Journal of Molecular Catalysis B: Enzymatic 60 (2009) 76–81

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Derivatization of amino acids by fungal laccases: Comparison of enzymatic and chemical methods

Veronika Hahn[a,*], Annett Mikolasch[a], Katrin Manda[b], Dirk Gördes[c], Kerstin Thurow[c], Frieder Schauer[a]

[a] Institut für Mikrobiologie, Ernst-Moritz-Arndt-Universität Greifswald, F.-L.-Jahnstr. 15, 17487 Greifswald, Germany
[b] Universitätsklinikum Rostock AöR, Zentrum für Radiologie, Klinik und Poliklinik für Strahlentherapie, Südring 75, 18059 Rostock, Germany
[c] Center for Life Science Automation Celisca, Friedrich-Barnewitz-Strasse 8, 18119 Rostock, Germany

ARTICLE INFO

Article history:
Received 8 October 2008
Received in revised form 9 January 2009
Accepted 2 April 2009
Available online 11 April 2009

Keywords:
Laccase
Sodium iodate
Quinone
Amino acids
Cross-coupling

ABSTRACT

Derivatization of the unprotected amino acids L-phenylalanine and L-tryptophan can be achieved by laccase-catalyzed cross linking to *para*-dihydroxylated compounds. The use of amino acids in laccase-catalyzed aminations may provide the basis of new adhesives modeled on mussel adhesive proteins. We have used laccases from *Pycnoporus cinnabarinus* and *Myceliophthora thermophila* for the enzymatic derivatization and compared its effectiveness to chemical catalysis by sodium iodate. Both types of catalysis resulted in the formation of mono- or diaminated products, depending on the degree of substitution of the dihydroxylated substances. However there were considerable differences in the courses of the chemically and enzymatically catalyzed reactions. Thus, the laccase-catalyzed reaction of 2,5-dihydroxyacetophenone with L-phenylalanine and L-tryptophan resulted in mono- and diaminated coupling products (yields 40–60%) while no transformation products were recovered from the reaction catalyzed by 6 mM sodium iodate. In this case the laccase-catalyzed derivatization is clearly more efficient than the chemically catalyzed counterpart.

1. Introduction

Laccases [E.C. 1.10.3.2] are enzymes which currently excite great interest for their use in organic synthesis. As polyphenoloxidases laccases are able to oxidize dihydroxylated aromatic substances to reactive radicals, which can undergo non-enzymatic coupling reactions [1–6]. The enzymatic catalyzed reaction of dihydroxylated compounds and amines results in the formation of aminobenzoquinones connected via stable C—N bonds [7–11]. Laccases have been used for the synthesis of novel antibiotics [8,12–14], for compounds with antineoplastic activity [15] and for the derivatization of unprotected amino acids [7,16]. The use of amino acids as coupling partner for laccase-catalyzed aminations might serve as the basis for the synthesis of new adhesives modeled on mussel adhesive proteins [17,18] and such potentially biocompatible adhesives would be of interest for many medical applications. To broaden the spectrum of laccase substrates which might be used in this way and to determine the enzyme's reactivity towards different potential substrates we have used 2,5-dihydroxyacetophenone, 1,4-hydroquinone and 2,3-dimethyl-1,4-hydroquinone as substrates.

For this study we exploited laccases of *Pycnoporus cinnabarinus* and *Myceliophthora thermophila* to catalyze the reaction of these substrates with L-phenylalanine and L-tryptophan to produce mono- or diaminated products. To compare enzymatic and chemical methods we attempted in parallel to catalyze the same reactions with sodium iodate.

2. Experimental

2.1. Chemicals

L-phenylalanine and L-tryptophan were purchased from Serva Feinbiochemica GmbH & Co. (Heidelberg, Germany). 1,4-Hydroquinone and 2,5-dihydroxyacetophenone were from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and 2,3-dimethyl-1,4-hydroquinone was obtained from Arcos Organics (NJ, USA).

2.2. Enzymes

Fungal strain. *Pycnoporus cinnabarinus* SBUG-M 1044 was isolated from an oak tree in northern Germany. This white rot fungus is deposited in the strain collection of the Department of Biology of the University Greifswald (SBUG).

* Corresponding author. Tel.: +49 3834 864225; fax: +49 3834 864202.
E-mail address: veronikahahn@gmx.at (V. Hahn).

1381-1177/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2009.04.002

Cultivation of *Pycnoporus cinnabarinus* SBUG-M 1044.: *P. cinnabarinus* was initially cultivated on malt agar plates that were incubated for 7 days at 30 °C and then kept at 4 °C. The liquid culture was prepared by inoculating a nitrogen-rich medium containing 5 g glucose, 1 g KH_2PO_4, 0.52 g L-asparagine, 0.5 g yeast extract, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 50 mL mineral salt solution and 50 mL $FeSO_4$ solution (0.2 g L^{-1}) with three 1 cm^2 agar culture fragments. The mineral salt solution contained 1 g $Ca(NO_3)_2 \cdot 4H_2O$, 0.06 g $CuSO_4 \cdot 5H_2O$, and 0.04 g $ZnSO_4 \cdot 7H_2O$ per liter modified according to Braun-Lüllemann et al. [19]. Incubation was performed without shaking at 30 °C for 7 days. A uniform inoculum was obtained by homogenization of this culture with an Ultra-Turrax homogenizer T25 (IKA Labortechnik, Staufen, Germany) at 8000 rpm. For the production of the ligninolytic enzyme laccase, 40 mL medium inoculated with 2 mL of the homogenized pre-culture was incubated in 100 mL Erlenmeyer flasks for 7 days with 3,4-dimethoxybenzyl alcohol (10 mM), a known inducer of laccase. Cultures were shaken in a water bath (GFL model 1092, Burgwedel, Germany) at 30 °C and 158 rpm.

