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Electro-oxidation of synthetic lignin with linoleic acid and co-catalyst

Bing Xie^{1,2}, Yuki Tobimatsu² and Toshiyuki Takano^{1*}

Abstract

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Linoleic acid (LA) was investigated as an electron transfer catalyst in biomimetic lignin degradation with an electrolytic mediator system (EMS). Initial attempts of electro-oxidations of synthetic lignin, guaiacyl (G)-type dehydrogenation polymer (G-DHP), with LA alone at 0.8 V and 1.8 V, resulted in limited G-DHP conversion, suggesting that LAmediated EMS is not effective when LA was used alone. Next, electro-oxidations of G-DHP with LA with O_2 -sparging as well as in the presence of co-catalysts, such as *p*-coumaric acid (PCA), tris(2,2'-bipyridine) iron(II) [Fe(bpy)₃], and 1-hydroxybenzotriazole (HBT) were performed. The combined use of oxygen and co-catalysts appeared to be effective to promote G-DHP conversion. In particular, G-DHP conversion proceeded effectively in the electro-oxidation with LA/HBT at 0.6 V, as demonstrated by structural analysis of the electrolysis residues using nuclear magnetic resonance. HBT was also found to be a vital mediator when used alone in the present system. Overall, the combined use of oxygen and co-catalysts was essential for the EMS oxidation with LA, as reported in laccase mediator system (LMS) oxidation with LA.

Keywords Electrolytic mediator system, Electro-oxidation, Electron transfer catalyst, DHP, Lignin

Introduction

Lignin, which is derived primarily from methoxylated p-hydroxycinnamyl alcohol (monolignol) building blocks, is the second most abundant natural polymer and could be regarded as the largest source of aromatic chemicals to replace fossil resources [1–4]. However, the present industrial use of lignin is limited mostly to thermal recycling by combustion of black liquor from Kraft pulping, and thereby lignin is vastly underutilized as a material. One of the important approaches to value-added lignin utilization is the conversion of lignin into useful low-molecular-weight aromatic compounds [5–8]. In particular, lignin degradation under mild conditions is strongly

desirable. In nature, white-rot fungi effectively degrade lignin by employing oxidative enzymes such as lignin peroxidase, manganese peroxidase (MnP), versatile peroxidase, and laccase [9-12]. Efficient biodegradation of lignin by these oxidative enzymes relies on indirect oxidation via an electron transfer carrier with low molecular weight (for example, "redox mediator"), without the direct contact of an enzyme with the lignin substrate.

The laccase mediator system (LMS) has been studied for many years as a biocatalytic reaction system mimicking the lignin biodegradation reaction by white-rot fungi [13–18]. Several mediators such as *N*-hydroxyphthalimide (NHPI), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and 1-hydroxybenzotriazole (HBT) have been well studied in LMS. An alternative biomimetic lignin degradation system is the MnP/lipid peroxidation system (MLS) [19–29]. In MLS, unsaturated fatty acids such as linoleic acid (LA) serve as electron transfer carriers to facilitate lignin oxidation by MnP. Previous studies have shown that MLS can be effectively used for the degradation of non-phenolic lignin model compounds



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[23–29] and also for wood pulp bleaching [30]. In LAmediated MLS, LA radical is considered as an oxidant rather than a redox catalyst, because LA radical can be degraded to various products during MLS reactions [31]. Further, peroxidation of unsaturated fatty acids has also been performed in LMS using laccase mediators such as p-hydroxycinnamic acid (PCA) [32], HBT [33, 34] and 4-hydroxybenzoic acid [35]. LMS and MLS have been applied not only to lignin degradation, but also to the degradation of recalcitrant aromatic compounds such as polycyclic aromatic hydrocarbons [36, 37] and natural rubber [38–40]. However, LMS and MLS are still not practical processes for industrial applications mainly due to the use of costly enzymes and limitations of the reaction conditions.

