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Letter

Enzymatic synthesis and curing of polycardol from renewable resources

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ABSTRACT

For the first time, oxidative polymerization of cardol derived from cashew nut shell liquid (CNSL), which is a cheap, useful, and renewable substance, has been carried out using a fungal peroxidase from *Coprinus cinereus* (CiP). Cardol, one of the major components of CNSL, is a resorcinol derivative mainly having a C15 unsaturated hydrocarbon chain with 1–3 double bonds at a meta position. To date cardol has not been completely exploited as a monomer for enzymatic polymerization. Enzymatic polymerization of cardol proceeded with higher yield in an equivolume mixture of *tert*-butanol and phosphate buffer (pH 7.0). The yield and molecular weight of polycardol depended on the hydrogen peroxide concentration. Polycardol was rapidly cured at room temperature within 4 h to give harden dry and dark brown color coatings. Pencil scratch hardness data indicated that the curing rate of polycardol was superior to those of polycardanol. Thermogravimetric analysis implied that the cured product from polycardol was thermally more stable than that from polycardanol. We expect that polycardol from renewable resources, which is similar to or superior to polycardanol, can find many applications in the near future.

1. Introduction

Enzyme-catalyzed polymerization has drawn much attention as a new methodology of polymer syntheses [1–5]. Major advantages of the enzymatic polymerization of phenol derivatives are (a) enzyme-catalyzed polymerization provides new polymeric materials, which are difficult to be obtained by conventional chemical methods; (b) enzymatic polymerization of phenols proceeds under mild conditions without the use of toxic reagents such as formaldehyde; (c) the phenolic moiety can be chemoselectively reacted in the peroxidase-catalyzed polymerization; (d) the solubility and structure of the polymer can be readily controlled by changing the reaction conditions [6–8]. Peroxidase-catalyzed polymerization has been successfully applied to synthesis of conducting polymers [9,10] and to removal of phenols from wastewater [11–14]. Recently, bisphenol A was enzymatically polymerized to form a new type of photoresist resin [15].

There is increasing interest in polymers which can be synthesized from renewable resources rather than petroleum-based feedstocks. This is evidence of a global requirement for sustainability without resource depletion [16]. Cashew nut shell liquid (CNSL) occurs as a greenish-yellow viscous liquid in the soft honeycomb of the shell of the cashew nut. CNSL, a side-product of the cashew industry, is a cheap and renewable substance. CNSL as a monomer for industrial polymer products can be very attractive in view of its low cost, abundant availability, and chemically reactive nature. Resins derived from CNSL are employed widely in the fields of friction materials, automobiles, surface coatings, adhesives, laminates, rubber compounding, and have several miscellaneous applications [17].

Commercially available technical CNSL contains mainly cardanol and cardol [18]. Cardanol, which is a phenol derivative having a meta substituent of a C15 unsaturated hydrocarbon chain mainly with 1–3 double bonds, has been successfully polymerized using soybean peroxidase (SBP) in aqueous organic solvents [19,20]. We first showed that horseradish peroxidase, which was known to be inactive toward cardanol, was able to catalyze polycardanol synthesis when appropriate mediators were used [21]. We also succeeded in polymerizing cardanol using microbial peroxidases, which are much more economical than plant peroxidases such as SBP [22]. Recently, we have synthesized epoxide-containing polycardanol from cardanol using lipase and peroxidase [23]. Cardol, another main component of technical CNSL, is a resorcinol derivative mainly having a C15 unsaturated hydrocarbon chain with 1–3 double bonds at a meta position. In contrast to cardanol, cardol has not been employed as a monomer for enzymatic polymerization to the best of our knowledge.

The current study has examined the enzymatic polymerization of cardol using a fungal peroxidase from *Coprinus cinereus* for the first time. Curing behavior and thermal stability of polycardol have been explored and compared to those of polycardanol.

2. Experimental

2.1. Materials

Technical grade of CNSL (Product No. AF6155, Palmer International, USA) is a dark brown colored liquid composed of cardanol (67 wt%), cardol (29 wt%) and the rest (4 wt%). Cardol was isolated from the CNSL by Kumar method [18], and its purity (93 wt%) and identity were confirmed by HPLC, ^{1}H NMR, and FT-IR. A fungal peroxidase from *Coprinus cinereus* IFO 8371 (CiP) was prepared as previously reported [22]. Other reagents were commercially available and used as received.

