Reactivity of syringyl quinone methide intermediates in dehydrogenative polymerization. Part 1. High yield production of synthetic lignins (DHPs) in horseradish peroxidase-catalyzed polymerization of sinapyl alcohol in the presence of nucleophilic reagents.

Yuki Tobimatsu, Toshiyuki Takano*, Hiroshi Kamitakahara, Fumiaki Nakatsubo

Division of Forest & Biomaterials Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan

*Corresponding author: Mailing Address: Division of Forest & Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto, 606-8502, Japan; Tel: +81-75-753-6254; Fax: +81-75-753-6300; E-mail: takatmys@kais.kyoto-u.ac.jp

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Abstract

It is known that the conventional dehydrogenative polymerization of sinapyl alcohol (S-alc) gave syringyl synthetic lignins (S-DHPs), but in extremely low yields. In this article, to examine the contribution of syringyl quinone methide intermediates (S-QM) on S-DHP production, horseradish peroxidase (HRP)-catalyzed dehydrogenative polymerization of S-alc was carried out in the presence of nucleophilic reagents that promote the rearomatization of S-QM. First, the HRP-catalyzed polymerization of sinapyl alcohol γ-*O*-β-D-glucopyranoside (isosyringin, iso-S), which allows us to monitor the polymerization process in a homogeneous aqueous phase, was utilized for screening of a nucleophile used as an S-QM scavenger. Monitoring of the iso-S polymerization in the presence of various nucleophilic reagents by UV spectroscopy and gel permeation chromatography with photodiode array detection (GPC-PDA) revealed a high ability of azide ion to convert oligomeric S-QM efficiently to S-DHP. Accordingly, azide ion was utilized as an S-QM scavenger in HRP-catalyzed polymerization of S-alc, which resulted in high yield production of S-DHPs (~83%), as expected. The ^{1}H-, ^{13}C- and 2D-HSQC NMR investigations on the resulting S-DHPs clearly demonstrated that azide ion efficiently performed nucleophilic additions to the C-α of S-QM during the polymerization. These results provide experimental proof that the low reactivity of S-QM with nucleophiles (such as water, phenolic and aliphatic hydroxyl groups) in the conventional polymerization system critically impedes the production of S-DHPs from S-alc.

Introduction

The last stage of lignin formation in plant cell wall can be mimicked *in vitro* by the enzymatic dehydrogenative polymerization of monolignols [*p*-coumaryl alcohol (H-alc); coniferyl alcohol (G-alc); sinapyl alcohol (S-alc)], leading to the lignin polymer models (dehydrogenation polymers, DHPs).[1-3] As reviewed by several authors,[4-8] much of what is now known about the lignin polymerizations is based on the studies of this system. However, a satisfactory synthesis of DHPs structurally resembling native lignins has not been achieved yet, implying that the polymerization process is not fully understood. One of the open questions with this regard is the peculiar polymerization behavior of S-alc, being completely different from those of H-alc and G-alc. Many researchers have reported that enzymatic dehydrogenative polymerization of S-alc afforded syringyl (S)-DHPs, but with low molecular masses in low yields, while H-alc and G-alc readily gave *p*-hydroxyphenyl (H)- and guaiacyl (G)-DHPs, respectively, with high molecular masses in high yields.[9-15]

As well established, the dehydrogenative polymerization of S-alc basically consisting of three reaction steps as depicted in Fig. 1: step 1: enzymatic radical formations; step 2: radical couplings; step 3: rearomatization of syringyl quinone methide intermediates (S-QM) by nucleophilic additions of nucleophiles in the polymerization system. Several problems in each reaction step have been discussed in connection with the low polymerizability of S-alc: the low reactivity of common oxidants such as horseradish peroxidase (HRP) / hydrogen peroxide to S-type phenolic compounds for step 1[16-20] and preferential β-β coupling reactions to β-*O*-4 for step 2.[20-22] So far, little attention has been paid to step 3 in connection with the low polymerizability of S-alc *in vitro*.

Recently, we have investigated on the HRP-catalyzed polymerization of sinapyl alcohol γ-*O*-β-D-glucopyranoside [isosyringin (iso-S), Fig. 2] as a model reaction system to study the polymerization behavior of S-alc.[15,23-27] Owing to the presence of a highly hydrophilic sugar unit attached to S-alc, the polymerization of iso-S gives water-soluble products in a homogeneous aqueous phase, whereas the conventional polymerization of S-alc gives water-insoluble products in a heterogeneous way. It was also confirmed that the reactivity and polymerization behavior of iso-S in the dehydrogenative polymerization are well reflected by those of S-alc. This unique polymerization system based on iso-S enabled us to follow the time-course of S-DHP formation in a homogeneous aqueous media by such as UV spectroscopy[26] and gel permeation chromatography with photodiode array detection (GPC-PDA).[27] Importantly, our approach has revealed that oligomeric S-QM accumulates stably during the HRP-catalyzed polymerization of iso-S. The low reactivity of S-QM can be explained by the presence of two electron-donating methoxyl groups, which reduce the positive charge density at the α-positions. It is reported that the analogous quinone methide, 2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone also reacts very slowly in aqueous media. Bolton et al. pointed out that this low reactivity is due to the lack of hydrogen bonding between the shielded oxo group and water molecules, suppressing charge separation of the quinone methide.[28,29] The same explanation can be applied for the low reactivity of S-QM.

