Laccase from *Melanocarpus albomyces* binds effectively to cellulose

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Abstract A laccase from the thermophilic fungus *Melanocarpus albomyces* was shown to bind to softwood and pure microcrystalline cellulose. The binding isotherm fitted well the Langmuir type one-site binding model. The adsorption parameters indicated that *M. albomyces* laccase binds with high affinity to cellulose with a relatively low maximum binding capacity, as compared to the values for various cellulases. The binding was shown to be reversible and not influenced by non-specific protein or 0.1–0.5 M Na_2SO_4. No binding was detected with laccases from *Trametes hirsuta* or *Mauginiella* sp., which suggests that binding to cellulose is typical for only some laccases.

Keywords: Cellulose binding; Bacterial microcrystalline cellulose; *Melanocarpus albomyces* laccase

1. Introduction

Laccases (EC 1.10.3.2) are multicopper oxidases catalyzing oxidation of various phenolic compounds, aromatic amines, and even certain inorganic compounds by a one-electron transfer mechanism. The electron withdrawn from the substrate is transferred via four copper atoms to molecular oxygen [1]. Laccases are very common in nature, especially in plants and fungi [2,3]. Fungal laccases participate in plant pathogenesis, pigment production, and lignin biodegradation [2,4]. Mainly because of the broad substrate specificity range of laccases, they possess great biotechnological potential. Promising applications for laccases include e.g., textile dye bleaching, pulp bleaching and bioremediation [2,5].

We have recently purified and characterized from the thermophilic fungus *Melanocarpus albomyces* a laccase, which has high thermostability and a pH optimum at a neutral and slightly alkaline pH range [6]. The three-dimensional structure of *M. albomyces* laccase has been solved as one of the first complete laccase structures including all four coppers [7]. In this article, a novel feature for *M. albomyces* laccase is demonstrated: its effective binding to cellulose. Binding to cellulose has been shown for many enzymes involved in modification of lignocellulose, including various cellulases (for reviews, see [8,9]), hemicellulases [10–12], a β-glucosidase [13], and some cellobiose dehydrogenases [14–17]. In addition to cellulose-binding enzymes, binding to solid substrates has been reported with several chitinases [18,19] and glucoamylases [20,21]. We demonstrate in this article for the first time that a laccase binds to cellulose with high affinity.

2. Materials and methods

2.1. Enzymes and cellulosic materials

The enzymes used in this study were: recombinant *M. albomyces* laccase purified from *Trichoderma reesei* [22], native *M. albomyces* laccase [6], *Trametes hirsuta* laccase [23] and *Mauginiella* sp. laccase [24]. Steam-pretreated softwood (SPS) from *Picea abies* [25], Avicel PH 101 (SERVA Electrophoresis) and bacterial microcrystalline cellulose (BMCC) from *Acetobacter xylinum* [26] were used as cellulosic adsorbents.

2.2. Adsorption studies with steam-pretreated softwood

Laccases were added into 1 ml of 10 g l^{-1} steam-pretreated softwood (SPS) suspension in 50 mM citrate buffer (pH 5) giving initial enzyme dosages of 100 and 1000 nkat g^{-1}. After gentle mixing in an end-over-end rotary shaker at 22 °C for 1 h, the samples were centrifuged (10 000×*g*, 22 °C, 5 min). The remaining laccase activity in the supernatant was measured spectrophotometrically with 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) [ABTS, Roche Diagnostics] as substrate [27]. All measurements described in this study were repeated two to four times and the results were calculated as mean values from the parallel samples.

