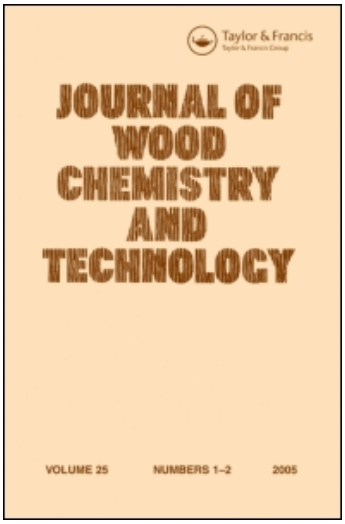


Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Studies on the Dehydrogenative Polymerizations of Monolignol β-glycosides. Part 3: Horseradish Peroxidase-Catalyzed Polymerizations of Triandrin and Isosyringin

Yuki Tobimatsu[a]; Toshiyuki Takano[a]; Hiroshi Kamitakahara[a]; Fumiaki Nakatsubo[a]
[a] Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Japan

To cite this Article Tobimatsu, Yuki , Takano, Toshiyuki , Kamitakahara, Hiroshi and Nakatsubo, Fumiaki(2008) 'Studies on the Dehydrogenative Polymerizations of Monolignol β-glycosides. Part 3: Horseradish Peroxidase-Catalyzed Polymerizations of Triandrin and Isosyringin', Journal of Wood Chemistry and Technology, 28: 2, 69 — 83
To link to this Article: DOI: 10.1080/02773810802124787
URL: http://dx.doi.org/10.1080/02773810802124787

Journal of Wood Chemistry and Technology, 28: 69–83, 2008

ISSN: 0277-3813 print / 1532-2319 online
DOI: 10.1080/02773810802124787

Taylor & Francis
Taylor & Francis Group

Studies on the Dehydrogenative Polymerizations of Monolignol β-glycosides. Part 3: Horseradish Peroxidase–Catalyzed Polymerizations of Triandrin and Isosyringin

Yuki Tobimatsu, Toshiyuki Takano, Hiroshi Kamitakahara, and Fumiaki Nakatsubo
Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Japan

Abstract: Horseradish peroxidase–catalyzed dehydrogenative polymerizations of the *p*-hydroxyphenyl monolignol glucoside (triandrin (**1P**)) and the syringyl monolignol glucoside (isosyringin (**1S**)) resulted in the formation of water-soluble lignin-like polymers (DHPs). The polymerization of **1P** gave highly polymerized DHPs in high yields as did previously reported polymerization of the guaiacyl monolignol glucoside (isoconiferin (**1G**)). It was shown that the hydrophilic D-glucose units of **1G** and **1P** contribute to a marked increase in the molecular weights of the resulting DHPs. On the other hand, the homogeneous phase polymerization of **1S**, similar to the polymerization of sinapyl alcohol, gave DHPs with extremely low molecular masses in poor yields. Structural characterization indicated that the DHPs from **1P** and **1S** were lignin-like polymers containing glucosidic units on their sidechains. It was also confirmed that D-glucosyl units introduced onto the γ-position of monolignols do not significantly affect the electrochemical oxidizability and the kinetics of the HRP-catalyzed initial monomer consumption.

Keywords: Cyclic voltammetry, dehydrogenative polymerization, dehydrogenation polymer (DHP), horseradish peroxidase (HRP), lignin, monolignol β-D-glucoside

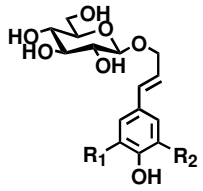
INTRODUCTION

Enzymatic dehydrogenative polymerizations of monolignols *in vitro*, mainly using peroxidase/H_2O_2 as a catalytic system, have been well investigated

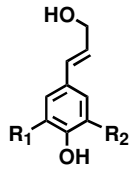
This research was supported by a Grant-in-Aid for Scientific Research (No. 18580162), from the Ministry of Education, Science, Sports and Culture, Japan.

Address correspondence to Yuki Tobimatsu, Division of Forest & Biomaterials Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, 606-8502, Japan. E-mail: tobikko@kais.kyoto-u.ac.jp

as model reactions for lignin biosynthesis and also as preparation methods of lignin model polymers known as dehydrogenation polymers (DHPs).[1–4] In the course of the dehydrogenative polymerizations, γ-hydroxyl groups of monolignols are not involved in radical couplings, although they contribute to the nucleophilic additions to the quinone methide intermediates resulting in the formation of so-called resinol units or tetrahydrofuran units for example. Thus, by the enzymatic polymerizations of monolignol glycosides such as monolignol γ-*O*-β-D-glucosides (isoconiferin (**1G**); triandrin (**1P**);



R_1=H, R_2=OMe: isoconiferin: **1G**
R_1=R_2=OMe: isosyringin: **1S**
R_1=R_2=H: triandrin: **1P**



R_1=H, R_2=OMe: coniferyl alcohol: **2G**
R_1=R_2=OMe: sinapyl alcohol: **2S**
R_1=R_2=H: *p*-coumaryl alcohol: **2P**

Scheme 1. Structures of monolignol glucosides (**1G**, **1P** and **1S**) and monolignols (**2G**, **2P** and **2S**)

isosyringin (**1S**)) (Scheme 1),[5] DHPs consisting of lignin structures as main polymer chains and hydrophilic D-glucose units as pendant units would be obtained, which are even less likely to mimic native lignins. Nevertheless, the effects of the hydrophilicity or chirality of the sugar unit on the reactivity of the monolignols and on the structures of the resulting DHPs have interesting faces of our fundamental knowledge about the monolignol polymerizations *in vitro*.

