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ORIGINAL PAPER

Enzymatic synthesis of polyphenols with longer conjugation lengths

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Abstract Polyphenols containing Schiff base pendent groups, poly(4-[benzylidene-amino]-phenol) (PBP), and poly(4-[(anthracen-9-ylmethylene)-amino]-phenol) (PAP) have been synthesized through the combination of Schiff base reaction and enzymatic polymerization using horseradish peroxidase (HRP) as catalyst. The polymers were characterized by GPC, FTIR, ^{1}H NMR, and UV spectroscopy. It shows that PBP and PAP are composed of polyphenol main chains bearing Schiff base pendent side groups. The PBP exhibits better solubility than PAP in some common solvents. The PAP has a large red-shift of 86 nm compared with polyphenols, indicating the Schiff base pendent groups remarkably increase the conjugation lengths of polyphenols.

Keywords Schiff base · Enzymatic polymerization · Polyphenol · Conjugation length

Introduction

Polyphenols have attracted considerable attention due to its conjugated structures and thermal oxidative stabilization properties [1–3]. This causes the potential applications in some important fields such as the microelectronics, nonlinear optical materials, and sensors.

However, polyphenols containing longer conjugation length cannot be easily synthesized by traditional organic chemistry [4, 5] and electro-chemistry [6, 7] methods. The synthetic process is complex, which generates a large number of by-products and is difficult to scale.

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Recently, the advents of enzymatic polymerization provide a promising alternative method to synthesize polyphenols [8]. Enzymatic polymerization is advantageous in that they can offer simple and environmentally benign reaction conditions, and obtain conjugated product that can hardly be produced by other methodology. In order to improve the electro-optical properties, a series of polyphenols with longer conjugated segments have been synthesized. Premachandran et al. [9] reported the synthesis of naphthol-based polymers to increase the conjugation lengths in the backbone. The polyphenols with longer conjugation lengths in the side groups have also been explored. Xu et al. [10] synthesized poly(4-phenylphenol) with phenyl pendent groups, and Liu et al. [11] synthesized poly(4-phenylazophenol) with phenylazo pendent groups.

It is well known that Schiff base can be very easily synthesized and has conjugated structure. If the Schiff base was attached to the benzene rings of polyphenols, the conjugation lengths could be increased. In this study, we describe a facile synthesis of polyphenols with longer conjugation length through the combination of Schiff base reaction and enzymatic polymerization. Specially, the monomers with Schiff base groups, 4-(benzylidene-amino)-phenol (BP) and 4-[(anthracen-9-ylmethylene)-amino]-phenol (AP), were synthesized using 4-aminophenol and benzaldehyde/anthracene-9-carboxaldehyde, respectively. Subsequently, enzymatic polymerization employing horseradish peroxidase (HRP) as catalysts has been used, and two kind of polyphenols containing Schiff base pendent groups, poly(4-[benzylidene-amino]-phenol) (PBP) and poly(4-[(anthracen-9-ylmethylene)-amino]-phenol) (PAP), were obtained.

Experimental

Materials

Horseradish peroxidase (HRP) was purchased form Shanghai Sanjie Biotechnology Ltd. THF was refluxed with potassium and benzophenone until a characteristic blue color was evident. 30% hydrogen peroxide and other reagents were used as received.

Synthesis of 4-(benzylidene-amino)-phenol (BP)

To a 150-mL three-necked flask, 5.45 g (50 mmol) 4-aminophenol, 85 mL ethanol, and 5.0 mL (50 mmol) benzaldehyde were added. After refluxing for 5 h, the mixture was cooled down and filtered (7.19 g, yield: 73.2%). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.3 ppm (*o*–*H*–Ar–N), 6.7 ppm (*m*–*H*–Ar–N), 7.2 ppm (*o*–*H*–Ar–C), 7.8 ppm (*m*–*H*–Ar–C), 8.5 ppm (N=C*H*), 9.5 ppm (O–*H*). FTIR (KBr, cm^{-1}): 1624, 2221 cm^{-1} (ν C=N), 1500, 1450, 1600 cm^{-1} (ν C=C of Ar).

