Peroxidase-Catalyzed Oxidative Polymerization of Cresols to a New Family of Polyphenols

Hiroshi Uyama, Hideharu Kurioka, Junji Sugihara, Izuru Komatsu, and Shiro Kobayashi*

Department of Molecular Chemistry and Engineering, Faculty of Engineering, Tohoku University, Aoba, Sendai 980-77

(Received May 29, 1995)

Enzymatic oxidative polymerization of cresols has been performed by using peroxidase catalyst in two types of polymerization solvent: an aqueous organic solution and a reverse micellar solution, to produce polymeric materials. Peroxidases derived from horseradish and soybean were employed as catalyst. The resulting polymers were partly soluble in DMF and DMSO, but, insoluble in water and other common organic solvents. The molecular weight of the DMF-soluble part was measured by GPC as ca. 5000. The solubility and molecular weight were dependent upon the polymerization condition and monomer structure. From IR and UV analyses, the polymer was found to be composed of a mixture of phenylene and oxyphenylene units. The polymer possessed no clear glass transition or melting points. TG analysis exhibited that the present polymers have relatively high thermal stability. The stability of the polymer from *o*- and *m*-cresols was better than that from the *p*-isomer. The polymer remained in around 40 weight % yield at 1000 °C under nitrogen.

Recently, polymerization catalyzed by an enzyme ("enzymatic polymerization") has received much attention not only as a new methodology for polymer synthesis but also a new aspect of enzymology.[1,2] Characteristic properties of enzymes are expected to induce production of polymers with high selectivity and/or with novel structure. Typical examples of enzymatic polymerization are the first in vitro synthesis of cellulose via non-biosynthetic path by cellulase-catalyzed polymerization of β-cellobiosyl fluoride[3] and the enantioselective polymerization of an epoxide-containing diester with a diol catalyzed by lipase, yielding an optical active polyester.[4]

Phenol-formaldehyde resins such as novolaks and resols are widely used in industrial fields.[5] These resins show excellent toughness and temperature-resistant properties. However, the toxicity problem of formaldehyde has hindered in their manufacture and use. Therefore, an alternative process for preparation of phenol polymers without using formaldehyde is strongly desired.

For several years, enzymatic synthesis of polyaromatics has been extensively explored.[6—13] Oxidative polymerization of *o*-phenylenediamine catalyzed by horseradish peroxidase (HRP) produced a soluble polymer having an iminophenylene unit with a molecular weight of 2×10^4,[6] which is hard to obtain by conventional oxidative polymerizations.

Klibanov and his co-workers first reported the enzymatic synthesis of polyphenols by oxidative polymerization of various phenol derivatives catalyzed by HRP.[7] The polymerization was carried out in an aqueous organic solution in order to increase the solubility of the polymer. Recently, we have synthesized a new class of polyphenols from phenol and alkylphenols using HRP catalyst,[8,9] which are composed of a mixture of phenylene and oxyphenylene units. In the polymerization of alkylphenols, the position and chain length of the alkyl substituent enormously affected the polymerization.

Reverse micellar systems are widely employed in biotechnology fields such as separation and purification of proteins.[14,15] Enzymatic synthesis of poly(*p*-ethylphenol) was conducted by the polymerization in the reverse micellar system using bis(2-ethylhexyl) sodium sulfosuccinate (AOT) as surfactant.[10] This system afforded a polymer with higher molecular weight in a higher yield than that obtained by the polymerization in the aqueous organic solvent. However, this system involved the use of much detergent to stabilize the micelle.

p-Cresol has been reported to be polymerized by HRP catalyst in a mixture of 1,4-dioxane and buffer.[7,9] Dordick et al. explored the kinetics of HRP-catalyzed oxidation of *p*-cresol in various aqueous organic solvents.[16] The characterization of the resulting polymer, however, has not been well conducted. To our knowledge, there has no report on the enzymatic oxidative polymerization of other cresol isomers, *o*- and *m*-

cresols. This article describes the peroxidase-catalyzed polymerization of *o*-, *m*-, and *p*-cresols in the aqueous organic and reverse micellar solutions and the thermal properties of the resulting polymers.