In the previous article, we demonstrated that horseradish peroxidase (HRP)–catalyzed polymerization of the guaiacyl glucoside **1G** produced a water-soluble DHP in a homogeneous aqueous phase, while the conventional DHP from coniferyl alcohol (**2G**) was obtained as precipitate in a heterogeneous polymerization system. The degree of polymerization (DP) of DHP obtained from **1G** under the optimal conditions was much higher than that of the DHP from **2G**, indicating that the homogeneous polymerization system of **1G** contributed to a increase in the molecular mass of the resulting DHP.[6]

On the other hand, it is well-known that there are significant differences among the dehydrogenative polymerization behaviors of the three monolignols due to the differences in the aromatic structures.[7–11] In this article, HRP-catalyzed polymerizations of the *p*-hydroxyphenyl glucoside **1P** and the syringyl glucoside **1S** were examined to deduce the influence of D-glucosyl units introduced onto the γ-positions of the monolignols on their

polymerization behaviors. Furthermore, redox potentials and HRP-catalyzed oxidation rates of the monolignol glucosides and the monolignols are discussed.

EXPERIMENTAL

Materials

Monolignol glucosides **1G**, **1P**, and **1S** were synthesized as previously reported.[5] Monolignols **2G**, **2P**, and **2S** were prepared according to Quideau and Ralph.[12] HRP (100 U mg^{-1} was purchased from Wako Chemical Co. (Osaka, Japan) and used without further purification. Other chemicals were purchased from Nacalai Tesque Inc. (Kyoto, Japan) and used as received.

Dehydrogenative Polymerizations of Monolignol Glucosides 1P and 1S (Entries 4 and 6 in Table 1)

Three solutions were prepared: Solution A: 0.6 mg of HRP in 30 ml of 10 mM phosphate buffer (pH 6.5); Solution B: 0.5 mmol of the glucoside (**1P** or **1S**) in 120 ml of distilled water; Solution C: 120 ml of 0.019% hydrogen peroxide (0.6 mmol) aqueous solution. Solution B and C were added drop-wise to Solution A over a period of 24 h and after finishing the addition of the solutions another 0.6 mg of HRP was added to the reaction mixture (total amount of HRP: 2.4 mg per 1 mmol monomer). The mixture was kept at room temperature for 24 h, then heated in a boiling water bath for 2 min and lyophilized. The product was purified by gel filtration (gel: Biogel-P2 (Bio-RAD); column dimension: 2.5 × 80 cm; eluent: H_2O), followed by precipitation in ethanol to afford the corresponding DHP (**1P**-DHP from **1P** as a white powder; **1S**-DHP from **1S** as a brown powder).

Dehydrogenative Polymerizations of Monolignols 2P and 2S (Entries 4 and 6 in Table 1)

The polymerization of monolignol **2P** or **2S** was carried out under the same conditions as for **1P**-DHP (or **1S**-DHP) except the solvent for Solution B, in which 2 ml of acetone was mixed because of the poor solubility of the monomer in water. The precipitate of the resulting polymer was collected by centrifugation (12,000 rpm, 10 min), washed twice with distilled water, and lyophilized to obtain the corresponding DHP (**2P**-DHP from **2P** as a white powder; **2S**-DHP from **2S** as a brown powder).

Acetylation of 2P- and 2S-DHPs

2P-DHP (or **2S**-DHP) (9–12 mg) was dissolved in 2 ml of a mixed solvent consisting of acetic anhydride and pyridine (1:1, v/v) containing 4-*N*,*N*-dimethylaminopyridine (Input: half weight of DHP). The reaction mixture was stirred overnight at 50°C and concentrated under vacuum. The product was dissolved in 1 ml of *N*,*N*-dimethylformamide and the solution was added drop-wise into distilled water. Resulting precipitate was collected by centrifugation (12,000 rpm, 10 min), washed twice with distilled water, and dried under vacuum to afford the corresponding acetylated DHP (yield: 102–110%, w/w).

SEC Analysis

Size exclusion chromatography (SEC) analyses of the DHPs were conducted on a Shimadzu LC-10 system equipped with a UV-Vis detector (SPD-10AVP, monitoring at 280 nm) and a refractive index detector (RID-10A). The conditions for SEC analyses of **1P**- and **1S**-DHP were as follows: Columns: TSK gel α-M and α-2500 (Tosoh, Japan); eluent: 0.1 M NaCl aq.; flow rate: 1.0 ml min^{-1}; calibration: polyethylene oxide standards. The conditions for SEC analyses of the acetylated samples of **2P**- and **2S**-DHP were as follows: Columns: K-802, K-802.5, and K-805 (Shodex, Japan); eluent: $CHCl_3$; flow rate: 1.0 ml min^{-1}; calibration: polystyrene standards.