The data in our previous studies strongly suggest that the low reactivity of S-QM with nucleophiles in the conventional polymerization system (at step 3 in Fig. 1) may retard the subsequent polymerization for S-DHP formation from S-alc. Based on this concept, if suitable nucleophiles with high nucleophilicity towards S-QM are added to the conventional polymerization system, they can perform nucleophilic additions to promote the rearomatization of S-QM and the subsequent polymerization steps in Fig. 1 would repeatedly proceed to yield S-DHPs efficiently. In the preliminary study, we showed that the HRP-catalyzed polymerization of S-alc in the presence of nucleophilic azide ion gave S-DHPs in significantly high yield.[30] In this article, further data on S-DHP formations in the presence of nucleophilic reagents are presented.

Experimental

Materials

Iso-S,[23] S-alc,[31] and syringylglycerol-β-syringyl ether [1-(4-hydroxy-3,5-dimethoxyphenyl)-2-(2,6-dimethoxyphenoxy)-propane-1,3-diol (**1**)][32,33] were synthesized according to the method described in literature. HRP (100 U mg^{-1}) was purchased from Wako Pure Chemicals (Kyoto, Japan) and used without further purification. Wakogel C-200 (Wako Pure Chemicals) was used in silica gel column chromatography. Other chemicals were purchased from Nacalai Tesque Inc. (Kyoto, Japan) or Wako Pure Chemicals and used as received.

Screening of nucleophile for HRP-catalyzed polymerization of S-alc by monitoring iso-S polymerization in the presence of nucleophilic reagents

UV spectroscopic monitoring of HRP-catalyzed polymerization of iso-S in the presence of nucleophiles was carried out as follows.[26] The solution (3 ml) consisting of 100 μM iso-S, 1500 μg l^{-1} HRP and 100-1000 μM nucleophilic reagents (D-glucronic acid, ethyl amine, sodium sulfite, pottasium iodide, cysteine and sodium azide) in 50 mM sodium phosphate buffer (pH6.5) and the same solution without monomer were placed in a sample cell and a reference cell, respectively. The cells were set in a JASCO V-560 spectrophotometer and kept at 25°C under stirring. The polymerization was initiated by adding 25 μl 0.024% hydrogen peroxide aqueous solution (final concentration: 60 μM) to the sample cell, and UV spectra were recorded at a regular time interval (scan rate, 2000 nm min^{-1}; scan region, 250-400 nm; data interval, 1 nm; response mode, quick).

GPC-PDA monitoring of the HRP-catalyzed polymerization of iso-S in the presence of azide ion was done as follows.[27] Three solutions were prepared for the polymerization of iso-S: solution A, 2.0 mg of HRP in 500 μl of 0.05 M phosphate buffer (pH 6.5); solution B, 20 μmol of the glycosides in 2500 μl of the buffer; solution C, 2500 μl aqueous solution containing sodium azide (20 μmol) and hydrogen peroxide (24 μmol). Polymerization was initiated by adding solutions B and C simultaneously to solution A at a constant rate (2.5 ml h^{-1}; monomer addition time, 60 min). After initiating the polymerization, reaction mixtures (100 μl) were periodically sampled and mixed with 900 μl of 0.1M LiCl in dimethylformamide (DMF) to terminate the reaction, immediately cooled at 0°C, and subjected to the GPC-PDA analyses within 15 min after withdrawing from the reaction mixture. The GPC-PDA analyses were performed on a Shimadzu LC-20A LC system (Shimadzu, Japan) equipped with a SPD-M20A photodiode array detector. Elution conditions were as follows: column, TSK gel α-M (Tosoh, Japan); eluent, 0.1 M LiCl in DMF; flow rate, 0.5 ml min^{-1}; column oven temperature, 40°C; injection volume, 20 μl. Conditions for PDA detection were as follows: scan region: 260-400 nm; band width, 4 nm; response, 1280 ms. Molecular weight calibration was made using polystyrene standards (Shodex, Japan). Data acquisition and computation utilized LCsolution version 1.22 SP1 software (Shimadzu, Japan).

HRP-catalyzed polymerization of S-alc in the presence of azide ion

Two solutions were prepared for polymerization of S-alc: solution A, 120 ml of distilled water containing 0.5 mmol S-alc and 3-12 mg of HRP; solution B, 120 ml of 0.019% hydrogen peroxide (0.6 mmol) aqueous solution containing 0.5 mmol sodium azide. Solutions A and B were added drop-wise to 30 ml of 0.1 M phosphate buffer over a period of 0.5-48 h. The precipitate of the resulting polymer was collected by centrifugation (12000 rpm, 10 min), washed twice with distilled water and lyophilized to obtain S-DHP.

S-DHPs were acetylated with standard protocols[15] and subjected to GPC and nuclear magnetic resonance (NMR) analyses. GPC was performed with a Shimadzu LC-10 system equipped with a UV-Vis detector (SPD-10Avp, monitoring at 280 nm) under the following conditions: columns, K-802, K-802.5 and K-805 (Shodex, Japan); eluent, $CHCl_3$; flow rate, 1.0 ml min^{-1}; column temperature, 40 °C. The system was calibrated with polystyrene standards (Shodex). ^{1}H-, ^{13}C-, and two-dimensional (2D)-heteronuclear single quantum coherence (HSQC) NMR spectra were collected with a Varian INOVA300 FT-NMR spectrometer (300 and 75.5 MHz for ^{1}H and ^{13}C nuclei, respectively) in chloroform-*d* with tetramethylsilane as the internal standard (0.0 ppm). Chemical shifts (δ) and coupling constants (J) were given in δ-values (ppm) and hertz (Hz), respectively.

