NOTES

Synergistic effects of cellobiose dehydrogenase and manganese-dependent peroxidases during lignin degradation

HUANG Feng[1], FANG Jing[1], LU Xuemei[1], GAO Peiji[1] & CHEN Jiaxiang[2]

1. State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, China;
2. State Key Lab of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510641, China

Correspondence should be addressed to Gao Peiji (e-mail: gaopj@sdu.edu.cn)

Abstract **The synergistic effects of cellobiose dehydrogenase (CDH) and manganese-dependent peroxidases (MnP) on the degradation of kraft pulp cellulolytic enzyme lignin (CEL) were investigated. Addition of CDH significantly increased the amount of water-soluble products reduced from CEL by MnP. CDH facilitated the reduction of the contents of methoxyl, carboxyl, phenolic hydroxyl and total hydroxyl groups of CEL by MnP. ^{1}H-NMR analysis showed that addition of CDH also decreased further the amount of protons of CEL degraded by MnP. The results proved for the first time that CDH could promote degradation of lignin by MnP and suggest that CDH could not only promote degradation of cellulose but also is an important part of the lignin biodegradation system.**

Keywords: cellobiose dehydrogenase, manganese-dependent peroxidase, lignin degradation.

Lignin is the most abundant renewable aromatic polymer in the biosphere. It comprises 20%—30% of woody plant cell walls and gives strength and protection against biodegradation. In recent years the mechanism of lignin degradation has been elucidated to a large extent. White-rot fungi are the only known organisms that can degrade lignin to any extent. Lignin biodegradation is mainly oxidative and the most important lignin degrading enzymes are lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase. However, it was found that these enzymes could not degrade lignin thoroughly *in vitro*. As research has advanced, the perception of lignin degradation has changed from that of an oxidative depolymerization process to a process of concerted oxidative and reductive conversions in which different classes of enzymes participate[1, 2]. Besides the enzymes mentioned above, cellobiose dehydrogenase (CDH, formerly named cellobiose oxidase) and cellobiose:quinone oxidoreductase (CBQ) have been suggested to play a role in the process of wood decay[3, 4]. CDH is a hemoflavoenzyme produced by some cellulolytic fungi. CDH contains one heme b and one FAD per molecule and it is organized into two domains: a heme-containing domain and a FAD-containing domain[5]. CBQ is a flavoenzyme, which is believed to be the protelysis product of CDH[6].

CDH can oxidize cellobiose and higher cellodextrins to the corresponding lactones. The enzyme has a wide variety of electron acceptors such as cytochrome c, dichlorophenol-indophenol[7], Fe^{3+} [4], MnO_2[8], and O_2[9]. Besides, it can also reduce lignin-related quinones, as well as cation and phenoxyl radicals in the presence of cellobiose[7, 10, 11]. Based on the current knowledge of the properties of CDH, the possible functions of the enzyme are suggested as follows: ⅰ) promotion of cellulose degradation through hydroxyl radical production by CDH mediated Fe^{3+} reduction in the presence of H_2O_2[12]; ⅱ) creation of Mn^{3+}-complexing agents to promote MnP activity[13]; ⅲ) prevention of lignin repolymerization after an oxidative enzyme attack. CDH-mediated reduction of phenoxyl and cation radicals favors the lignin degradation by *Phanerochaete* peroxidases[3, 4]. All the suggested possible functions of CDH are needed for the further experiments.

Research to develop a cell-free biological system that can bleach and delignify the kraft pulp efficiently is being conducted in many laboratories. The possible application of ligninases in kraft pulp delignification has been studied extensively and the application of CDH in delignification of kraft pulp has also been concerned. In this work, the effects of CDH of the white-rot fungus *Schizophyllum commune* on kraft pulp lignin degradation by manganese-dependent peroxidases from *Phanerochaete chrysosporium* were investigated.