Structural Characterizations of DHPs

The DHPs (prepared based on entries 2, 4, and 6 in Table 1) were subjected to FT-IR and ^{13}C-NMR spectroscopic characterization, alkaline nitrobenzene oxidation, and optical rotation measurements. FT-IR spectroscopy was performed by the KBr pellet method with a Shimadzu 8600 PCs FT-IR spectrophotometer (resolution mode: 4.0; number of scans: 100). ^{13}C-NMR spectra were collected with a Varian INOVA300 FT-NMR spectrometer (75.5 MHz) at 22°C using DMSO-d_6 as solvent, with chemical shifts referenced to TMS (0.0 ppm). Alkaline nitrobenzene oxidations were conducted according to the modified method reported by Katahira and Nakatsubo.[13] Specific optical rotations were measured in H_2O at 25°C using a Jasco DIP 1000 polarimeter.

Cyclic Voltammetry

Cyclic voltammetry was performed on an ALS electrochemical analyzer (Model 650B) under the following conditions: working electrode: a platinum electrode; reference electrode: a saturated calomel electrode (SCE); counter

Table 1. HRP-catalysed polymerizations of monolignol glucosides (**1G**, **1P** and **1S**) and monolignols (**2G**, **2P** and **2S**)

			DHP from monolignol glucosides					DHP from monolignols				
Entry	Monomer addition time (h)	HRP[a] (mg)	Monomer	Yield (%)	M_n $\times 10^{-3}$	M_w/M_n	DP_n^b	Monomer	Yield (%)	M_n^c $\times 10^{-3}$	M_w/M_n^c	DP_n^b
1	0.5	2.4	**1G**	78.0	9.3	1.6	27	**2G**	80.4	2.6	2.1	9.8
2	24	2.4	**1G**	80.5	15	1.3	43	**2G**	77.5	3.0	2.0	11
3	0.5	2.4	**1P**	84.5	5.1	1.6	16	**2P**	88.8	2.4	1.8	10
4	24	2.4	**1P**	86.3	13	1.3	41	**2P**	72.8	2.6	2.2	11
5	0.5	2.4	**1S**	8.5	1.2	1.7	3.2	**2S**	4.2	1.4	2.5	4.8
6	24	2.4	**1S**	22.8	2.5	1.8	6.7	**2S**	20.9	1.9	2.1	6.5
7	24	0.24	**1S**	8.6	1.2	1.6	3.2	—	—	—	—	—
8	24	4.8	**1S**	26.8	2.1	1.3	5.6	—	—	—	—	—
9	48	2.4	**1S**	22.2	1.9	1.3	5.1	—	—	—	—	—

[a] Per 1 mmol monomer
[b] Calculated based on monomer's molecular weight
[c] Determined after acetylation

electrode: a platinum wire electrode; sweep rate 0.1 V s^{-1}; solvent: MeOH/0.1 M phosphate buffer (pH 6.5) (10:90, v/v) containing 0.1 M KCl; sample concentration: 0.03 M.

Measurements of the HRP-Catalyzed Monomer Consumption Rates

A reaction mixture (10 ml) consisting of 0.1 mM monomer and 2.4 μg l^{-1} HRP in 0.1 M phosphate buffer (pH 6.5) was prepared and kept at 25°C. The polymerization was initiated by adding 50 μl of 0.82% H_2O_2 aq. to the reaction solution (final concentration of H_2O_2: 0.12 mM). Reaction mixtures (600 μl) were sequentially sampled and mixed with 300 μl of 6% trifluoroacetic acid in acetonitrile/water (3:7, v/v) and cooled in an ice bath to terminate the reaction.[14] The mixture was subjected to a high-performance liquid chromatography (HPLC) on a Shimadzu LC-10 system equipped with a UV-Vis detector (SPD-10AVP, monitoring at 265 nm) under the following conditions: column: Cosmosil 5C18MS (4.6 × 250 mm, Nacalai Tesque Inc., Japan); eluent and flow rate: water/acetonitrile (90:10, v/v) with 1.0 ml min^{-1} for the polymerizations of monolignol glucosides **1P**, **1G**, and **1S**, water/acetonitrile (88:12, v/v) with 1.2 ml min^{-1} for the polymerizations of monolignols **2P**, **2G**, and **2S**.

RESULTS AND DISCUSSION

Dehydrogenative Polymerization

We previously reported that the HRP-catalyzed polymerization of guaiacyl glucoside **1G** by the so-called bulk (monomer addition time: 0.5 h) and end-wise (monomer addition time: 24 h) polymerization methods[15,16] under the optimal conditions gave DHP (**1G**-DHP) with number average degree of polymerization (DP_n) of 27 and 43, respectively (Table 1, entries 1 and 2).[6] The HRP-catalyzed polymerizations of *p*-hydroxyphenyl glucoside **1P** and syringyl glucoside**1S** by the bulk and end-wise polymerization methods were conducted according to the polymerization conditions for **1G**.

