Enzymatic Synthesis and Thermal Properties of a New Class of Polyphenol

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(Received August 7, 1995)

Enzymatic oxidative polymerization of phenol has been carried out in an aqueous organic solvent using horseradish peroxidase as catalyst. The polymerization in a mixture of 1,4-dioxane and phosphate buffer (pH 7.0) (80 : 20 vol%) produced powdery polymeric materials, which were partly soluble in DMF and DMSO. The molecular weight of the DMF-soluble part determined by GPC was 3.5×10^4. Polymerization conditions have been investigated with respect to the polymer yield, solubility, and molecular weight. The solvent composition enormously affected the polymerization. The polymer structure was estimated by IR and NMR spectroscopies and found to contain a mixture of phenylene and oxyphenylene units. From TG analysis, the polymer was found to possess high thermal stability; 43 weight % of the polymer remained up to 1000 °C under nitrogen. DSC measurement showed no clear glass transition temperature or melting point.

Phenol–formaldehyde resins are widely used in industrial fields. Generally, such resins are produced by curing treatment of novolaks and/or resols, which are obtained by poly(addition-condensation) of phenol with formaldehyde. The cured materials are tough, temperature-resistant, and have a low void content.[1] However, the concern over the toxicity of formaldehyde has resulted in limitations on their production and use. Therefore, an alternative process for preparation of phenol polymers, without use of formaldehyde is desired.

There has been much interest in enzymatic polymerizations because of their high potential for the synthesis of novel structures and properties.[2,3] Recently, it has been reported that oxidative polymerizations of phenol and aniline derivatives using bilirubin oxidase or peroxidase as catalyst afforded a new class of polyaromatics.[4—12] Horseradish peroxidase (HRP) induced the polymerization of *o*-phenylenediamine to produce an iminophenylene polymer, which is difficult to be synthesized by conventional oxidative polymerizations. In a previous communication, we have reported enzymatic synthesis of a novel polyphenol by the HRP-catalyzed oxidative polymerization of phenol.[9] The present paper describes comprehensive results on the enzymatic polymerization behavior of phenol and the thermal properties of the resulting polymer.

Results and Discussion

Enzymatic Oxidative Polymerization of Phenol. The HRP-catalyzed oxidative polymerization of phenol was performed at room temperature (20 °C) for 24 h under air. Hydrogen peroxide was employed as oxidizing agent. The polymerization in a phosphate buffer (pH 7.0) did not produce the polymer (Entry 7 in Table 1). This is because the resulting oligomer was not soluble in the buffer solution, thereby preventing the further formation of higher molecular weight polymer.

Table 1. Enzymatic Polymerization of Phenol in Aqueous 1,4-Dioxane of Different Buffer Contents[a]

Entry	Content of buffer	Polymer		
		Yield[b]	DSP[c]	M_n[d]
	%	%	%	$\times10^{-4}$
1	0	0	—	—
2	20	75	25	3.5
3[e]	20	0	—	—
4	40	100	0	—
5	60	95	15	0.62
6	80	77	55	1.4
7	100	0	—	—

a) Polymerization of phenol using HRP catalyst in a mixture of 1,4-dioxane and phosphate buffer (pH 7.0) at 20 °C for 24 h. b) Methanol-insoluble part. c) DMF-soluble part of polymer. d) Determined by GPC. e) Without enzyme.

Then, the polymerization was carried out in a mixture of the phosphate buffer and 1,4-dioxane. Table 1 shows results of the polymerization in the aqueous dioxane. In the mixed solvent, the polymeric material was formed. The polymerization in 60% 1,4-dioxane gave the polymer (methanol-insoluble part) quantitatively (Entry 4); it was insoluble in common organic solvents and water. The polymer was a pale-yellow powdery material. Above or below this content of 1,4-dioxane, the yield decreased (Fig. 1). In only 1,4-dioxane, the monomer was not reacted by HRP catalyst (Entry 1). This is probably because HRP is insoluble in 1,4-dioxane and/or is denaturated by the solvent. A similar behavior was observed in the enzymatic polymerization of *p*-phenylphenol.[4] In the polymerization without the enzyme under the similar conditions (control experiment, Entry 3),