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Table 1
Enzymatic polymerization of cardol

Entry	Organic solvents	H_2O_2 mmol (%)	Polymer yield (%)	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n
1	2-Propanol	1.0 (10%)	28	3.2	6.9	2.1
2	1,4-Dioxane	1.0 (10%)	15	1.6	2.8	1.8
3	Acetone	1.0 (10%)	16	2.1	4.2	2.0
4	*tert*-Butanol	0.5 (5%)	54	6.2	16.4	2.6
5	*tert*-Butanol	1.0 (10%)	66	5.6	13.5	2.4
6	*tert*-Butanol	1.5 (15%)	31	3.3	5.4	1.6
7	*tert*-Butanol	3.0 (30%)	13	3.1	4.4	1.4

CiP-catalyzed polymerization of cardol (2.0 mmol) in a mixture of organic solvents (12.5 mL) and 0.1 M phosphate buffer (12.5 mL, pH 7.0).

2.2. Enzymatic polymerization of cardol

Unless otherwise mentioned, the enzymatic polymerization of cardol was carried out as follows. 0.632 g (2.0 mmol) of cardol was dissolved in a mixture of 12.5 mL *tert*-butanol and 12.5 mL phosphate buffer (0.1 M, pH 7.0). Six thousand units of CiP was then added to the reaction mixture. H_2O_2 (10 vol% aqueous solution, 340 μL) was continuously added by a perfusion pump for 5 h at room temperature under gentle stirring. After 24 h, the reaction mixture was concentrated under reduced pressure. Ethyl acetate (30 mL) and water (10 mL) was added to the residue and the organic layer was separated and solvent evaporated under reduced pressure. Methanol was poured into the oily residue. The methanol-insoluble portion was separated by centrifugation and dried in a vacuum. The polycardol yield was determined by measuring the dry weight of the polymer precipitated by the methanol addition.

2.3. Analysis

The activity of the enzyme was measured as previously described [22]. One unit of peroxidase was defined as the amount of enzyme required to catalyze the conversion of 1 μmol of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) or ABTS ($\varepsilon = 35\,mM^{-1}\,cm^{-1}$ at 405 nm) per min at 25 °C. Gel permeation chromatograph (GPC) analysis was carried out using a refractive index detector under the following conditions: PL4 mixed BB columns (Tosoh, Japan) and tetrahydrofuran as solvent at 1 mL/min. The calibration curves for GPC analysis were obtained using polystyrene standards. ^{1}H NMR and FT-IR spectra were recorded on a 500 MHz AVANCE 500 spectrometer (Bruker Co., Germany) and FT/IR-6100 spectrometer (JASCO International Co., Japan), respectively. The film hardness was evaluated using a pencil scratch apparatus (ASTM D 3363-74). Thermogravimetric analysis (TGA) was carried out using a TGA 2950 thermobalance (TA instruments, Inc., USA). The temperature was increased to 800 °C at a heating rate of 10 °C/min in N_2 atmosphere.

3. Results and discussion

3.1. Enzymatic polymerization of cardol

Enzymatic polymerization of cardol using CiP was performed in an equivolume mixture of water-miscible organic solvents and phosphate buffer (0.1 M, pH 7.0) at room temperature for 24 h. Hydrogen peroxide was used as oxidizing agent. Even though the peroxidase activity in buffer was much higher than that in aqueous organic solvent, the yield of phenolic polymers obtained by peroxidases in buffer has been reported to be far lower than that obtained in a mixture of buffer and organic solvent. As a rule, a high yield was achieved in the buffer content of about 50% [24]. As summarized in Table 1, the higher yield of polycardol was obtained in *tert*-butanol/phosphate buffer than in aqueous 2-propanol, 1,4-dioxane, and acetone. This is interesting, considering that CiP-catalyzed polymerization of cardanol did not proceed in *tert*-butanol/phosphate buffer and the highest yield of polycardanol was obtained in 2-propanol/buffer solution [22]. Similarly, even when CiP was replaced with SBP for the cardanol polymerization, the yield was the highest in 2-propanol/phosphate buffer (0.1 M, pH 7.0) and polycardanol was not obtained in *tert*-butanol/buffer [20]. From these results, it appears that solvent effects on the polymer yield are more closely related to monomer nature than peroxidase type. Table 1 also shows effects of the H_2O_2 concentration on the polymer yield and molecular weight in an equivolume mixture of *tert*-butanol and pH 7.0 phosphate buffer. The optimum H_2O_2 concentration is expected to exist because hydrogen peroxide acts as an enzyme denaturant as well as an oxidant for peroxidase-catalyzed polymerization. The yield of polycardol was the highest at 1.0 mmol of hydrogen peroxide. It is quite likely that little H_2O_2 is not sufficient for polycardol synthesis and much H_2O_2 is not good for CiP activity. These results imply that selection of appropriate solvents and H_2O_2 concentration is essential for successful oxidative polymerization catalyzed by peroxidases. Number average molecular weight (M_n) and weight average molecular weight (M_w) of the polycardol obtained at the highest yield were 5600 and 13500 Da, respectively, which were higher than those of the polycardanol

