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HRP-catalyzed synthesis of water-soluble and redox poly(catechol) at room temperature

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Abstract: Catalytic oxidative polymerization of catechol was performed using the horseradish peroxidase (HRP) as catalyst. The reaction was carried out in the presence of sulfonated polystyrene (SPS) as a template with a stoichiometric amount of catechol. Also, hydrogen peroxide was utilized as an oxidant at pH 4.3 in 0.01 M sodium phosphate buffer solution medium. Formation of water-soluble poly(catechol) was characterized by comparing FT-IR spectroscopy of catechol and poly(catechol). FT-IR and TGA were employed to investigate the structure and thermal behavior of synthesized poly(catechol). It was found that catechol units connected to each other by ether linkage and TGA result ensures the high thermal stability of the poly(catechol) for high-temperature applications. Cyclic voltammetry measurements demonstrate that the polymer has convenient electroactivity.

Keywords: Poly(catechol); oxidative polymerization; enzyme HRP; water-soluble polymer; redox.

Introduction

In the recent years, extensive studies have focused on phenols and aromatic amine-based polymers due to their wide applications such as making resins for different purposes [1,2]. Poly(catechol) is a valuable redox polymer which its redox moiety and polymer matrix functionality contribute to many of its properties and applications [3]. Catechols are also versatile electroactive species, which are generally soluble in aqueous solutions at pH 7 and exhibit pseudoreversible electrochemistry [4]. Repetitive cycling of catechol can lead to the formation of polymeric materials for unique films, which are being exploited in a number of biosensor applications [5,6]. Several researchers have explored this alternative method using

enzyme for the synthesis of a new class of polyphenols and polyamines [7,8].

Enzymatic polymerization has drawn much attention for the formation of polymers whose synthesis is otherwise difficult; this process possesses much potential to give a polymer of novel structure and properties [9,10]. Akkara *et al*., while evaluating in general the enzymatic polymer synthesis of various substituted aromatic amines and phenols, sparsely mentioned catechol as one of the monomer candidates for enzymatic HRP polymerization [11].

Only a few enzymes, including horseradish peroxidase (HRP) [12,13], soybean peroxidase (SBP) [14,15], biluribin oxidase [16], tyrosinase [17], laccase [18,19] and lipase [20,21], have been shown to catalyze polymerization of monomers such as phenols and their derivatives into polymers. Of these enzymes, HRP and SBP, in the presence of hydrogen peroxide have been utilized the most for the polymerization of phenolic monomers [22]. Polymers with well-defined structures can be prepared by enzyme-catalyzed in an environmentally benign process [23,24].

The HRP is a Fe containing porphyrin type structure which is shown in Figure 1. The enzyme HRP in the presence of hydrogen peroxide catalyzes the polymerization of phenol and aromatic amines [25]. This polymerization has been carried out in presence of a polyelectrolyte template such as poly(4-styrene sulphonate) (SPS) or poly(vinylphosphonic acid) (PVP) under mild conditions in pH 4.3 phosphate buffer [26,27]. Recently, we have reported the enzymatic polymerization of aniline derivatives [28].

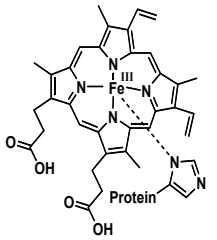

Fig 1. The structure of active site of HRP

In view of the above, it was worthwhile to study in detail the preparation of poly(catechol) by oxidative polymerization using peroxidase (HRP) and evaluate the electrochemical property of enzymatically prepared poly(catechol). The study also includes the thermal and electrochemical properties of the prepared polymer.

Materials and Methods

Materials

Poly(sodium 4-styrene sulphonate) (MW of 70000), which used in this study was purchased from Aldrich Chemical Co. (Milwaukee, WI) and used has been without any further purification. HRP (EC 1.11.1.7) (about 170 units/mg), hydrogen peroxide (H_2O_2) (30 wt %), and catechol were obtained from Merck.

