

Poly(acrylic acid) Template-guided Enzymatic Synthesis of Water-soluble Poly(*o*-toluidine)

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ABSTRACT

It was recently reported that water-soluble conducting polyaniline may be prepared using a new template-guided enzymatic approach. It was found that the template provides a necessary type of local environment where the pH and charge density near the template molecules are different from that of the bulk solution. A strong acid polyelectrolyte, such as sulphonated polystyrene (SPS), is the most favourable because it provides a lower, local pH environment that serves to both charges and preferentially align the monomers through electrostatic and hydrophobic interactions to promote the desirable head-to-tail coupling. Poly(acrylic acid) (PAA) partial sodium salt is used as an acid polyelectrolyte in this work. Biocatalytic polymerization using a naturally occurring enzyme is advantageous because it is a simple, one-step and environmentally compatible method. The horseradish peroxidase (HRP) enzyme is used in polymerization of *o*-toluidine in the presence of poly(acrylic acid) partial salt as a template and camphor sulphonic acid as a dopant. The synthesis is simple and the conditions are mild. Best results are obtained in pH 4.3 phosphates buffered aqueous solutions with stoichiometric amount of monomer, template and oxidant and catalytic amount of enzyme. The synthesized polymers have been characterized by UV-vis and FTIR spectroscopy and cyclic voltammetry. These studies confirm that electroactive and conducting forms of poly(*o*-toluidine) (POT) are obtained in the presence of PAA.

Key Words:

template-guided;
horseradish peroxidase;
water-soluble;
poly(*o*-toluidine);
conducting polymer.

INTRODUCTION

Conducting polymers are an attractive class of polymers that have attracted more interest in the last decade [1,2]. Among the group of conducting polymers, polyaniline (PANI) and derivatives are the most studied because of their most

promising applicability [3,4]. Recent investigations have focused on exploring methods to augment the electrical properties, processability and environmental compatibility of these polymers for numerous technological applications

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[5,6]. The commercial use of PANI has been limited due to poor processability [7]. Many research works have focused on improving the processability of PANI by different methods [8-10]. Recently, an enzymatic approach has been developed to synthesize polyaniline in the presence of the template under mild conditions [11]. Horseradish peroxidase (HRP) has been used as a catalyst for the synthesis of water-soluble conducting PANI. HRP is an oxidoreductase which catalyzes the radical polymerization of aniline in the presence of hydrogen peroxide as an oxidizing reagent.

A major limitation of the enzymatic oxidation of aniline is that as soon as polymer begins to precipitate only very low molecular weight polymers are formed [12]. To obviate this problem and improve processability, the enzymatic polymerization is performed in the presence of a polyelectrolyte template [13]. This approach is based on the preferential electrostatic alignment of aniline monomer onto an anionic template to minimize branching and promote a linear chain growth.

Recently, we have reported an enzymatic technique for polymerization of aniline derivatives in the presence of a sulphonated polystyrene (SPS) as a template [14-16]. This template provides a type of nanoreactor that serves to both emulsifications of the monomers and provides a localized lower pH environment that promotes the *para*-directed coupling of the monomers [13]. So far, polyelectrolyte such as poly(vinyl phosphonic acid) and DNA have been demonstrated as good templates for enzymatic synthesis of conducting polyaniline [17,18].

Wang et al. synthesized water-soluble conducting polyaniline in the presence of poly(acrylic acid) (PAA) as a template by a chemical method under low-pH conditions [19]. Although these approaches have been successful toward the polymerization of PANI/PAA complexes, the harsh chemical conditions preclude the use of more delicate biological-based methods. In order to obtain additional insight into polyaniline, methyl substituents have been used to block the *ortho* position of aromatic ring of aniline.

In this paper, we report the template assisted PAA, enzymatic synthesis and characterization of water-soluble conducting poly(*o*-toluidine). The polymerization has been carried out in mild conditions of

aqueous pH 4.3 phosphate buffer solution. The reaction was initiated by the addition of the H_2O_2 , and the progress of the reaction was monitored spectroscopically.

EXPERIMENTAL

Materials

Poly(acrylic acid) partial sodium salt 25 wt% (M_w of 240,000), purchased from Aldrich Chemical Co. Hydrogen peroxide (30 wt%), HRP (EC 1.11.1.7) (about 170 units/mg), (+) 10-camphor sulphonic acid and *o*-toluidine were obtained from Merck.