EMS (electrolytic mediator system) is an alternative biomimetic reaction system that may circumvent the intrinsic problems derived from the sensitivity of the enzymes (pH, temperature, inhibitors and so on) in LMS and MLS [41–44]. The mediator is one of the important reaction factors to influence the reaction efficiency and selectivity in EMS. Various mediators that had been studied in LMS have been studied in EMS [45–53], but the knowledge of mediators in EMS is still insufficient. In particular, there is no knowledge concerning EMS with unsaturated fatty acids although unsaturated fatty acids are also promising candidates as electron transfer catalysts in EMS, because they acted efficiently in LMS and MLS as described above.

In this study, we investigated EMS using a fatty acid as an oxidant. In particular, we used LA as an electron transfer catalyst because it is the predominant unsaturated fatty acid produced by wood-rot fungi [54]. Electro-oxidations of synthetic lignin, guaiacyl (G)-type dehydrogenation polymer (G-DHP), with LA alone and in combination with a co-catalyst for LA oxidation, such as PCA, tris(2,2'-bipyridine) iron(II) [Fe(bpy)₃] perchlorate and HBT were investigated.

Materials and methods

Materials

The LA and co-catalysts used in the current study are shown in Fig. 1. LA, PCA and HBT were purchased from Nacalai Tesque Inc. (Kyoto, Japan) or Tokyo Chemical Industry Co. (Tokyo, Japan). Fe(bpy)₃(ClO_4)₂ was synthesized as previously described [52]. G-DHP was prepared using horseradish peroxidase-catalyzed dehydrogenative polymerization of coniferyl alcohol as described previously [55]. Other chemicals used in this study were purchased from commercially available sources and used without further purification.



Fig. 1 Chemical structures of linoleic acid and co-catalysts

Cyclic voltammetry

Cyclic voltammetry (CV) measurements of LA, cocatalysts (PCA, Fe(bpy)₃, and HBT), and their mixtures [LA/PCA, LA/Fe(bpy)₃, LA/HBT (5/1, mol/mol)] were performed with an ALS electrochemical analyzer (ALS 600E, BAS, Tokyo, Japan) at room temperature using an undivided cell with a platinum disk (1.6 mm in diameter) working electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode in 0.1 mol/L LiClO₄ in 70% CH₃CN/H₂O (v/v) as an electrolyte at a sweep rate of 50 mV/s.

Bulk electrolysis of G-DHP

Bulk electrolysis of G-DHP at fixed potential was performed in the anode chamber of a divided cell on the ALS electrochemical analyzer equipped with a power booster (ALS 680C, BAS, Tokyo, Japan). Other conditions were as follows. In the anode chamber, the working electrode was a carbon felt $(2.0 \times 3.0 \text{ cm}^2; \text{ thickness: } 0.3 \text{ cm})$ electrode, the reference electrode was an Ag/AgCl reference electrode and the anolyte was 0.1 mol/L LiClO₄ in 70% CH₃CN/H₂O. In the cathode chamber, the counter electrode was a platinum wire electrode and the catholyte was 0.1 mol/L LiClO₄ in 70% CH₃CN/H₂O. Briefly, G-DHP (37.5 mg/15 mmol) was electro-oxidized with the electron transfer catalyst [LA alone: 5 mmol or LA/cocatalyst (PCA, Fe(bpy)₃, HBT): 5 mmol/1 mmol or HBT alone: 5 mmol] at a fixed potential (Table 1) in 0.1 mol/L $LiClO_4$ in 70% CH₃CN/H₂O (10 mL) in the anode chamber. In some electro-oxidations of G-DHP, O2-sparging with 93% O₂ purity was performed using a BOSWELL oxygen generator (KJP-11, Anhui Kangjuren Intelligence Science & Technology Co., Hefei, China). Bulk electrolysis of G-DHP was performed for 6 h, and the slightly cloudy reaction solution in the anode chamber was concentrated in vacuo, and resultant suspension was washed with CH₂Cl₂ (35 mL, three times). The resulting precipitate was collected by filtration, washed with distilled