Experimental

Materials. HRP and soybean peroxidase (SBP) were purchased from Wako Chemical Co. and Sigma Chemical Co., respectively. These enzymes were employed without further purification. Other reagents and solvents were commercially available and were used as received.

Enzymatic Polymerization of Cresols in Aqueous Organic Solution. A typical run was as follows (Entry 6 in Table 1). *p*-Cresol (0.54 g, 5 mmol) and SBP (20 mg) in a mixture of 20 mL of 1,4-dioxane and 5 mL of 0.1 M phosphate buffer (pH 7) were placed in a 50 mL flask (1 M=1 mol dm^{-3}). 30% Hydrogen peroxide (28 μL, 0.25 mmol) was added to the mixture every 15 min for 20 times at room temperature under air. After 24 h, the solvent in the reaction mixture was evaporated under reduced pressure. The residue was washed successively with methanol, water, and methanol, followed by drying in vacuo to give 0.19 g of the polymer (yield 35%): ^{1}H NMR (DMSO-d_6) δ=2.2 (br, C$\underline{H}_3$-Ar), 7.0 (br, Ar).

Enzymatic Polymerization of Cresols in Reverse Micellar System. A typical run was as follows (Entry 6 in Table 2). *p*-Cresol (0.32 g, 3.0 mmol) and AOT (1.34 g, 3.0 mmol) were dissolved in 20 mL of isooctane (2,2,4-trimethylpentane). SBP (20 mg) in 0.82 mL of 0.1 M phosphate buffer (pH 7) was slowly added to the solution under stirring. 30% Hydrogen peroxide (28 μL, 0.25 mmol) was added to the mixture every 15 min for 16 times at room temperature under air. After 24 h, the resulting precipitate was collected by filtration; it was washed with water and then with isooctane. The residue was dried in vacuo for several hours and then dispersed in methanol. The insoluble part was collected by filtration, followed by drying in vacuo to give 0.12 g of the polymer (yield 39%).

Measurements. GPC analysis was carried out using a Toso SC8010 apparatus with a refractive index (RI) detector under the following conditions: TSKgel G4000H$_{HR}$ or G2500H$_{HR}$ column and DMF containing 0.02 M LiCl eluent at a flow rate of 0.5 mL/min. The calibration curves for GPC analysis were obtained using polystyrene standards.

Table 2. Enzymatic Polymerization of Cresols in Reverse Micellar System[a)]

Entry	Catalyst	Monomer	Polymer		
			Yield[b)]	M_n[c)]	M_w/M_n[c)]
			%	$\times10^{-3}$	
1	HRP	*o*-Cresol	28	4.6	2.4
2	HRP	*m*-Cresol	12	7.8	2.8
3	HRP	*p*-Cresol	18	2.7	1.6
4	SBP	*o*-Cresol	48	8.7	3.1
5	SBP	*m*-Cresol	30	6.6	3.1
6	SBP	*p*-Cresol	39	2.9	1.6

a) Polymerization of cresol in a mixture of isooctane, phosphate buffer (pH 7), and AOT at room temperature for 24 h. b) Methanol-insoluble part. c) Determined by GPC. Values for DMF-soluble part of the polymer.

Monomer conversion was determined using the GPC system fitted with TSKgel G1000H$_{HR}$ column (GPC column for analysis of low molecular weight compounds) of DMF eluent. HPLC analysis was performed using a Hitachi 655A-12 pump and 655A UV monitor under the following conditions: Toso ODS-80Ts column and aqueous methanol eluent (water : methanol = 25 : 75 vol%) at a flow rate of 0.6 mL min^{-1}. ^{1}H NMR spectra were recorded on a 250 MHz Bruker AC-250T spectrometer. IR and UV spectra were recorded on Shimadzu IR-460 and UV-160 spectrometers, respectively. DSC measurement was made at a 10 °C min^{-1} heating rate under nitrogen using a Seiko SSC/5200 differential scanning calorimeter calibrated with an indium reference standard. TG analysis was performed using a Seiko SSC/5200 apparatus for thermogravimetry/differential thermal analysis at a heating rate of 10 °C min^{-1} in a gas flow rate of 300 mL min^{-1}.