3-azido-3-(4-hydroxy-3,5-dimethoxyphenyl)-2-(2,6-dimethoxyphenoxy)-1-propanol (**3**)

The quinone methide **2** was prepared from syringylglycerol-β-syringyl ether (**1**) by the method in literature.[34,35] Briefly, compound **1** (380 mg, 1.0 mmol) was dissolved in 10 ml of dichloromethane and to this solution 260 μl of trimethylsilyl bromide (2.0 mmol) was added with stirring under nitrogen at room temperature. After 1 min, the solution was poured into a separation funnel and extracted twice with 30 ml saturated sodium bicarbonate aqueous solution. The organic layer was dried over sodium sulfate and evaporated to dryness. Obtained raddish colored solid of compound **2** was dissolved in 5 ml of anhydrous dioxane and added dropwise into 4 ml of dioxane/water solution (1:1, v/v) containing sodium azide (650 mg, 10 mmol) at 0 °C under nitrogen. After 1 h, the reaction mixture was extracted with ethyl acetate and washed twice with saturated sodium chloride aqueous solution, and dried over sodium sulfate. Evaporation in vacuo produced an orange oil, which was purified by a silica gel column chromatography [eluent, ethyl acetate/n-hexane (3:2, v/v)] to give compound **3** as white solid (192.7 mg, 48% yield, *erythro*/*threo* = ~1.0). Stereochemical assignments were made from ^{1}H-NMR signals of propyl side-chain protons in analogy with the data of β-O-4 lignin model compounds in literature.[36,37]

Acetate of compound **3**; ^{1}H-NMR (in $CDCl_3$): δ 1.96 (3H, s, C_{γ}-OCOCH_{3}, *erythro* isomer), 1.98 (3H, s, C_{γ}-OCOCH_{3}, *threo* isomer), 2.33 (3H, s, C_4-OCOCH_{3}), 3.77-3.82 (3.77, 3.80, 3.81, 3.82) (12H, s, Ar-OMe), 3.84-3.93 (1H, m, $H_{\gamma1}$), 4.25-4.33 (1H, m, $H_{\gamma2}$), 4.39-4.53 (1H, m, H_{β}), 4.91 (0.5H, d, J = 6.6 H_{α}, *erythro* isomer), 5.01 (1H, d, J = 4.8 H_{α}, *threo* isomer), 6.55 (2H, d, J = 3.0, $H_{2'}$ and $H_{6'}$, *threo* isomer), 6.58 (2H, d, J = 3.0, $H_{2'}$ and $H_{6'}$, *erythro* isomer), 6.65 (2H, s, H_2 and H_6, *threo* isomer), 6.71 (2H, s, H_2 and H_6, *erythro* isomer), 7.00 (1H, t, J = 8.7, $H_{1'}$, *erythro* isomer), 7.01 (1H, t, J = 8.7, $H_{1'}$, *threo* isomer). ^{13}C-NMR: δ 20.3, 20.6 (COCH_3), 55.8, 56.0, 56.1 (Ar-OMe), 62.7 (C_{γ}, *erythro* isomer), 63.1 (C_{γ}, *threo* isomer), 66.2 (C_{α}, *erythro* isomer), 66.7 (C_{α}, *threo* isomer), 81.2 (C_β, *erythro* isomer), 81.9 (C_β, *threo* isomer), 103.8 (C_2 and C_6, *threo* isomer), 104.2 (C_2 and C_6, *erythro* isomer), 104.6 ($C_{2'}$ and $C_{6'}$, *erythro* isomer), 104.9 ($C_{2'}$ and $C_{6'}$, *threo* isomer), 124.1 ($C_{1'}$), 128.1 (C_4, *erythro* isomer), 128.5 (C_4, *threo* isomer), 134.7 (C_1), 135.1 ($C_{4'}$, *erythro* isomer), 135.2 ($C_{4'}$, *threo* isomer), 151.9 (C_3 and C_5, *eryhtro* isomer), 152.0 (C_3 and C_5, *threo* isomer), 153.2 ($C_{3'}$ and $C_{5'}$), 168.5 (Ar-O*C*OCH$_3$), 170.3, 170.7 (C_γ-O*C*OCH$_3$)

Results and Discussions

Screening of nucleophile for HRP-catalyzed polymerization of S-alc

In previous studies, we successfully detected and characterized S-QM formed in the HRP-catalyzed polymerization of iso-S using UV spectroscopic[26] and GPC-PDA[27] measurements. In the present study, these techniques were applied for screening of nucleophile used as an S-QM scavenger in the polymerization of S-alc.

UV spectroscopic monitoring of HRP-catalyzed polymerization of iso-S in the presence of nucleophilic reagents

Figure 3A shows the time-depended changes in UV spectra during the HRP-catalyzed polymerization of iso-S without nucleophiles. As the reaction time increased, the absorbance peak at 274 nm decreased, indicating that iso-S was oxidized by HRP. Formation and accumulation of stable S-QM were clearly indicated by the appearance of the absorption peak at 325 nm, as evidenced in our previous study.[26] A suitable nucleophile should not retard the HRP-catalyzed oxidation of iso-S, which can be evaluated by the decrease of the absorption at 274 nm (A_{274}), and the one should suppress the accumulation of S-QM, which can be evaluated by the increase of absorptions at 325 nm (A_{325}). Representative nucleophilic reagents investigated here are carboxyl acid (D-glucronic acid), amine (ethyl amine), sulfite ion (sodium sulfite), iodide ion (pottasium iodide), thiol (cysteine) and azide ion (sodium azide). Figure 4 displays plots of A_{274} and A_{325} during iso-S polymerizations in the presence of the nucleophilic reagents. In polymerization with carboxylic acid, amine, sulfite ion and iodide ion, A_{274} decreased smoothly, but A_{325} increased significantly, indicating high levels of S-QM accumulation (Fig. 4B-E). These results indicate that the nucleophilicity of these compounds toward S-QM is not sufficient under the present conditions. In several reports, highly nucleophilic thiol compounds[38-40] and azide ion[41-43] were used to trap QM species formed as reactive intermediates in various chemical reactions. On the other hands, both these are well-known peroxidase inhibitors.[44] When iso-S polymerization was conducted in the presence of cysteine (Fig. 4F), the decreasing of A_{274} was much slower at the initial stage of polymerization (~30min), while the increase in A_{325} was suppressed in this period. After a period of reaction time, A_{274} suddenly dropped and then A_{325} started increasing. Thiol compounds are reported to be substrates for HRP.[45,46] The result obtained here can be explained by that HRP-catalyzed oxidation of cysteine took place in advance of the oxidation of iso-S. Thus, thiol compounds seem to be unsuitable as a S-QM scavenger used in HRP-catalyzed polymerization. On the other hand, A_{274} decreased smoothly in the presence of sodium azide, whereas the increasing in A_{325} was hardly observed (Figs. 3B and 4G). The results indicated that azide ion efficiently scavenges S-QM without significant inhibition to the catalytic ability of HRP. Therefore, azide ion was concluded to be the most suitable nucleophile as a S-QM scavenger used in HRP-catalyzed polymerization of S-alc.