Entry	Mediators		02 ^{b)}	Applied potential	Products	yield A ^{c)}	yield B ^{d)}	Nomä subst	alized : ructur	signal i es ^{e)}	ntensi	ties of I	lignin		M _n (× 10 ³)	$M_{\rm w}$ ($ imes$ 10 ³)	M _w /M _n
		(MM)		ŝ		(wt%)	(wt%)	_¤	Πa	Πa	×	X'	Χ" _α	", "			
0	G-DHP (before EMS)	I	I	I		72.5	100	11	19	1	24	m	I	I	2.65	10.62	4.0
	LA	5	I	0.8	LA-0.8	78.2	108	10	14	13	18	£	I	I	3.75	8.28	2.2
2	LA	5	I	1.8	LA-1.8	53.6	74	4	10	10	10	2		I	3.66	10.62	4.1
e	LA	5	+	0.8	$LA-0.8O_2$	33.9	47	10	6	7	16	2	I	I	2.19	11.91	5.4
4	LA	5	+	1.8	LA-1.80 ₂	53.3	74	7	10	9	7	, -	-	I	3.51	13.47	3.8
5	I	0	+	0.8	control-0.80 ₂	71.5	66	11	1	2	22	2	, -	I	2.76	12.38	4.5
9	I	0	+	1.8	control-1.80 ₂	45.3	62	5	, -	7	, -	2		I	3.00	11.58	3.9
7	LA/PCA	5/1	T	0.8	LA/PCA-0.8	59.5	82	13	14	9	17	2	-	I	3.27	9.12	2.8
8	LA/Fe(bpy) ₃	5/1	I	1.2	LA/Fe-1.2	41.6	57	15	16	8	16	£		I	3.08	10.38	3.4
6	LA/HBT	5/1	I	0.6	LA/HBT-0.6	27.2	36	9	8	6	16	2	2	m	1.29	9.55	7.4
10	LA/HBT	5/1	+	0.6	LA/HBT-0.6O ₂	62.9	87	9	13		10	4	I	I	1.55	12.27	7.9
11	PCA	5	Т	0.8	PCA-0.8	64.5	89	10	14	6	18	2	2	I	3.24	9.1	2.8
12 ^{f)}	Fe(bpy) ₃	5	I	1.2	Fe-1.2	47.8	66	5	I	I	I	I	4	I	1.30	6.98	5.4
13	HBT	5	I	0.6	HBT-0.6	42.1	58	9	12	6	11	7	2	I	1.74	9.52	5.5
14	HBT	5	+	0.6	HBT-0.60 ₂	64.3	89	5	11	12		, -			1.67	13.47	8.1
^a Other e	lectro-oxidation conditior	IS: G-DHP (3	1.5 mg/	15mL), anolyte: 0	1.1 mol/L LiClO4 in 70	%-CH ₃ CN/H ₂ C), at room temp	arature,	for 6 h								

Table 1 Reaction conditions of the electro-oxidations and chracterization of the electrolysis residues^{a)}

^b +: O₂-sparging

 $^{\rm c}$ Based on the starting G-DHP

 $^{\rm d}$ Base on the G-DHP after $\rm CH_2 Cl_2$ extraction

^e The signal intensitities were normalized based on the intensity of methoxy groups (OMe) signals

^f Data cited reference [<mark>52</mark>]

water (30 mL, twice) and then freeze-dried to give an electrolysis residue as a light brown solid (product names and yields A based on the starting G-DHP are listed in Table 1). The bulk electrolysis was performed twice.

As a control experiment, G-DHP (37.5 mg/15 mmol) was dissolved in in 0.1 mol/L LiClO₄ in 70% CH₃CN/H₂O (10 mL), and recovered by the same work-up method (concentration, washing with CH₂Cl₂, and filtration with distilled water, freeze-drying) to give G-DHP (27.2 mg, 72.5% yield) [51]. The yields of the bulk electrolysis residue base on the G-DHP after the work-up method (yields B in Table 1) were also determined and used in "Results and discussion" part.