Table 2
Pencil scratch hardness of products cured by catalysis of cobalt naphthenate

Sample	Time							
	2 h	4 h	1 d	3 d	5 d	9 d	14 d	20 d
Cardol	–	–	–	–	–	–	–	–
Cardanol	–	–	–	–	–	–	–	–
Polycardol[a]	TF[c]	HD[e]	1H	3H	4H	5H	6H	7H
Polycardanol[b]	DF[d]	DF	2B	2H	4H	4H	4H	5H
Commercial lacquer tree paint	–	–	–	HB	H	2H	3H	4H

[a] Soluble polycardol obtained from entry 5 in Table 1.
[b] Soluble polycardanol (M_n 5600) obtained using CiP in aqueous 2-propanol in our laboratory.
[c] Touch free dry.
[d] Dust free dry.
[e] Harden dry.

Table 3
Pencil scratch hardness of products cured by thermal treatment

Sample	Time				
	0.5 h	1 h	2 h	4 h	1 d
Cardol	–	–	–	3H	9H
Cardanol	–	–	–	–	HD
Polycardol	4B	2B	2H	3H	9H
Polycardanol	TF	HD	1H	3H	9H

Thermal treatment of film was prepared on glass slide using applicator to form a film of 100 μm thickness at 150 °C.

reported previously [20,22]. The formed polycardol was soluble in ethyl acetate, xylene, tetrahydrofuran, 2-butanone, and chloroform, and was insoluble in methanol, acetone, and water.

3.2. Curing properties

The curing was examined by two methods: catalysis of cobalt naphthenate and thermal treatment. The sample coating was prepared on a glass slide using an applicator to produce a coating of 100 μm thickness at room temperature. The curing was monitored by pencil scratch hardness. Table 2 shows the curing behaviors by cobalt naphthenate (0.18 wt%) and methyl ethyl ketone peroxide (MEKP, 0.32 wt%). Cardol and cardanol were not cured, while their polymers were cured and the hardness increased with time. However, the curing rates of polycardol and polycardanol were different. For example, when cured at room temperature for 4 h, polycardol gave harden dry and dark brown color coatings. By contrast polycardanol was dust free dry and yellow transparent. Thus, the curing rate of polycardol was higher than that of polycardanol. With regard to hardness, polycardol showed higher pencil scratch hardness than polycardanol after the 20 d curing: 7H for polycardol and 5H for polycardanol. Oriental lacquer film, which was examined for reference, showed the lowest curing rate and hardness. In Table 3, the curing behaviors by thermal treatment (150 °C) are shown. The curing by thermal treatment proceeded more rapidly than that by cobalt naphthenate. The pencil scratch hardness of polycardol increased at a higher rate compared to that of polycardanol. From these results, it appears reasonable to conclude that the curing rate of polycardol is higher than that of polycardanol irrespective of curing methods. This may be because cardol has higher degree of unsaturation at the side chain than cardanol: the compositions of triene constituent of cardol and of cardanol are 65 wt% and 41 wt%, respectively [25]. More unsaturated groups of cardol may give rise to the higher curing rate. The curing rate of polymers is one of important factors for industrial and commercial applications. In this respect, the higher curing rate may enable polycardol to have more applications compared to polycardanol.