Synthesis of 4-[(anthracen-9-ylmethylene)-amino]-phenol (AP)

To a 250-mL three-necked flask, 2.18 g (20 mmol) 4-aminophenol, 4.12 g (20 mmol) anthracene-9-carbaldehyde, 180 mL ethanol, and 35 mL THF were

added. After refluxing for 5 h, the mixture was cooled down and then filtered (4.80 g, yield: 80.8%). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.7 ppm (*o*–*H*–Ar–N), 7.3 ppm (*m*–*H*–Ar–N), 8.1 ppm (1,2,6,7-PA-*H*), 8.7 ppm (3,5,8,10-PA-*H*), 8.9 ppm (4-PA-*H*), 9.54 ppm (N=C*H*), 9.68 ppm (O–*H*). FTIR (KBr, cm^{-1}): 1624 cm^{-1} (v C=N), 1503, 1600, 1450 cm^{-1} (v C=C of Ar).

Enzymatic oxidative polymerization

A typical run was as follows [10]: monomers and Horseradish peroxidase (HRP) in a mixture of dioxane and 0.1 M phosphate buffer (pH = 7) were placed in a 100 mL flask. 2.0 mL of 30% hydrogen peroxide (18 mmol) was added dropwise over 5 h. The mixture was stirred for 24 h at room temperature under air. The precipitated materials were collected by filtration, washed with water and methanol, and dried in vacuum.

Instrumentation

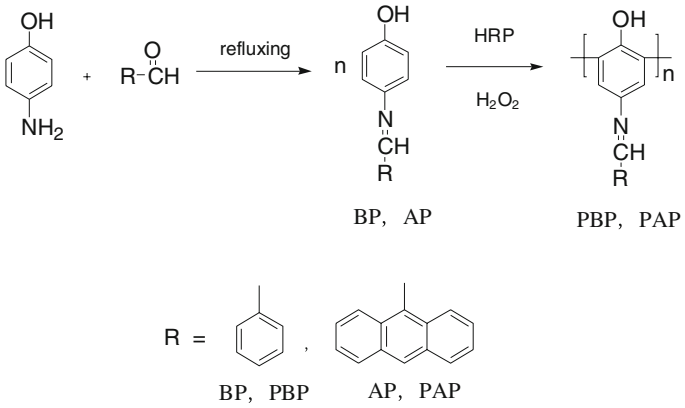
^{1}H NMR spectra were recorded on a Bruker AV400 spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS) as the internal reference. FTIR spectra were recorded on a 20SX spectrometer using KBr pellets. Gel permeation chromatography (GPC) were carried out in THF (1 mL min^{-1}) at 40 °C using a Waters 515 liquid chromatography equipped with three styragel columns (HR 3, 4, and 6) and 2414 refractive index detector. Monodisperse PS samples were used as standards for calibration.

Results and discussion

Synthesis and characterization PBP and PAP

Scheme 1 illustrates our strategy for the preparation of PBP and PAP. The often used enzymatic catalysts are HRP, soybean peroxidase, and garden enzymes. Among them, HRP shows the highest catalytic activity and monomer selectivity in enzymatic polymerization. Therefore, we choose HRP as the catalyst. Hydrogen peroxide is used as oxidizing agent. The enzymatic polymerization was carried out in a mixture of methanol and phosphate buffer (pH = 7) for 24 h at room temperature under air. The powdery precipitates were collected by filtration. The yields of polymerization were high, indicating an effective catalysis in a benign reaction condition.