Results and Discussion

Enzymatic Polymerization of Cresols in Aqueous Organic Solution. Peroxidases are enzymes whose primary function is to oxidize a variety of hydrogen donors such as phenol and aniline derivatives at the expense of hydrogen peroxide. Peroxidases used in this study were derived from horseradish and soybean (HRP and SBP, respectively). SBP has been first em-

Table 1. Enzymatic Polymerization of Cresols in Aqueous Organic Solvent[a)]

Entry	Catalyst	Monomer		Polymer			
		Structure	Conv.[b)]	Yield[c)]	DSP[d)]	M_n[b,e)]	M_w/M_n[b,e)]
			%	%	%	$\times10^{-3}$	
1	HRP	*o*-Cresol	96	44	3	—	—
2	HRP	*m*-Cresol	95	72	1	—	—
3[f)]	HRP	*p*-Cresol	99	6	8	4.4	1.8
4	SBP	*o*-Cresol	94	64	12	5.6	2.0
5	SBP	*m*-Cresol	78	42	3	—	—
6	SBP	*p*-Cresol	93	35	37	5.1	1.8

a) Polymerization of cresol in a mixture of 1,4-dioxane and phosphate buffer (pH 7.0) (80 : 20 vol%) at room temperature for 24 h. b) Determined by GPC. c) Methanol-insoluble part. d) DMF-soluble part of the polymer. e) Values for DSP. f) Data from Ref. 9.

ployed as catalyst for the enzymatic oxidative polymerizations. SBP is anionic peroxidase isozyme composed of glycoprotein.[17]

Enzymatic polymerization of cresols using peroxidase catalyst was performed in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (80:20 vol%) at room temperature under air. Table 1 summarizes results of the polymerization in the aqueous dioxane. In the HRP-catalyzed polymerization of *p*-cresol (Entry 3), the monomer was consumed quantitatively to produce pale yellow powdery polymers, but, the yield of the polymer (methanol-insoluble part) was low. The polymer was partly soluble in *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). The ratio of the DMF-soluble part of the polymer was 8%. The molecular weight of the polymer was estimated by gel permeation chromatography (GPC). The molecular weight of the DMF-soluble part was 4400, which was lower than that from phenol under similar reaction conditions.[8]

In our previous study, *o*- and *m*-isopropylphenols were found not to be polymerized by HRP catalyst in the aqueous 1,4-dioxane.[9] This may be due to the steric hindrance of the substituent on the monomer. On the other hand, *o*- and *m*-cresols were polymerized by HRP catalyst to produce polymeric materials (Entries 1 and 2), indicating that these monomers are recognized and oxidized by this enzyme. The solubility of the resulting polymers from *o*- and *m*-cresols toward DMF was very low, so, the molecular weight could not be measured by GPC.

SBP also catalyzed the polymerization of cresols to produce the polymer. The SBP-catalyzed polymerization of cresols produced a polymer showing higher solubility toward DMF than that obtained using HRP catalyst. The yield of the polymer from *o*- and *p*-cresols by SBP catalyst was larger than that by HRP; on the other hand, the opposite tendency was observed in the polymerization of *m*-cresol catalyzed by these peroxidases.

Enzymatic Polymerization of Cresols in Reverse Micellar System. Bis(2-ethylhexyl) sodium sulfosuccinate (AOT) was used as detergent for reverse micellar system. The molar ratio of AOT and water was around 15. Polymerization results are shown in Table 2. In the reversed micellar system, a precipitate was formed during the polymerization. The precipitate was collected by filtration to give the powdery compound in 90% yield in the case of the *p*-cresol polymerization. The precipitate was further separated by washing with methanol to give a methanol-insoluble polymer in 18% yield (Entry 3). The molecular weight of DMF-soluble part was determined by GPC as 2700, which is smaller than that obtained by the polymerization in the aqueous organic solvent.