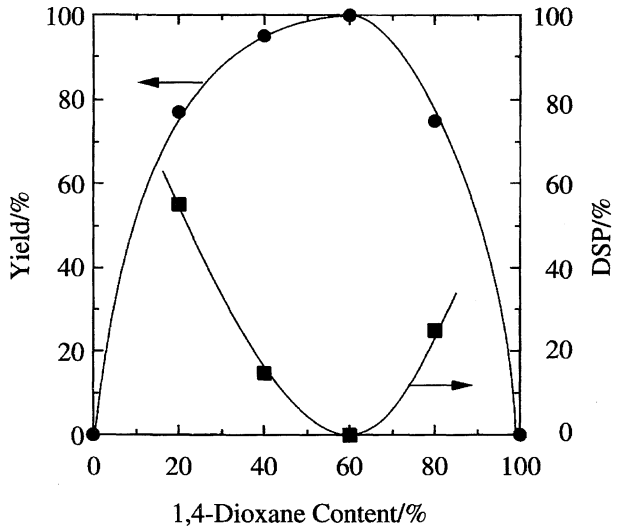


Fig. 1. Effect of 1,4-dioxane content in the solvent upon polymer yield and DMF-soluble part (DSP).

Table 2. Enzymatic Polymerization of Phenol in Aqueous 1,4-Dioxane of Different Buffers[a)]

Entry	Buffer		Polymer		
	Salt	pH	Yield[b)]	DSP[c)]	M_n[d)]
			%	%	$\times 10^{-4}$
1	Acetate	4.0	67	35	3.2
2	Acetate	5.0	80	24	2.9
3	Phosphate	6.0	29	14	2.2
4	Phosphate	7.0	75	25	3.5
5	Sulfite	7.0	0	—	—
6	HEPES[e)]	7.3	60	75	2.5
7	Phosphate	8.0	64	31	3.6
8	Borate	9.0	15	23	1.3
9	Carbonate	10.0	20	12	1.9
10	Carbonate	11.0	12	5	1.2

a) Polymerization of phenol using HRP catalyst in a mixture of 1,4-dioxane and buffer (80 : 20 vol%) at 20 °C for 24 h. b) Methanol-insoluble part. c) DMF-soluble part of polymer. d) Determined by GPC. e) 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonate.

the monomer was not consumed, indicating that the present oxidative polymerization of phenol proceeds through the enzyme catalysis. The resulting polymer was partly soluble in *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). The composition of the solvent also affected the ratio of the DMF-soluble part (DSP); there was a minimum point of DSP in the content of 1,4-dioxane = 60%.

The molecular weight of the DMF-soluble part was evaluated by gel permeation chromatography (GPC) using DMF containing 0.02 M lithium chloride (1 M = 1 mol dm^{-3}) as eluent. Among the polymers obtained in the mixture of 1,4-dioxane and the phosphate buffer, the solvent of 80% 1,4-dioxane afforded the highest molecular weight = 3.5×10^4 (molecular weight distribution = 3.5). In case of the polymerization in 60% 1,4-dioxane, the molecular weight of the polymer could not be evaluated because of its insolubility toward DMF.

Next, the effect of the buffer on the polymerization has been examined in 80% 1,4-dioxane (Table 2). The polymerization in acetate buffer of pH 5.0 produced the polymer in the highest yield (Entry 2). The yield slightly decreased as the pH of acetate buffer decreased (Entry 1). The polymerization in alkaline buffers (pH≧9) produced the polymer in low yields (Entries 8—10). The yield, DSP, and molecular weight of the polymer obtained in the carbonate buffer of pH 11.0 were the lowest. In the case of the polymerization using a phosphate buffer, the buffer of pH 7.0 or 8.0 produced the polymer in a relatively high yield, but, the yield decreased using the buffer of pH 6.0.

Among buffers of pH around 7 (Entries 4—6), the phosphate buffer produced the polymer in high yield. In the polymerization using 4-(2-hydroxyethyl)piperazine-1-ethanesulfonate (HEPES) buffer of pH 7.3, the polymer having high solubility toward DMF was obtained. Phenol monomer was recovered unreactedly in a sulfite buffer of pH 7.0, resulting in no formation of polyphenol. These data indicate that the buffer salt affected the polymer yield and solubility. This may be due to the difference of the enzyme activity in these buffers.