Polymerization of Monolignol Glucoside **1P**

The HRP-catalyzed polymerization of **1P** proceeded in a homogeneous aqueous phase without any precipitate and produced the water-soluble **1P**-DHPs in high yields (entries 3 and 4). The slow addition of monomer contributed to a marked increase in the molecular weight of **1P**-DHP. The polymerization of **1P** with 0.5 h and 24 h of the monomer addition time afforded **1P**-DHPs with DP_n of 16 and 41, respectively. On the other hand, the DP_n of **2P**-DHPs obtained in a conventional heterogeneous system from *p*-coumaryl

alcohol **2P** were only around 10. The results indicated that the introduction of a hydrophilic D-glucosyl unit into the monomer promotes the propagation of the polymer chains in the polymerizations of *p*-hydroxyphenyl-type monomers in an aqueous media, as well as in the polymerizations of guaiacyl-type monomers.

Polymerization of Monolignol Glucoside **1S**

The polymerizations of **1S** also proceeded homogeneously and the water-soluble **1S**-DHPs were obtained (entries 5 and 6). However, the yields of **1S**-DHPs were extremely low. The DP_n of **1S**-DHPs obtained with 0.5 h and 24 h of the monomer addition time were only 3.2 and 6.7. We reported in the previous article that the HRP amount and the monomer addition time significantly influence the yield and DP of DHP in the polymerization of **1G**.[6] Polymerizations of **1S** under other polymerization conditions were carried out. However, no significant increase in the yields and DPs of **1S**-DHP was observed (entries 7–9). The polymerizations of sinapyl alcohol **2S** also gave **2S**-DHPs with low DPs in low yields as observed in earlier reports.[7–10,17] It was found that the behavior of **1S** in the HRP-catalyzed polymerization is entirely the same as that of **2S**, even in a homogeneous polymerization system.

Structural Characterizations of 1P-DHP and 1S-DHP

Figure 1 shows the FT-IR spectra of **1P**- and **1S**-DHPs together with those of the corresponding **2P**- and **2S**-DHPs. In the spectra of **1P**- and **1S**-DHPs, strong bands at 3400–3200 cm^{-1} (hydroxyl groups in D-glucosyl units) and at 1200–1000 cm^{-1} (glucosidic linkage, ring, and C-OH vibrations of D-glucosyl units)[18] can be observed. Characteristic bands from lignin aromatic vibrations at 1000–1600 cm^{-1},[9,19,20], which were observed in the spectra of **2P**- and **2S**-DHPs, were visible, indicating **1P**- and **1S**-DHPs had typical lignin structures as well as **2P**- and **2S**-DHPs.

In the ^{13}C-NMR spectra of **1P**- and **1S**-DHPs (Figure 2), the peaks assigned to glucosyl units (C_1 (g1) at around 101–106 ppm and C_2–C_6 (g2-g6) at 60–80 ppm) and aromatic carbons (at 110–160 ppm) are present. In the lignin side-chain region (40–100 ppm), broad signals, overlapped by strong signals from glucose carbons, appeared at around 50 and 78–90 ppm. Based on NMR data of natural and synthetic lignins[9,21–23] and those of neolignan γ-*O*-β-D-glucosides,[24–29] signals from inter-monomeric structures could be assigned. For **1P**-DHP, the predominant substructure appeared to be of β-5 structure (δ at 85.7 and 49.6 ppm for carbons α and β, respectively). A small contribution from β-*O*-4 structures might be indicated by the shoulder peaks at around 80 ppm (for carbon α). For **1S**-DHP, the predominance of β-β structures was indicated by the signals at 84.3 and 54.7 ppm for carbons α and β, respectively. The β-*O*-4 structures were also indicated by the signal at 80.6 ppm for carbon α In

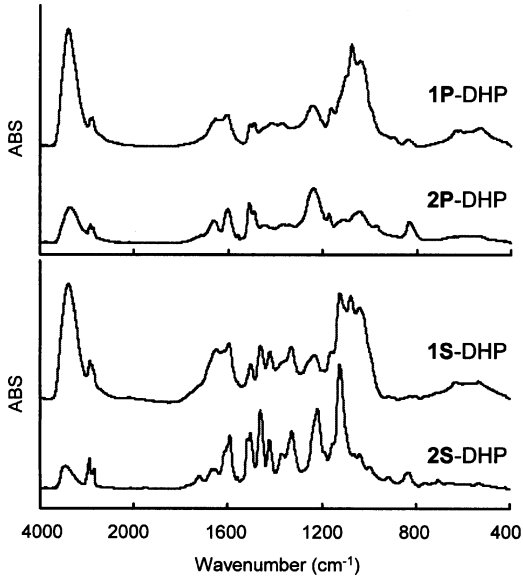


Figure 1. FT-IR spectra of **1P**- and **2P**-DHP (prepared based on entry 4 in Table 1) and **1S**- and **2S**-DHP (prepared based on entry 6 in Table 1).

the spectrum of **1S**-DHP, weak signals were observed in the carbonyl regions (160–200 ppm), suggesting the possible presence of α-carbonyl moieties (194.9 ppm) and quinone structures (176.2 ppm).[23,30]

These spectra confirmed that DHPs from the monolignol glucosides had lignin inter-monomeric structures with D-glucosyl units attached to the lignin sidechains.