Preparation of laccase from *Pycnoporus cinnabarinus* SBUG-M 1044.: Under these culture conditions *P. cinnabarinus* produced laccase as a single extracellular enzyme with an activity of 500 nmol mL^{-1} min^{-1}. The culture medium was filtered through a glass fiber filter in a Büchner funnel to separate the medium from whole cells. The cell-free culture medium was stirred with DEAE-Sephacel (Sigma, Steinheim, Germany) for 1 h and the adsorbed enzymes were eluted from the DEAE-Sephacel with 20 mM sodium acetate buffer (pH 5). The enzyme extract was desalted using a Sephadex G-25 Superfine column (Pharmacia, Freiburg, Germany). This enzyme preparation contains only isoenzymes of laccase, but no other enzymes and was used in 20 mM sodium acetate buffer (SAB) pH 5.0 which is close to its pH optimum [20–22].

Laccase from *Myceliophthora thermophila* (expressed in genetically modified *Aspergillus* sp.) was obtained from Novozymes (Bagsvaerd, Denmark). It was used as received (activity 1000 U g^{-1}; substrate: syringaldazine) in citrate phosphate buffer (CPB, 18 mM citrate, 165 mM phosphate) at its pH optimum of pH 7.0 [21,23].

2.3. Measurement of laccase activity

The activity of laccases was determined spectrophotometrically at 420 nm with ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) as substrate [24] using the method described by Jonas et al. [22]. 1 U is defined as 1 μmol mL^{-1} min^{-1}.

2.4. Experimental procedures

For analytical experiments the educts L-phenylalanine or L-tryptophan (1 mM, 10 mM or 100 mM) and the respective dihydroxylated compound (1 mM) were incubated with laccase (activity 0.5 U). In controls the respective compounds were incubated in SAB or CPB without laccase. For the chemical catalysis we used a final concentration of sodium iodate of 6 mM or 320 mM in distilled water [11,25–27].

2 mL reaction mixtures were incubated with agitation at 200 rpm in 5-mL-brown-glass-bottles at room temperature.

2.5. Analytical HPLC

For routine analysis, the reaction mixtures were analyzed using an HPLC system LC-10AT VP (Shimadzu, Germany) consisting of a FCV-10AL VP pump, SPD-M10A VP diode array detector, and a SCL-10A VP control unit controlled by Class-VP version 6.12 SP5. The separation of the substances was achieved on an endcapped, 5 μm, LiChroCART® 125-4 RP18 column (Merck, Darmstadt, Germany) at a flow rate of 1 mL min^{-1}. A solvent system consisting of methanol (eluent A) and 0.1% phosphoric acid (eluent B), starting from an initial ratio of 10% A and 90% B and reaching 100% methanol within 14 min, was used.

2.6. Isolation of transformation products

For the isolation of **4a**, a RP18 silicagel column (60 mL, 10 g adsorbent material, phenomenex, Strata, Germany) was charged with 8 mL of reaction mixture. Excess L-phenylalanine was eluted with 50 mL citrate phosphate buffer, 20 mL 30% methanol in water and 7 mL 100% methanol. The cross-coupling product was eluted with additional 7 mL 100% methanol. The isolated product was dried by lyophilization prior to mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.

The isolation of **3c**, **4c** and **3d** was as described by Hahn et al. [16].

2.7. Characterization of the products

The products were characterized by liquid chromatography/mass spectrometry (LC/MS). The standard atmospheric pressure ionization (API) mass spectrometry experiments were performed using an Agilent Series 1100 HPLC system and an Agilent 1946C quadrupole mass spectrometer (Waldbronn, Germany). The MS was used with both, atmospheric pressure chemical ionization (APCI) and electrospray ionization (API-ES) sources. HPLC separation was performed on a LiChroCART® 125-4, LiChrosphere® 100 RP-18e column (Merck, Darmstadt, Germany) at 25 °C at a flow rate of 1 mL min^{-1} within a 14-min gradient from 10% to 100% methanol in 0.1% aqueous formic acid. APCI/API-ES MS conditions (positive and negative ion mode) were as follows: nebulizer and drying gas, nitrogen; nebulizer pressure, 30 psig; drying gas flow, 10 L min^{-1}; vaporizer temperature (for APCI), 350 °C; drying gas temperature, 250 °C; capillary voltage, 4 kV; corona current (for APCI), 4 μA.