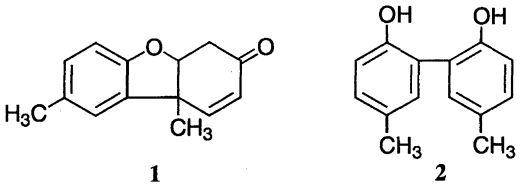
Polymeric materials were also obtained by the HRP-catalyzed polymerization of *o*- and *m*-cresols in the reverse micellar solution (Entries 1 and 2). The polymerization of *m*-cresol using HRP and SBP catalysts in the aqueous 1,4-dioxane produced insoluble products (Table 1), on the other hand, poly(*m*-cresol) obtained in the reverse micellar system was partly soluble in DMF. The molecular weight of the DMF-soluble part was evaluated by GPC as 7800, which was higher than that from other cresols (Entry 2).

HPLC Analysis of Methanol Soluble Products. It is known that Pummerer's ketone (**1**) and 2,2′-dihydroxy-5,5′-dimethylbiphenyl (biphenyl, **2**) are the main products in the chemical oxidation of *p*-cresol (Scheme 1). **1** is formed by *ortho–para* coupling of two molecules of *p*-cresol, followed by isomerization. Recently, the distribution of these compounds in the HRP-catalyzed oxidation of *p*-cresol in various solvents has been explored in detail.[18] In an aqueous solution, **1** was mainly formed, on the other hand, the main product was **2** in organic solvents containing a small amount of water and the ratio of the two products was dependent upon the water content.

As shown in Table 1, the monomer conversion was very high in the enzymatic polymerization of *p*-cresol in the aqueous organic solvent; however, the polymer yield was low, i.e., the low-molecular weight products (methanol-soluble part) were mainly formed. The methanol-soluble part was then analyzed by HPLC using an inverse-phase silica gel column. In the case of the SBP-catalyzed polymerization, peaks due to a number of other low-molecular weight products besides dimers **1** and **2** were observed in the HPLC chart (Fig. 1). These peaks overlapped, so other products could not be separated by HPLC.

Product yields of **1** and **2** determined by HPLC are shown in Table 3. In all cases, Pummerer's ketone **1** was mainly obtained and the yield of the *ortho–ortho* dimer was 2—4% based on the monomer. This tendency is similar to that with the HRP-catalyzed oxidation in an aqueous solution.[18] The methanol-soluble part obtained by the polymerization of *o*- and *m*-cresols was also analyzed by HPLC. There were several peaks in the HPLC chart and the main peaks were overlapping with each other.

Structure of Poly(cresol)s. The product polymer showed low solubility toward various organic solvents. Therefore, the structure of the polymer was estimated mainly by IR analysis. Figure 2(C) shows IR spectrum of poly(*p*-cresol)s obtained by the SBP-catalyzed polymerization in the aqueous 1,4-dioxane. A broad peak centered at 3370 cm^{-1} is ascribed to the vibration of the phenolic O–H bond. The absorption



1 **2**

Scheme 1.

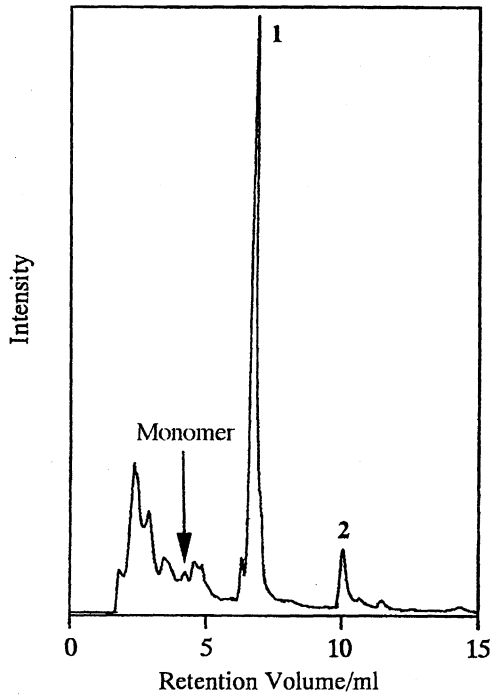


Fig. 1. HPLC chart of methanol soluble part obtained by HRP-catalyzed polymerization of *p*-cresol in aqueous 1,4-dioxane.