2.3. Adsorption of M. albomyces laccase on pure cellulose

Recombinant *M. albomyces* laccase (0.09–5 μM) was added into 0.25–1 ml of cellulose suspension containing 10 g l^{-1} Avicel or 0.1 g l^{-1} BMCC in 50 mM sodium citrate buffer (pH 5) at 22 °C. In order to reduce non-specific adsorption of laccase, 0.5% of bovine serum albumin (BSA; Sigma) was added to the BMCC-containing reaction mixtures [28,29]. The remaining laccase activity in the supernatant was measured after 1 h as described above. The corresponding protein concentrations were calculated by using the specific activity of 600 nkat mg^{-1} (measured on ABTS in citrate buffer) and the molecular weight of 71 000 Da for recombinant *M. albomyces* laccase [22]. The amount of bound laccase was calculated from the difference between the initial and free enzyme concentrations. A control sample without cellulose was also measured each time to ensure that the laccase remained active during the treatment. The non-linear regression curves for binding isotherms were calculated with GraphPad Prism 3.02 program (GraphPad Software).

The activity of bound laccase was measured with an end-point activity assay after binding. The supernatant was removed and the cellulose fraction containing the bound laccase was suspended in citrate buffer to restore the initial volume. The cellulose suspension was

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Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate); BMCC, bacterial microcrystalline cellulose; BSA, bovine serum albumin; CBD, cellulose-binding domain; SPS, steam-pretreated softwood

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Table 1
Melanocarpus albomyces, *Mauginiella* sp. and *Trametes hirsuta* laccase activities in supernatant after mixing with 1% steam-pretreated softwood (SPS) for 1 h at 22 °C, pH 5

Laccase	Initial activity (nkat ml^{-1})	Activity in the supernatant after 1 h treatment (nkat ml^{-1})	
		Sample with SPS	Control without SPS
Melanocarpus albomyces	1	0.02 ± 0.01	1.0 ± 0.2
	10	0.14 ± 0.01	10.0 ± 0.8
Mauginiella sp.	1	0.74 ± 0.01	<0.01
	10	7.0 ± 0.6	6.3 ± 0.6
Trametes hirsuta	1	0.9 ± 0.1	<0.01
	10	10.0 ± 0.2	8 ± 1

further diluted and ABTS (20 mM solution) was added to give a final concentration of 5 mM [27]. The reaction was allowed to proceed for exactly 2 min, after which the solution was filtrated through a 0.2 μm sterile FP 30 filter (Scleicher and Schuell) and the absorbance at 436 nm was immediately measured.

Reversibility of binding was determined by dilution experiments. *M. albomyces* laccase was first allowed to adsorb on BMCC for 1 h. Subsequently, the mixture was diluted fivefold with the sample buffer. Formation of a new equilibrium was monitored by removing small samples from the mixture after 1, 15, 30, 60 and 120 min. The samples were filtrated through a 0.2 μm GHP filter (Scleicher and Schuell), and the concentration of laccase at the new equilibrium was determined by activity measurements as described above.

The effect of non-specific adsorption on binding to BMCC was studied by omitting BSA in the binding experiments and the role of ionic interactions in adsorption was analyzed by adding 0.1 or 0.5 M Na_2SO_4 into the reaction mixture. The adsorption of *T. hirsuta* laccase on BMCC was studied at two protein concentrations (0.1 and 0.4 μM) as described above.

3. Results

The adsorption of *M. albomyces*, *T. hirsuta* and *Mauginiella* sp. laccases on steam-pretreated softwood was tested at two enzyme concentrations, 1 and 10 nkat ml^{-1}. The difference in binding between different laccases was very obvious; only *M. albomyces* laccase showed extensive binding, whereas *T. hirsuta* and *Mauginiella* laccases did not adsorb on softwood (Table 1). Interestingly, SPS seemed to stabilize *Mauginiella* and *T. hirsuta* laccases, as it prevented significant loss of activity that was detected in the control samples containing low concentrations of these laccases in buffer without SPS. Steam-pretreatment of softwood generates swollen wood fibers consisting of cellulose (42%) and lignin (51%) [25]. The stabilizing effect of SPS may be related to the exposed lignin, because various lignin-derived phenolic compounds have been shown to enhance the stability of *Trametes versicolor* laccase in citrate buffer [30]. In the case of *M. albomyces* laccase, the loss of activity was not observed even in very low protein concentrations.