GPC-PDA monitoring of HRP-catalyzed polymerization of S-alc in the presence of azide ion

The HRP-catalyzed polymerization of iso-S in the presence of azide ion was monitored by GPC-PDA to obtain further confirmation of the ability of azide ion to trap S-QM. This method permits to follow the changes of the molecular weights of S-DHP intermediates as well as the formation of oligomeric S-QM during the course of the iso-S polymerization.[27] Figure 5 shows the GPC-PDA profiles of the iso-S polymerization in the absence and presence of azide ion. In the absence of azide ion, the presence of oligomeric S-QM was clearly indicated by the intense peak detected at 344 nm at 19.2 min of elution time (peak top MW = 1700) (Fig. 5A). As Fig. 5C shows, in the absence of azide ion, the peak area detected at 344 nm rose significantly just after initiating the polymerization and then decreased very slowly as reaction time progressed, indicating the transient but stable presence of oligomeric S-QM. In contrast, during the polymerization in the presence of azide ion, the peak area from S-QM remained constantly low, indicating that accumulations of the oligomeric S-QM were effectively suppressed (Fig. 5B,C). This finding agrees well with the results in UV spectroscopic monitoring of the polymerization described above. Formation of polyphenolic S-DHP could be followed by the absorption at 274 nm. The product molecular weights calculated based on PDA detection at 274 nm are plotted against reaction time in Fig. 5D. Clearly, the addition of azide ion to the polymerization system resulted in efficient formation of polyphenolic S-DHP, as the product molecular mass increased faster in polymerization with azide ion than without azide ion. These results are readily rationalized if the oligomeric S-QM are rapidly converted to the corresponding phenolics by azide addition and the resulting phenolics react further to produce S-DHP.

HRP-catalyzed polymerization of S-alc in the presence of azide ion

HRP-catalyzed polymerization of S-alc in the presence of azide ion, which serves as a S-QM scavenger, was carried out under various polymerization conditions. Figure 6 shows the effect of azide ion on the yield of isolated S-DHPs. The yield of S-DHPs prepared according to the so-called bulk polymerization method,[2] in which the monomer is added to the polymerization system drop-wise but rapidly in 0.5 h, was much affected by the amount of sodium azide added to the polymerization system (Fig. 6A). As expected from earlier studies,[9-15] in the absence of azide ion, the yield of S-DHP was quite low (~5%). As amount of sodium azide was upped to 1 eq. for S-alc, the yield of S-DHP greatly increased to 54 %. When excess amount of sodium azide for S-alc was applied, however, the yield of S-DHP dropped again probably due to inactivation of HRP induced by azide ion. Then, the so-called end-wise polymerization method,[2] in which the monomer is added to the polymerization system slowly for 24-48 h, was employed with 1eq. of sodium azide for S-alc. Figure 6B shows the effect of monomer addition time on the yield of S-DHP. The yield of S-DHP with azide ion further increased to 83% as the monomer addition time increased to 48 h, while the yield of S-DHP without azide ion also increased but to no more than 12%. Table 1 lists the average molecular weights (M_n and M_w) and their distributions (M_w/M_n) of S-DHPs. The M_n values of the acetylated samples of S-DHPs prepared with azide ion were 1300-1800 (degree of polymerization, DP = 4-6), which are in the same range as those reported for the conventional

DHPs.[47,48] The end-wise polymerization method contributed to an increase in the molecular mass of S-DHP. It was observed that M_n and M_w/M_n values of the isolated S-DHPs prepared with azide ion were slightly lower than those for S-DHPs prepared without azide ion. This may be explained by structural differences between them, as discussed in the next section. Nevertheless, it is obvious that appropriate amount of azide ion (1 eq. for S-alc) significantly promotes the production of S-DHP, indicating that the low reactivity of S-QM with nucleophiles is critically responsible for the low yield of S-DHP in the conventional polymerization system.