Polymerization in a mixture of a phosphate buffer (pH 7.0) and various organic solvents (80% organic solvent) has been carried out (Table 3). Acetone and 1,4-dioxane afforded the polymer in high yields (Entries 2 and 3). The polymer obtained in acetone or acetonitrile possessed bimodal peaks in the chromatograms (Entries 1 and 2), one of which in a lower elution volume was minor and showed very high molecular weight. The formation of the bimodal peaks may be because a branched structure was contained in the resulting polymer. The reaction in an aqueous alcohol solution yielded the polymer in low yields (Entries 5 and 6). The polymerization in a mixture of methanol and the phosphate buffer gave a polymer soluble in DMF (DSP = 100%), whose molecular weight was relatively high ($M_n = 2.0\times10^4$), on the other hand, the DSP value and molecular weight of the polymer obtained in

Table 3. Enzymatic Polymerization of Phenol in a Mixture of Phosphate Buffer and Various Organic Solvents[a)]

Entry	Organic solvent	Polymer		
		Yield[b)]	DSP[c)]	M_n[d)]
		%	%	$\times 10^{-4}$
1	Acetonitrile	38	31	37[e)] 8.4[e)]
2	Acetone	71	11	>40[e)] 7.4[e)]
3	1,4-Dioxane	75	25	3.5
4	DMF	0	—	—
5	Ethanol	31	5	0.72
6	Methanol	38	100	2.0
7	THF	15	23	0.3

a) Polymerization of phenol using HRP catalyst in a mixture of organic solvent and phosphate buffer (pH 7.0) (80 : 20 vol%) at 20 °C for 24 h. b) Methanol-insoluble part. c) DMF-soluble part of polymer. d) Determined by GPC. e) Bimodal peaks.

the aqueous ethanol were quite low. The use of tetrahydrofuran (THF) resulted in the formation of the polymer with very low molecular weight in a low yield (Entry 7). The polyphenol was not obtained in a mixture of DMF and the phosphate buffer (Entry 4). These results indicate that the yield, solubility, and molecular weight of the polymer were dependent upon the nature of organic solvents used. The difference of such polymerization behaviors may be due to the polymer solubility toward the reaction solvent and the enzyme activity in the solvent.

The polymerization temperature also affected the polymer formation (Table 4). In the range from 20 to 40 °C, the polymer yield was almost constant. The polymerization at 50 °C gave the polymer in a lower yield. As the polymerization temperature increased, the molecular weight decreased. These behaviors may be due to the lower selectivity to elongate the polymer chain as well as to the lower enzymatic activity at a higher temperature at 50 °C.

Structural Analysis of Polyphenol. In our previous paper, the structural analysis of polyphenol using IR and ^{1}H NMR spectrometers was briefly described.[9] Here, the detailed characterization on the polyphenol structure was performed by these spectroscopies as well as by ^{13}C NMR. Figure 2 shows IR spectra of DMF-soluble and -insoluble parts of the polymer obtained in a mixture of 1,4-dioxane and phosphate buffer (pH 7.0) (80 : 20 vol%) at room temperature. The absorption peaks at 1611, 1490, 866, 833, 753, and 692 cm^{-1} in the spectrum of the DMF-soluble part 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 1202 cm^{-1}. A peak at 1101 cm^{-1} corresponds to the symmetric vibration of the ether bond. These data indicate that the polymer structure is composed of a mixture of phenylene and oxyphenylene units (Scheme 1). A peak at 1660 cm^{-1} may be attributed to the C=O stretching vibration of the quinone. The quinone moiety may be formed when the phenolic hydroxyl groups at the ends of the chain are oxidized.[13] In electrochemical oxidative polymerization of aniline and phenol derivatives, such an absorption was observed.[13,14]

The spectrum of the DMF-insoluble part exhibits a similar pattern to that of the DMF-soluble part, implying that the DMF-insoluble part possesses higher molecular weight and/or slightly contains branching and crosslinking structures. IR spectra of the polymers obtained in the solvent of different pH are similar to each other, indicating that the pH of the buffer did not affect the polymer structure.

Table 4. Enzymatic Polymerization of Phenol in Aqueous 1,4-Dioxane at Different Temperatures[a]

Entry	Temperature	Polymer		
		Yield[b]	DSP[c]	M_n[d]
	°C	%	%	$\times 10^{-4}$
1	20	75	25	3.5
2	30	78	35	3.1
3	40	78	34	1.6
4	50	40	36	1.3

a) Polymerization of phenol using HRP catalyst in a mixture of 1, 4-dioxane and phosphate buffer (pH 7.0) (80 : 20 vol%) for 24 h. b) Methanol-insoluble part. c) DMF-soluble part of polymer. d) Determined by GPC.