Binding of *M. albomyces* laccase was also analyzed on relatively pure cellulose, Avicel, to elucidate whether the binding to SPS was truly caused by cellulose present in wood fibres. The adsorption studies with 1% Avicel clearly indicated that *M. albomyces* laccase was effectively bound to purified cellulose (Table 2). Activity of the Avicel-bound laccase was also

Table 2
Amount of *Melanocarpus albomyces* laccase in the supernatant and in the solid fractions after mixing with 1% Avicel at 22 °C for 1 h at pH 5

Initial dosage (nkat)	Supernatant fraction (nkat)	Cellulose fraction (nkat)
10	0.2 ± 0.1	10 ± 1
0.8	<0.01	0.8 ± 0.1

determined in order to clarify whether the bound laccase was still active. As shown from the results (Table 2), all laccase activity could be recovered from the Avicel fraction, indicating that binding does not inactivate the enzyme.

The macroscopic structure of cellulose in Avicel preparations is relatively heterogeneous [26,31], therefore more thorough binding analyses were conducted with bacterial microcrystalline cellulose (BMCC). The adsorption isotherm of *M. albomyces* laccase on BMCC is shown in Fig. 1. In comparison, the adsorption of *T. hirsuta* laccase was studied at two points on the isotherm. No adsorption of *T. hirsuta* laccase occurred, since all laccase activity was detected in the supernatant after 1 h mixing with BMCC (Fig. 1).

The data points on the binding isotherm of *M. albomyces* laccase fitted well the classical Langmuir-type binding model represented by the Eq. (1)

$$Y = B_{max}[L]/(K_d + [L]), \quad (1)$$

where [L] is the concentration of free enzyme in equilibrium, B_{max} is the maximum binding capacity and K_d is the dissociation constant (= the reciprocal of the association constant K_a) [32]. B_{max} and K_d were solved by non-linear regression, and the curve fit yielded values of 1.94 ± 0.05 μmol g^{-1} and 0.006 ± 0.001 μM, respectively. The relative partition coefficient (K_p) was calculated using the slope of the isotherm in low enzyme concentrations. The slope was calculated from the first derivative of the isotherm Eq. (1) as the concentration of free enzyme approaches zero [33]. Thus, K_p was calculated by substituting the values for B_{max} and K_d into the Eq. (2)

$$K_p = \lim_{[L]\to 0} Y'([L]) = B_{max}/K_d. \quad (2)$$

Eq. (2) yielded $K_p = 320 \pm 80$ l g^{-1}.

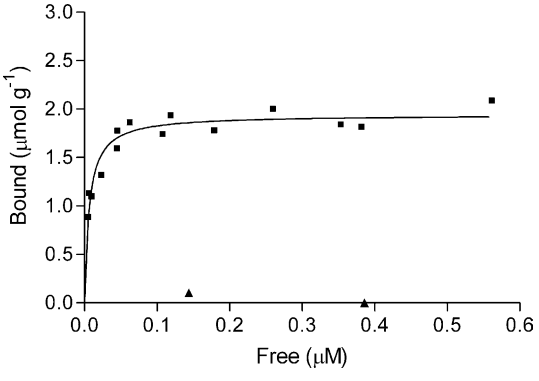

Fig. 1. The adsorption isotherm of *Melanocarpus albomyces* (■) and *Trametes hirsuta* (▲) laccases obtained with bacterial microcrystalline cellulose at 22 °C, pH 5.

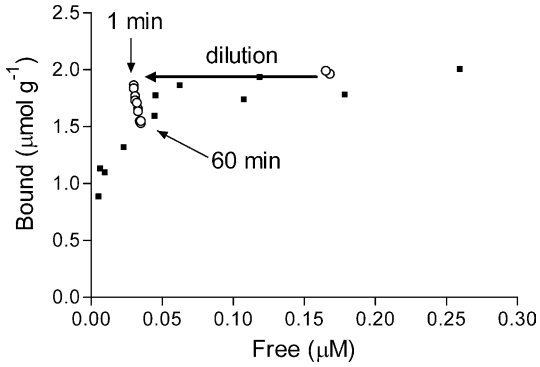

Fig. 2. Reversibility of *Melanocarpus albomyces* laccase by dilution when bound to bacterial microcrystalline cellulose at 22 °C, pH 5. (■) isotherm points, (○) dilution studies.