LC high-resolution mass spectrometry experiments were performed on an Agilent Series 1200 HPLC system and an Agilent 1969A time-of-flight mass spectrometer (Waldbronn, Germany). The TOF-MS conditions (negative and positive ion mode) with a dual sprayer API-ES source were as follows: nebulizer and drying gas, nitrogen; nebulizer pressure, 40 psig; drying gas flow, 10 L min^{-1}; drying gas temperature, 350 °C; capillary voltage, 4 kV; fragmentor voltage, 175 V; skimmer voltage, 60 V; octopole voltage, 250 V; mass reference (*m/z*), 121.05087 and 922.00979 in positive ion mode, and 112.98558 and 1033.98810 in negative ion mode. HPLC separation was performed on a Zorbax Eclipse XDB-C8, 4.6 mm × 50 mm, 3.5 μ, column (Agilent, Germany) at 25 °C at a flow rate of 0.5 mL min^{-1} within a 14-min gradient from 10% to 100% methanol in 0.1% aqueous formic acid.

The nuclear magnetic resonance (NMR) spectra for product **4a** (^1H, ^{13}C NMR, HMBC) was recorded on a Bruker Avance 600 instrument (Karlsruhe, Germany) at 600 MHz in MeOH-d_4. Tetramethylsilane was used as internal standard.

2.7.1. 2-(2-Acetyl-3,6-dioxocyclohexa-1,4-dienylamino)-3-phenylpropionic acid (*3a*)

Yield 29.67%. R_f (HPLC) 11.84 min, UV–vis (MeOH) λ_{max} 204, 277, 445 nm.

MS (LC/MS): *m/z* (relative intensity): API-ES, positive ion mode 314 ($[M+H]^+$, 92), 336 ($[M+Na]^+$, 85), 268 ($[M—CO_2H—H]^+$, 100).

HRMS for **3a**: $[M+Na]^+$ ($C_{17}H_{15}NO_5Na$): calculated: 336.08424; found: 336.08425 (+0.1 ppm).

*2.7.2. 2-(2-Acetyl-3,6-dioxocyclohexa-1,4-dienylamino)-3-(1H-indol-3-yl)-propionic acid (**3b**)*

Yield 38.20%. R_f (HPLC) 11.31 min, UV–vis (MeOH) λ_{max} 216, 279, 447 nm.

MS (LC/MS): *m/z* (relative intensity): API-ES, positive ion mode 391 ($[M+K]^+$, 15), 375 ($[M+Na]^+$, 70), 353 ($[M+H]^+$, 8).

HRMS for **3b**: $[M+H]^+$ ($C_{19}H_{17}N_2O_5$): calculated: 353.11319; found: 353.11335 (+0.4 ppm). $[M+Na]^+$ ($C_{19}H_{16}N_2O_5Na$): calculated: 375.09514; found: 375.09538 (+0.6 ppm).

*2.7.3. 2-(3,6-Dioxocyclohexa-1,4-dienylamino)-3-phenylpropionic acid (**3c**)*

3c was only present in a mixture with **4c**, due to the fast reaction of **3c** to **4c**. Yield for **3c** and **4c** 66.80%. R_f (HPLC) 10.52 min, UV–vis (MeOH) λ_{max} 211, 260, and 477 nm. Structural characterization was previously published by Hahn et al. [16]

*2.7.4. 2-(4,5-Dimethyl-3,6-dioxocyclohexa-1,4-dienylamino)-3-phenylpropionic acid (**3d**)*

Dark red to purple solid. Yield 60.50%. R_f (HPLC) 12.95 min, UV–vis (MeOH) λ_{max} 204, 289, and 477 nm. Structural characterization was previously published by Hahn et al. [16].

*2.7.5. 2-[2-Acetyl-4-(1-carboxy-2-phenylethylamino)-3,6-dioxocyclohexa-1,4-dienylamino]-3-phenylpropionic acid (**4a**)*

Synthesis and isolation as described above. Brown solid. Yield 39.52%. R_f (HPLC) 12.85 min, UV–vis (MeOH) λ_{max} 209, 338, and 479 nm.

MS (LC/MS): *m/z* (relative intensity): API-ES, positive ion mode 975.3 ($[2M+Na]^+$, 35), 499.2 ($[M+Na]^+$, 23), 477 ($[M+H]^+$, 100), 431 ($[M-CO_2H-H]^+$, 20).

HRMS for **4a**: $[M+H]^+$ ($C_{26}H_{25}N_2O_7$): calculated: 477.16562; found: 477.16556 (−0.1 ppm). $[M+Na]^+$ ($C_{26}H_{24}N_2O_7Na$): calculated: 499.14756; found: 499.14727 (−0.6 ppm).

^{1}H NMR (methanol-d_4) δ 7.23 (m, 2H, H-5, H-9), 7.17 (m, 2H, H-5", H-9"), 7.21 (m, 2H, H-6", H-8"), 7.19 (m, 2H, H-6, H-8), 7.15 (dd, *J* = 6.5, 6.6 Hz, 2H, H-7, H-7"), 5.6 (s, 1H, H-5′), 5.55 (dd, *J* = 4.4, 8.3 Hz, 1H, H-2), 4.02 (dd, *J* = 5.0, 6.4 Hz, 1H, H-2"), 3.35 (dd, *J* = −13.8, 4.3 Hz, 1H, H-3), 3.02 (dd, *J* = −13.8, 8.6 Hz, 1H, H-3), 3.23 (dd, *J* = −13.7, 4.6 Hz, 1H, H-3"), 3.07 (dd, *J* = −13.7, 6.9 Hz, 1H, H-3"), 2.47 (s, 3H, H-8′).