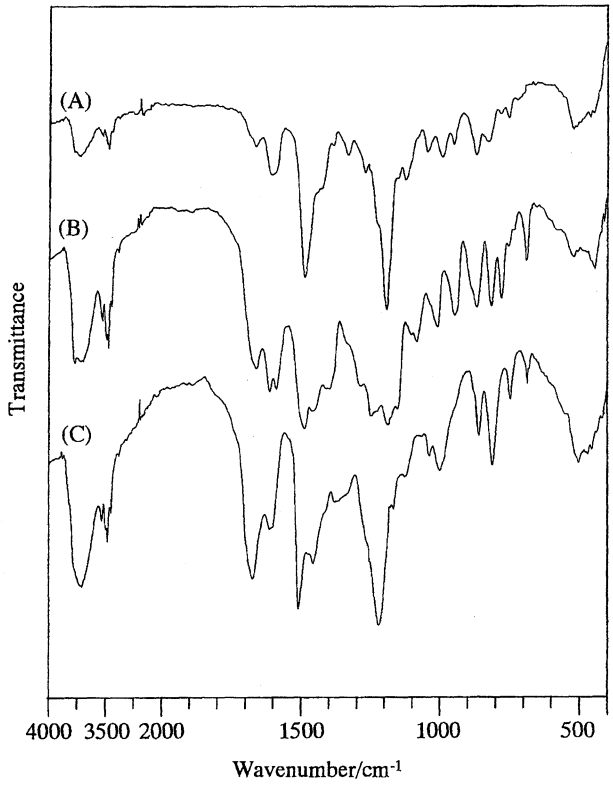


Fig. 2. IR spectra of polymers obtained by SBP-catalyzed polymerization in aqueous 1,4-dioxane: (A) poly(*o*-cresol); (B) poly(*m*-cresol); (C) poly(*p*-cresol).

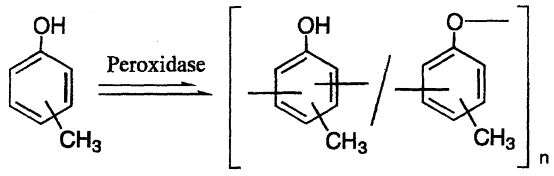
Table 3. HPLC Analysis of Methanol-Soluble Part Obtained by Enzymatic Oxidative Polymerization of *p*-Cresol

Entry	Polymerization[a]		Yield[b]	
	Catalyst	Solvent	**1**	**2**
			%	%
1	HRP	Aqueous 1,4-dioxane	16	4
2	HRP	Reverse micellar solution	19	2
3	SBP	Aqueous 1,4-dioxane	27	3
4	SBP	Reverse micellar solution	7	2

a) Polymerization condition, see footnote of Tables 1 and 2. b) Based on monomer. Determined by HPLC.

peaks at 1603, 1490, 811, and 752 cm^{-1} are characteristic of the various vibration modes of the C–H and C–C bonds of aromatic nuclei. A peak due to the asymmetric vibrations of the C–O–C linkage and a peak ascribed to the C–OH vibration are overlapping at 1213 cm^{-1}. These data indicate that the polymer structure is composed of a mixture of phenylene and oxyphenylene units (Scheme 2).

A strong and broad peak at 1661 cm^{-1} is attributed to the C=O stretching vibration of the quinone. The quinone moiety may be formed when the phenolic hydroxyl group at the ends of the chain is oxidized. In



Scheme 2.

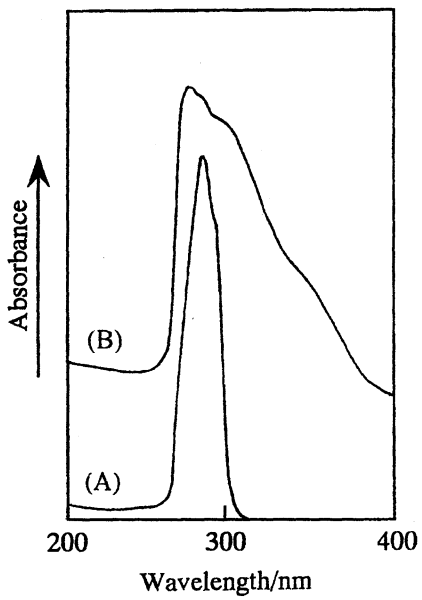


Fig. 3. UV spectra in DMF: (A) *p*-cresol; (B) poly(*p*-cresol) obtained by SBP-catalyzed polymerization in aqueous 1,4-dioxane.