1 Experimental

(ⅰ) Microorganisms and enzymes. Peroxidases were prepared from the white-rot fungus LIP14, a mutant of *P. chrysosporium* ME446 (ATCC 34541). The excellular enzymes contents MnP 127 U/L and none of LiP, CDH and Laccase activities were detected. CDH of *Schizophyllum commune* AS5.391 was prepared and purified according to our previous work[8]. The purified CDH has a specific activity of 11.5 μmol • min^{-1} • mg^{-1}. Cellulases of *Trichoderma pseudokoningii* S38 were prepared according to ref. [14].

(ⅱ) Preparation of cellulase enzyme lignin (CEL) from kraft pulp. An industrial eucalyptus kraft pulp with a kappa number of 30.8 was used. Isolation and purification of the residual lignin (CEL) from the pulp was performed according to ref. [15]. Cellulases were used to remove the carbohydrates of the pulp in this isolation process.

(ⅲ) Degradation of CEL by MnP and CDH. CEL (1 g) was added in 5 mL 40 mmol/L, pH 4.8 acetate buffer containing 0.4 mL LIP14 enzyme, 50 μg CDH, 40 μL

1 mmol/L H_2O_2, 20 μL 10 mmol/L $MnSO_4$, and 200 μL 1 mmol/L cellobiose. It was incubated in a rotary shaker (150 r/min) at 28℃ for 72 h, the reaction mixture was supplemented with 20 μL 1 mmol/L H_2O_2 and 50 μL 1 mmol/L cellobiose every 12 h. After 72 h, the reaction mixture was centrifuged (10000×*g*) for 10 min. The supernatant was used for UV spectrum analysis. The precipitate was washed with pure water ten times and recovered by freeze-drying and stored in a vacuum desiccator over phosphorus pentoxide. The experiment using addition of LIP14 enzyme and inactivated CDH was carried out in the same conditions as control. Freeze-dried precipitates were subjected to further analysis.

(ⅳ) Analysis of CEL samples. Residual carbohydrate in CEL was measured by an HPLC system with refractive index detector and SC1011 column (8 mm × 300 mm). Pure water at flowing rate of 1 mL/min was used as the mobile phase. C, H and N were determined in the elemental analyzer of Heraeus CHN-O (Germany). S was determined through the combustion method. The amount of O was calculated from the data of C, H, N, and S. The average molecular weight of CEL samples were determined by gel permeation chromatography (GPC). The contents of methoxyl-[16], carboxyl-[17], phenolic hydroxyl-[18] and carbonyl groups[19] in CEL samples were determined according to refs. [16—19]. FTIR determination was carried out by using a Nicolet 20SX type FTIR spectrometer with resolution of 4 cm^{-1}, scan number of 32 and scan range of 4000—400 cm^{-1}. Samples were pressed into tablets with KBr before analysis. 10—20 mg acetylated CEL samples were dissolved in 0.5 mL of deuteriochloroform respectively and examined by NMR spectroscopy using a Bruker DRX400 model spectrometer .

2 Results and discussion

(ⅰ) Isolation and purification of CEL. To isolate the residual lignin from kraft pulp by using cellulase could remain the structure of lignin in maximum permissible limit. In this experiment, a ball mill was used to treat the kraft pulp that suspended in tolan for distruction of the celludextran and promotion of hydrolization. The treatment system was kept under 30℃. The super speed freeze centrifuge and vacuum freeze drier were used to promote the purification. The yield of isolated and purified residual lignin was 66.28%. The carbohydrate content of CEL was 2.61%. The result of HPLC analysis showed that the main hydrolysate of the residual carbohydrate was xylose. The result of element analysis indicated that the nitrogen content was 0.52%.

Table 1 The effect of redoxase on CEL structure

CEL sample	OCH_3 (%)	*t* value	Empirical formula	Unit molecular weight*M*	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w / \overline{M}_n$
Control	15.01±0.09	A. $t = 9.67 > t_{(4)0.01}$, $p < 0.01$	$C_9H_{12.49}O_{3.71}(OCH_3)_{1.04}$	212.31	8283	4738	1.748
LIP14	14.72±0.12	B. $t = 17.14 > t_{(4)0.01}$, $p < 0.01$	$C_9H_{11.03}O_{3.79}(OCH_3)_{1.01}$	211.19	8174	4664	1.753
LIP14+CDH	13.52±0.05		$C_9H_{10.26}O_{3.43}(OCH_3)_{0.92}$	201.86	8105	4643	1.746