Structural characterization of S-DHPs

The ^{1}H-, ^{13}C-, and 2D-HSQC NMR spectra of acetylated S-DHPs prepared in the absence and presence of azide ion (prepared based on Table 1 entry 3) are shown in Fig. 7. Nucleophilic attacks of azide ion to S-QM during the polymerization are clearly demonstrated by the appearance of the signals from β-*O*-4/α-N_3 structure (**I**), which are identical to the data for α-azide model compound **3** synthesized according to Fig. 8. All the spectra indicate that the contributions from β-*O*-4/α-OH (**II**) and β-*O*-4/α-ether substructures (**III**) are negligibly small for S-DHP obtained with azide ion, whereas the both structures are abundant for the conventional S-DHP prepared without azide ion. This result suggests that during the polymerization of S-alc with nucleophilic azide ion, the β-*O*-4 S-QM are exclusively quenched by azide ion but not by water, phenolic or aliphatic hydroxyl groups. A series of peaks from β-β resinol structure (**IV**) is also observed in the spectra of S-DHP obtained with azide ion, indicating that β-β S-QM are rapidly trapped by intramolecular γ–hydroxyl groups even in the presence of azide ion. Our preliminary data of Fourier transform-infrared (FT-IR) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS analyses of S-DHPs also support this result.[30] As expected from these data, S-DHP prepared in the presence of azide ion is a simply linear polymer made up mainly of structure **I** and **IV**. As already mentioned, the S-DHPs prepared with azide ion tend to have slightly lower molecular masses and narrower molecular mass distributions than those of convetional S-DHPs (see Table 1). This difference might be explained by the lack of the structure **III** in the S-DHPs prepared with azide ion, as the branching structure **III** is formed by nucleophilic attacks of oligomeric phenolics onto β-*O*-4 S-QM.

Conclusion

To examine the contribution of reactivity of S-QM on S-DHP production from S-alc, HRP-catalyzed dehydrogenative polymerization in the presence of nucleophilic reagents was investigated. The HRP-catalyzed polymerization of iso-S, which permits to monitor the formation of S-QM in a homogeneous aqueous phase, was successfully utilized for screening of nucleophile used as a S-QM scavenger in the polymerization of S-alc. UV spectroscopic monitoring of iso-S polymerization in the presence of various nucleophiles revealed the high ability of azide ion to trap S-QM without significant inhibition to HRP activity. GPC-PDA monitoring of the polymerization of iso-S also demonstrated that the oligomeric S-QM efficiently converted to S-DHP in the presence of azide ion. Accordingly, azide ion was applied as a S-QM scavenger in HRP-catalyzed polymerization of S-alc, resulting in production of S-DHPs in remarkably high yields. Although azide ion dramatically promotes the production of S-DHP, the molecular masses of the isolated

S-DHPs was not improved so much, which is partly explained by the lack of branching α-O-4 structures (**III**) in the S-DHPs prepared with azide ion. NMR analyses on S-DHPs clearly demonstrated that azide ion efficiently performed nucleophilic additions to the C-α of the S-QM during the polymerization. It was demonstrated that, in the HRP-catalyzed polymerization of S-alc in the presence of strongly nucleophilic azide ion, S-QM are readily rearomatized by azide addition. Then, subsequent polymerization steps, initiated by the oxidation of the re-generated phenolic hydroxyl groups, can proceed repeatedly to yield S-DHPs efficiently. Consequently, these data provide experimental proof that the low reactivity of S-QM with nucleophiles in the conventional polymerization system is a crucial cause of the low efficiency in the dehydrogenative polymerization of S-alc *in vitro*. Because there seems to be no evidence that any particular nucleophilic reagents operate in lignin formations *in vivo*, subsequent studies should focus on the reactions of S-QM under various polymerization conditions without the use of strongly nucleophilic reagents. Such studies are expected to provide new clues for understanding the factors controlling lignin polymerization in the plan cell.

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Figure and scheme legends

Figure 1 The dehydrogenative polymerization of sinapyl alcohol (S-alc) via β-*O*-4 couplings.

Figure 2 Chemical structures of sinapyl alcohol (S-alc) and isosyringin (iso-S) (sinapyl alcohol γ-*O*-β-D-glucopyranoside).

Figure 3 UV spectra of the polymerization mixtures during the HRP-catalyzed polymerizations of isosyringin (iso-S). **A** In the absence of nucleophiles. Reaction time: 0, 2, 6, 10, 16, 20 and 30 min. **B** In the presence of azide ion (1eq. for iso-S). Reaction time: 0, 2, 6, 10, 16, 10, 20 and 30 min.

Figure 4 Changes of the absorbance at 274 nm (A_{274}, ○) and at 344 nm (A_{325}, ●) during the HRP-catalyzed polymerizations of iso-S (100 μM) in the presence of various nucleophilic reacgents: A: none (control); B: D-Glucronic acid (1000 μM); C: Ethyl amine (1000 μM); D: Sodium sulfite (500 μM); E: Pottasium iodide (1000 μM); F: Cysteine (100 μM); G: Sodium azide (NaN_3, 100 μM).

Figure 5 GPC-PDA monitoring of the HRP-catalyzed polymerization of iso-S. **A** Three-dimensional (3D) PDA plots in polymerization without nuceophilic regents.

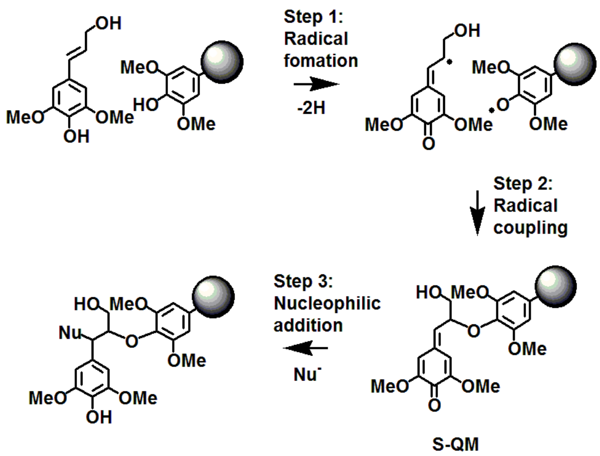
B 3D PDA plots in polymerization with azide ion (1eq. for iso-S). **C** Plots of peak area detected at 344 nm over reaction time. **D** Plots of number and weight average molecular weights (M_n and M_w) calculated based on PDA detection at 274 nm over reaction time.