Nuclear magneic resonance (NMR) spectroscopy

The two-dimensional (2D) heteronuclear single quantum coherence (HSQC) NMR spectra were recorded on a Varian FT-NMR (500 MHz) spectrometer (Agilent Technologies, Santa Clara, CA, USA) interfaced with the Varian VnmrJ 3.2 software. G-DHP and its electrolysis residues were dissolved in DMSO- d_6 /pyridine- d_5 (4/1) (v/v). Adiabatic HSQC experiments were performed using the Varian standard implementation "HSQCAD", and the experimental parameters were set by referring to previous studies [acquired from 10 to 0 ppm in F2 (¹H) with F2 acquisition time 200 ms, and 200 to 0 ppm in F1 (^{13}C) with F1 acquisition time 8 ms and typically with 180 scans with a 1.5-s interscan delay [55, 56]. Data processing was performed using the Bruker Topspin 4.1 (Mac) software (Bruker, Biospin, Billerica, MA, USA) with typical matched Gaussian apodization in F2 (LB = -0.5, GB=0.001) and squared cosine-bell and one level of linear prediction (32 coefficients) in F1. For volume integration, linear prediction was turned off and no correction factors were used; therefore, the reported data represent volume integrals only. For the analysis of the inter-monomeric linkage types, the well-resolved $C\alpha$ -H α signals from β -O-4 (**I**_{α}), β -5 (**II**_{α}) and β - β (**III**_{α}) substructures were integrated and C_{α} signals were logically halved. For the analysis of the aromatic unit signals, C2–H2 signals from non-oxidized G units (G_2) and oxidized G units $(\mathbf{G'}_2)$ were integrated. For the analysis of the end-unit signals, Cy–Hy signals from the coniferyl alcohol (X_y) and coniferyl aldehyde (X'_{γ}) end-units and C α -H α signals from the benzaldehyde end-units (X''_{a}) were integrated and X_v signals were logically halved [51, 52].

Gel permeation chromatography (GPC)

The electrolysis residues and original G-DHP were acetylated with Ac_2O /pyridine at room temperature overnight before GPC measurements for molecular weight determination. GPC analysis was performed on a Shimadzu LC-10 system (Shimadzu) equipped with a Shimadzu UV–visible detector (SPD-10Avp) under the following conditions: columns: Shodex K-802, K-802.5 and K-805 connected in series (Showa Denko K. K., Tokyo, Japan); column temperature: 40 °C; eluent: CHCl₃; flow rate: 1.0 mL/min; sample detection: 280 nm; standards: polystyrene (Shodex, Showa Denko K. K., Tokyo, Japan).

Gas chromatography-Mass spectroscopy (GC-MS)

After the reaction of bulk electrolysis with LA/HBT (entry 9 in Table 1) was completed, acetovanillone was added to the reaction mixture as an internal standard. Following work-up procedures as described above, an aliquot of the CH₂Cl₂-soluble fraction was derivatized in N,O-bis(trimethylsilyl)acetamide and subjected to GC-MS analysis on a Shimadzu GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) with the following conditions: column, Agilent DB-5MS-0.25 (30 m×0.25 mm, Agilent); carrier gas, He; injection temperature, 200 °C; oven temperature, 60 °C at t=0, then ramped to 100 °C at 50 °C min⁻¹, then ramped to 220 °C at 9.5 °C min⁻¹, and held at 220 °C for 1 min; ion source temperature, 250 °C; interface temperature, 250 °C; ionization, electronimpact mode (70 eV); MS detection by scan (total ion chromatogram, TIC) mode at m/z = 50-500 and selective ion mode (SIM) at m/z=207 (HBT), 224 (vanillin), 238 (acetovanillone, internal standard), 312 (vanillic acid), and 337 (LA). For the quantification of each compound, calibration curves, based on the relative SIM signal intensities of each compound compared to the acetovanillone internal standard, were constructed using authentic standards.