(ⅱ) Effect of the redoxase on CEL structure. Table 1 shows the effect of the redoxase on CEL structure. During the treatment few of other additives were induced except the enzymes and the detection result of lignin structure would not be interfered. When eucalyptus kraft pulp lignin was degraded by LIP14 enzyme, the content of methoxyl groups decreased. The content of methoxyl group in untreated CEL was 15.01±0.09. After treatment with LIP14 enzyme, the content of the methoxyl group was 14.72±0.12. The unit molecular weight, weight-average molecular weight ($\overline{M}_w$) and number-average molecular weight ($\overline{M}_n$) were decreased while the polydispersity tended to increase at the same time. If CDH was added to the reaction, the content of methoxyl group was further decreased to 13.52±0.05. The unit molecular weight, $\overline{M}_w$, $\overline{M}_n$ and polydispersity all tended to decrease. The results showed that LIP14 enzyme has the ability to degrade CEL. Increase of polydispersity could be the indication of polymerization of the derived radicals in some degree. The statistical analyses indicated that the changes of the methoxyl group content were significant. Addition of CDH could facilitate the decrease of methoxyl groups by LIP14 enzyme. The formulae of the unit structure of the samples calculated from the above mean values are shown in table 1. When CEL was treated by LIP14 enzyme, addition of CDH could further decrease the unit molecular weight of CEL. The absorbance spectra of the water-soluble products solubilized from CEL by LIP14 enzyme, and by LIP14 enzyme plus CDH are shown in fig. 1. The absorbance (around 280 nm) of the water-soluble products generated by LIP14 enzyme and CDH (broken line) was much higher than that by LIP14 enzyme alone (solid line). From the difference spectrum, the solutes that come from lignin degradation have absorbancy at around 240, 260 and 280 nm. These results suggest that addition of CDH could increase the water-soluble products solubilized from CEL by LIP14 enzyme.

(ⅲ) Effect of the redoxase on auxochromes and chromophores in CEL structure (1) Carboxyl group. From table 2 we can see that after the treatment by LIP14 enzyme, the carboxyl group content of CEL decreased from 23.97±0.11 to 6.71±0.08. 72.0% of carboxyl

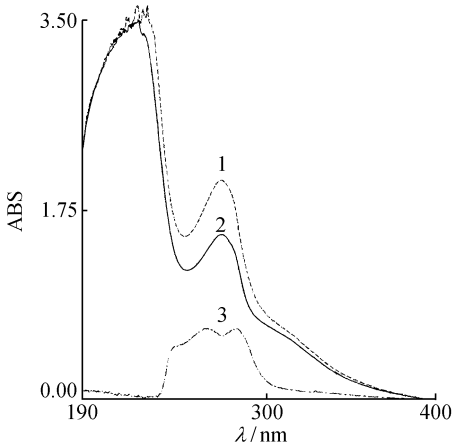

Fig. 1. Ultraviolet absorbance spectra of water-soluble products of CEL degraded by *Phanerochaete* peroxidases and *Phanerochaete* peroxidases with CDH. 1, Absorbance spectrum of water-soluble products of CEL degraded by *Phanerochaete* peroxidases and CDH. 2, Absorbance spectrum of water-soluble products of CEL degraded by *Phanerochaete* peroxidases. 3, Absorbance difference spectrum of water-soluble products of CEL degraded by *Phanerochaete* peroxidases and CDH against that by *Phanerochaete* peroxidases.

Table 2 Content of carboxyl group

CEL Sample	COOH/CEL (%)	*t* value	Change of carboxyl group content (%)
Control	23.97±0.11		–
LIP14	6.71±0.08		–72.0
LIP14+CDH	5.67±0.04		–76.3

groups were decreased. If CDH was added, the carboxyl group content of CEL decreased further to 5.67±0.04. The decrease of carboxyl groups was 76.3%. Obviously, CDH enhanced the decarboxylation of CEL by LIP14 enzyme. As we know, there are few carboxyl groups in protolignin. But, some carboxyl groups will be formed if the pulp was treated chemically. The decrease in carboxyl groups indicated that the decomposition of CEL occurred. The statistical analyses showed significant decreases of carboxyl groups in the enzyme-treated CEL samples.