^{13}C NMR (methanol-d_4) δ 130.7 (C-5, C-9); 130.7 (C-5", C-9"); 129.4 (C-6", C-8"); 129.4 (C-6, C-8); 127.7 (C-7, C-7"), 98.2 (C-5′), 64.6 (C-2), 60.2 (C-2"), 41.6 (C-3), 39.0 (C-3"), 32.9 (C-8"), 201.4 (C-7′), 180.2 (C-6′), 177.9 (C-3′), 176.9 (C-1), 176.3 (C-1"), 158.4 (C-1′), 151.3 (C-4′), 138.4 (C-4"), 138.9 (C-4), 107.4 (C-2′).

HMBC ^{1}H–^{13}C correlations H-5, H-9 (C-3, C-7, C-5, C-9); H-5", H-9" (C-3", C-7", C-5", C-9"); H-6", H-8" (C-4", C-6", C-8"); H-6, H-8 (C-4, C-6, C-8); H-7, H-7" (C-5, C-9, C-5", C-9′); H-5′ (C-4′, C-1′, C-3′); H-2 (C-3, C-4, C-1′, C-1); H-2" (C-3", C-4", C-4′, C-1"); H-3 (C-2, C-5, C-9, C-4, C-1); H-3" (C-2", C-5", C-9", C-4", C-1").

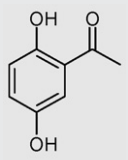
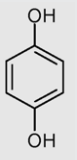
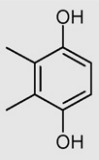
*2.7.6. 2-{2-Acetyl-4-[1-carboxy-2-(1H-indol-3-yl)-ethylamino]-3,6-dioxo-cyclohexa-1,4-dienylamino}-3-(1H-indol-3-yl)-propionic acid (**4b**)*

Yield 37.60%. R_f (HPLC) 12.18 min, UV–vis (MeOH) λ_{max} 218, 282, and 337 nm.

MS (LC/MS): *m/z* (relative intensity): API-ES, positive ion mode 577.1 ($[M+Na]^+$, 100), 555.1 ($[M+H]^+$, 51).

HRMS for **4b**: $[M+H]^+$ ($C_{30}H_{27}N_4O_7$): calculated: 555.18742; found: 555.18689 (−0.9 ppm). $[M+Na]^+$ ($C_{30}H_{26}N_4O_7Na$): calculated: 577.16946; found: 577.16827 (−2.1 ppm). $[2M+Na]^+$ ($C_{60}H_{52}N_8O_{14}Na$): calculated: 1131.34950; found: 1131.34687 (−2.3 ppm).

Table 1
Coupling of aromatic substrates (**1a**—**c**) and educts (**2a**, **2b**) and formation of products (**3a**—**4c**).

	Aromatic substrates		
	 (**1a**)	 (**1b**)	 (**1c**)
L-phenylalanine (2a)	**3a**, **4a**	**3c**, **4c**	**3d**
L-tryptophan (2b)	**3b**, **4b**	–[a]	–[a]

[a] No reaction performed.

*2.7.7. 2-[4-(1-Carboxy-2-phenylethylamino)-3,6-dioxocyclohexa-1,4-dienylamino]-3-phenylpropionic acid (**4c**)*

Red brown solid. Yield 71.30%. R_f (HPLC) 12.95 min, UV–vis (MeOH) λ_{max} 205, 342, and 460 nm. Structural characterization was previously published by Hahn et al. [16].

3. Results and discussion

The unprotected amino acids L-phenylalanine **2a** and L-tryptophan **2b** were subjected to laccase-catalyzed transformation with *p*-hydroquinone and two different substituted *p*-hydroquinones **1a**–**c** (Table 1).

Using 1 mM 2,5-dihydroxyacetophenone (**1a**) and 10 mM concentration of the amino acids (**2a**, **2b**) one monoaminated product per reaction (**3a**, **3b**) was detected after 20 min by HPLC (Fig. 1: A1, A2 and B1, B2). Extending the reaction time up to 24 h yielded one diaminated product (**4a**) in reactions using either of the laccases. Product **4b,** in contrast, was only detected in reactions in which the laccase from *Myceliophthora thermophila* was used. The C–N coupling products, consisting of *p*-quinone and amino acid moieties, were structurally characterized by HPLC using a diode array detector, by MS and NMR resulting in the structures **3a**, **3b** and **4a**, **4b** illustrated in Fig. 2.

The laccase substrate 2,5-dihydroxyacetophenone will be oxidized by laccase. The one-electron oxidation of the dihydroxylated aromatic substrate is accompanied by reduction of molecular oxygen to water by transfer of four electrons. The oxidation of a reducing substrate by laccase typically involves the loss of a single electron and the generation of a free radical [3,5]. The free radicals of the laccase substrate have been described to create new heteromolecular hybrid molecules (Fig. 2). Two molecules of the radical disproportionate to the laccase substrate and a quinone structure, which reacts with the amino acids forming aminated quinone derivatives **3a** and **3b**.