Alkaline nitrobenzene oxidation is one of the important methods to characterize lignin structure.[31,32] Alkaline nitrobenzene oxidations of **1G**-, **1P**-, and **1S**-DHPs gave the corresponding *p*-hydroxybenzaldehydes, indicating that **1G**-, **1P**-, and **1S**-DHPs are composed of lignin-like structures. The yields of the aldehydes released per lignin structures from **1G**-, **1P**-, and **1S**-DHPs, however, were found to be lower than those from **2G**-, **2P**-, and **2S**-DHPs (Table 2). These results suggest two possibilities: (1) the levels of condensed structure of the DHPs from monolignol glucosides were higher than those of the conventional DHPs from monolignols; (2) The presence of sugar moiety contributes to a resistance to degradation in alkaline degradations or nitrobenzene oxidations. Further studies on the detailed chemical structures of **1G**-, **1P**-, and **1S**-DHPs are required to clarify this point.

Specific optical rotations of the DHPs are also listed in Table 2. DHPs from monolignol glucosides have negative $[\alpha]_D$ values, which are different from the values of the corresponding monomer glucosides. It is still unknown

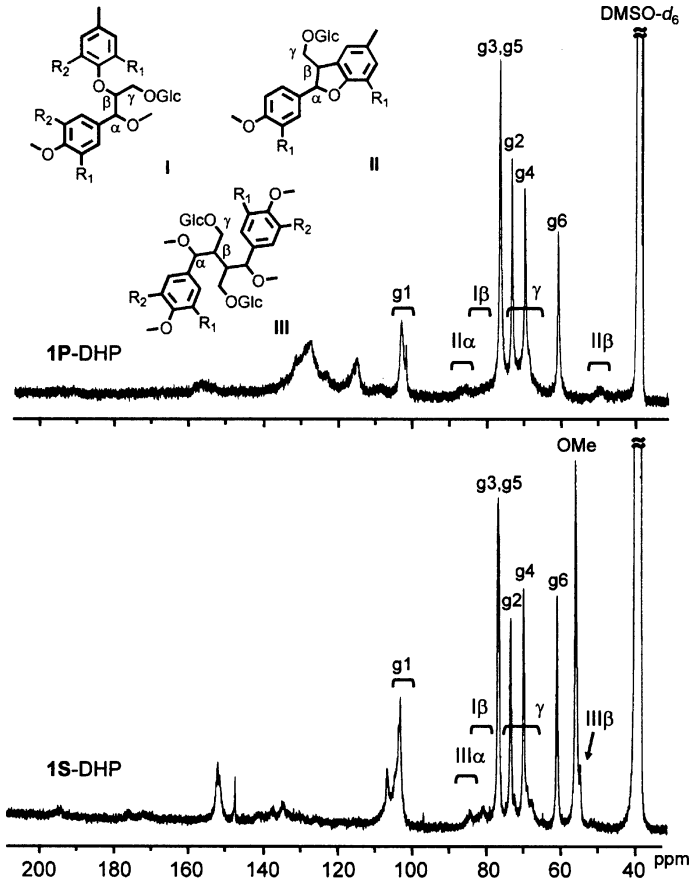


Figure 2. ^{13}C-NMR spectra of **1P**-DHP (prepared based on entry 4 in Table 1) and **1S**-DHP (prepared based on entry 6 in Table 1).

yet whether the phenylpropanoid units are optically active or not, as their optical rotations should be measured after the optically active D-glucosyl units were removed from the DHPs.

Reactivity of Monolignol Glucosides in the Dehydrogenative Polymerizations

The redox potentials and HRP-catalyzed initial polymerization rates of the glucosides were investigated as basic parameters for the dehydrogenative polymerizations.

Table 2. Nitrobenzene oxidation yields and optical rotations of DHPs

Compounds	Nitrobenzene oxidation released aldehyde (μmol/g lignin)	Optical rotations[a] (deg.) Polymer (monomer)
1G-DHP	489[b]	−22.7 (−43.3)
1P-DHP	389[b]	−15.2 (−42.6)
1S-DHP	1445[b]	−14.0 (−22.1)
2G-DHP	860	—
2P-DHP	624	—
2S-DHP	2252	—

[a]In H_2O, c = 0.1.

[b]The proportions of lignin structures were calculated based on monomer structures.

Redox Potentials

We used cyclic voltammetry to evaluate the oxidation potentials of the monomers in a buffer solution. Cyclic voltammograms of the monomers in MeOH/0.1 M phosphate buffer (pH 6.5) (10:90, v/v) are shown in Figure 3. All voltammograms showed one anodic peak but no corresponding cathodic peak. Similar irreversible voltammograms were reported in anodic oxidations of monolignols and their analogues.[11,33,34] These irreversible voltammograms suggest that the polymerizations may occur at the electrode very rapidly with respect to the sweep rate. In this study, the reactivity in the electrochemical oxidations were estimated by the anodic peak potential (E_{pa}).[11] The E_{pa} values of the monolignol glucosides decreased with decreases in methoxyl substitutions, indicating that the order of the reactivities in the electrochemical oxidations is syringyl (**1S**) > guaiacyl (**1G**) > *p*-hydroxyphenyl glucoside (**1P**). The result agreed with the order of the E_{pa} values of the monolignols and can be explained by the electron donating effect of the methoxyl groups. The E_{pa} value of each glucoside is close to that of the corresponding monolignol.