Polymerization Reaction

An amount of 42 μ l (0.40 mmol) of *o*-toluidine was dissolved in 10 mL of 0.01 M potassium phosphate buffer. Next 0.288 g (1 mmol, per monomer unit, M_w 240,000) of poly(acrylic acid) partial sodium salt (25 wt%) was added and the mixture was stirred for 6 h. Then to this adduct solution, 110 μ l of 2 M (+) 10-camphor sulphonic acid (CSA) was added as a chiral dopant and adjusted to pH 4.3. Catalytic amount of HRP (1 mg in 0.5 mL of deionized H_2O) was added and continuously stirred. The reaction was then initiated with addition of 0.6 mL of 0.02 M H_2O_2 under vigorous stirring over a period of 1 h. A dark green solution was formed that was an indication of the formation of doped POT/PAA complex. The reaction was then left stirring for at least 4 h and the final solution was dialyzed overnight against deionized water.

FTIR, UV-vis spectroscopy and cyclic voltammetry were used in the characterization of the complex.

Characterization

UV-Vis spectra were obtained using a Shimadzu UV-2100 spectrophotometer. FTIR Measurements were carried out on a Bruker IFS 66/S FTIR spectrometer in the form of dried POT/PAA complex using KBr pellets. The cyclic voltammetry (CV) measurements were performed using a Metrohm Polarograph model 746 VA Trace Analyzer. Cyclic voltammograms were recorded at room temperature by employing a three-electrode cell with platinum as an auxiliary electrode, an Ag/AgCl as the reference electrode and a Pt foil (1 cm^2 surface area) as the working electrode. The

cyclic voltammograms were obtained in 1.0 M HCl electrolyte in polymer solutions and scanned between 0 to 1 V at scan rates from 50 to 200 mV/s.

RESULTS AND DISCUSSION

It was recently reported that water-soluble conducting PANI may be prepared using a new enzymatic approach. To minimize branching, Samuelson et al. developed polyelectrolyte-assisted polymerization of aniline [11]. To address the mechanistic role of the template in this reaction, various templates were investigated [13]. It was found that the template provides a necessary type of local environment where the pH and charge density near the template molecule are different from that of the bulk solution. The template promotes the *para*-directed polymerization and provides the requisite counterions for doping of the polymer. When monomer is added to a template solution at pH about 4.0, the monomer molecules become protonated and the electrostatic interaction between the protonated monomer and the template results in close association of the protonated monomer with the template.

Previous works have shown that enzymatic polymerization of aniline in the presence of weak acid templates such as poly(glutamic acid) and poly(aspartic acid) produced strong absorption at 400-600 nm with only a weak tail at 800-1200 nm due to the polaron transition band [13]. However, these results are indicative of a branched, low molecular weight form of polyaniline. When the polymerization is car-

ried out in the presence of strong acid polyelectrolyte such as SPS, dark green solutions are formed which show strong polaron absorption bands at about 400 nm and 800-1200 nm [19].

PAA was found to be a suitable template in the chemical polymerization of aniline [20]. The synthetic strategy involves two steps: (1) physically adsorb aniline molecules onto PAA to form adduct $(An)_x/PAA$ in aqueous solution and (2) an oxidant to polymerize the adsorbed aniline monomers to form a doped interpolymer complex. Recently, Cholli et al. have synthesized water-soluble chiral conducting polyaniline by an enzymatic method [21]. Horseradish peroxidase (HRP) was used as a catalyst and polymerization was carried out in the presence of PAA as a template.

The polymerization of aniline in the presence of different types of the template produced different forms of PANI (conducting or insulating). These results have clearly shown that there is a direct dependence on the template structure and the type of polymer formed. The template assisted (PAA) in the enzymatic polymerization of *o*-toluidine is presented in this paper and the UV-vis spectrum of POT/PAA is presented in Figure 1. In the UV-vis spectra we observed three bands at 325 nm, 410 nm and 740 nm. The band at 325 nm is assigned to the π - π^* electronic transition, while bands at 410 and 740 nm are connected with creation of polarons [22]. This UV-vis spectrum is in good agreement with spectra obtained from polymerization of *o*-toluidine in the presence of SPS [14].