(2) Determination of carbonyl groups. The carbonyl groups were detected by the reduction difference absorbance spectrometry and the content is summarized in table 3. Generally, phenyl aldehyde type carbonyl, *o*-quinone carbonyl and more α-carbonyl were included in technical lignin. The most common carbonyl groups in CEL were phenolic α-carbonyl and etherified γ-carbonyl groups. Treated by LIP14 enzyme, the quantities of these two kinds of carbonyl groups were increased, but the phenolic γ-carbonyl and etherified α-carbonyl groups were decreased. If CDH was added, more etherified γ-carbonyl groups were formed. Meanwhile, CDH also increased the quantities of phenolic γ-carbonyl and etherified α-carbonyl groups compared with those by LIP14 enzyme alone. In view of the total amount, addition of CDH increased the content of carbonyl groups. The results of statistical analysis showed variations in the data. Increase of the total carbonyl groups suggests that the possible new chromophoric groups would be produced in the enzyme-treated CEL. This is important for kraft pulp bleaching. When eucalyptus kraft pulp was bleached by LIP14 enzyme in the presence of CDH, its kappa number was decreased; for example, from 9.9 to 7.6, but the brightness of the pulp was increased little, from 56.0 ISO% to 56.6 ISO%. Therefore, we should not draw a hasty conclusions just according to the brightness change of the pulp in kraft pulp bleaching.

Table 3 Content variation of carbonyl groups (unit: 100 C_9)

CEL sample	Phenolic α-carbonyl group	Phenolic γ-carbonyl group	Etherified α-carbonyl group	Etherified γ-carbonyl group	Total of carbonyl groups	*t* value (total of carbonyl groups)
Control	2.18±0.02	0.09±0.01	0.20±0.01	0.34±0.02	2.80±0.03	A. $t = 2.00 < t_{(4)0.01}$,
LIP14	2.31±0.01	0.08±0.01	0.04±0.01	0.41±0.01	2.84±0.01	$0.01 < p < 0.05$
LIP14+CDH	2.14±0.02	0.11±0.01	0.13±0.02	0.80±0.01	3.19±0.05	B. $t = 8.75 > t_{(4)0.01}, p < 0.01$

Table 4 Content variation of phenolic groups

CEL sample	Content of phenolic groups (%)			*t* value (254 nm)
	254 nm	296 nm	365 nm	
Control	2.12±0.01	2.66±0.02	0.47±0.01	
LIP14	2.11±0.03	2.87±0.01	0.46±0.02	A. $t = 0.50 < t_{(4)0.01}$, $p > 0.05$
LIP14+CDH	1.90±0.02	2.81±0.04	0.46±0.01	B. $t = 21.00 > t_{(4)0.01}$, $p < 0.01$

(3) Determination of phenolic hydroxyl groups. The contents of phenolic hydroxyl groups derived from the ionization difference spectra are summarized in table 4. The phenomenon was complex. The content of the phenolic hydroxyl group corresponding to 254 nm was changed a little, from (2.12±0.01)% to (2.11±0.03)%, when CEL was degraded by LIP14 enzyme, while when CDH was added, the content was reduced to (1.90±0.02)%. The content of phenolic hydroxyl group corresponding to 296 nm was increased from (2.66±0.02)% to (2.87±0.01)% when CEL was treated by LIP14 enzyme, and after addition of CDH, the content was (2.81±0.04)%. The absorbance around 296 nm was mainly caused by non-conjugated phenoxy ions. The carbonyl groups con-

jugated with phenoxy ions also had effect on the absorbance. So the reason for the change of absorbance at 296 nm was complicated. The absorbance at 365 nm was likely caused by conjugated phenoxy ions, which is a minority of the hydroxyl groups and changes little. The absorbance at 254 nm was caused by conjugated and non-conjugated phenoxy ions, which represents the majority of phenoxy hydroxyl groups. We chose this group of data to do the statistical analyses and the results showed that the variation in the data was significant.