HRP-Catalyzed Monomer Consumption Rates

Figure 4 shows the monomer conversion curves during HRP-catalyzed polymerizations of monolignol glucosides and monolignols. In these experiments, the polymerization reactions were carried out at lower enzyme concentration compared to those for the DHP preparation experiments to detect the monomer consumptions. The HRP-catalyzed oxidation rates of monolignol glucosides are *p*-hydroxyphenyl (**1P**) ≈ guaiacyl (**1G**) >> syringyl glucoside (**1S**). The

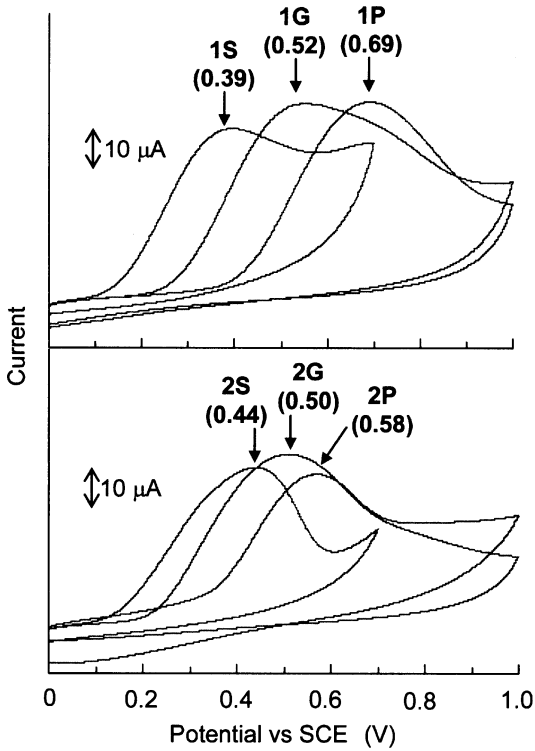


Figure 3. Cyclic voltammograms of monolignol glucosides (**1G**, **1P**, and **1S**) and monolignols (**2G**, **2P,** and **2S**). The values in parentheses indicate the anodic peak potentials (E_{pa}).

result was not consistent with the order of the electrochemical reactivities estimated by cyclic voltammetry. The syringyl glucoside **1S** was a relatively poor HRP substrate as well as sinapyl alcohol **2S**. This could be interpreted by the low accessibility of syringyl-type monomers to HRP due to the steric and hydrophobic effects of two methoxyl groups.[11,35] There were not significant differences in monomer consumption rate between the monolignol glucoside and the corresponding monolignol.

Consequently, it was confirmed that a D-glucosyl unit introduced onto the γ-position does not affect the redox potentials and the kinetics of the HRP-catalyzed oxidation of monolignols. It was previously reported that γ-acylations of monolignols do not affect their HRP-catalyzed coupling reactions much either.[30,36] These observations indicate that the aromatic structures of monolignol analogs fundamentally decide their reactivity in dehydrogenative polymerizations.

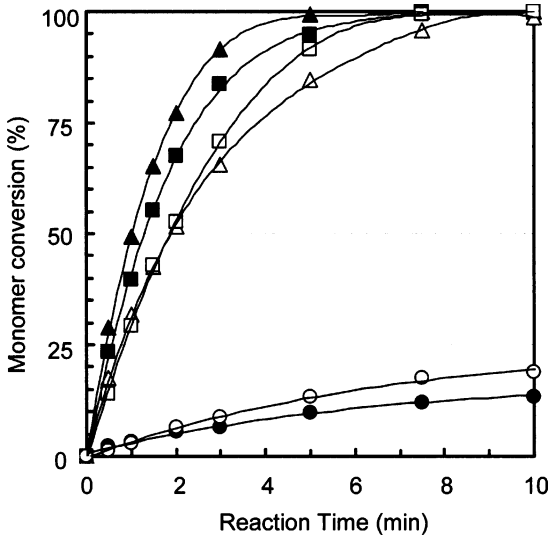


Figure 4. Monomer conversions during HRP-catalyzed polymerizations of monolignol glucosides (**1G** (■); **1P** (▲); **1S** (●)) and monolignols (**2G** (□); **2P** (△); **2S** (○)).

CONCLUSION

HRP-catalyzed dehydrogenative polymerizations of *p*-hydroxyphenyl glucoside **1P** and syringyl glucoside **1S** were carried out, resulting in the production of water-soluble DHPs in a homogeneous aqueous phase, similar to the polymerization of the guaiacyl glucoside **1G**. It was confirmed that the D-glucosyl unit of monolignol glucosides conferred water-solubility to the resulting DHPs, as expected. The polymerizations of **1P** gave **1P**-DHPs with high DPs in high yields, as did the polymerizations of **1G**. It was shown that the hydrophilic D-glucosyl units introduced to guaiacyl and *p*-hydroxylphenyl monolignols contributed to an increase in the molecular masses of the resulting DHPs. On the other hand, the yields and DPs of **1S**-DHPs from **1S** were extremely low. The polymerization behavior of **1S** was, even in a homogeneous phase, similar to the peculiar polymerization behavior of **2S**, as is well reported. Spectroscopic analyses indicated that **1P**- and **1S**-DHPs had typical lignin structures, although there were differences between the DHPs from the glucosides and those from monolignols in the yields of *p*-hydroxybenzaldehydes from alkaline nitrobenzene oxidations. It was also confirmed that D-glucosyl units introduced onto the γ-position do not fundamentally affect the basic parameters for the reactivities of monolignols, such as the redox potentials and the HRP-catalyzed oxidation rates. The HRP-catalyzed polymerizations of the monolignol glucosides (**1G**, **1P**, and **1S**) therefore fundamentally reflected those of the monolignols (**2G**, **2P**, and **2S**) and could be useful model reaction systems proceeding in