Optimization of Reaction Conditions and Stoichiometry of the POT/PAA Complex

As the polymerization is carried out in the presence of strong acid polyelectrolyte such as sulphonated polystyrene, the protonation of the monomers is performed fast and the electrostatic interaction between the protonated monomers and sulphonated groups results in close association of the protonated monomer with template. When *o*-toluidine monomer is added to a PAA partially sodium salt solution, as a convenient acid polyelectrolyte, the monomer molecules become protonated gradually. Consequently, for enough protonation of the monomers we must stir the mixture for 6 h.

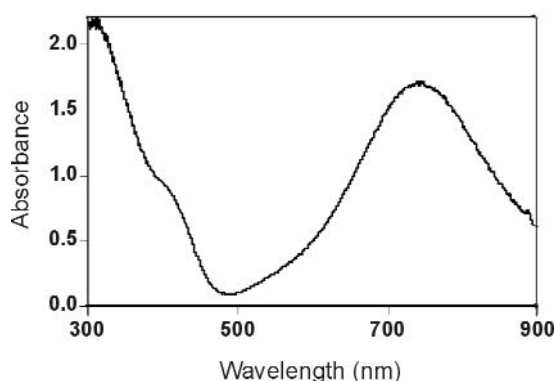


Figure 1. UV-Vis spectrum of the POT/PAA complex.

The proton concentration around the carboxylate groups provides a unique local environment that permits polymerization of *o*-toluidine at a higher pH than the conventional chemical synthetic methods and facilitates a predominately *para*-directed coupling that deters parasitic branching during the polymerization [13].

If the polymerization is allowed to proceed too far and a critical chain length is attained, the complex precipitates or "salts out" of solution. In an attempt to optimize the conditions required for determining the ratio of carboxylic acid repeat unit to monomer repeat unit in the complex just prior to precipitation, a matrix of reaction systems was set up. In these experiments, the molar ratio of PAA: *o*-toluidine in the reaction medium was varied from 0.5:1 to 5:1 while the amount of enzyme was kept constant in all cases. The polymerization was controlled by incremental addition of H_2O_2 until the onset of precipitation. In all cases the reaction mixture contained 10 mL of buffer, 0.28 mg of HRP and 5 mM of *o*-toluidine. A 0.3% of H_2O_2 solution was used to carry out the reaction and 25 μ l of this solution was added to the reaction mixtures every 1.5 min until precipitation of the complex was observed. Figure 2 is a plot of the amount of H_2O_2 required to precipitate the POT/PAA complex with 5 mM of *o*-toluidine in the reaction medium, while varying only the concentration of PAA. These results show that the amount of H_2O_2 required for precipitation increases linearly up to 2.5:1 of PAA: *o*-toluidine. After this maximum ratio, the amount of H_2O_2 required for precipitation decreases and then

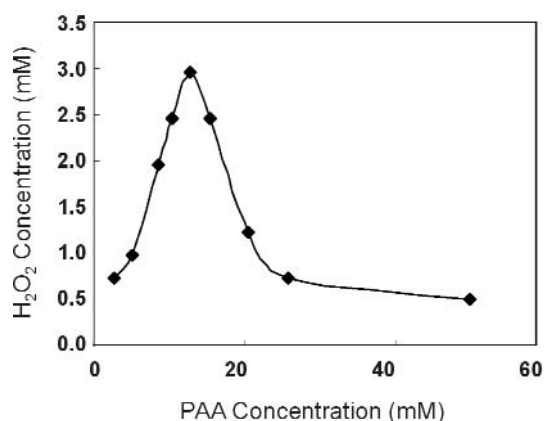


Figure 2. PAA Concentration versus H_2O_2 concentration required for precipitation of the POT/PAA complex.

eventually levels off as the PAA: *o*-toluidine ratio continues to increase.

Considering the mechanism of HRP-catalyzed polymerization, it is reasonable to expect a linear relationship between the amount of H_2O_2 added and the amount of monomer radicals formed [23]. When the molar ratio of PAA: *o*-toluidine is increased beyond the 2.5:1 peak ratio, the excess PAA in the system is no more able to contribute to the solubility of the complex. These results suggest that in the linear region of the curve up to 25 mM concentration of PAA, the POT/PAA precipitate has a constant composition of PAA and POT.