Figure 1a shows the GPC traces of BP and PBP. The trace of PBP exhibits multiple peaks and broad distribution, indicating the mixture of polymer chains with different lengths. This is caused by the chain propagation mechanism of radical coupling in enzymatic polymerization. Under the action of H_2O_2 and HRP, phenoxy/phenylic radicals are formed. The polymer chains then grow through radical–radical coupling reaction of phenoxy/phenylic radicals. The final polymers



Scheme 1 The synthetic route of PBP and PAP

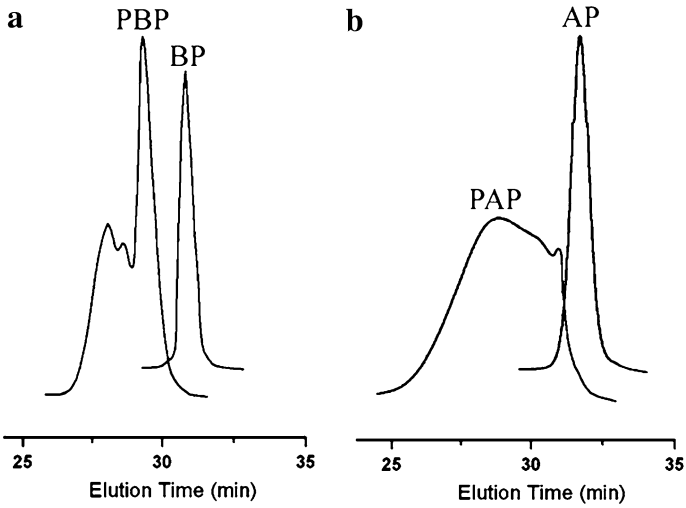
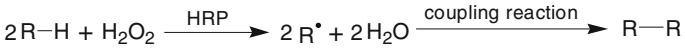


Fig. 1 GPC traces of monomers BP, AP and polymers PBP, PAP

are composed of the dimer, trimer, and the tetramer, etc., (see Scheme 2). Figure 1b indicates that PAP also propagated through the similar mechanism.

The M_n values of tetrahydrofuran-soluble part of PBP and PAP calculated from GPC are 2,900 and 2,400, respectively. Therefore, the polymers can be formulated as PBP_{21} and PAP_{13}. The polydispersity index (PDI) of PBP and PAP are 1.50 and 1.57, respectively.

Figure 2 shows the FTIR spectra of BP and PBP_{21}. The peak at 1,624 cm^{-1} is assigned to the characteristic absorption of C=N vibration. The bands at 650–900 cm^{-1} correspond to different substitution patterns in the aromatic ring [12, 13]. In the case of BP, the peak at 833 cm^{-1} shows the presence of two adjacent aromatic hydrogen atoms. In the case of PBP_{21}, the peak intensity at 833 cm^{-1}



Scheme 2 Chain propagation mechanism of the enzymatic polymerization. Where R is phenol or polyphenol oligomer, and R* is the radical species

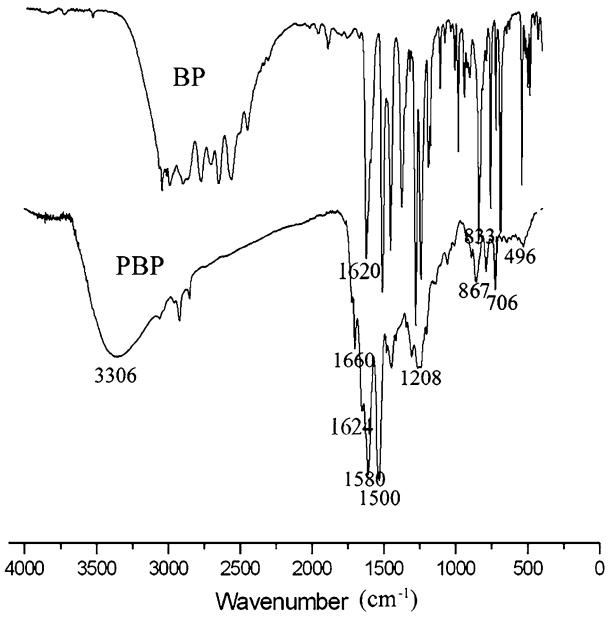


Fig. 2 FTIR spectra of BP and PBP_{21}

decreases considerably, and another new peak at 867 cm^{-1}, corresponding to characteristic absorption of hydrogen atoms located in 3- and 5- positions of benzene ring appears. This suggests that the polymerization proceeds primarily through ortho–ortho coupling.