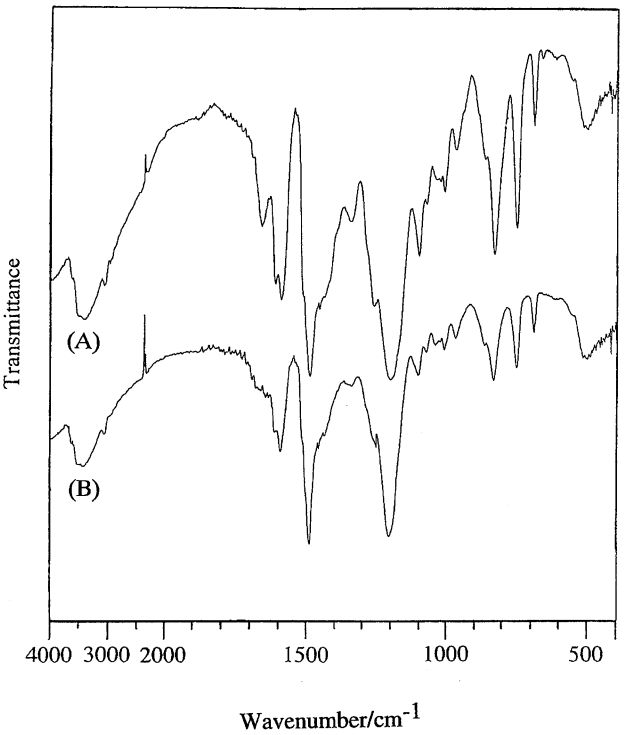
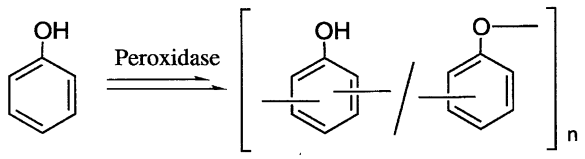


Fig. 2. IR spectrum of polymer (Entry 2 in Table 1): (A) DMF-soluble part; (B) DMF-insoluble part.



Scheme 1.

^{1}H NMR spectrum of the DMF-soluble part shows two broad peaks. The peak at $\delta = 6.2$—8.0 corresponds to the protons of aromatics, the peak at $\delta = 8.8$—9.7 to the proton of the phenolic hydroxyl group. From the integrated ratio of these peaks, the ratio of phenylene unit to oxyphenylene unit was found to be 5 : 6. The peak due to the phenonlic hydroxyl group disappeared by the addition of deuterium oxide in the NMR sample.

There are 6 broad peaks in the ^{13}C NMR spectrum of the polymer. Peaks at $\delta = 115$, 123, 130, and 154 correspond to the carbons of 2,6-disubstituted polyphenylene from phenol.[15] Peaks at $\delta = 119$ and 157 are ascribed to the carbons of 1,4-disubstituted poly(oxyphenylene). No peaks are observed in the region of $\delta = 140$—150, indicating that an ortho-coupled oxyphenylene unit is ruled out. From these spectral data, the polymerization is considered to be through mainly ortho-carbon–carbon and para-carbon–oxygen linkages.

Thermal Property of Polyphenol. Thermal properties of the enzymatically synthesized polyphenol were evaluated by using thermogravimetry (TG) and differential scanning calorimetry (DSC). Figure 3 shows the TG chart of the polyphenol. The measurement was performed under air and nitrogen. In the first step, a slight gradual weight loss of the polymer (less than 10% of the weight loss) was observed below 300 °C. This may be due to the evaporation and/or evolution of low molecular compounds. Temperature at 10% weight loss of the polymer under air was 335 °C, which was lower than that under nitrogen (387 °C). These data indicate high thermal stability of the present polyphenol.

Under air, the polymer was completely decomposed at 571 °C. Forty-three weight % of the polymer remained at 1000 °C under nitrogen. Polymer compositions before and after the measurement under nitrogen are as follows: before the measurement, C 73.01; H 4.13%; after the measurement, C 82.35; H 0.91%. From the data of the elemental analysis, the ratio of carbon to hydrogen in the polymer after TG analysis was found to become larger than that before the heating, indicating that carbonization of the polymer took place by the heating under nitrogen to produce graphite-like polymers. Conventional phenol resins are known to be precursors for carbonized products such as polyacene and graphite.[16]

Figure 4 shows the DSC chart of the polymer measured under nitrogen. In the chart of the first scan, an exothermic peak was observed at 238 °C. The crosslinking and/or branching of the polymer may take place at this point. There was no defined peak in the charts of the second (not shown in Fig. 4) and third scans, suggesting that the present polyphenol exhibited no clear glass transition temperature or melting point below 300 °C.