3.3. FT-IR and ^{1}H NMR spectroscopy

The chemical structure of polycardol was examined using FT-IR and ^{1}H NMR spectroscopy. Fig. 1(a) and (b) show the FT-IR spectra of cardol and polycardol, respectively. The broad peak at 3400 cm^{-1} is due to the vibration of the O—H linkage of phenolic group. This peak was observed in the FT-IR spectrum of polycardol. The absorption bands between 1300 and 1000 cm^{-1} are characteristic for phenyl ether and vinyl ethers [26]. In Fig. 2, ^{1}H NMR spectra of cardol and polycardol are shown. Most peaks of polycardol became broader than those of cardol as shown in cardanol and polycardanol. These data reveal that polycardol formed by CiP consisted of a mixture of phenylene and oxyphenylene units like polycardanol [19]. The proposed chemical structure of polycardol synthesized by the enzyme is shown in Fig. 3. FT-IR spectroscopy can be also used for monitoring of the curing behavior. Fig. 1(c) indicates the FT-IR spectrum of

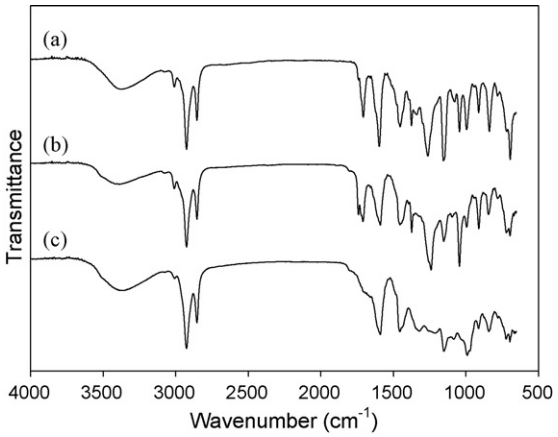

Fig. 1. FT-IR spectra of (a) cardol, (b) polycardol, and (c) polycardol cured for 4 h.

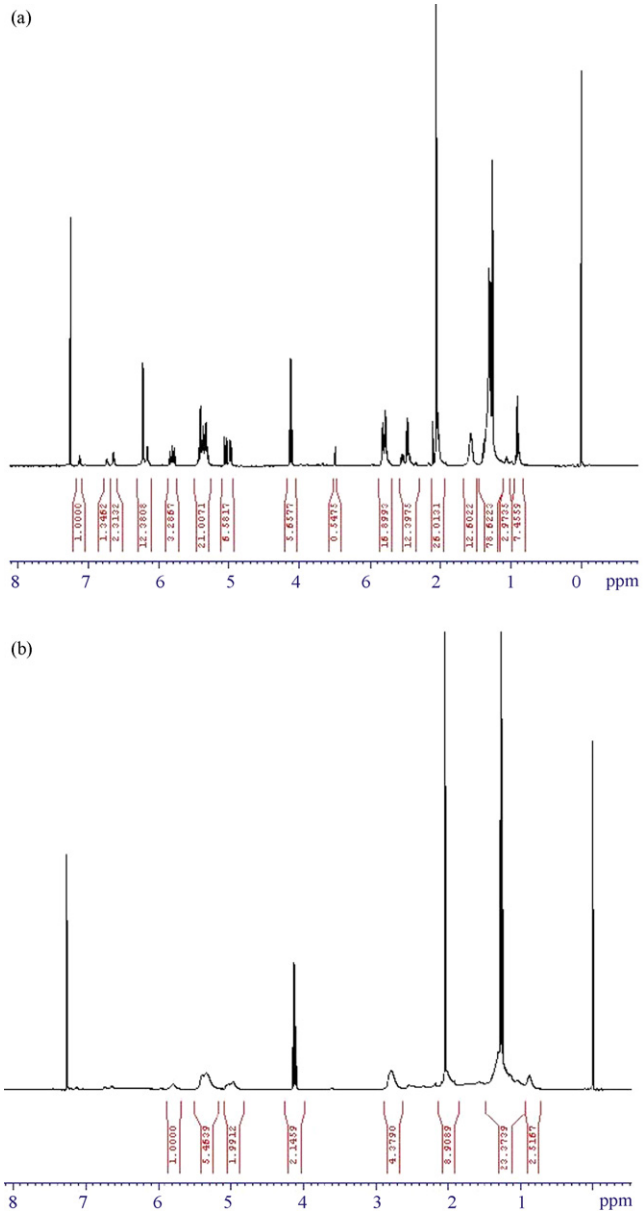

Fig. 2. ^{1}H NMR spectra of (a) cardol and (b) polycardol.