Data points on the isotherm were also measured with higher enzyme concentrations than presented in Fig. 1 to ensure that the saturation level evident in Fig. 1 was truly valid [34]. No increase in the amount of bound enzyme per gram of cellulose was seen with data points of up to free enzyme concentration of 4.3 μM (data not shown). In high enzyme concentrations (>1 μM) the random error of data points substantially increased, when the relative proportion of bound enzyme versus free enzyme decreased, as has previously been discussed by Bothwell and Walker [32].

The reversibility of binding was determined by dilution experiments. The equilibrium between laccase and cellulose was unbalanced by adding buffer, and the desorption of laccase was monitored by activity measurements of the supernatant fraction. The desorption data showed that a new equilibrium was established on the same isotherm (Fig. 2). In order to elucidate the nature of interactions affecting the adsorption of *M. albomyces* laccase on BMCC, the effect of non-specific protein (BSA) and ionic strength on binding were studied. BSA was added to the reaction mixtures in adsorption studies to prevent non-specific adsorption of the laccase [28,29], and the effect of non-specific adsorption was studied by omitting BSA in the binding experiments at three points on the isotherm. Omission of BSA did not cause changes in the adsorption behaviour of *M. albomyces* laccase (Fig. 3). In addition, the effect of 0.1 and 0.5 M Na_2SO_4 on adsorption was studied at three points on the isotherm. The results showed that the addition of salt did not alter the adsorption of *M. albomyces* laccase on BMCC (Fig. 3).

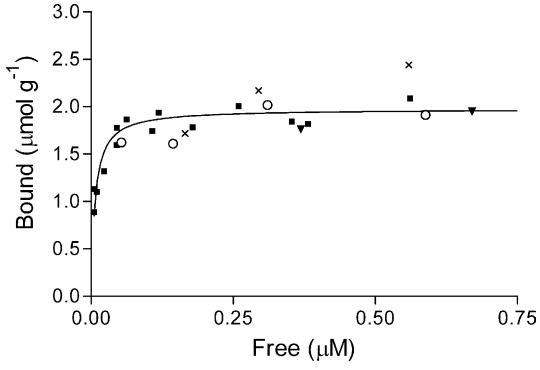

Fig. 3. The effect of 0.1 M (○) or 0.5 M (▼) Na_2SO_4 or omission of bovine serum albumin (BSA) (×) on binding of *Melanocarpus albomyces* laccase to bacterial microcrystalline cellulose. (■) isotherm points with BSA and without salt.

4. Discussion

The adsorption of a laccase on cellulose was demonstrated for the first time in this study; laccase from the ascomycete *M. albomyces* was shown to effectively bind to steam-pretreated softwood, Avicel and bacterial microcrystalline cellulose. Furthermore, it was demonstrated that the cellulose-bound laccase retained its full activity. The adsorption on cellulose was also studied with two other fungal laccases from *T. hirsuta* and *Mauginiella* sp. The results clearly indicated that neither of these laccases were able to bind to cellulose. These results thus suggest that laccases can be divided into two groups based on their affinity for cellulose.