HRP-catalyzed or electrochemical polymerization of phenol derivatives, the absorption due to the quinone was observed[19,20]

Figure 3 shows UV spectra of *p*-cresol and the DMF-soluble part of the poly(*p*-cresol), which was obtained by the SBP-catalyzed polymerization in the aqueous organic solvent. The monomer shows a sharp peak at 272 nm. In the spectrum of the polymer, the broadening of the peak is observed, its maximum being at the same wavelength. There is no absorption in the region of wavelength longer than 400 nm, supporting the conclusion that the present polymer is not composed of single unit of a phenylene, but of a mixture of phenylene and oxyphenylene units. A phenylene polymer from 1,5-dihydroxynaphthalene showed the UV absorption in the range of 300—470 nm.[13]

IR spectrum of the polymer from *o*-cresol is shown in Fig. 2(A). A broad peak due to the phenolic O–H bond is observed at 3430 cm^{-1}. A strong characteristic peak at 1187 cm^{-1} corresponds to the asymmetric vibration of the C–O–C linkage and the C–OH vibration. In the spectrum of poly(*m*-cresol) (Fig. 2(B)), a peak due to the asymmetric vibration of the ether bond is overlapping with a peak due to the C–OH vibration at 1188 cm^{-1}. A peak at 1098 cm^{-1} corresponds to the symmetric vibration of the ether bond. UV spectra of these polymers have broader absorption widths than those of the corresponding monomers. The absorption maxima of the polymers are not changed from those of the monomers and there is no absorption in the area of wavelength longer than 350 nm. From these data, the polymers from *o*- and *m*-cresols are supposed to be also composed of a mixture of phenylene and oxyphenylene units.

Thermal Properties of Poly(cresol)s. Thermal properties of the product polymers were evaluated by differential scanning calorimetry (DSC) and thermogravimetry (TG). Figure 4 shows DSC traces of the polymer obtained by the SBP-catalyzed polymerization of *p*-cresol in 1,4-dioxane-phosphate buffer. In the first scan (A), an exothermic peak is observed at 150 °C. This peak may correspond to the branching and/or crosslinking of the polymer. Similar DSC traces were obtained in the polymers prepared by the HRP-catalyzed polymerization of phenol[19] and *p*-phenylphenol.[11] There are no clear glass transition or melting points (T_g and T_m, respectively) below 250 °C in the second (not shown) and third (B) scans. The polymers from *m*- and *o*-cresols also shows no clear T_g or T_m.

Figure 5 shows TG traces of poly(*p*-cresol) obtained by using HRP catalyst in the aqueous 1,4-dioxane. The measurement was performed under nitrogen and air. The polymer gradually decomposed below 250 °C.

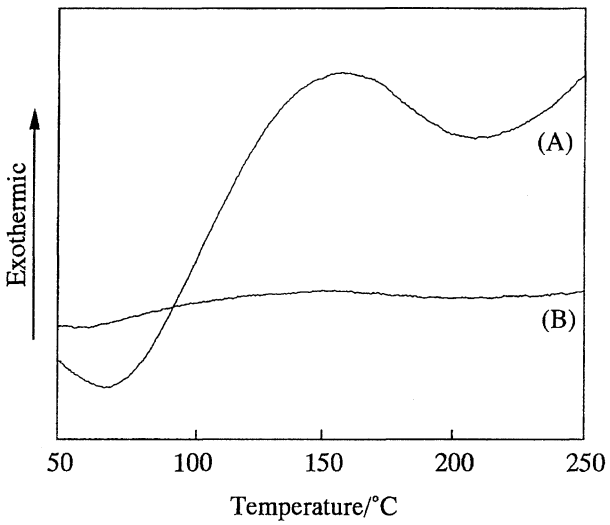


Fig. 4. DSC traces of poly(*p*-cresol) obtained by SBP-catalyzed polymerization in aqueous 1,4-dioxane: (A) first scan; (B) third scan.