a homogeneous phase to study enzymatic dehydrogenative polymerizations and especially to elucidate the factors affecting the peculiar polymerization behaviors of the syringyl-type monomers.

REFERENCES

1. Freudenberg, K. Lignin. Its constitution and formation from *p*-hydroxycinnamyl alcohols. Science **1965**, *148*, 595–600.
2. Higuchi, T. Lignin biochemistry: biosynthesis and biodegradation. Wood Sci. Tech. **1990**, *24*, 23–63.
3. Ralph, J.; Lundquist, K.; Brunow, G.; Lu, F.; Kim, H.; Schatz, P.F.; Marita, J.M.; Hatfield, R.D.; Ralph, S.A.; Christensen, J.H.; Boerjan, W. Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids. Phytochem. Rev. **2004**, *3*, 29–60.
4. Monties B. Biological variability of lignins. Cellulose Chem. Tech. **2005**, *39*, 341–367.
5. Takano, T.; Tobimatsu, Y.; Hosoya, T.; Hattori, T.; Ohnishi, J.; Takano, M.; Kamitakahara, H.; Nakatsubo, F. Studies on the dehydrogenative polymerizations of monolignol β-glycosides. Part 1. Synthesis of monolignol β-glucosides, (*E*)-isoconiferin, (*E*)-isosyringin, and (*E*)-triandrin J. Wood Chem. Tech. **2006**, *26*(3), 215–229.
6. Tobimatsu, Y.; Takano, T.; Kamitakahara, H.; Nakatsubo, F. Studies on the dehydrogenative polymerizations of monolignol β-glycosides. Part 2: horseradish peroxidase-catalyzed dehydrogenative polymerization of isoconiferin. Holzforschung **2006**, *60*, 513–518.
7. Freudenberg, K.; Reznik, H.; Boesenberg, H.; Rasenack, D. The enzyme system participating in lignification. Chem. Ber. **1952**, *85*, 641–647.
8. Sterjiades, R.; Dean, J.F.D.; Gamble, G.; Himmelsbach, D.S.; Eriksson, K.E.L. Extracellular laccases and peroxidases from sycamore maple (*Acer pseudoplatanus*) cell-suspension cultures. Reactions with monolignols and lignin model compounds. Planta **1993**, *190*, 75–87.
9. Weymouth, N.; Dean, J.F.D.; Eriksson, K.E.L.; Morrison, W.H., III; Himmelsbach, D.S.; Hartley, R.D. Synthesis and spectroscopic characterization of *p*-hydroxyphenyl, guaiacyl and syringyl lignin polymer models (DHPs). Nord. Pulp Paper Res. J. **1993**, *8*, 344–349, 383.
10. Yoshida, S.; Chatani, A.; Tanahashi, M.; Honda, Y.; Watanabe, T.; Kuwahara, M. Preparation of synthetic lignin by manganese peroxidase of *Bjerkandera adusta* in organic solvents. Holzforschung **1998**, *52*, 282–286.
11. Kobayashi, T.; Taguchi, H.; Shigematsu, M.; Tanahashi, M. Substituent effects of 3,5-disubstituted *p*-coumaryl alcohols on their oxidation using horseradish peroxidase-H_2O_2 as the oxidant. J. Wood Sci. **2005**, *51*, 607–614.
12. Quideau, S.; Ralph, J. Facile large-scale synthesis of coniferyl, sinapyl, and *p*-coumaryl alcohol. J. Agr. Food Chem. **1992**, *40*, 1108–1110.
13. Katahira, R.; Nakatsubo, F. Determination of nitrobenzene oxidation products by GC and ^{1}H-NMR spectroscopy using 5-iodovanillin as a new internal standard. J. Wood Sci. **2001**, *47*, 378–382.