The adsorption isotherm of *M. albomyces* laccase on BMCC was found to fit the Langmuir model, which is commonly used to describe the binding of cellulases to cellulose [35–38]. The curve fitting facilitated the calculation of the adsorption parameters for maximum binding capacity and binding affinity. The maximum binding capacity of *M. albomyces* laccase (1.94 μmol g^{-1}) was relatively low as compared to the values for various cellulases with binding capacities for BMCC ranging from 6 to 20 μmol g^{-1} [39,40]. However, similar binding capacity of 2.1 μmol g^{-1} has previously been reported for a cellobiose dehydrogenase from *Phanerochaete chrysosporium* [14]. On the other hand, the affinity of *M. albomyces* laccase represented by the partition coefficient of 320 l g^{-1} was clearly higher than reported for cellulases. For example, the partition coefficients of cellobiohydrolases Cel6A and Cel7A from *Trichoderma reesei* were 3.4 and 18 l g^{-1}, respectively [29], and of the cellulases CenA and Cex from *Cellulomonas fimi* 40.5 and 33.3 l g^{-1}, respectively [26]. The combination of high affinity and relatively low capacity of binding suggests that *M. albomyces* laccase is able to bind very effectively to BMCC, but only on relatively few binding sites.

The differences in binding parameters between cellulases and *M. albomyces* laccase may be related to the absence of a cellulose-binding domain (CBD) in the latter. A separate CDB, which in most cellulases dominates the binding characteristics, cannot be located either in the amino acid sequence [41] or the crystal structure of *M. albomyces* laccase [7]. In the case of cellulases, the available structures suggested specific sites on the protein that putatively interact with cellulose [42]. These were subsequently confirmed by experimental mutagenesis studies, which showed that the binding of CBDs is mediated by several aromatic amino acids forming a planar surface on the binding face of the domain [42,43]. An analysis of the *M. albomyces* laccase structure did not, however, reveal any hydrophobic surface patches on the protein that could obviously be assigned as the cellulose-binding site. Similarly to other laccases, the crystal structure of *M. albomyces* showed three cupredoxin-like domains, none of which contained regions with increased hydrophobicity on the outer surfaces. These observations are consistent with the results obtained with *P.*

chrysosporium cellobiose dehydrogenase, which binds effectively to cellulose but does not contain a separate CBD or any other evident cellulose-binding substructure [14,44].

One of the assumptions in a Langmuir-type binding model is full reversibility of adsorption. Our results from dilution studies of bound *M. albomyces* laccase showed that the binding was fully reversible, thus reinforcing the applicability of a Langmuir-type binding model for calculating binding constants from our data. Reversibility of binding is not a straightforward issue among enzymes adsorbing on cellulose, as both reversible and irreversible binding have been demonstrated [29,35,39,45,46]. The observed irreversible binding of cellulases may be related to their two-domain structure, because both domains participate in the binding [28,45]. Adsorption of *M. albomyces* laccase on cellulose was not affected by non-specific protein (BSA), which indicated that binding was not due to unspecific protein binding to solid substrate. In addition, the binding was unaltered by the presence of 0.1–0.5 M salt. As ionic interactions are weakened by increasing ionic strength, it can be concluded that electrostatic forces are not the main cause for the observed binding [46].

Interestingly, Paice et al. [47] reported a preliminary observation of *Myceliophthora thermophila* laccase adsorption on Kraft pulp that is mostly composed of cellulose. *Mt. thermophila* laccase is highly homologous to *M. albomyces* laccase, having a level of amino acid sequence identity of 73% [41]. The result suggests that the adsorption on cellulose might be a common feature among some fungal laccases. The role of the binding of *M. albomyces* laccase to cellulose may be related to total hydrolysis of lignocellulose, because *M. albomyces* is also known to produce several cellulose- and hemicellulose-degrading enzymes [48,49]. However, the detailed binding mechanism as well as the possible role of the binding need to be further elucidated. The ability of a laccase to bind to cellulose might be exploited in applications. It would be interesting, for example, to analyze whether the dosage of the enzyme can be decreased in textile or pulp applications, when a laccase that adsorbs on cellulose is used. On the other hand, the adsorption on inexpensive cellulosic materials could possibly be utilized for immobilization and recycling purposes.