The coupling of **1a** with **2a** and **2b** respectively occurs in *ortho*-position of the $COCH_3$-group of **1a**, as described by Manda et al. [9] and Niedermeyer et al. [10] for the reactions of *para*-dihydroxylated benzoic acid derivatives with 4-aminobenzoic acid. Prolonged incubation and variation of the incubation conditions can lead to oligomerization reactions. After the formation of the monoaminated products **3a** and **3b** a second molecule of the amino partner may react *para* to the first amine group resulting in diaminated products (**4a**, **4b**) as described for other diaminations [10,28,29].

To determine the influence of the amount of amino partner on the product yields, we varied the concentration of L-phenylalanine (**2a**) (Fig. 3). The highest product concentration 0.33 mg mL^{-1} was

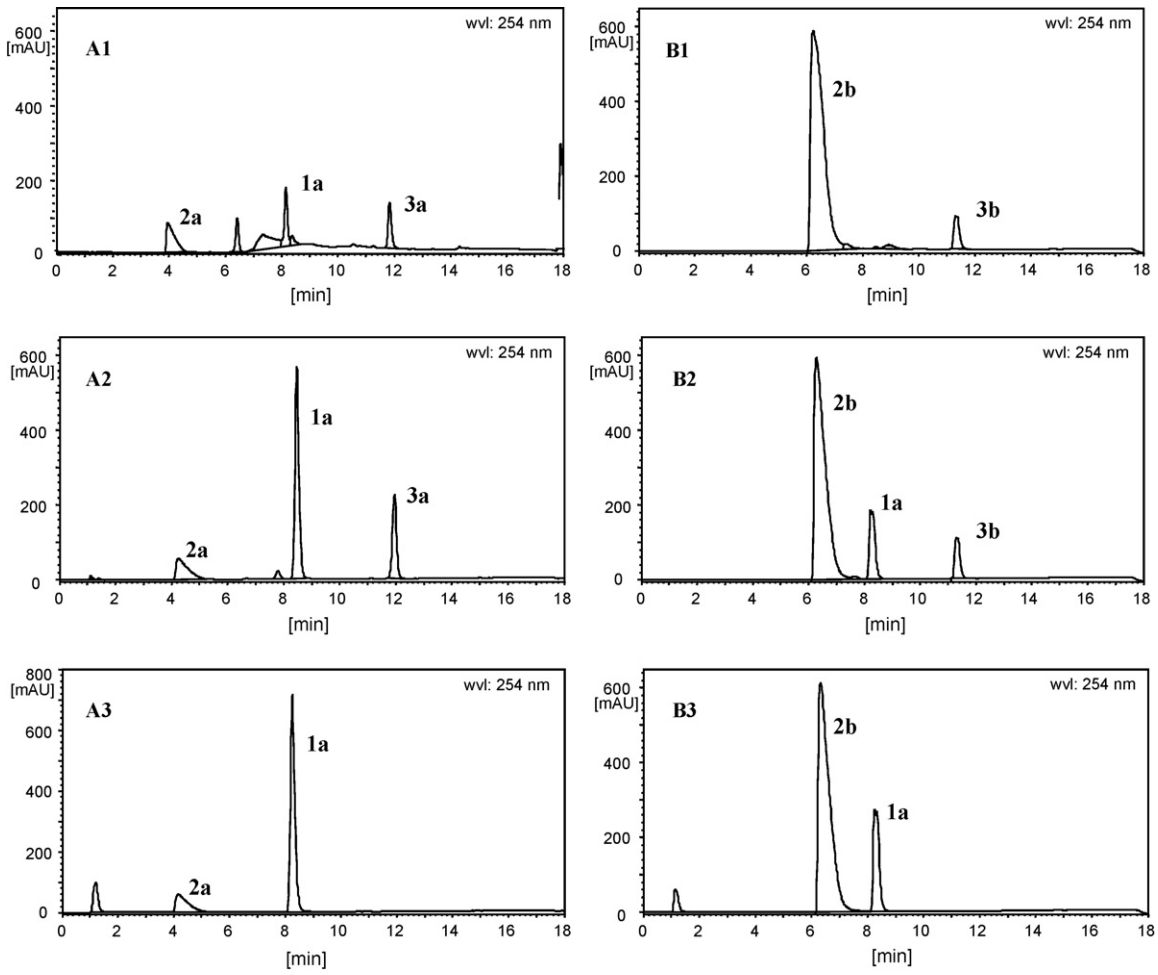


Fig. 1. HPLC chromatograms of the reaction of 2,5-dihydroxyacetophenone (**1a**) with (A) L-phenylalanine (**2a**) (injection volume: 20 μL) and (B) L-tryptophan (**2b**) (injection volume: 10 μL) respectively (1:10 mM) catalyzed by (1) *Pycnoporus cinnabarinus* laccase after 20 min reaction time; (2) *Myceliophthora thermophila* laccase after 20 min reaction time; (3) 6 mM sodium iodate after 120 min reaction time.