Instrumentation characterization

The FT-IR measurements were carried out with the help of a BOMEM MB-Series FT-IR spectrometer in the form of KBr pellets. Samples were analyzed by TGA (TGAQ50, TA Instruments) 20 ˚C/min for heating rate under air. The cyclic voltammetry (CV) measurements were performed with a Metrohm Polarograph model 746 VA Trace Analyzer. The cyclic voltammograms were recorded at room temperature using a three electrode cell: platinum as an auxiliary electrode, Ag/AgCl as the reference electrode and Pt foil (0.2 cm^2 surface area) as the working electrode. The cyclic voltammograms were obtained in a 1.0 M HCl electrolyte and scanned from 0 to 0.9 V at various scan rates in the range of 50–500 mV/s.

Polymer Synthesis

A procedure for the preparation of the poly(catechol) is as follows: Typically, 5 mg catechol (0.045 mmol) and 0.009 g SPS (0.045 mmol) (based on monomer repeat unit) were added to 10 mL 0.01 M sodium phosphate buffer solution (pH 4.3) at room temperature under

constant stirring. The mixing was followed by the addition of a catalytic amount of the enzyme (1 mg HRP). To initiate the reaction, 2.3 mL of diluted hydrogen peroxide (0.02 M) was added dropwise under vigorous stirring over a period of 1 h. The reaction was then left to stir 24 h at the ambient temperature. The final solution was dialyzed (molecular cutoff = 3,000) overnight to remove any unreacted monomers and oligomers. A homogeneous, reddish-brown, water-soluble poly(catechol) was obtained.

Results and Discussion

In the present investigation, HRP is used for oxidative polymerization of catechol. The polymerization of catechol was performed by the HRP in the phosphate buffer 0.01 M pH = 4.3. After the addition of the hydrogen peroxide drops, the reaction media of catechol developed a reddish-brown colour within several minutes indicating a fast catechol oxidation and the water-soluble form of poly(catechol) were obtained.

Catalytic mechanism of HRP

HRP is classified as an oxidoreductase due to the cyclic reduction and oxidation of the heme group which gives rise to its enzymatic activity. In brief, HRP reduces hydrogen peroxide to form a complex which can oxidize a variety of organic and inorganic substrates [29].

HRP catalyzes the oxidation reaction of catechol with H_2O_2 in phosphate buffer solution. From the catalytical cycle of HRP in reaction [30], the processes of the enzyme-catalyzed reaction can be expressed as Figure 2. The catalytic mechanism of HRP has been studied extensively and can be seen as two one-electron reduction steps that generate radical species. The generation of radical species creates a complex profile of reaction catechol, resulting in the catechol cation radical. This cation radical attacks other cation radicals of the monomer to form a dimmer, with elimination of two protons [31,32].

Primary dimer can also act as peroxidase substrates which in turn produce additional radical species that take part in further coupling reactions. As a result, the oxidation of catechol by HRP produces polymers.

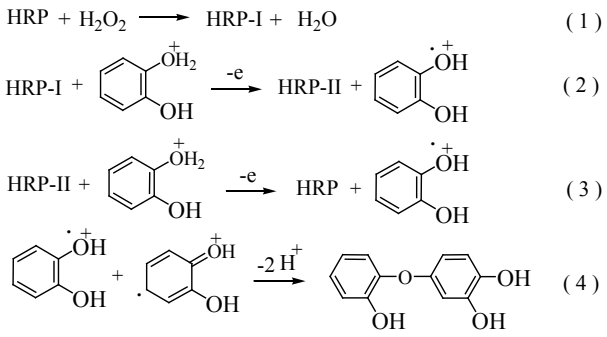

Fig 2. The catalytical cycle of HRP in reaction with catechol. HRP-I and HRP-II are the intermediates of HRP.