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References

[1] Messerschmidt, A. (1997) in: Comprehensive Biological Catalysis (Sinnott, M., Ed.), vol. 3, pp. 401–426, Academic Press, London.
[2] Gianfreda, L., Xu, F. and Bollag, J.-M. (1999) Biochem. J. 3, 1–25.
[3] Mayer, A.M. and Staples, R.C. (2002) Phytochemistry 60, 551–565.
[4] Thurston, C. (1994) Microbiology 140, 19–26.
[5] Xu, F. (1999) in: The Encyclopedia of Bioprocessing Technology: Fermentation, Biocatalysis and Bioseparation (Flickinger, M.C. and Drew, S.W., Eds.), pp. 1545–1554, John Wiley & Sons, New York.
[6] Kiiskinen, L.-L., Viikari, L. and Kruus, K. (2002) Appl. Microbiol. Biotechnol. 59, 198–204.
[7] Hakulinen, N., Kiiskinen, L.-L., Kruus, K., Saloheimo, M., Paananen, A., Koivula, A. and Rouvinen, J. (2002) Nat. Struct. Biol. 9, 601–605.
[8] Linder, M. and Teeri, T.T. (1997) J. Biotechnol. 57, 15–28.
[9] Lynd, L.R., Weimer, P.J., van Zyl, W.H. and Pretorius, I.S. (2002) Microbiol. Mol. Biol. Rev. 66, 506–577.
[10] Irwin, D., Jung, E.D. and Wilson, D.B. (1994) Appl. Environ. Microbiol. 60, 763–770.
[11] Poole, D.M., Hazlewood, G.P., Huskisson, N.S., Virden, R. and Gilbert, H.J. (1993) FEMS Microbiol. Lett. 106, 77–83.
[12] Tenkanen, M., Buchert, J. and Viikari, L. (1995) Enz. Microb. Technol. 17, 499–505.
[13] Lymar, E.S., Li, B. and Renganathan, V. (1995) Appl. Environ. Microbiol. 61, 2976–2980.
[14] Henriksson, G., Salumets, A., Divne, C. and Pettersson, G. (1997) Biochem. J. 324, 833–838.
[15] Henriksson, G., Johansson, G. and Pettersson, G. (2000) J. Biotechnol. 78, 93–113.
[16] Roy, B.P., Dumonceaux, T., Koukoulas, A.A. and Archibald, F.S. (1996) Appl. Environ. Microbiol. 62, 4417–4427.
[17] Subramaniam, S.S., Nagalla, S.R. and Renganathan, V. (1999) Arch. Biochem. Biophys. 365, 223–230.
[18] Arakane, Y., Zhu, Q., Matsumiya, M., Muthukrishnan, S. and Kramer, K.J. (2003) Insect. Biochem. Mol. Biol. 33, 631–648.
[19] Tjoelker, L.W., Gosting, L., Frey, S., Hunter, C.L., Trong, H.L., Steiner, B., Brammer, H. and Gray, P.W. (2000) J. Biol. Chem. 275, 514–520.
[20] Coutinho, P.M. and Reilly, P.J. (1994) Protein Eng. 7, 393–400.
[21] Dalmia, B.K. and Nikolov, Z.L. (1991) Enz. Microb. Technol. 13, 982–990.
[22] Kiiskinen, L.-L., Kruus, K., Bailey, M., Ylösmäki, E., Siika-aho, M. and Saloheimo, M. Microbiology, in press.
[23] Rittstieg, K., Suurnäkki, A., Suortti, T., Kruus, K., Guebitz, G. and Buchert, J. (2002) Enz. Microb. Technol. 31, 403–410.
[24] Palonen, H., Saloheimo, M., Viikari, L. and Kruus, K. (2003) Enz. Microb. Technol. 33, 854–862.
[25] Palonen, H. and Viikari, L. (2004) Biotechnol. Bioeng. 86, 550–557.
[26] Gilkes, N.R., Jervis, E., Henrissat, B., Tekant, B., Miller Jr., R.C., Warren, R.A. and Kilburn, D.G. (1992) J. Biol. Chem. 267, 6743–6749.
[27] Niku-Paavola, M.-L., Karhunen, E., Salola, P. and Raunio, V. (1988) Biochem. J. 254, 877–884.
[28] Linder, M. and Teeri, T.T. (1996) Proc. Natl. Acad. Sci. USA 93, 12251–12255.
[29] Palonen, H., Tenkanen, M. and Linder, M. (1999) Appl. Environ. Microbiol. 65, 5229–5233.
[30] Mai, C., Schormann, W., Milstein, O. and Hüttermann, A. (2000) Appl. Microbiol. Biotechnol. 54, 510–514.
[31] Ek, R., Alderborn, G. and Nyström, C. (1994) Int. J. Pharm. 111, 43–50.
[32] Bothwell, M.K. and Walker, L.P. (1995) Biores. Technol. 53, 21–29.
[33] Linder, M., Salovuori, I., Ruohonen, L. and Teeri, T.T. (1996) J. Biol. Chem. 271, 21268–21272.
[34] Klotz, I.M. (1989) in: Protein Function – A Practical Approach (Creighton, T.E., Ed.), pp. 25–54, Oxford University Press, Oxford.
[35] Beldman, G., Voragen, A.G.J., Rombouts, F.M., Searle-van Leeuwen, M.F. and Pilnik, W. (1987) Biotechnol. Bioeng. 30, 251–257.
[36] Dijkerman, R., Vervuren, M.B., Op den Camp, H.J. and van der Drift, C. (1996) Appl. Environ. Microbiol. 62, 20–25.
[37] Jung, H., Wilson, D.B. and Walker, L.P. (2002) Biotechnol. Bioeng. 80, 380–392.
[38] Kyriacou, A., Neufeld, R.J. and MacKenzie, C.R. (1988) Enz. Microb. Technol. 10, 675–681.
[39] Jung, H., Wilson, D.B. and Walker, L.P. (2003) Biotechnol. Bioeng. 84, 151–159.
[40] Reinikainen, T., Teleman, O. and Teeri, T.T. (1995) Proteins 22, 392–403.
[41] Kiiskinen, L.-L. and Saloheimo, M. (2004) Appl. Environ. Microbiol. 70, 137–144.