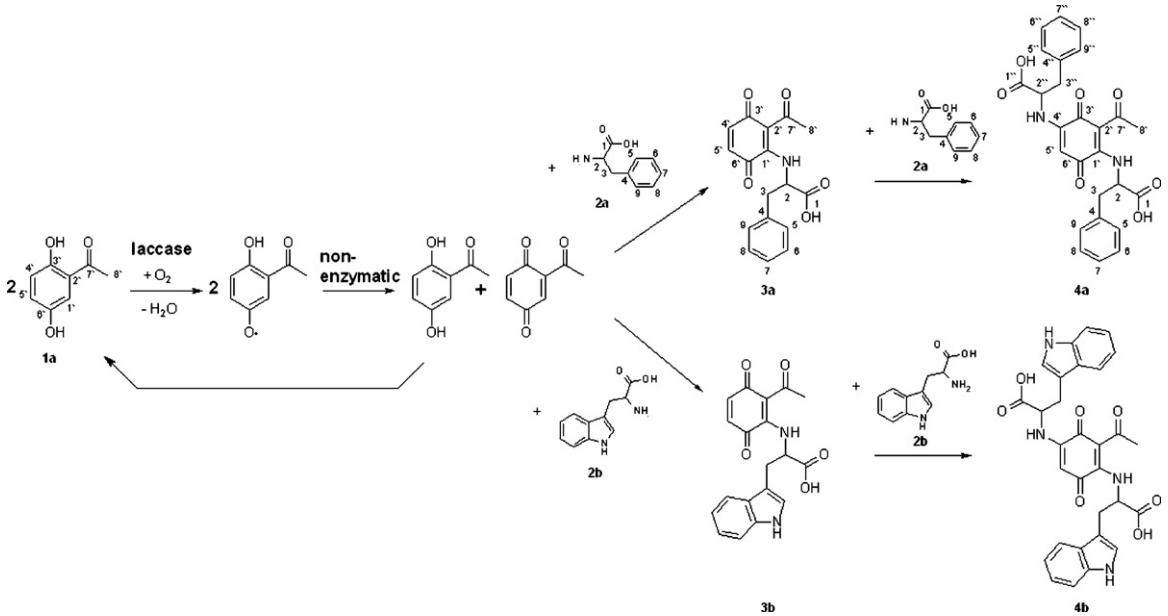


Fig. 2. 2,5-Dihydroxyacetophenone (substrate **1a**) with L-phenylalanine (**2a**) and L-tryptophan (**2b**) and the products (**3a, 3b**), (**4a, 4b**).

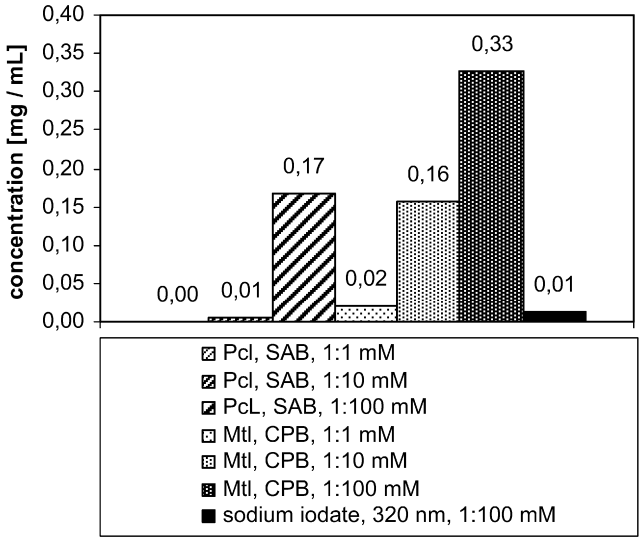


Fig. 3. Comparison of the product formation of **4a** for the reaction of 2,5-dihydroxyacetophenone (**1a**) and L-phenylalanine (**2a**) with different educt concentrations (1:1 mM; 1:10 mM; 1:100 mM) and catalysts. **Pcl SAB** reactions catalyzed by *Pycnoporus cinnabarinus* laccase in sodium acetate buffer; **Mtl CPB** reactions catalyzed by *Myceliophthora thermophila* laccase in citrate phosphate buffer.