FT-IR Spectroscopy

Figure 3 shows FT-IR spectra of (a) poly(catechol)/SPS complex and (b) catechol monomer, in the region from 400 to 4000 cm^{-1}. In Figure 3b, broad doublet peaks at 3430 cm^{-1} and 3330 cm^{-1} belong to characteristic hydrogen-bonded phenolic O–H vibration bands for catechol. Four absorption peaks between 1480 cm^{-1} and 1635 cm^{-1} are attributed to the aromatic ring C=C vibration bands [32]. C–O vibration bands for catechol are at 1250 cm^{-1} and 1100 cm^{-1}. The other absorption bands at 860 cm^{-1} and 736 cm^{-1} are ascribed to out-of-plane bending of –C–H bonds of an aromatic ring [33].

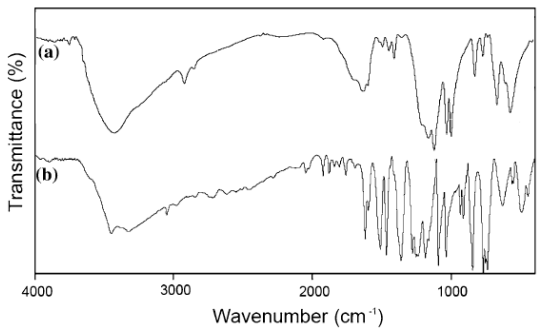

Fig. 3. FT-IR spectra of (a) HRP -synthesized

poly(catechol) in complex with SPS (b) catechol monomer

Figure 3a shows the IR spectrum of poly(catechol) obtained by HRP-catalyzed polymerization in the phosphate buffer solution. A broad peak centered at 3430 cm^{-1} is due to phenolic O–H bond [34]. The absorption bands between 1410 cm^{-1} and 1640 cm^{-1} are due to the C–C vibration of the aromatic ring. The intense phenyl ether bond absorption is also seen at 1130 cm^{-1} and the peak at 1210 cm^{-1} is assigned to the overlapping peaks of asymmetric vibration of C–O–C linkage and C–OH vibrations [35]. The peaks at 820 cm^{-1} and 700 cm^{-1} belong to out-of plane bending of –C–H bonds of an aromatic ring. Also, the peaks observed at 1000 and 1030 cm^{-1}, corresponding to symmetric and asymmetric S=O stretching, confirm the presence of SPS in the complex [33].

The above data indicate that in the presence of SPS as a template, catechol units in the enzymatically produced polymer structure connected to each other by ether linkage, and there were still a high amount of phenolic O–H functional groups on the poly(catechol). Conclusively, the proposed chemical structure of poly(catechol) is presented in Figure 4.

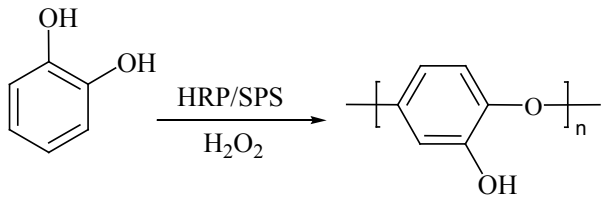

Fig. 4. The proposed chemical structure of HRP catalyzed poly(catechol).

Thermal properties

The thermal behaviors of the poly(catechol) sample were investigated by TGA, and the results are shown in Figure 5. The plot shows the mass percentage as a function of the sample temperature of the poly(catechol)/SPS complex. Herein the sample was heated at a rate of 20 ˚C/ min.

The TG plot of poly(catechol) obtained by HRP-catalyzed polymerization (Figure 5) shows the two stages of weight loss. At about 120 ˚C, the polymer starts to evolve the small amount of moisture, which is found to be 8.6% by TGA. The TGA results show that poly(catechol) undergoes thermal degradation beginning at about 200 ˚C and with a total mass loss of 22%. A weight loss about 400 ˚C is observed in poly(catechol) may be due to the elimination of sulfonic group of the SPS that is in complex with polymer. The mass loss of poly(catechol) at 550 °C is about 90% of the initial poly(catechol), and 10% of the polymer remained which is stable up to 800°C. The thermal stability of the catechol increased in the poly(catechol) polymeric structure effectively [33]. This result ensures the high thermal stability of the poly(catechol) for high-temperature applications.