Redox Reversibility

Figure 3 displays the evolution of absorption spectra of the POT/PAA complex in aqueous solution with increasing pH from 3 to 12 by titrating with 1 M of NaOH. At pH 3, the complex is in doped state as indicated by the presence of the three bands at 325 nm (π - π^* transition of the benzenoid rings), 410 and 700 nm (polaron bands transition). The synthesized POT/PAA complex shows reversible reduction/oxidation behaviour in the absorption spectra with varying pH, which is similar to that observed in the POT/PAA solution.

As the pH of the solution is increased, the polaron bands gradually disappear and a strong absorption due to exciton transition of the quinoid rings at about 550 nm is emerged. At pH 12, the colour of the solution is blue and the POT is completely dedoped to give emeraldine base form.

These processes are completely reversible and

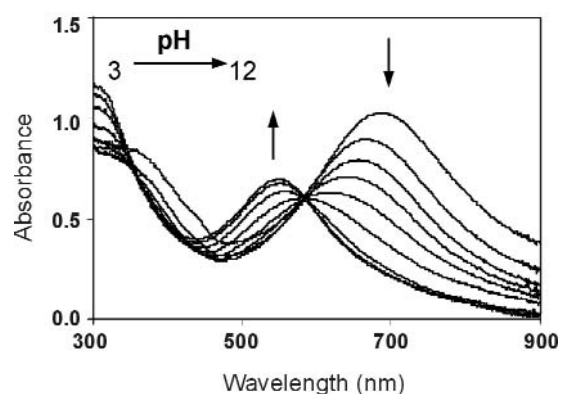


Figure 3. UV-vis spectra of POT/PAA complex during titration with base in pH range of 3-12.

dedoped POT can be redoped by titration with 1 N HCl, and a reversible colour change is observed (data are not shown). Also isobestic point at 610 nm is observed clearly. This pH induced redox reversibility demonstrates that the electroactive poly(*o*-toluidine) is enzymatically synthesized in the aqueous solutions.

Cyclic Voltammetry

The electrochemical properties of the enzymatically synthesized poly(*o*-toluidine) are characterized by cyclic voltammetry. Figure 4 shows the cyclic voltammograms of the (a) blank solution containing PAA and HRP and (b) POT/PAA complex. The cyclic voltammograms were recorded in 1 M HCl solutions at the scan rate of 100 mV/s. Only one broad peak at $E_{1/2} \sim -0.55$ V is dominant in Figure 4b. Similar results were obtained previously in POT/SPS complex [14]. It seems that the presence of PAA had similar influence on electrochemical properties of the complex.

On recording the cyclic voltammograms at different scan rates, we observed an appreciable change in the anodic and cathodic peak current values. The electrochemical characterization of POT/PAA complex is shown in Figure 5 at different scan rates between 50 and 200 mV/s. Focke et al. have reported the scan rate dependence of the reduction and oxidation peaks earlier [24]. The peak potentials for redox process vary linearly with scan rates. Figure 6 shows a linear relationship between anodic peak current vs. scan rates for POT/PAA complex. The slope of these curves indicated the electroactivity of the polymer.

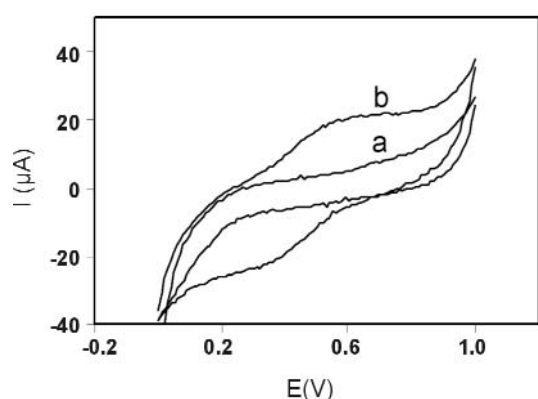


Figure 4. Cyclic voltammograms of (a) blank solution and (b) POT/PAA complex in 1.0 M HCl at 100 mV/s scan rate.