Figure 6 Yields of syringyl dehydrogenation polymers (S-DHPs) in the HRP-catalyzed polymerization of S-alc in the presence of azide ion. **A** Effect of the amount of sodium azide (HRP = 6 mg for 1 mmol S-alc; monomer addition time = 0.5 h). **B** Effect of the monomer addition time (HRP = 24 mg for 1 mmol S-alc; sodium azide = 1 eq. for S-alc).

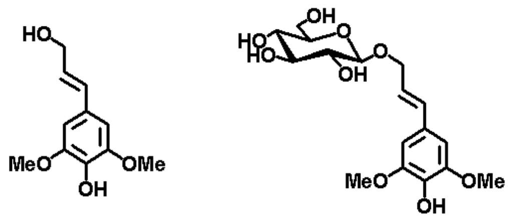
Figure 7 Nuclear magnetic resonance (NMR) characterizations of acetylated S-DHPs from S-alc synthesized in the presence of azide ion. **A** ^{1}H-NMR spectra. **B** ^{13}C-NMR spectra. **C** 2D-heteronuclear single quantum coherence (HSQC) spectra.

Figure 8 Synthetic scheme for model compound **3**. TMS, trimethylsilyl

(Figure 1)

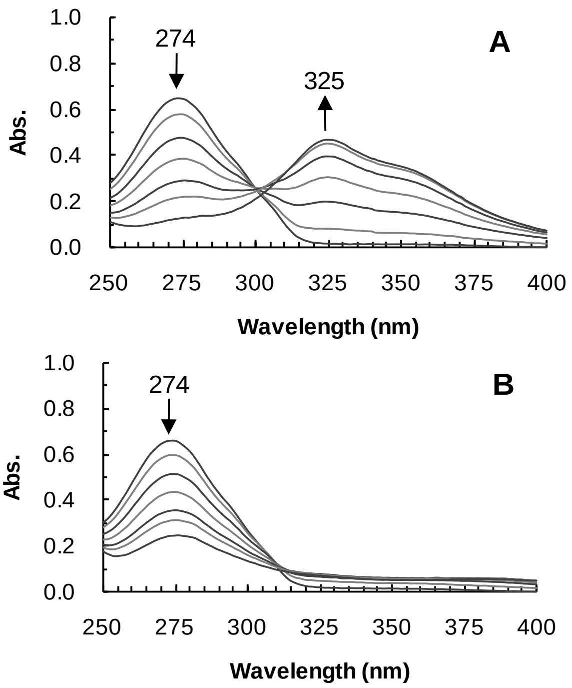

OH
MeO
OMe
OH
MeO
HO
OMe
Step 1:
Radical
fomation
-2H
OH
MeO
OMe
O
MeO
O
OMe
Step 2:
Radical
coupling
Step 3:
Nucleophilic
addition
Nu⁻
HO
MeO
Nu
O
OMe
MeO
OMe
OH
HO
MeO
O
OMe
MeO
OMe
O
S-QM

(Figure 2)

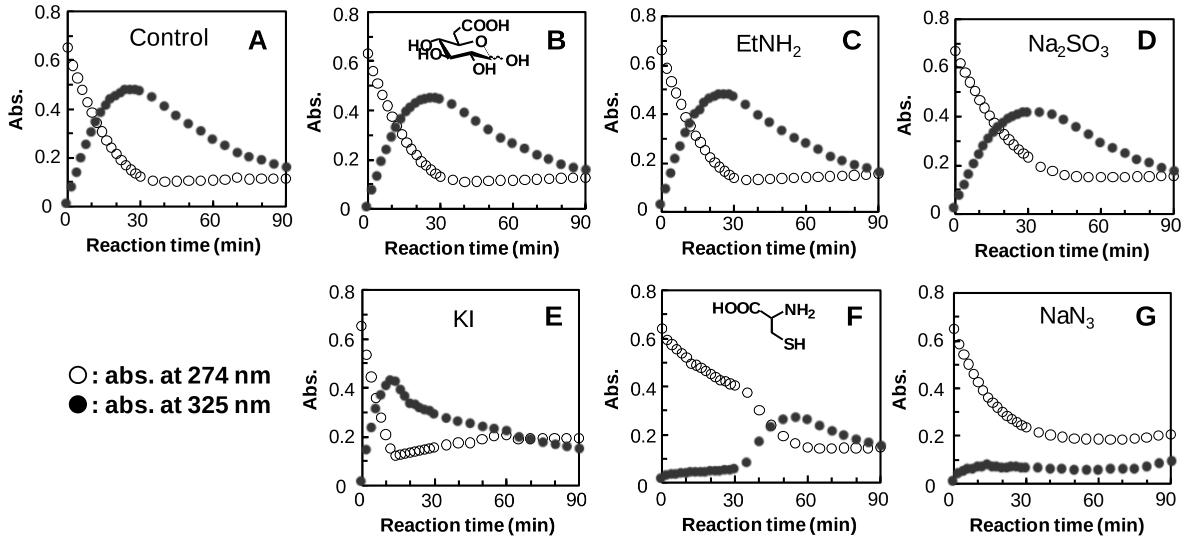


Sinapyl alcohol (**S-alc**) Isosyringin (**iso-S**)

(Figure 3)