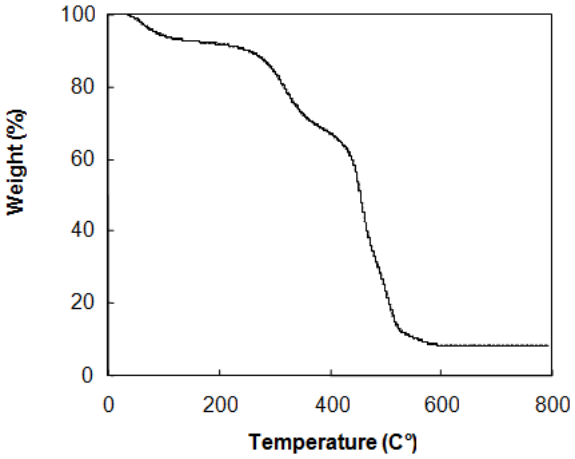

Fig. 5. TGA thermogram of HRP catalyzed poly(catechol)

Cyclic voltammetry

The electrochemical properties of the enzymatic synthesized poly(catechol) are characterized by cyclic voltammetry. Figure 6 shows the cyclic voltammograms for poly(catechol) in the complex with SPS in 1.0 M HCl at different scan rates.

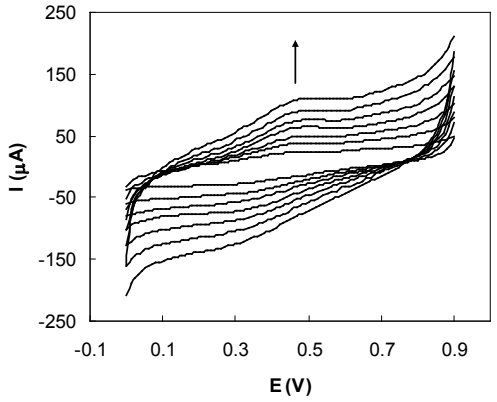

Fig. 6. Cyclic voltammetry of a solution of poly(catechol) catalyzed by HRP in 1.0 M HCl at different scan rates between 50 and 500 mV/s.

The enzymatic polymerization of poly(catechol) exhibits one oxidation peak; similar results also have been observed previously [36]. One oxidation peak was noticed in the anodic sweep at 0.48 V versus Ag/AgCl sweep between 0 and 0.9 V. On recording the cyclic voltammograms at different scan rates between 50 and 500 mV/s, appreciable changes in the anodic and cathodic peaks current values were observed. Linear relationships between the anodic peak current and the scan rates of the poly(catechol)/SPS complexes are presented in Figure 7. These recorded CV curves suggest that the poly(catechol)/SPS complexes produced by enzyme HRP are electrochemically active.

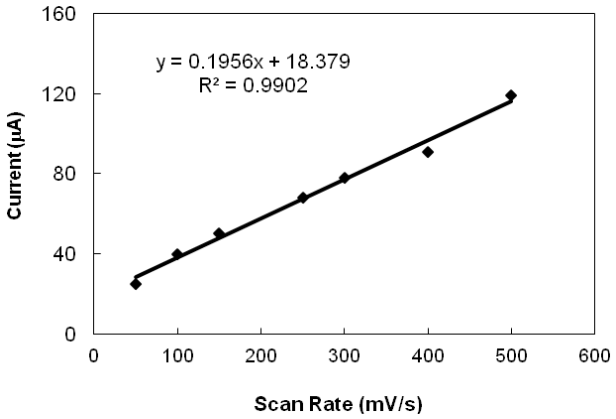

Fig. 7. Plot of the anodic peak current versus the scanning rate for poly(catechol)

Conclusion

The poly(catechol) in complex with SPS was successfully synthesized with the catalytic polymerization of catechol by enzyme HRP. The FT-IR data indicate that in the presence of SPS as a template, catechol units in the polymer structure connected to each other by ether linkage, and there were still a high amount of phenolic functional groups. The TGA data ensures the high thermal stability of the poly(catechol) for high-temperature applications. The CV results suggest that the poly(catechol)/SPS complexes produced by enzyme HRP are electrochemically active. In conclusion, this method is simple and economical for the preparation of water-soluble poly(catechol).

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