Conclusion. HRP-catalyzed oxidative polymerization of phenol in the aqueous organic solvent produced powdery polymeric materials. The solubility and molecular weight of the polymer was very dependent upon the solvent composition. The polymerization in 60% dioxane produced the polymer quantitatively. A polymer of higher molecular weight

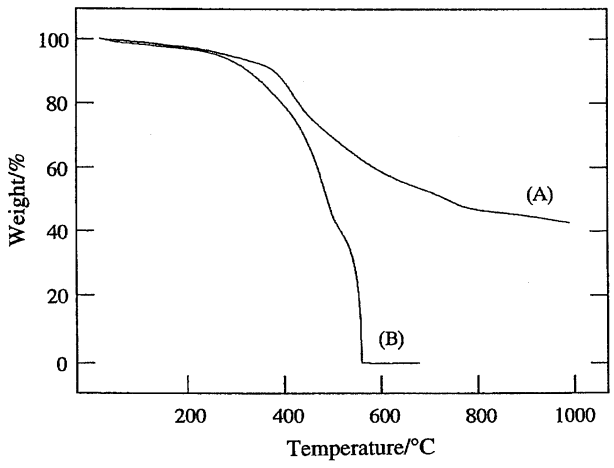


Fig. 3. TG traces of polymer (Entry 2 in Table 1): (A) under nitrogen; (B) under air.

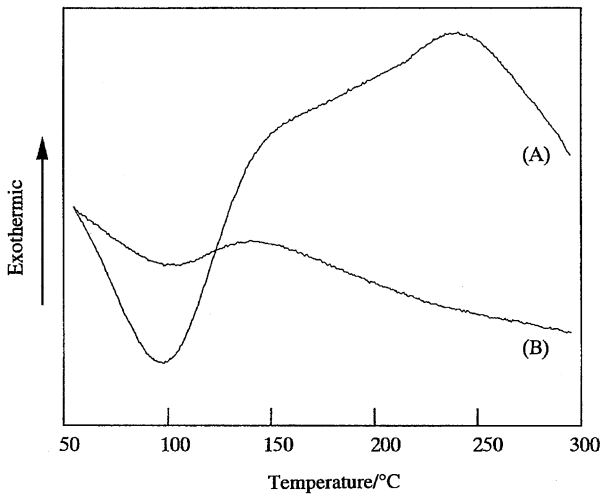


Fig. 4. DSC traces of polymer (Entry 2 in Table 1): (A) first scan; (B) third scan.

was obtained in an aqueous acetone or acetonitrile. The use of methanol induced the formation of a polymer exhibiting complete solubility toward DMF. From IR and NMR analyses, the polymer structure was estimated to be a mixture of phenylene and oxyphenylene units. The polymer was stable around 350 °C and around 40% of the polymer remained at 1000 °C in the TG measurement under nitrogen.

The present polyphenol can be conveniently synthesized in one-pot under mild conditions. Furthermore, its synthesis does not involve the use of toxic formaldehyde. Therefore, the enzymatically synthesized polyphenol is expected to be a new class of polyphenols showing high performance. Further investigations on the regioselective synthesis of the polymer (poly(phenylene) or poly(oxyphenylene)) and the mechanism of polymer formation are under way in our laboratory.

Experimental

Materials. Phenol was purified by distillation. Horseradish peroxidase (HRP) was purchased from Wako Chemical Co. and employed without further purification. Other reagents and solvents were used as received.

Enzymatic Oxidative Polymerization of Phenol. A typical run was as follows (Entry 2 in Table 1). Phenol (0.47 g, 5 mmol) and HRP (10 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. Hydrogen peroxide (30% aq solution, 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 0.35 g of the polymer (yield 75%).

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^{-1}. The calibration curves for GPC analysis were obtained using polystyrene standards. ^{1}H and ^{13}C NMR spectra were recorded on a 250 MHz Bruker AC-250T spectrometer. IR spectra were recorded on Shimadzu IR-460 spectrometer. TG analysis was performed us-

ing 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}. 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.

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

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