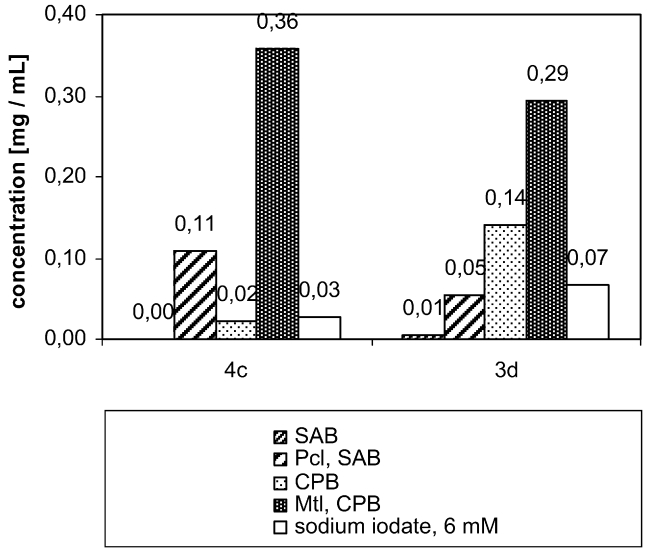


Fig. 4. Comparison of the formation of **4c** and **3d** formed in the 1:100 mM reactions of 1,4-hydroquinone (**1b**) and 2,3-dimethyl-1,4-hydroquinone (**1c**) with L-phenylalanine (**2a**) respectively without a catalyst, catalyzed by laccases or sodium iodate after 24 h.
SAB reactions without a catalyst in sodium acetate buffer; **CPB** reactions without a catalyst in citrate phosphate buffer; **Pcl SAB** reactions catalyzed by *Pycnoporus cinnabarinus* laccase in sodium acetate buffer; **Mtl CPB** reactions catalyzed by *Myceliophthora thermophila* laccase in citrate phosphate buffer.

achieved with the laccase from *M. thermophila* and a ratio of reactants of 1 mM **1a** to 100 mM **2a**. Whereas an educt concentration of 1:10 mM yielded only 0.16 mg mL^{-1}. Product yields are dependent on the amount of amino partner as described before [10,16]. In the case of L-tryptophan (**2b**) a ratio of 1:10 mM was the maximum that could be achieved because of its poor solubility.

In contrast to the enzymatic reaction, chemical catalysis using sodium iodate yielded only the untransformed reactants **1a** and **2a** or **2b** (Fig. 1: A3, B3). Extending the reaction time to 24 h did not lead to any detectable product formation, though increasing the catalyst concentration up to saturating levels (320 mM) did result in the detection of small amounts of the products **3a**, **3b** and **4a** (Fig. 3).

The laccase-catalyzed reaction of unprotected L-phenylalanine with 1,4-hydroquinone (**1b**) and 2,3-dimethyl-1,4-hydroquinone (**1c**) (Table 1) proceeds as described for 2,5-dihydroxyacetophenone (**1a**).

Whereas only one monoaminated product **3d** was formed during the reaction with **1c**, in the reaction mixture with **1b** two products (**3c** monoaminated product, **4c** diaminated product) were detected. A second amination of **3d** is hindered because of the two methyl groups on **1c**.

Aminated products were also detected using sodium iodate (6 mM) as catalyst (Fig. 4). However, the yield of **4c** was 73% higher when *Pycnoporus cinnabarinus* laccase was used and 92% higher with laccase from *Myceliophthora thermophila* than in the reaction catalyzed by sodium iodate. Whereas the yield of **3d** was similar for the *P. cinnabarinus* laccase and sodium iodate catalyzed reactions, the concentration achieved with *M. thermophila* laccase was 76% higher than in the reaction with sodium iodate.

In summary we demonstrate that the reaction of 2,5-dihydroxyacetophenone (**1a**) with L-phenylalanie (**2a**) or L-tryptophan (**2b**) was more efficiently catalyzed by laccase than by sodium iodate whereas with the compounds **1b** or **1c** the advantage of laccase as a catalyst was less pronounced. In contrast to our results Niedermeyer and Lalk [11] reported that laccase-catalyzed amination of simple *p*-hydroquinones with simple primary aromatic amines can be accomplished more conveniently using the well-known chemical oxidant sodium iodate. This underscores the important point that for each reaction undertaken the optimal catalyst and reaction conditions must be sought.

The laccase-catalyzed reaction can be optimized by variation of the concentration of the amino reaction partner, by variation of the pH value of the used buffer and the amount of the enzyme. Higher enzymatic activity of the laccase results in a faster reaction and thereby higher product yields can be obtained. Furthermore both the amount of enzyme and the type of laccase are variable. More than 60 laccases isolated from plants, insects, bacteria and fungi are conceivable [3] so that different types of laccase can be used to perform reactions, resulting in different product pattern.

In respect for the advantage of laccase it is worth noting that the laccase process is a catalytic cycle [30] (Fig. 2), whereas sodium iodate is needed in stoichometric amounts that means the chemical catalyst is consumed during reaction time. The interest in the use of laccases for derivatization of amino acids is mainly stimulated by the simple one-step coupling reaction and the gentle transformation of sensitive starting materials using environmental friendly conditions. The high stability of laccases in solution, the mild reaction conditions used in laccase-catalyzed reactions and the avoidance of toxic solvents, additives or catalysts make laccases attractive for derivatization of natural compounds e.g. amino acids.

4. Conclusions

In the presence of laccases it is possible to derivatize unprotected amino acids like **2a** and **2b**. Whereas with sodium iodate the product formation was very slow and resulted in little amount of products, the product formation with laccase mostly proceeded in 20 min and especially the reactions with the laccase of *M. thermophila* showed comparatively high product yields. There are many ways for the derivatization of compounds and stoichiometric chemical methods are in general not favorable. We could show that the enzymatic catalysis is more efficient than the chemical one for derivatization of amino acids with 2,5–dihydroxyacetophenone.

