, 1998)	51[b]	nd[c]	116
B. adusta MnP1 (Heinfling et al., 1998)	20	160	4000
B. adusta MnP2 (Heinfling et al., 1998)	22	180	4500
P. eryngii MnPL1 (Martínez et al., 1996a)	16	200	3500
P. eryngii MnPL2 (Martínez et al., 1996a)	14	300	3000
P. eryngii MnP SSF1 (Martínez et al., 1996b)	40	200	3500
Pleurotus ostreatus MnPL (Sarkar et al., 1997)	18	950	4100
Pleurotus pulmonarius MnPL (Martínez et al., 1996b)	12	250	3800
Pleurotus pulmonarius MnPSSF (Martínez et al., 1996b)	22	250	3800

[a] ns, no substrate.
[b] Carried out at pH 4.5.
[c] nd, not determined.

only differ in phosphorylation and carbohydrate moiety (Rothschild et al., 1997).

Peroxidases with Mn^{2+}-independent activity on phenolic and non-phenolic aromatic compounds were first reported from *Pleurotus* species (Martínez et al., 1996a; Camarero et al., 1996; Sarkar et al., 1997). These enzymes have been considered as MnP, despite differences in catalytic properties with *P. chrysosporium* MnP, because they show the highest affinity for Mn^{2+} and the existence of a characteristic Mn^{2+}-interaction site has been shown in the molecular model of *P. eryngii* Mn^{2+}-oxidizing peroxidase (Ruiz-Dueñas et al., 1999). In conclusion, we confirm the existence of two types of Mn^{2+}-oxidizing peroxidases, those that strictly require Mn^{2+} as the MnP from *P. chrysosporium* and those that, showing high Mn^{2+} affinity, also have Mn^{2+}-independent activity such as those from *Bjerkandera* and *Pleurotus* species. In the case of *Bjerkandera* sp. the Mn^{2+}-oxidizing peroxidases could be the enzymes responsible for biobleaching of *Eucalyptus globulus* Kraft pulp in the absence of Mn^{2+} (Moreira et al., 1997b, 1998).

Acknowledgements

The authors thank Dr J.A. Field (Wageningen Agricultural University, The Netherlands) for providing the *Bjerkandera* BOS55 strain used in this study and Dr F. Guillén for comments and discussions. This research has been partially funded by the projects BIO95-377 and BIO96-393 of the Spanish Biotechnology Programme.

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