(iv) FTIR analysis. The FTIR analysis spectra of CEL samples are shown in fig. 2. The data in table 5 show that the relative intensities of bands at 3450 and 3000 cm^{-1} were decreased after synergistc treatment by LIP14 enzyme and CDH. Both of the bands were attributable to the stretch vibration of hydroxyl group, so the result means that the contents of hydroxyl groups in CEL were decreased after treatment by the enzymes. The band at 1731 cm^{-1} was attributable to acetyl group and the ester structure. The intensity of the band was further decreased when CDH was added to the degradation system, indicating that the degradation of acetyl group and break of ester bond occurred. Beside these, decrease of carboxyl groups also weakens the intensity of the band at 1731 cm^{-1}. It coincides with the analysis result that content of carboxyl groups in CEL was decreased after treatment by redoxases. The band at 1662 cm^{-1} was attributable to the stretch vibration of conjugated carbonyl group in side chain of benzene cycle. Decrease of the band intensity indicated that part of this chromophore structure in CEL was destroyed after synergistic treatment by LIP14 enzyme and CDH. The bands at 1328, 1224 and 1124 cm^{-1} were concern of the syringyl unit. The redoxases treatment affected

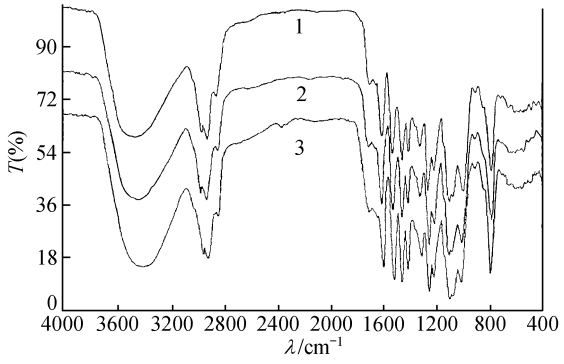

Fig. 2. FTIR spectra of CEL samples. 1, CEL; 2, *Phanerochaete* peroxidases treated CEL; 3, *Phanerochaete* peroxidases and CDH treated CEL.

little to the structure. The result was identical with that of GC-MS analysis[20].

(v) ^{1}H-NMR analysis. The ^{1}H-NMR analysis spectra of CEL samples are shown in figs. 3 and 4. The assignment of the signals in the ^{1}H-NMR spectra was according to Lundquist[21], Chen and Robert[22]. When CEL was treated with LIP14 enzyme, the amount of protons in various structures was decreased. Addition of CDH strengthened the decrease of protons in degradation by LIP14 enzyme (table 6). It is known that the amount of protons in lignin will decrease if lignin is degraded by ligninases. In this work, the fact that addition of CDH

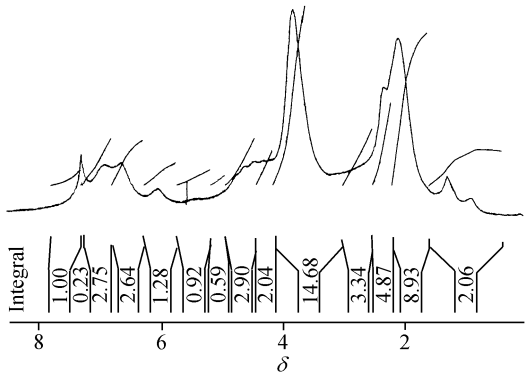

Fig. 3. ^{1}H-NMR spectrum of acetylated CEL.