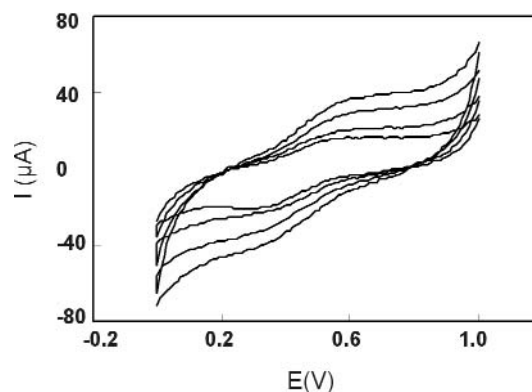


Figure 5. Cyclic voltammograms of a solution of POT/PAA complex at different scan rates between 50 to 200 mV/s.

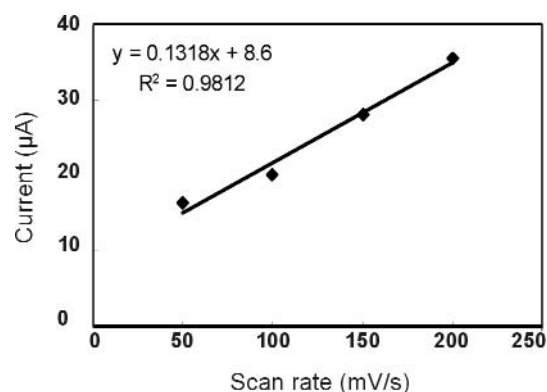


Figure 6. Plots of anodic peak current vs. scan rate for POT/PAA complex.

FTIR

To further characterization of the complex, we measured the infrared spectra of PAA and POT/PAA. Figure 7 shows the FTIR spectra of PAA (Figure 7a) and POT/PAA complex (Figure 7b). The characteristic bands of poly(acrylic acid), for example a broad absorption from 3600 to 2300 cm^{-1} corresponds to the O-H stretching and a strong band at 1720 cm^{-1} for carbonyl stretching were observed in the PAA spectra. Also in the FTIR spectra of the complex characteristic bands are observed.

For example, the peak at 1570 cm^{-1} is assigned to ring stretched of quinoid form and the other peak at 1485 cm^{-1} is due to benzenoid ring [25].

The peak observed at 1310 cm^{-1} corresponds to the C-H bending of the benzenoid ring and the peak at 1330 cm^{-1} belongs to C-N stretching of a secondary aromatic amine.

The peaks at 810 and 880 cm^{-1} are due to a 1,2,4-

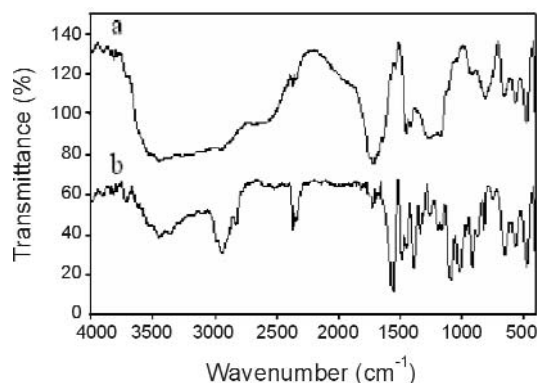


Figure 7. FTIR Spectra of (a) poly(acrylic acid) and (b) POT/PAA complex.

trisubstitution pattern, indicating that the monomeric unit is linked to *para* position of the ring. The peak at 2940 cm^{-1} is ascribed to the C-H stretching of the aliphatic group on the complex. FTIR Spectroscopy provides an evidence that the POT/PAA is mixed at the molecular level. These FTIR spectra are in good agreement with spectra obtained previously [26].

CONCLUSION

We have demonstrated the feasibility of the template-guided enzymatic synthesis of water-soluble, poly(*o*-toluidine) in the presence of poly(acrylic acid) partially sodium salt as a convenient acid polyelectrolyte. The pH value of the polymerization must be controlled around 4.3 to form continuous and conductive poly(*o*-toluidine), because the pH value has a significant effect on both the catalytic activity of the enzyme and the formation of alignment of *o*-toluidine monomers along the poly(acrylic acid) templates. This process is a one step, chemically mild, environmentally benign and requires minimal separation and purification.

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