Acknowledgements

Financial support by means of a scholarship from the government of Mecklenburg-Vorpommern is gratefully acknowledged. We thank M. Lalk (Institute of Pharmacy, University of Greifswald) for providing NMR data and R. Jack (Institute of Immunology, University of Greifswald) for help in preparing the manuscript.

References

[1] J.M. Bollag, in: H. Sigel, A. Sigel (Eds.), Metal Ions in Biological Systems, 28 ed., Marcel Dekker Inc., New York, 1992, pp. 205–217.
[2] C.F. Thurston, Microbiology 140 (1994) 19–26.
[3] L. Gianfreda, F. Xu, J.M. Bollag, Bioremediat. J. 3 (1999) 1–25.
[4] S.G. Burton, Curr. Org. Chem. 7 (2003) 1317–1331.
[5] H. Claus, Arch. Microbiol. 179 (2003) 145–150.
[6] L.B. Davin, N.G. Lewis, Curr. Opin. Biotech. 16 (2005) 398–406.
[7] K. Manda, E. Hammer, A. Mikolasch, D. Gördes, K. Thurow, F. Schauer, Amino Acids 31 (2006) 409–419.
[8] A. Mikolasch, T.H.J. Niedermeyer, M. Lalk, S. Witt, S. Seefeldt, E. Hammer, F. Schauer, M. Gesell, S. Hessel, W.-D. Jülich, U. Lindequist, Chem. Pharm. Bull. 54 (2006) 632–638.
[9] K. Manda, E. Hammer, A. Mikolasch, T. Niedermeyer, J. Dec, A.D. Jones, A.J. Benesi, F. Schauer, J.M. Bollag, J. Mol. Catal. B-Enzym. 35 (2005) 86–92.
[10] T.H.J. Niedermeyer, A. Mikolasch, M. Lalk, J. Org. Chem. 70 (2005) 2002–2008.
[11] T.H.J. Niedermeyer, M. Lalk, J. Mol. Catal. B-Enzyme 45 (2007) 113–117.
[12] H. Agematu, T. Tsuchida, K. Kominato, N. Shibamoto, T. Yoshioka, H. Nishida, R. Okamoto, T. Shin, S. Murao, J. Antibiot. 46 (1993) 141–148.
[13] A. Mikolasch, T.H.J. Niedermeyer, M. Lalk, S. Witt, S. Seefeldt, E. Hammer, F. Schauer, M. Salazar, S. Hessel, W.-D. Jülich, U. Lindequist, Chem. Pharm. Bull. 55 (2007) 412–416.
[14] A. Mikolasch, A. Matthies, M. Lalk, F. Schauer, Appl. Microbiol. Biotechnol. 80 (2008) 389–397.
[15] D. Poeckel, T.H.J. Niedermeyer, H.T.L. Pham, A. Mikolasch, S. Mundt, U. Lindequist, M. Lalk, O. Werz, J. Med. Chem. 2 (2006) 591–595.
[16] V. Hahn, A. Mikolasch, K. Manda, D. Gördes, K. Thurow, F. Schauer, Amino Acids (2008), doi:10.1007/s00726-008-0154-2.
[17] L.O. Burzio, V.A. Burzio, T. Silva, L.A. Burzio, J. Pardo, Curr. Opin. Biotechnol. 8 (1997) 309–312.
[18] L.A. Burzio, J.H. Waite, Biochemistry 39 (2000) 11147–11153.
[19] A. Braun-Lüllemann, A. Majcherczyk, A. Hüttermann, Appl. Microbiol. Biotechnol. 47 (1997) 150–155.
[20] C. Eggert, U. Temp, K.E.L. Eriksson, Appl. Environ. Microbiol. 62 (1996) 1151–1158.
[21] B.B. Feng, M. Voehler, L. Zhou, M. Passarelli, C.M. Harris, T.M. Harris, M.P. Stone, Biochemistry 35 (1996) 7316–7329.
[22] U. Jonas, E. Hammer, F. Schauer, J.M. Bollag, Biodegradation 8 (1998) 321–328.
[23] R.M. Berka, P. Schneider, E.J. Golightly, S.H. Brown, M. Madden, K.M. Brown, T. Halkier, K. Mondorf, F. Xu, Appl. Environ. Microbiol. 63 (1997) 3151–3157.
[24] R. Bourbonnais, M.G. Paice, FEBS Lett 267 (1990) 99–102.
[25] W. Schaefer, A. Aguado, Angew. Chem. Internat. Edit. 10 (1971) 405–406.
[26] M. Pardo, K. Joos, W. Schäfer, Liebigs Ann. Chem. (1979) 503–521.
[27] T. Torres, S.V. Eswaran, W. Schäfer, J. Heterocyclic Chem. 22 (1985) 697–699.
[28] T. Yamaoka, S. Nagakura, Bull. Chem. Soc. Jpn. 44 (1971) 2971–2975.
[29] S. Berger, P. Hertl, A Rieker, Physical and chemical analysis of quinones, in: S. Patai, Z. Rappoport (Eds.), The chemistry of the Quinoid Compounds, 2 ed., John Wiley and Sons Ltd, New York, 1988, pp. 29–86.
[30] S. Riva, Trends Biotechnol. 24 (2006) 219–226.