It should be noted that the oxyphenylene (C–O) linkages might exist since these linkages have been observed previously in the HRP-catalyzed polymerization of phenol [14]. However, no evident characteristic bands at 1,270–1,280 cm^{-1} [12, 13] for these functionalities were observed in FTIR spectrum of PBP_{21}. Therefore, the polymerization proceeds primarily through C–C linkages. The peaks at 1,660 cm^{-1} attributed to the carbonyl (−C=O) stretching vibration appeared, indicating that the quinone or ketonic structures exist due to the partial oxidization of phenolic hydroxyl groups [7, 15]. Figure 3 shows the FTIR spectra of AP and PAP_{13}, which display the similar phenomena.

The compositions of THF-soluble part and- insoluble part of PBP and PAP were also determined by FTIR spectra. The THF-insoluble part exhibits a similar pattern to that of the THF-soluble part, implying that the THF-insoluble part possesses higher molecular weight and/or contains branching and crosslinking structures [15].

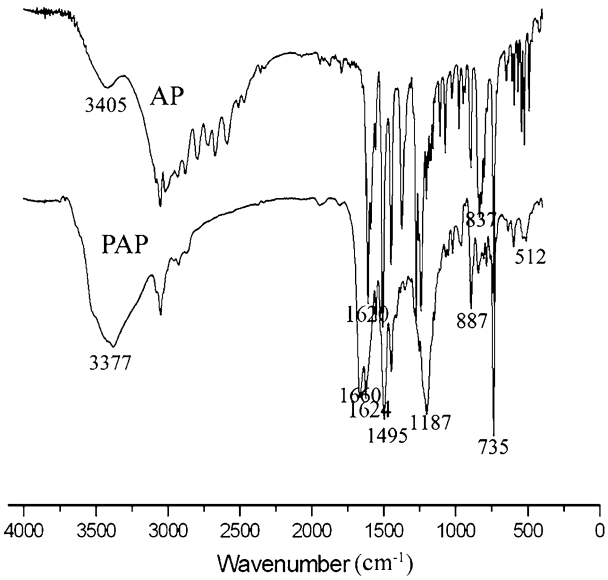


Fig. 3 FTIR spectra of AP and PAP_{13}

Figure 4 shows ^{1}H NMR spectra of BP and PBP_{21} in DMSO-d_6. The resonance at 6.3 ppm that ascribed to the *o*-positions of benzene ring (*o*–*H*–Ar–N) weakened significantly in the PBP_{21}, which could also suggest that the polymerization proceeds primarily through ortho–ortho coupling. The resonances at 9.5 ppm (a, a′) and 8.5 ppm (b, b′) are ascribed to hydroxyl protons and methine protons of Schiff base moiety (–CH=N), respectively. From the integrated ratio of the aromatic protons and the methine protons of the Schiff base, the ratio of the oxyphenylene unit to phenylene unit could be found. The calculated results show that no oxyphenylene linkages are found. This also confirms the observation in FTIR spectra.

Figure 5 shows ^{1}H NMR spectra of AP and PAP_{13} in DMSO-d_6. The resonance at 6.7 ppm that ascribed to the *o*-positions of benzene ring (*o*–*H*–Ar-N) also weakened in the PAP_{13}, which could suggest the ortho–ortho coupling. The resonances at 9.68 and 9.54 ppm for AP monomer are ascribed to the hydroxyl and methine protons, respectively. After polymerization, the resonance of methine protons of PAP_{13} overlaps with that of hydroxyl protons (g′, h′). However, the characteristic peaks of the methine protons and hydroxyl protons can be clearly identified in the FTIR spectra (Fig. 3), indicating that PAP_{13} still contains Schiff base and hydroxyl groups.