Results and discussion

Cyclic voltammetry of LA and co-mediators

CV is a powerful and popular electrochemical technique commonly employed to investigate the oxidation and reduction processes of various molecular species [57]. It can also be easily applied to determine the applied potential and to evaluate the catalytic efficiency of the mediator for oxidizing lignin model compounds. On the other hand, it is not suitable for the determination of an oxidation potential at more than 1.0 V in a common aqueous electrolyte due to the limitation of the potential window of the aqueous electrolyte [58]. Figure 2a shows the cyclic voltammogram of LA alone from 0 V to 2.0 V in 0.1 mol/L LiClO₄ in 70% CH₃CN/H₂O, in which no clear oxidation/reduction peaks were observed. This result suggested that LA was not easily converted to LA radical in the present electrolyte. The applied potentials of the bulk electrolysis with LA alone were chosen as 0.8 V and 1.8 V. The former, corresponding to the maximum oxidation potential of laccase [14], was chosen because LA peroxidation has been reported in LMS [29-32], and the



Fig. 2 Cyclic voltammograms of the mediators (black line) and LA/mediator mixture (5/1) (red line) in 0.1 mol LiClO₄ in 70%-CH₃CN/H₂O (scan rate: 50 mV/s)

latter was based on the oxidation potential of LA alone in CH₃CN (1.76 V (vs SCE)) [59]. Figure 2b–c shows the cyclic voltammograms of PCA, HBT and their LA mixtures from 0 V to 1.0 V in 0.1 mol/L LiClO₄ in 70% CH₃CN/H₂O.

The peak patterns of the voltammograms of the LA mixtures were similar to those of co-catalysts alone. On the other hand, the cyclic voltammogram of $Fe(bpy)_3$ in the electrolyte has been reported in the previous paper [52], the peak pattern of the voltammogram of LA/ $Fe(bpy)_3$ was also similar to that of $Fe(bpy)_3$ alone (data not shown). Then, the applied potentials of the bulk electrolysis with the LA mixtures were selected 0.8 V, 1.2 V, and 0.6 V, which corresponds to the oxidation potentials of the co-catalysts, PCA, $Fe(bpy)_3$, and HBT, respectively.

Electro-oxidations of G-DHP with LA alone

First, electro-oxidation of G-DHP with LA alone at 0.8 V was performed. The polymeric electrolysis residue (LA-0.8) was obtained in 108% yield (entry 1, Table 1). The number averaged molecular weight (M_n) of LA-0.8 as determined by GPC was higher than that of the original G-DHP. These results suggested recondensation of G-DHP during the electrolysis. The chemical structure of LA-0.8 was comparatively characterized with original

G-DHP by the HSQC NMR. Despite slight decreases in the signal intensities of β -5 units (**II** α) and coniferyl alcohol end-units (**X** γ), the HSQC NMR spectra of the electrolysis residue LA-0.8 and the original G-DHP remained largely unchanged (Fig. 3). This suggests that G-DHP conversion during the electro-oxidation with LA at 0.8 V proceeded with minimal reactivity and selectivity for the major inter-monomeric linkages in lignin.

Electro-oxidation of G-DHP with LA at an elevated voltage of 1.8 V produced the electrolysis residue (LA-1.8) in 74% yield (entry 2, Table 1). The M_n of LA-1.8 was similar to that of LA-0.8. The HSQC NMR spectrum of LA-1.8 exhibited further decreased signals from β -5 units (II_{α}) and coniferyl alcohol end-units (X_{γ}) compared to those in the HSQC NMR spectrum of LA-0.8 (Fig. 3 and Table 1). In addition, the aldehyde signals from the benzaldehyde units (X''_{α}) newly appeared in the aldehyde region of the HSQC spectrum of LA-1.8. These results suggested that G-DHP conversion in the electro-oxidation with LA alone at 1.8 V resulted in some preferential loss of β -5 units albeit still with a moderate efficiency.