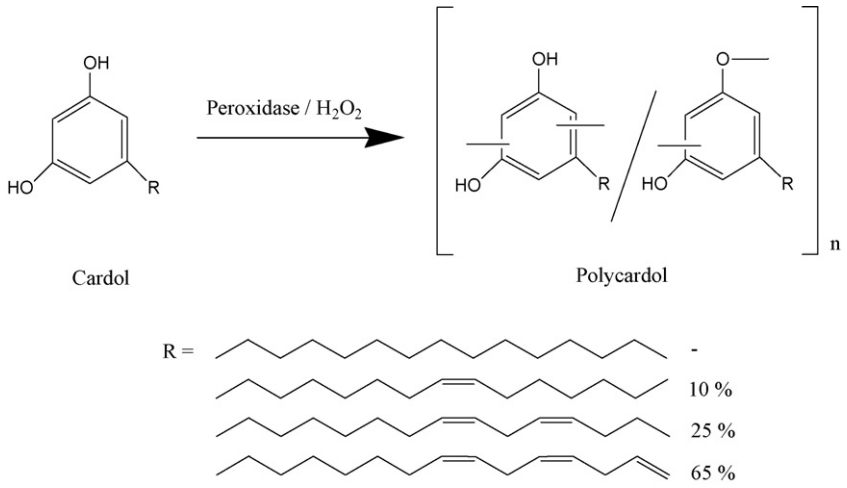

Fig. 3. Peroxidase-catalyzed polymerization of cardol and the proposed chemical structure of the resultant polycardol.

polycardol cured at room temperature for 4 h. A peak at 3010 cm^{-1} due to the unsaturated group became small when polycardol was cured. Intensity of peak at 910 cm^{-1} ascribed to C-H vibration of the terminal vinyl group also decreased with curing [19].

3.4. Thermal stability

Thermal stability of the product polymers was evaluated by thermogravimetric analysis. The measurement was performed under N_2 atmosphere. Thermal properties of cured polycardol in this study are influenced mainly by phenol derivative having a long alkyl side chain at meta position, phenolic hydroxyl group, the configuration of the molecule, the molecular weight between crosslinks, the degree of segments in curing hardness, the bond degree of phenylene and oxyphenylene [27]. Fig. 4 shows TGA thermograms of polycardol and polycardanol cured by the catalyst at room temperature for 30 d. Weight loss rose gradually until 400 °C and then increased sharply. The characteristic thermal data are also provided with the temperatures at the various percentage weight losses as shown in Table 4. The temperatures at 10% decomposition for polycardol and polycardanol were 289 and 178 °C, respectively (Table 4). From the TGA data, it appears that the cured product from polycardol has higher thermal stability than that from polycardanol. This might be because cardol has the higher degree of unsaturation at a meta position, leading to higher cross-linking density than cardanol. The higher cross-linking density results in the higher thermal stability [28]. This result indicates that the polycardol may be more proper for high-temperature applications than polycardanol.

Table 4
Thermal decomposition of the polycardol and polycardanol cured at room temperature

	Temperature (°C) at the various percent weight losses				
	10	20	30	50	80
Polycardol	289	398	426	451	489
Polycardanol	178	271	389	445	475

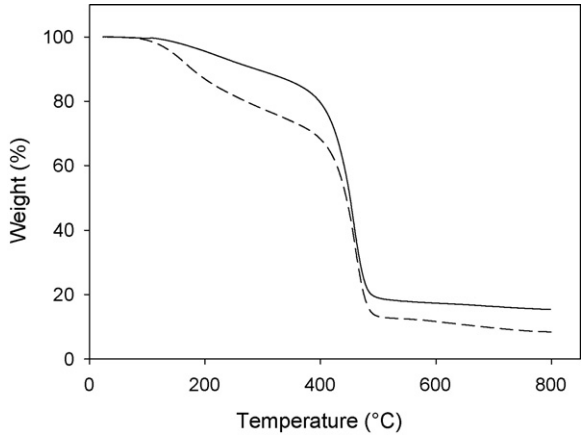

Fig. 4. Thermogravimetric traces of polycardol (solid line) and polycardanol (broken line) by catalyst curing at room temperature for 30 d.

4. Conclusions

For the first time, oxidative polymerization of cardol derived from a renewable resource has been carried out using *Coprinus cinerius* peroxidase. The polycardol synthesized was investigated with GPC, FT-IR, ^{1}H NMR, and TGA. Under appropriate reaction conditions, the polycardol was successfully obtained in 66% yield and M_w of 13500 Da. Compared to polycardanol, polycardol was rapidly cured at room temperature within 4 h to give harden dry and dark brown color coatings. The curing rate of polycardol was higher than that of polycardanol irrespective of curing methods. The TGA data revealed that polycardol was more thermostable than polycardanol when they were cured at room temperature.

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