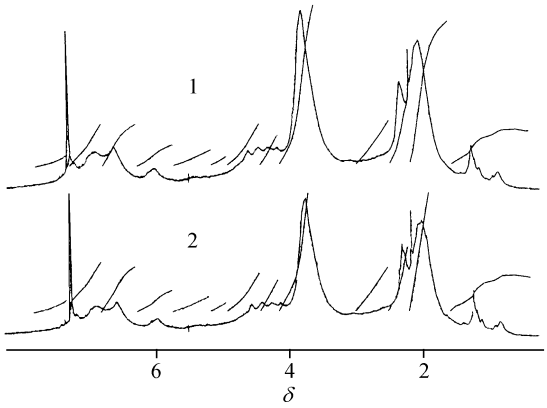

Fig. 4. ^{1}H-NMR spectra of acetylated CEL samples. 1, *Phanerochaete* peroxidases treated CEL; 2, *Phanerochaete* peroxidases and CDH treated CEL.

supported the decrease of protons in degradation by LIP14 enzyme suggests that CDH could facilitate CEL degradation by the ligninases.

The contents of the groups calculated from the ^{1}H-NMR analysis are summarized in table 7. The integration values are often on the high side due to broad and overlap others of the signals[23—25]. Similar to the results from periodate oxidation and ultraviolet spectrophotometry, the ^{1}H-NMR analysis results showed that the contents of methoxyl and phenolic hydroxyl groups of CEL were decreased after treatment by LIP14 enzyme, and the tendency would be furthered if the treatment by the enzymes is combined with CDH.

On the basis of these experiments and above analyses, for generality, we try to propose a mechanism for the ligninolysis by LIP14 enzyme that after treatment the contents of carboxyl-, phenolic hydroxyl- and total hydroxyl-groups of the eucalyptus kraft pulp CEL were all

decreased. Addition of CDH significantly increased the amount of water-soluble products reduced from CEL by LIP14 enzyme. CDH facilitated the reduction of the contents of methoxyl, carboxyl, phenolic hydroxyl and total hydroxyl groups of CEL by LIP14 enzyme. LIP14 is a new Lip defective mutant induced from the wild strain ME446, a typical ligninolytic bacterial strain. Only MnP activity was detected from the extracellular peroxidase[26]. As is seen from the present result, our previous work[20] indicated that the lignin peroxidase (Lip) is not the only key enzyme in lignin biodegradation as might be expected before. Protolignin of lignocellular material contains little carboxyl groups, while in treatment by white-rot fungi the function groups were produced by oxidation of the extracellular peroxidase. During the pulping and paper-making process, some of the lignin degraded and dissolved under the high temperature, alkali and pressure conditions and during these processes breaking of the chemical bonds and opening of the phenolic rings made the residual lignin have considerable content of carboxyl groups. The end of enzymatic mineralization and depolymerization of lignin is changing into CO_2 and H_2O. One of the lignin degradation actions is to form CO_2 by decarboxyilation. The carboxyl groups content of the lignin

Table 5 Relative band intensities of CEL samples

Wave number/cm^{-1}	Relative band intensity (A_i/A_{1506})			Wave number/cm^{-1}	Relative band intensity (A_i/A_{1506})		
	control	LIP14	LIP14+CDH		control	LIP14	LIP14+CDH
3450	0.9800	1.0069	0.8238	1462	1.0307	1.0233	1.0273
3000	0.5625	0.5784	0.4528	1422	0.8750	0.8759	0.8727
2938	0.8767	0.8841	0.7810	1328	0.8077	0.8076	0.8019
2880	0.6091	0.6192	0.4999	1267	1.1336	1.1241	1.1558
2850	0.6346	0.6465	0.5181	1224	1.0745	1.0618	1.0865
1731	0.4644	0.4578	0.4082	1124	1.3883	1.3659	1.4158
1662	0.5320	0.5388	0.4951	1034	1.2264	1.1997	1.2384
1591	0.8204	0.8391	0.8240	922	0.3618	0.3458	0.3309
1506	1.0000	1.0000	1.0000	830	0.5344	0.5347	0.5138