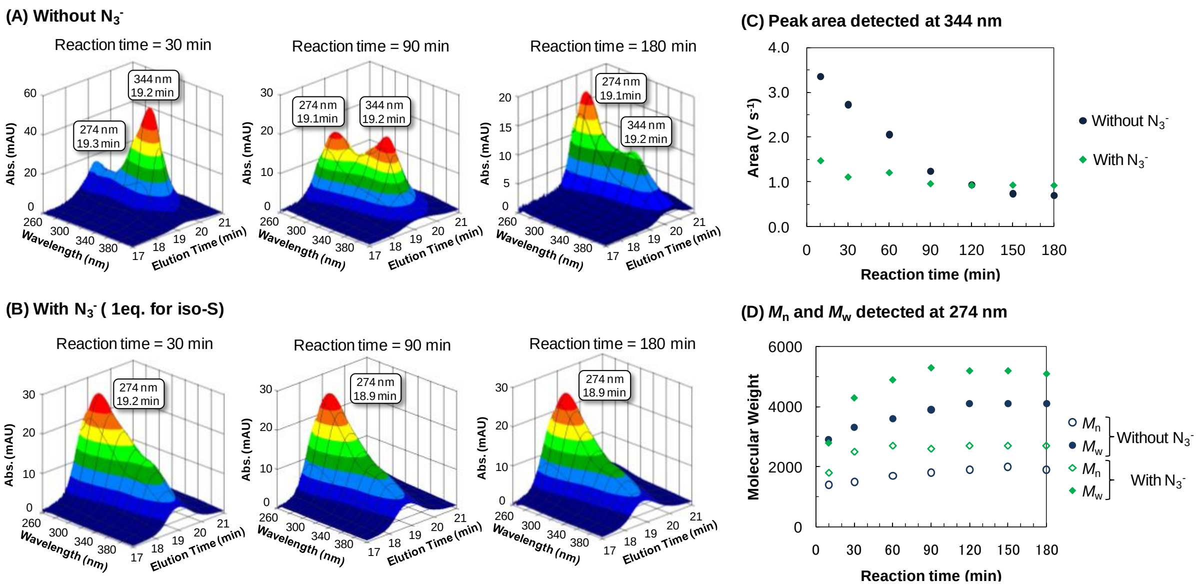

A
274
325
Abs.
1.0
0.8
0.6
0.4
0.2
0.0
250
275
300
325
350
375
400
Wavelength (nm)
B
274
Abs.
1.0
0.8
0.6
0.4
0.2
0.0
250
275
300
325
350
375
400
Wavelength (nm)

(Figure 4)

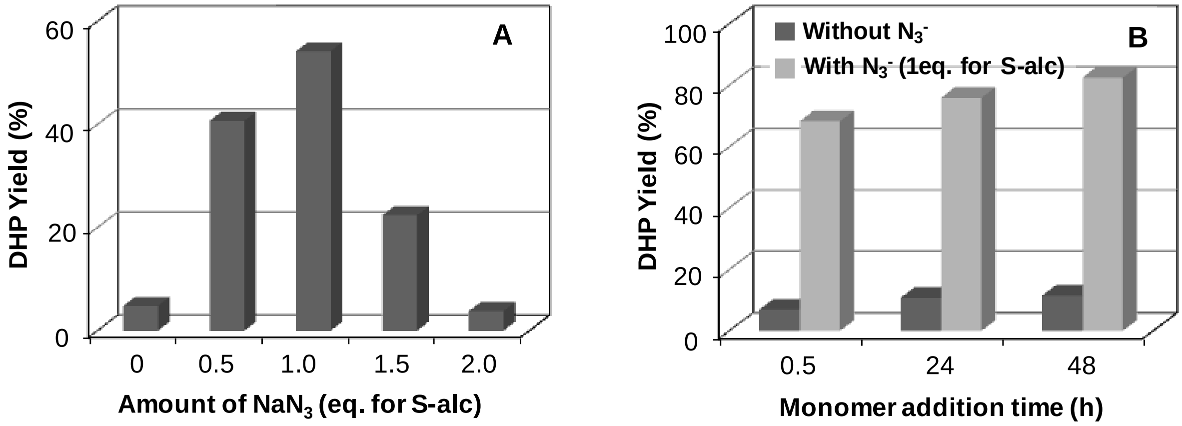


Control
A
B
COOH
HO
HO
O
OH
OH
EtNH2
C
Na2SO3
D
KI
E
HOOC
NH2
SH
F
NaN3
G
Abs.
Reaction time (min)
○: abs. at 274 nm
●: abs. at 325 nm

(Figure 5)