14. Sasaki, S.; Nishida, T.; Tsutsumi, Y.; Kondo, R. Lignin dehydrogenative polymerization mechanism: a poplar cell wall peroxidase directly oxidizes polymer lignin and produces in vitro dehydrogenative polymer rich in β-O-4 linkage. FEBS Lett. **2004**, *562*, 197–201.
15. Sarkanen, K.V. Precursors and their polymerization. In *Lignins-Occurrence, Formation, Structure, and Reactions.* Sarkanen, K.V., Ludwig, C.H., Eds.; Wiley-Interscience, New York, **1971**, 95–163.
16. Cathala, B.; Saake, B.; Faix, O.; Monties, B. Evaluation of the reproducibility of the synthesis of dehydrogenation polymer models of lignin. Polym. Degrad. Stabil. **1998**, *59*, 65–69.
17. Tanahashi, M.; Higuchi, T. Effect of the hydrophobic regions of hemicelluloses on dehydrogenative polymerization of sinapyl alcohol. Mokuzai Gakkaishi **1990**, *36*, 424–428.
18. Kacurakova, M.; Capek, P.; Sasinkova, V.; Wellner, N.; Ebringerova, A. FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohyd. Polym. **2000**, *43*, 195–203.
19. Faix, O. Investigation of lignin polymer models (DHP's) by FTIR spectroscopy. Holzforschung **1986**, *40*, 273–280.
20. Faix, O. Fourier transform infrared spectroscopy. In *Methods in Lignin Chemistry*; Lin, S.Y., Dence, C.W., Eds.; Springer-Verlag, New York, **1992**, 233–241.
21. Lewis, N.G.; Newman, J.; Just, G.; Ripmeister, J. Determination of bonding patterns of carbon-13 specifically enriched dehydrogenatively polymerized lignin in solution and solid state. Macromolecules **1987**, *20*, 1752–1756.
22. Ralph, J.; Zhang, Y. A new synthesis of (*Z*)-coniferyl alcohol, and characterization of its derived synthetic lignin. Tetrahedron **1998**, *54*, 1349–1354.
23. Chen, C.-L. Characterization of milled wood lignins and dehydrogenative polymerizates from monolignols by carbon-13 NMR spectroscopy. In *ACS Symposium Series 697, Lignin and Lignan Biosynthesis*; Lewis, N.G., Sarkanen, S., Eds; American Chemical Society, Washington, DC, **1998**, vol. 697, 255–275.
24. Binns, A.N.; Chen, R.H.; Wood, H.N.; Lynn, D.G. Cell division promoting activity of naturally occurring dehydrodiconiferyl glucosides: do cell wall components control cell division? Proc. Nat. Acad. Sci. USA **1987**, *84*, 980–984.
25. Warashina, T.; Miyase, T.; Ueno, A. Phenylethanoid and lignan glycosides from *Verbascum thapsus*. Phytochemistry **1992**, *31*, 961–965.
26. Kanchanapoom, T.; Kamel, M. S.; Kasai, R.; Yamasaki, K.; Picheansoonthon, C.; Hiraga, Y. Lignan glucosides from *Acanthus ilicifolius*. Phytochemistry **2001**, *56*, 369–372.
27. Takara, K.; Matsui, D.; Wada, K.; Ichiba, T.; Nakasone, Y. New antioxidative penolic gycosides isolated from *Kokuto* non-centrifuged cane sugar. Biosci. Biotech. Biochem. **2002**, *66*, 29–35.
28. Takara, K.; Matsui, D.; Wada, K.; Ichiba, T.; Chinen, I.; Nakasone, Y. New phenolic compounds from Kokuto, non-centrifuged cane sugar. Biosci. Biotech. Biochem. **2003**, *67*, 376–379.
29. Yamauchi, H.; Kakuda, R.; Yaoita, Y.; Machida, K.; Kikuchi, M. Two new glycosides from the whole plants of *Glechoma hederacea* L. Chem. Pharm. Bull. **2007**, *55*, 346–347.

30. Lu, F.; Ralph, J.; Morreel, K.; Messens, E.; Boerjan, W. Preparation and relevance of a cross-coupling product between sinapyl alcohol and sinapyl *p*-hydroxybenzoate. Org. Biomol. Chem. **2004**, *2*, 2888–2890.
31. Creighton, R.H.J.; Hibbert, H. Lignin and related compounds. LXXVI. Alkaline nitrobenzene oxidation of corn stalks. Isolation of *p*-hydroxybenzaldehyde. J. Am. Chem. Soc. **1944**, *66*, 37–38.
32. Chen, C. L. Nitrobenzene and cupric oxide oxidations. In *Methods in lignin chemistry*; Lin, S.Y., Dence, C.W., Eds.; Springer-Verlag, New York, **1992**, 301–321.
33. Hapiot, P.; Pinson, J.; Francesch, C.; Mhamdi, F.; Rolando, C.; Schneider, S. One-electron redox potentials for the oxidation of coniferyl alcohol and analogs. J. Elect. Chem. **1992**, *328*, 327–331.
34. Hapiot, P.; Pinson, J.; Neta, P.; Francesch, C.; Mhamdi, F.; Rolando, C.; Schneider, S. Mechanism of oxidative coupling of coniferyl alcohol. Phytochemistry **1994**, *36*, 1013–1020.
35. Nielsen, K.L.; Indiani, C.; Henriksen, A.; Feis, A.; Becucci, M.; Gajhede, M.; Smulevich, G.; Welinder, K.G. Differential activity and structure of highly similar peroxidases. Spectroscopic, crystallographic, and enzymatic analyses of lignifying *Arabidopsis thaliana* peroxidase A2 and horseradish peroxidase A2. Biochemistry **2001**, *40*, 11013–11021.
36. Lu, F.; Ralph, J. Preliminary evidence for sinapyl acetate as a lignin monomer in kenaf. Chem. Comm. **2002**, 90–91.