Table 4. TG Analysis of Poly(cresol)s[a)]

Entry	Polymerization[b)]			Atmosphere	Polymer		
	Monomer	Solvent	Catalyst		T_{d10}[c)] °C	T_{d100}[d)] °C	Residue[e)] %
1	*o*-Cresol	Aqueous 1,4-dioxane	HRP	Nitrogen	395	—	38
2	*o*-Cresol	Aqueous 1,4-dioxane	HRP	Air	356	548	—
3	*o*-Cresol	Aqueous 1,4-dioxane	SBP	Nitrogen	385	—	36
4	*m*-Cresol	Aqueous 1,4-dioxane	HRP	Nitrogen	368	—	40
5	*m*-Cresol	Aqueous 1,4-dioxane	HRP	Air	340	544	—
6	*m*-Cresol	Aqueous 1,4-dioxane	SBP	Nitrogen	312	—	38
7	*p*-Cresol	Aqueous 1,4-dioxane	HRP	Nitrogen	277	—	40
8	*p*-Cresol	Aqueous 1,4-dioxane	HRP	Air	263	544	—
9	*p*-Cresol	Aqueous 1,4-dioxane	SBP	Nitrogen	262	—	39
10	*p*-Cresol	Aqueous 1,4-dioxane	SBP	Air	258	520	—
11	*p*-Cresol	Reverse micellar solution	HRP	Nitrogen	304	—	42
12	*p*-Cresol	Reverse micellar solution	SBP	Nitrogen	267	—	40

a) Measurement was performed at a heating rate of 10 °C min^{-1}. b) Polymerization condition, see footnote of Tables 1 and 2. c) Temperature at 10% weight loss. d) Temperature at complete decomposition. e) Weight % of residue at 1000 °C.

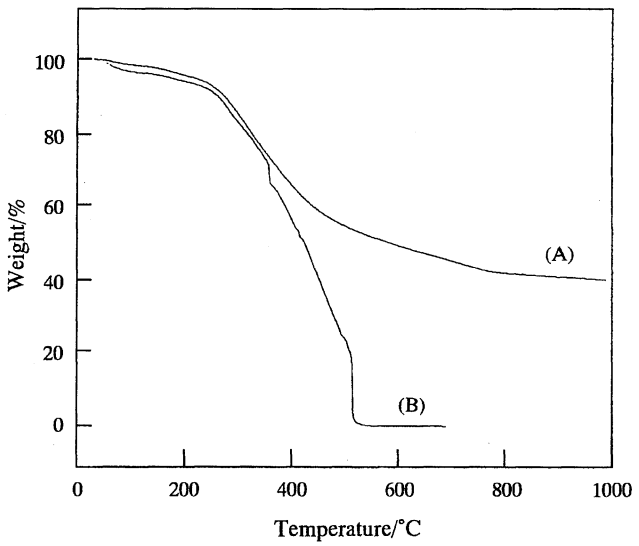


Fig. 5. TG traces of poly(*p*-cresol) obtained by HRP-catalyzed polymerization in aqueous 1,4-dioxane: (A) under nitrogen; (B) under air.

Temperature at 10% weight loss (T_{d10}) of the polymer measured under nitrogen was 277 °C, higher than that under air. These values were lower than that of the polymer obtained by the polymerization of phenol under the similar conditions (387 °C, measurement under nitrogen).[19] The polymer remained in 40 weight % in the measurement at 1000 °C under nitrogen. The residues may be carbonized products such as polyacene and graphite-like polymer.[21] Under air, the polymer was completely decomposed at 544 °C.

Table 4 summarizes TG analysis data of the polymers. T_{d10} value of the polymers prepared by using HRP catalyst was larger than that of the polymer prepared by using SBP. The polymerization of *p*-cresol in the reverse micellar system produced the polymer showing higher T_{d10} than that obtained by the polymerization in the aqueous 1,4-dioxane. T_{d10} values of the polymer from *o*- and *m*-cresols were higher than 300 °C. This value was higher than that of the polymer from *p*-cresol under the similar conditions. The temperature at complete decomposition of the polymer in the measurement under air was around 540 °C regardless of the monomer structure. All the polymers remained in ca. 40% at 1000 °C under nitrogen.