Table 6 Assignment of the signals in the ^{1}H-NMR spectra of acetylated CEL

Chemical shift (δ)	Type of protons	Proton (%)			Proton (1/C_9)		
		1[a)]	2[b)]	3[c)]	1[a)]	2[b)]	3[c)]
8.00—11.50	protons in carboxyl and aldehyde groups	–	–	–	–	–	–
7.30—7.80	aromatic protons in ρ-hydroxyphenol units	2.16	1.84	1.63	0.46	0.35	0.29
6.78—7.25	aromatic protons in guaiacyl units	6.45	6.74	6.75	1.38	1.29	1.20
6.28—6.78	aromatic protons in syringyl units	5.71	5.63	6.04	1.22	1.08	1.08
5.75—6.28	protons in β-O-4 and β-1 structures	2.77	2.82	2.90	0.59	0.54	0.52
5.19—5.75	H_α in β-5 structures	1.99	2.12	2.10	0.43	0.41	0.37
4.95—5.19	protons in carbohydrates	1.28	1.27	1.19	0.27	0.24	0.21
4.48—4.95	H_β and Hγ in β-O-4 structures	6.27	5.76	5.29	1.34	1.10	0.94
4.15—4.48	protons in C-β of acetylated β-O-4 structures, in C-γ of cinnamyl alcohol acetate moieties in aceylated substructures, and in C-α of acetylated β-β substructures	4.41	4.23	4.24	0.95	0.81	0.76
3.00—4.15	protons in methoxyl groups	31.75	30.08	29.77	6.80	5.76	5.31
2.52—3.00	protons on C-β in acetylated β-1 and β-β substructures	7.22	5.85	5.84	1.55	1.12	1.04
2.18—2.52	protons in aromatic acetate	10.53	9.55	8.69	2.26	1.83	1.55
1.60—2.18	protons in aliphatic acetate	19.32	19.57	20.98	4.14	3.75	3.74
0.38—1.60	hydrocarbon contaminant	4.46	4.53	4.57	0.96	0.87	0.82

a) Untreated CEL; b) CEL treated by LIP14 enzyme; c) CEL treated by LIP14 enzyme and CDH.

Table 7 Contents of the functional groups following ^{1}H-NMR analysis

Name of functional groups	Functional groups /C_9		
	1[a)]	2[b)]	3[c)]
Methoxyl group	2.27	1.92	1.77
Phenolic hydroxyl group	0.75	0.61	0.52
Aliphatic hydroxyl group	1.38	1.25	1.25
Total hydroxyl group	2.13	1.86	1.77

a), b) and c) are the same as in table 6.

sample treated by the oxidative enzymes was decreased. CDH could promote the degradation of lignin by MnP, and addition of CDH could increase the water-soluble products solubilized from CEL by LIP14 enzyme, both of which suggest that CDH could not only promote degradation of cellulose but also is an important part of the lignin biodegradation system.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant Nos. 20077015 and 29906005) and

by the Special Foundation of the Ministry of Education of China (Grant No. 200023).