Effects of reaction conditions on enzymatic polymerization

Table 1 shows the effects of dioxane content on the yield, molecular weight, and solubility of the final products [16, 17]. As the dioxane content increases from 10 to 70%, the yields and molecular weights of PBP increase, but the solubility decreases.

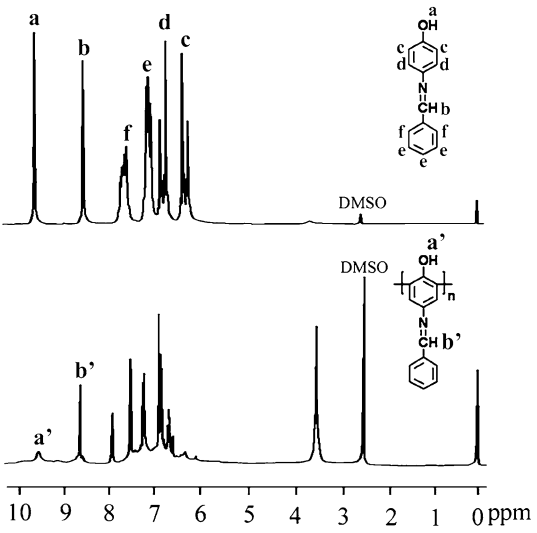


Fig. 4 ^{1}H NMR spectra of BP and PBP_{21} in DMSO-d_6

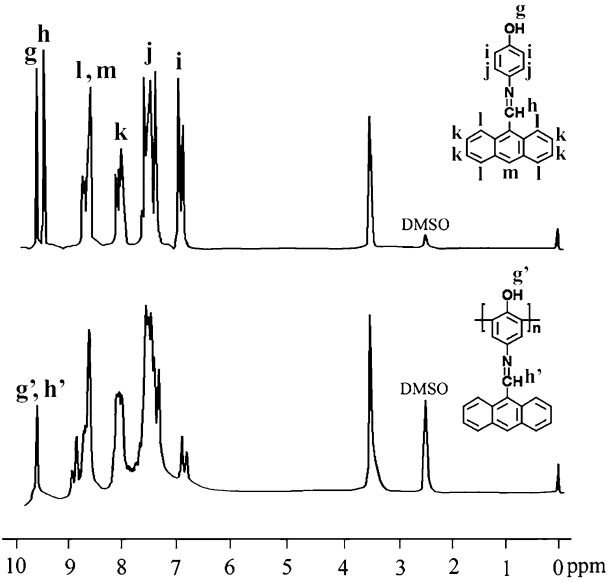


Fig. 5 ^{1}H NMR spectra of AP and PAP_{13} in DMSO-d_6

This is due to the fact that as the dioxane content increases so does the solubility of the reaction system. It can dissolve the polymer with longer chains to grow. Therefore, the yields and molecular weights of the product increase. However, the solubility of PBP is contrary to the polymer chain length. When the dioxane content reaches 70%, the highest yield (93%) and molecular weight (3,500) are obtained. Above 70% dioxane, the yields and molecular weights of PBP decrease. This is because the HRP is partly denatured when large amounts of dioxane are used.