Conclusion. Enzymatic oxidative polymerization of cresols was performed using two peroxidase enzymes. *p*-Cresol as well as *o*- and *m*-isomers were polymerized to produce powdery polymeric materials. The solubility and molecular weight of the polymer depended on the monomer structure and the polymerization conditions. The reverse micellar system afforded the polymer showing higher solubility. From IR analysis, the resulting polymer was composed of a mixture of phenylene and oxyphenylene units. TG analysis indicates that enzymatically synthesized polycresols had relatively high thermal stability. The stability of the polymer from *o*- and *m*-cresols was better than that from *p*-isomer.

The present method provides a convenient and safe pathway to produce a new class of polyphenol analogues, which is expected to be an alternative to the process of conventional phenol resins. Further investigations including regioselective synthesis and applications of the resulting polymers are now under way in our laboratory.

This work was partly supported by Grants-in Aid for Scientific Research (Nos. 06403026 and 07555289) from the Ministry of Education, Science and Culture and by Nissan Science Foundation.

References

1) S. Kobayashi, S. Shoda, and H. Uyama, *Adv. Polym. Sci.*, **121**, 1 (1995).
2) H. Ritter, *Trends Polym. Sci.*, **1**, 171 (1993).
3) S. Kobayashi, K. Kashiwa, T. Kawasaki, and S. Shoda, *J. Am. Chem. Soc.*, **113**, 3079 (1991).
4) J. S. Wallace and C. J. Morrow, *J. Polym. Sci., Polym. Chem. Ed.*, **27**, 2553 (1987).
5) P. W. Kopf, "Encyclopedia of Polymer Science and Engineering," 2nd ed, John Wiley & Sons, New York (1986), Vol. 11, pp 45—95.
6) S. Kobayashi, I. Kaneko, and H. Uyama, *Chem. Lett.*, **1992**, 393.
7) J. S. Dordick, M. A. Marletta, and A. M. Klibanov, *Biotechnol. Bioeng.*, **30**, 31 (1987).
8) H. Uyama, H. Kurioka, I. Kaneko, and S. Kobayashi, *Chem. Lett.*, **1994**, 423.
9) H. Kurioka, I. Komatsu, H. Uyama, and S. Kobayashi, *Macromol. Rapid Commun.*, **15**, 507 (1994).
10) A. M. Rao, V. T. John, R. D. Gonzalez, J. A. Akkara, and D. L. Kaplan, *Biotechnol. Bioeng.*, **41**, 531 (1993).
11) J. A. Akkara, K. J. Senecal, and D. L. Kaplan, *J. Polym. Sci., Polym. Chem. Ed.*, **29**, 1561 (1991).
12) H. Uyama, H. Kurioka, I. Kaneko, and S. Kobayashi, *Macromol. Reports*, **A31**, 421 (1994).
13) L. Wang, E. Kobatake, Y. Ikariyama, and M. Aizawa, *J. Polym. Sci., Polym. Chem. Ed.*, **31**, 2855 (1993).
14) G. A. Ayala, S. Kamat, E. J. Beckman, and A. J. Russell, *Biotechnol. Bioeng.*, **39**, 816 (1992).
15) V. M. Paradkar and J. S. Dordick, *Biotechnol. Prog.*, **7**, 330 (1991).
16) K. Ryu, D. R. Stafford, and J. S. Dordick, *ACS Symp.*, **389**, 141 (1989).
17) J. W. Gillikin and J. S. Graham, *Plant Physiol.*, **96**, 214 (1991).
18) P. Pietikäinen and P. Adlercreutz, *Appl. Microbiol. Biotechnol.*, **33**, 455 (1990).
19) H. Uyama, H. Kurioka, J. Sugihara, and S. Kobayashi, *Bull. Chem. Soc. Jpn.*, in press.
20) M.-C. Pham, J. Moslih, and P.-C. Lacaze, *J. Electroanal. Chem.*, **278**, 415 (1990).
21) K. Funabiki, M. Nakamura, and M. Tsuriya, *Netsukokasei Jushi*, **2**, 220 (1981).