[42] Linder, M., Mattinen, M.-L., Kontteli, M., Lindeberg, G., Ståhlberg, J., Drakenberg, T., Reinikainen, T., Pettersson, G. and Annila, A. (1995) Prot. Sci. 4, 1056–1064.
[43] Tormo, J., Lamed, R., Chirino, A.J., Morag, E., Bayer, E.A., Shoham, Y. and Steitz, T.A. (1996) EMBO J. 15, 5739–5751.
[44] Hallberg, B.M, Henriksson, G., Pettersson, G. and Divne, C. (2002) J. Mol. Biol. 315, 421–434.
[45] Bothwell, M.K., Wilson, D.B., Irwin, D.C. and Walker, L.P. (1997) Enz. Microb. Technol. 20, 411–417.
[46] Kyriacou, A., Neufeld, R.J. and MacKenzie, C.R. (1989) Biotechnol. Bioeng. 33, 631–637.
[47] Paice, M., Bourbonnais, R., Renaud, S., Labonté, S., Sacciadis, G., Berry, R., Amann, M., Candussio, A. and Müller, R. (2002) in: Biotechnology in the Pulp and Paper industry: 8th ICBPPI (Viikari, L. and Lantto, R., Eds.) Progress in Biotechnology, vol. 21, pp. 203–211, Elsevier, Amsterdam, New York.
[48] Miettinen-Oinonen, A., Londesborough, J., Joutsjoki, V., Lantto, R., Vehmaanperä, J. and Primalco Ltd. Biotec. (2004) Enz. Microb. Technol. 34, 332–341.
[49] Saraswat, V. and Bisaria, V.S. (2000) Biosci. Biotechnol. Biochem. 64, 1173–1180.