Table 1 The effects of dioxane content on the enzymatic polymerization of BP

Entry[a]	Dioxane content	PBP		
		Yield (%)	Mn[b]	DSP[c] (%)
1	10	73	750	90
2	50	80	1,300	80
3	60	84	1,500	70
4	70	93	3,500	20
5	80	80	2,900	60
6	90	70	900	90

[a] The concentration of BP is 0.15 mol L^{-1}, and the concentration of HRP is 0.4 g L^{-1}

[b] The THF-soluble part determined by GPC

[c] The DMF-soluble part of PBP

Table 2 The effects of monomer concentration on enzymatic polymerization of BP

Entry[a]	Monomer	Mn[b]	DMF-soluble	
	Concentration (mol L^{-1})		Part (%)	Yield (%)
1	0.10	1,200	85	70
2	0.15	2,900	60	80
3	0.20	3,100	30	90
4	0.25	3,800	20	80

[a] Dioxane content is 70%

[b] THF-soluble part of the polymer determined by GPC

Table 2 shows the effects of the monomer concentration on molecular weight, solubility, and the yield of the final products obtained from 70% dioxane content. As the monomer concentration increases so does the molecular weight of PBP, but the solubility of PBP decreases. These phenomena are similar to that observed in the enzymatic polymerization of polyphenol [15]. In all cases examined, the polymer yields are high between 70 and 90%. Considering these factors, we use the dioxane content of 70% and monomer concentration of 0.15 mol L^{-1} as the optimum reaction conditions in this investigation.

Properties of PBP and PAP

Table 3 shows the solubility of PBP and PAP. PAP was only partly soluble in DMF, DMSO, and was slightly soluble in THF. PBP has better solubility than PAP. It can be almost soluble in DMF, DMSO, and partly soluble in THF, but insoluble in water, ethanol, acetone, and dichloromethane etc.

Figure 6 shows the UV spectra of PBP_{21} and PAP_{13}, together with the UV spectrum of polyphenol for reference. The strong absorbance peaks at 320, 350, and

Table 3 The solubility of PBP and PAP

Solvent	PBP	PAP
Acetone	−	−
Chloroform	−	−
DMF	+	±
DMSO	+	±
THF	±	±
Ethanol	−	−
Water	−	−

+: soluble; ±: partly soluble; −: insoluble

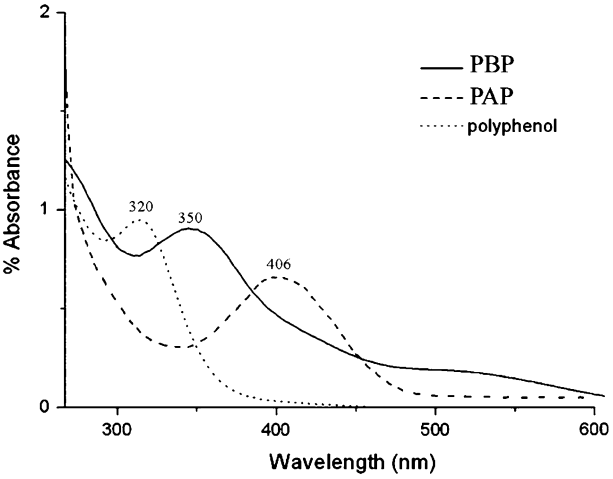


Fig. 6 UV spectra of PBP_{21}, PAP_{13} and polyphenol (Solvent: DMSO. Concentration: 0.1 g L^{-1})

406 nm correspond to π–π^* transitions of polyphenol, PBP_{21}, and PAP_{13}, respectively. Compared with polyphenols, PBP_{21} has 30 nm red-shifts and PAP_{13} has 86 nm red-shifts. This suggests that the incorporation of Schiff base groups remarkably increases the conjugated lengths of polyphenols, and the enhancement of Schiff base structure obtained from benzaldehyde is better than that from anthracene-9-carboxaldehyde.

Conclusions

In conclusion, polyphenols with Schiff base pendent groups, PBP and PAP, have been successfully synthesized through the combination of Schiff base reaction and enzymatic polymerization. The content of dioxane and the concentration of the monomers have great effects on the yield, solubility, and molecular weight of PBP and PAP. PBP exhibits better solubility than PAP in some common solvents. PAP has a large red-shift of 86 nm owing to the attachment of Schiff base groups with longer conjugation lengths. Those interesting properties of PBA and PAP will be benefit to their potential applications in electro-optics fields.

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