References

1. Ander, P., Marzullo, L., Sugar oxidoreductases and veratryl alcohol oxidase as related to lignin degradation, J. Biotechnol., 1997, 53: 115.
2. Tuor, U., Winterhalter, K., Fiechter, A., Enzymes of white-rot fungi involved in lignin degradation and ecological determinants for wood decay, J. Biotechnol., 1995, 41: 1.
3. Ander, P., The cellobiose-oxidizing enzymes CBQ and CBO as related to lignin and cellulose degradation——A review, FEMS Microbiol. Rev., 1994, 13: 297.
4. Eriksson, K. E. L., Habu, N., Samejima, M., Recent advances in fungal cellobiose oxidoreductases, Enzyme Microb. Technol., 1993, 15: 1002.
5. Henriksson, G., Pettersson, G., Johanson, G. et al., Cellobiose oxidase from *Phanerochaete chrysosporium* can be cleaved by papain into two domains, Eur. J. Biochem., 1991, 196: 101.
6. Wood, D., Wood, P. M., Evidence that cellobiose:quinone oxidoreductase from *Phanerochaete chrysosporium* is a breakdown product of cellobiose oxidase, Biochim. Biophys. Acta, 1992, 1119: 90.
7. Samejima, M., Eriksson, K. E. L., A comparison of the catalytic properties of cellobiose:quinone oxidoreductase and cellobiose oxidase from *Phanerochaete chrysosporium*, Eur. J. Biochem., 1992, 207: 103.
8. Fang, J., Liu, W., Gao, P. J., Cellobiose dehydrogenase from *Schizophyllum commune*: Purification and study of some catalytic, inactivation, and cellulose-binding properties, Arch. Biochem. Biophys., 1998, 353: 37.
9. Wilson, M. T., Hogg, N., Jones, D., Reaction of reduced cellobiose oxidase with oxygen, Biochem. J., 1990, 270: 265.
10. Roy, B. P., Archibald, F. S., Effects of kraft pulp and lignin on *Trametes versicolor* carbon metabolism, Appl. Environ. Microbiol., 1993, 59: 1855.
11. Roy, B. P., Dumonceaux, T., Koukoulas, A. A. et al., Purification and characterization of cellobiose dehydrogenases from the white-rot fungus *Trametes versicolor*, Appl. Environ. Microbiol., 1996, 62: 4417.
12. Kremer, S. M., Wood, P. M., Production of Fenton's reagent by cellobiose oxidase from cellulolytic cultures of *Phanerochaete chrysosporium*, Eur. J. Biochem., 1992, 208: 807.
13. Archibald, F. S., Bourbonnais, R., Jurasek, L. et al., Kraft pulp bleaching and delignification by *Trametes versicolor*, J. Biotechnol., 1997, 53: 215.
14. Ma, D. B., Gao, P. J., Wang, Z. N., Preliminary studies on the mechanism of cellulase formation by *Trichoderma pseudokoningii* S38, Enzyme Microb. Technol., 1990, 12: 631.
15. Jiang, J. E., Chang, H. M., Bhattacharjee, S. S. et al., Characterization of residual lignins isolated from unbleached and semi-bleached softwood kraft pulps, J. Wood. Chem. Technol., 1987, 7: 81.
16. Chen, C. L., Determination of methoxyl groups, in Methods in Lignin Chemistry (eds. Lin, S. Y., Dence, C. W.), Berlin: Springer-Verlag, 1992, 465—472.
17. Pobiner, H., Improved inflection points in the non-aqueous potentiometric titration of acid functionalities in lignin chemicals by using internal standardization and ion exchange, Anal. Chim. Acta, 1983, 155: 57.
18. Goldschmid, O., Determination of phenolic hydroxyl content of lignin preparations by ultraviolet spectrophotometry, Anal. Chem., 1954, 26: 1421.
19. Adler, E., Marton, J., Zur Kenntnis der Carbonyl-Gruppen in Lignin (Ⅰ), Acta Chem. Scand., 1959, 13: 75.
20. Huang, F., Gao, P. J., Chen, J. X., Using cyclic liquid-liquid extraction method for isolation and identification of relative compounds during lignin biodegradation, Science in China, Ser. E, 1999, 42(6): 644.
21. Lundguist, K., NMR studies of lignins (2): Interpretation of the ^{1}H NMR spectrum of acetylated birch lignin, Acta Chem. Scand., 1979, B33: 27.
22. Chen, C. L., Robert, D., Characterization of lignin by ^{1}H and ^{13}C NMR spectroscopy, Methods Enzymol., 1988, 161: 137.
23. Faix, O., Grünwald, C., Beinhoff, O., Determination of phenolic hydroxyl group content of milled wood lignins (MWLs) from different botanical origins using selective aminolysis, FTIR, ^{1}H-NMR, and UV spectroscopy, Holzforschung, 1992, 46: 425.
24. Faix, O., Argyropoulos, D. S., Robert, D. et al., Determination of hydroxyl groups in lignins evaluation of ^{1}H-,^{13}C-,^{31}P-NMR, FTIR and wet chemical methods, Holzforschung, 1994, 48: 387.
25. Vázquez, G., Antorrena, G., González, J. et al., FTIR, ^{1}H and ^{13}C NMR characterization of acetosolv-solubilized pine and eucalyptus lignins, Holzforschung, 1997, 51: 158.
26. Lu, X. M., Li, Y. Z., Wang, W. et al., Study of the role of lignin peroxidase produced by phanerochaete chrysosporium in depolymerization of natural lignin, Mycosystema (in Chinese), 1998, 17(2): 179.

(Received July 28, 2001)