LA radical may be converted by reaction with oxygen to LA peroxyl radical species, which may more efficiently oxidize lignin in MLS [19, 22]. The supply of oxygen has also been known to facilitate catalytic oxidations of lignin



Fig. 3 HSQC NMR spectra of electrolysis residues from G-DHP. Aliphatic, aldehyde (insert) sub-regions and aromatic sub-regions. Solvent: DMSO- $d_6/$ pyridine- d_5 (4/1)

[41, 60]. Accordingly, electro-oxidations of G-DHP with LA at 0.8 and 1.8 V with O₂-sparging were carried out, which afforded the electrolysis residues, $\mathrm{LA}\text{-}0.8\mathrm{O}_2$ and LA-1.8O₂, in 47% and 74% yields, respectively (entries 3 and 4, Table 1). Direct electro-oxidations of G-DHP without LA at 0.8 and 1.8 V with O₂-sparging were carried out as control experiments, which afforded the electrolysis residues, control-0.8O₂ and control-1.8O₂, in 99% and 62% yields, respectively (entries 5 and 6, Table 1). The yield of the residue LA-0.8O₂ was considerably lower than those of the residues LA-0.8 and control- $0.8O_2$, and the $M_{\rm n}$ of LA-0.8O₂ was lower than that of the original G-DHP. The signal intensities of all the major lignin units $(I_{\alpha}, II_{\alpha}, III_{\alpha} \text{ and } X_{\nu})$ decreased in the HSQC NMR spectrum of the residue LA-0.8O₂ (Fig. 3, Table 1), suggesting that O₂-sparging at 0.8 V facilitated the electro-oxidations of lignin linkages but did not exhibited selectivity for the major lignin inter-monomeric linkages. On the other hand, the yield of the residue LA-1.8O₂ was higher than those of LA-0.8O₂ and control-1.8O₂, and $M_{\rm n}$ of LA- $1.8O_2$ was higher than that of the original G-DHP. These results suggest that the combination of LA and oxygen was ineffective in the electro-oxidation of G-DHP at 1.8 V, differing from its performance at 0.8 V. On the other hand, our data for control-1.8O₂ suggested that the direct electro-oxidation with O₂-sparging at 1.8 V was somewhat effective for G-DHP conversion, warranting further investigation (entry 6, Table 1).

Electro-oxidations of G-DHP with LA in combination with co-catalyst

We also tested a combined use of LA with co-catalysts, i.e., PCA, $Fe(bpy)_3$, and HBT (Fig. 1), in attempts to use them as a co-oxidant for converting LA to LA radical at low applied potentials.

PCA has been reported to mediate LA peroxidation in LMS [32]. Electro-oxidation of G-DHP with LA/PCA at 0.8 V was carried out, and the electrolysis residue LA/ PCA-0.8 was obtained in 82% yield (entry 7, Table 1) which was lower than that of LA-0.8 prepared without PCA (entry 1, Table 1). Electro-oxidation of G-DHP with PCA alone at 0.8 V was also carried out as a control experiment, which produced the electrolysis residue PCA-0.8 in 89% yield (entry 11, Table 1). The $M_{\rm p}$ of LA/ PCA-0.8 was slightly lower than that of LA-0.8, although recondensation of G-DHP was suggested as M_n of LA/ PCA-0.8 was still higher than that of the original G-DHP. These results suggest that PCA may facilitate the formation of reactive LA radical as expected. The intensity of the signals from the β -5 units (II α), β - β units (III α) coniferyl alcohol end-units $(X\gamma)$ decreased somewhat in the HSQC NMR spectrum of LA/PCA-0.8, suggesting that G-DHP conversion proceed with preferential loss of $\beta\text{-}5$ and $\beta\text{-}\beta$ units in the electro-oxidation with LA/PCA at 0.8 V.