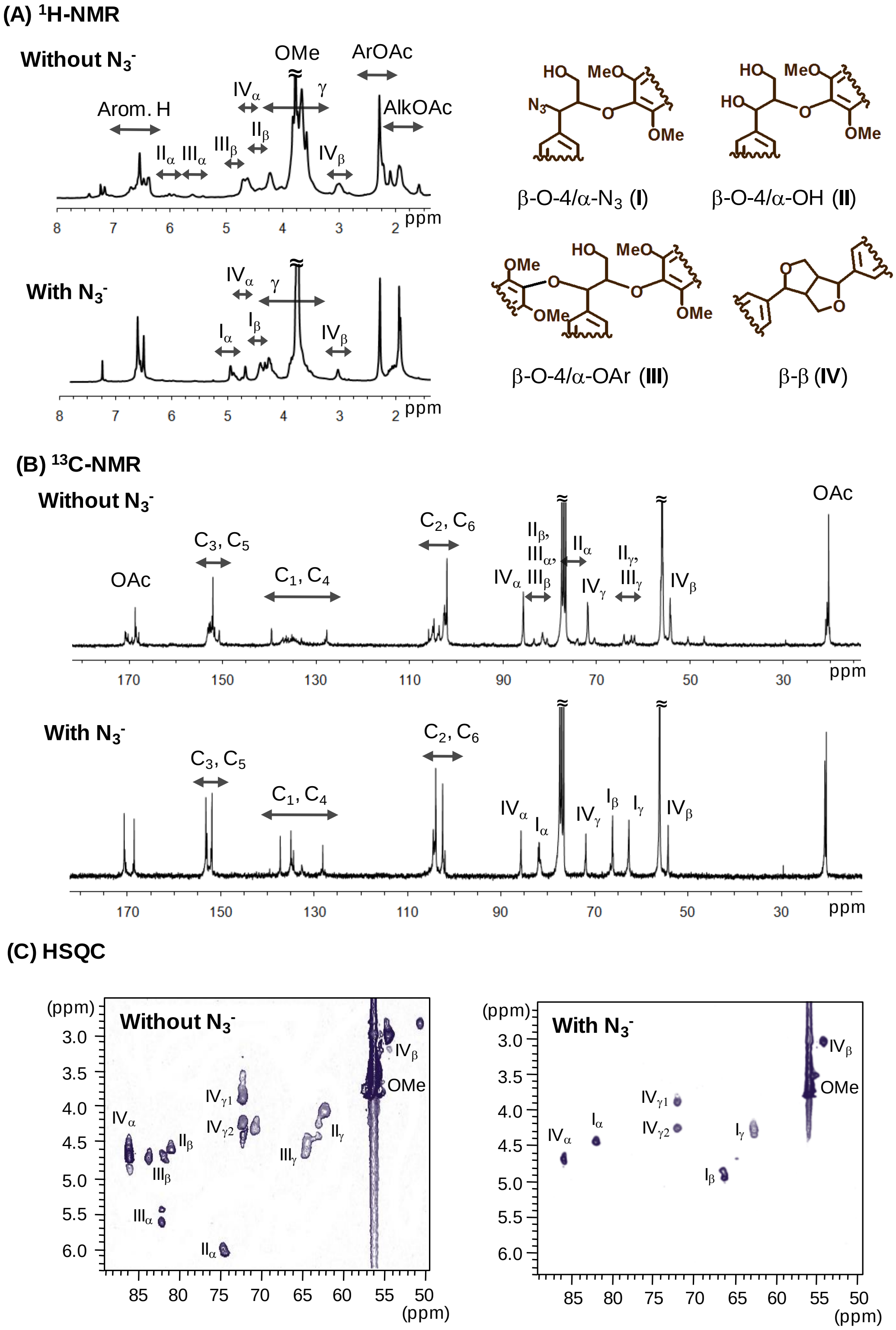

(A) Without N3-
Reaction time = 30 min
Reaction time = 90 min
Reaction time = 180 min
274 nm 19.3 min
344 nm 19.2 min
274 nm 19.1min
344 nm 19.2 min
274 nm 19.1min
344 nm 19.2 min
Abs. (mAU)
Wavelength (nm)
Elution Time (min)
(B) With N3- (1eq. for iso-S)
Reaction time = 30 min
Reaction time = 90 min
Reaction time = 180 min
274 nm 19.2 min
274 nm 18.9 min
274 nm 18.9 min
(C) Peak area detected at 344 nm
Area (V s-1)
Reaction time (min)
Without N3-
With N3-
(D) Mn and Mw detected at 274 nm
Molecular Weight
Reaction time (min)
Mn
Mw
Without N3-
With N3-

(Figure 6)

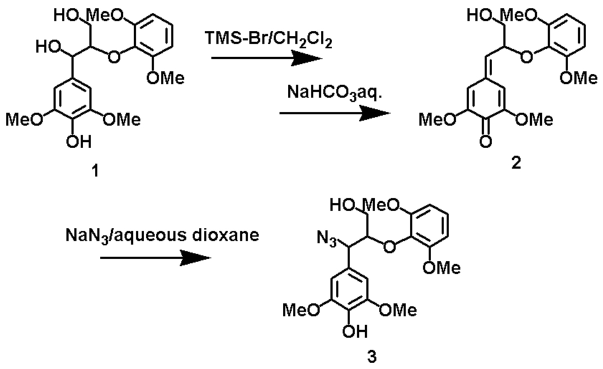


A
DHP Yield (%)
60
40
20
0
0.5
1.0
1.5
2.0
Amount of NaN_3 (eq. for S-alc)
B
Without N_3^-
With N_3^- (1eq. for S-alc)
100
80
60
40
20
0
0.5
24
48
Monomer addition time (h)

(Figure 7)


(A) ¹H-NMR
Without N3-
With N3-
OMe
ArOAc
AlkOAc
Arom. H
ppm
β-O-4/α-N3 (I)
β-O-4/α-OH (II)
β-O-4/α-OAr (III)
β-β (IV)
(B) ¹³C-NMR
Without N3-
With N3-
OAc
C3, C5
C1, C4
C2, C6
ppm
(C) HSQC
Without N3-
With N3-
OMe
(ppm)

(Figure 8)



(Table 1)

Table 1 HRP-catalyzed polymerizations of sinapyl alcohol (S-alc) in the presence and the absenceof sodium azide

Entry	HRP [a] (mg)	Monomer addition time (h)	Without N_3^-				With N_3^- (1eq. for S-alc)			
			Yield (%)	M_n [b] $\times 10^{-3}$	M_w/M_n [b]	DP_n [c]	Yield (%)	M_n [b] $\times 10^{-3}$	M_w/M_n [b]	DP_n [c]
1	6	0.5	4.8	1.4	2.5	4.8	54.2	1.3	1.3	4.4
2	24	0.5	7.0	1.8	2.1	6.1	68.3	1.4	1.3	4.8
3	24	24	10.8	2.1	1.9	7.1	76.0	1.6	1.2	5.4
4	24	48	11.5	2.1	2.0	7.1	82.5	1.8	1.2	6.1

[a] per 1 mmol of S-alc
[b] determined by GPC after acetylation
[c] calculated based on the molecular weight of sinapyl alcohol diacetate