Next, we used $Fe(bpy)_3$ as a co-catalyst for the electrooxidation with LA, as it has been reported that Fe(II) ion can initiate LA peroxidation [22, 61] and we also recently found that $Fe(bpy)_3$ could be a promising mediator in lignin EMS [52]. The electro-oxidation of G-DHP with $LA/Fe(bpy)_3$ at 1.2 V produced the electrolysis residue LA/Fe-1.2 in 57% yield (entry 8, Table 1). The yield was lower than those of the residues LA-0.8 and LA-1.8, and also lower than that of Fe-1.2 which was obtained by the electro-oxidation of G-DHP with $Fe(bpy)_3$ at 1.2 V as a control (entry 12, Table 1) [52]. The M_n of LA/Fe-1.2 was lower than those of LA-0.8 and LA-1.8, but higher than that of the original G-DHP. The NMR signal intensities of the β - β units (III_{α}) and coniferry alcohol end-units (X_v) decreased in the HSQC NMR spectrum of LA/Fe-1.2 (entry 8, Table 1) more prominently than those observed in the NMR spectrum of the Fe-1.2 control (entry 12, Table 1). These results suggested that $Fe(bpy)_3$ might also act as an effective co-catalyst for the formation of LA radical (or LA peroxyl radical).

Lastly, we tested the combined use of LA with HBT, which also has been reported to efficiently peroxidize LA in LMS [33, 34]. Electro-oxidation of G-DHP with LA/ HBT at 0.6 V afforded the electrolysis residue LA/HBT-0.6 in 36% yield (entry 9, Table 1). The $M_{\rm p}$ of LA/HBT-0.6 was lower than that of the original G-DHP. The intensities of the HSQC NMR signals from β -O-4 units (I_{α}), β -5 units (II_{α}) and coniferryl alcohol end-units (X_{ν}) largely decreased in the HSQC NMR spectrum of LA/HBT-0.6. Further, the HSQC NMR spectrum of LA/HBT-0.6 displayed signals from oxidized lignin substructures, i.e., the benzaldehyde $(\mathbf{X''}_{\alpha})$ as well as phenylcoumarone $\mathbf{II''}_{\mathbf{v}}$ [62]. These results suggested that G-DHP conversion proceeded with preferential loss of β -O-4 and β -5 units and oxidation of coniferyl alcohol end-units in the electro-oxidation with LA/HBT at 0.6 V.

We also analyzed the CH_2Cl_2 -soluble fraction extracted from the reaction mixtures obtained from the electrooxidation of G-DHP with LA/HBT at 0.6 V (entry 9, Table 1). GC–MS analysis detected vanillin (approximately 1.5%) and vanillic acid (trace amounts, less than 0.5%) as typical degradation products derived from G-type lignin, although yields were low (Figure S1). These findings suggest that C α -C β cleavages, which would yield these monomeric products, are not the dominant pathway in the current electro-oxidation using LA/ HBT. GC–MS analysis also detected HBT and LA with recovery yields of 85.3% and 70.3%, respectively (Figure S1). The relatively high recovery of HBT suggests limited decomposition or side reactions involving HBT during the electro-oxidation. However, the specific role of HBT in the electro-oxidation process remains unclear. It is plausible that HBT primarily acted as a co-mediator, facilitating electron transfer. The decrease in LA concentration during the electro-oxidation suggests that LA may act as an oxidant rather than as a redox catalyst. However, the present data do not reveal whether the recovered LA originated from unreacted starting material or from regenerated LA that functioned as a mediator. Notably, our HSQC NMR analysis of the electrolysis residues detected clear signals from LA residues, indicating that a portion of LA is incorporated into DHP during the electro-oxidation (Fig. 3). Consequently, G-DHP conversion proceeded the most effectively in the electro-oxidation of G-DHP with LA/HBT at 0.6 V. Overall, these data suggested that HBT can act as an efficient co-catalyst for the generation of LA radical, and the LA radical acts as an electro-transfer catalyst for the G-DHP oxidation, as shown in Fig. 4, although Fig. 4 is only one example of the reaction mechanism.

To further promote G-DHP conversion, LA/HBTmediated electro-oxidations of G-DHP with O₂-sparging were carried out, affording the electrolysis residue LA/ HBT-0.6O₂ in 87% yield (entry 10, Table 1). Although M_n of LA/HBT-0.6O₂ was similar to that of LA/HBT-0.6, the yield of LA/HBT-0.6O₂ was significantly higher than that of LA/HBT-0.6. The results suggested that oxygen may unexpectedly inhibit LA/HBT-mediated G-DHP conversion. The HSQC NMR spectrum of the residue LA/ HBT-0.6O₂ showed decreased signal intensities for all major lignin units (I_{α} , II_{α} , III_{α} , and X_{γ}), suggesting that $\rm O_2$ sparging might also alter the reaction selectivity of the LA/HBT-mediated electro-oxidation.

As a control experiment, electro-oxidation of G-DHP at 0.6 V with HBT alone was also performed, which produced the electrolysis residue HBT-0.6 in 58% yield (entry 13, Table 1). The $M_{\rm n}$ of HBT-0.6 was lower than that of the original G-DHP. The intensities of the signals from the β -O-4 units (I_{α}), β -5 units (II_{α}) and coniferyl alcohol end-units (X_v) decreased and coniferyl aldehyde end-units $(X_{\nu'})$ and benzaldehyde end-units $(X_{\alpha''})$ in the HSQC NMR spectrum of HBT-0.6, suggesting that G-DHP conversion proceed with some loss of β-O-4 and β -5 units preferentially in the electro-oxidation with HBT alone at 0.6 V. Overall, these results suggested that HBT was an effective mediator for the electro-oxidation of G-DHP. On the other hand, HBT-mediated electrooxidation of G-DHP at 0.6 V with O2-sparging produced the electrolysis residue HBT-0.6O₂ in 89% yield (entry 14, Table 1). Because this yield was higher than that of the residue HBT-0.6, oxygen may prevent HBT from acting as a mediator in the electro-oxidation as we observed in the LA/HBT-mediated electro-oxidation of G-DHP.

Conclusions

Electro-oxidation of G-DHP with LA alone and with LA in combination with co-catalyst was investigated as a lignin oxidation system mimicking MLS. While electro-oxidation of G-DHP with LA alone proceeded to some extent at both 0.8 V and 1.8 V, LA did not act as an effective oxidant. This might be attributed to inefficient LA



Fig. 4 An example of possible reaction mechanism of LA/HBT-catalyzed electro-oxidation

radical generation under the current conditions. To promote the formation of LA radical, we employed oxygensparging and combined the use of co-catalysts (PCA, Fe(bpy)₃, and HBT) with LA in the electro-oxidation of G-DHP. As expected, these additions enhanced G-DHP conversion, with LA/HBT at 0.6 V being particularly effective. Notably, HBT proved to be a good mediator even when used alone in this system. Although the detailed reaction mechanism remains unclear based on the present data, it is evident that the combined use of oxygen and co-catalysts is crucial for the effective electro-oxidation of G-DHP with LA, similar to its role in LMS oxidation with LA [32-34].

Abbreviations

CV EMS Fe[bpy]3 GC-MS G-DHP GPC HBT LA LMS MLS NMR	Cyclic voltammetry Electrolytic mediator system Tris(2,2'-bipyridine) iron(II) Gas chromatography–mass spectrometry Guaiacyl dehydrogenation polymer Gel permeation chromatograohy 1-Hydroxybenzotriazole Linoleic acid Laccase mediator system Manganese peroxidase/lipid peroxidation system Nuclear magnetic reasonance
NILS	Manganese peroxidase/lipid peroxidation system
	nuclear magnetic reasonance
1 C/1	p nydroxyennanne dela

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Author contributions

BX contributed to all experiments. YT supported BX's experiments. TT (corresponding author) designed this study and wrote this paper with BX.

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Availability data and materials

Not applicable.

Declarations

Competing interests

The authors declare that they have no competing interests.

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