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Extraction and characterizations of enzymatically synthesized conductive poly(3,4-ethylenedioxythiophene)

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ABSTRACT

Poly(3,4-ethylenedioxythiophene) doped with poly(4-styrenesulfonate) (PEDOT:PSS) has become the most applicable conductive polymer due to its promising processability. Recently, the conductive PEDOT has been successively synthesized by an enzyme-catalyzed approach. In this study, we have further verified the efficiency of a two-stage process for the enzymatic synthesis of PEDOT by employing a heating procedure after the horseradish peroxidase (HRP) catalyzed reaction in the presence of PSS template. An effective extraction to partially remove extra PSS as well as oligomers of PEDOT was also established with n-butanol as an organic solvent. Detail characterizations of enzymatically synthesized PEDOT:PSS included UV–vis spectrum, XPS, ^{1}H NMR, and TGA, which further confirmed the synthesis of conductive PEDOT with a quinoid structure and using PSS as the template.

1. Introduction

Poly(3,4-ethylenedioxythiophene) (PEDOT) is considered as the most attractive conductive polymer due to its excellent solubility in water, in addition to the features of low band-gap, great conductivity, pronounced electrochromic activity, and long-term air stability [1–4]. Chemically synthesized PEDOT has been commercialized using poly(4-styrenesulfonate) (PSS) as the template and shows many prospects and potential applications, such as antistatic layer for photographic films, electroluminescent devices, organic field effect transistors, and biosensor fabrications [3,5,6].

Because of the catalytic power of enzymes, enzyme-catalyzed synthesis of conductive polymers is known to be an environmentally friendly process with more efficiency [7]. We have previously utilized horseradish peroxidase (HRP) to successfully synthesize conductive polyaniline (PANI) that possessed excellent solubility in aqueous solution with good electrochemical properties [8,9]. Enzymes, such as HRP and soybean peroxidase (SBP), have also been reported for the synthesis of conductive PEDOT [10–12]. Lately, we have proposed a two-stage enzymatic process for the synthesis of PEDOT, where HRP acted as the catalyst to promote the generation of EDOT radical cations followed by the oligomerization under the room temperature in the presence of PSS, then a mild heating process was employed for the chain extension [13]. The final product exhibited a molecular structure similar to that of commercial PEDOT:PSS and had good electrochemical properties.

The electrochemical properties of PEDOT:PSS greatly depend on the molecular structure of PEDOT as well as its association with PSS. In this study, we provided further characterizations of PEDOT synthesized by an enzyme-catalyzed two-stage process. Our results presented additional evidences to verify the molecular structure of PEDOT and its interaction with PSS. In order to improve the electrochemical properties of PEDOT:PSS, various organic solvents were examined for the extraction of PEDOT:PSS by reducing the contents of PSS, EDOT and its oligomers. Meanwhile, molecular docking was employed to understand the possible mechanism involved in the HRP catalyzed polymerization of EDOT.

2. Experimental

2.1. Materials

Horseradish peroxidase (type II HRP, EC 1.11.1.7), 3,4-ethylenedioxythiophene (EDOT, 97%), poly(sodium 4-styrenesulfonate) (PSS), chemically synthesized PEDOT:PSS (2.8 wt.% dispersion in H_2O, low-conductivity grade) were purchased from Sigma–Aldrich Corp. (St. Louis, USA). Hydrogen peroxide (H_2O_2, 35% (v/v)) was obtained from Merck (KGaA Darmstadt, Germany). All other reagents that used for preparing buffers and standard solutions were purchased from various commercial sources and were of analytical grade.

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2.2. Analysis of HRP activity in different pH

1-Step^{TM} ABTS for HRP activity was purchased from PIERCE Chemical Co. (Rockford, IL, USA) and was performed according to manufactory's procedure. To investigate the effect of pH on HRP activity, we prepared 1 M phosphate–citrate buffer solutions with various pH in the range of pH 2–7. The same amount of HRP was diluted in a 500 μl of 0.2 M buffer solution with different pH to the final concentration of 0.5 mg HRP/ml and incubated under room temperature for one hour. 5 μl of each mixture was pipetted into 195 μl of ABTS stock solution (pH 7) and incubated under room temperature for 10 min before was analyzed at 405–410 nm with a Genesys 2 UV-vis spectrophotometer.

2.3. Enzymatic polymerization of EDOT

Polymerization of EDOT catalyzed by HRP was carried in a buffer with pH 2 in order to produce water-soluble PEDOT. A 45 ml of mixture containing 13.5 mmol PSS and 4.5 mmol EDOT in 0.2 M phosphate–citrate buffer (pH 2) was prepared by stirring under room temperature and HRP was added with gentle mixing. To initiate the polymerization, 1.8 ml of 0.1 M H_2O_2 was then added dropwisely with 8-min time interval until completion in order to eliminate its effect on HRP activity. The polymerization processed for 16 hours under room temperature with gentle agitation. The reaction was then transferred to a 60 °C water-bath for another 2.5 h. PEDOT was collected in aqueous phase (bottom) with n-butanol extraction and was lyophilized for about 24 h. The dark-blue PEDOT powder was then subjected to various characterizations.

2.4. Extraction of PEDOT

Organic solvents, including acetonitrile, methyl t-butyl ether, acetone, acetic anhydride, methyl iso-butyl ketone, pyridine, 1-chlorobutane, ethanol, toluen, hexanes, diethyl ether, isopropyl alcohol, methanol, n-butanol, DMSO, chloroform, were mixed with the same volume of PEDOT:PSS aqueous solution with gentle vortexing, then briefly centrifuged under room temperature. Stable phase separations were obtained with several organic solvents and both phases were then separated for further characterizations.

2.5. Characterizations of PEDOT:PSS

A Genesys 2 UV-vis spectrophotometer (Rochester, NY, USA) was routinely employed to monitor the polymerization of EDOT and samples were normally diluted 10 times before measurements. Proton nuclear magnetic resonance (^{1}H NMR) measurements were performed under room temperature with a Varian VNMRS 600 MHz NMR spectrometer (Varian, Inc., CA, USA). A lyophilized NaPSS or PEDOT:PSS sample was placed in a 5 mm Optima NMR tube (NES-600) and dissolved in 1 mL of deuterium oxide (D_2O) from Aldrich Chemical Co. A Q50 TGA instrument (TA Instruments Inc., DE, USA) under the nitrogen atmosphere with a heating rate of 10 °C/min was employed to characterize the thermal decomposition of a PEDOT:PSS powder. X-ray Photoelectron Spectroscopy (XPS) was carried out by a PHI Quantera SXM/Auger: AES 650 (ULVAC-PHI Inc., Kanagawa, Japan), where a lyophilized PEDOT:PSS sample was pressed into a pellet with 0.2 cm in thickness.

3. Results and discussion

3.1. The effect of pH on the HRP catalyzed polymerization of EDOT

Fig. 1A indicates that optimum pH for HRP activity in the phosphate–citrate buffer at the room temperature is around pH 4, where HRP remains 94% of its original activity after one hour incubation (line b). With pH 5 and pH 2, 1 h incubation leads to 26% and 52% loss of activity, respectively (line b). The activity of HRP with pH 4 is relatively stable with time as shown by lines a and b in Fig. 1A. We then carried out the HRP catalyzed polymerization of EDOT with different pH, however, pH 2 was preferred for the synthesis of PEDOT according to the UV–vis spectrums shown in Fig. 1B. The increased spectrum above 800 nm indicates the formation of conjugated PEDOT with a relative lower energy level corresponding to extra π–π^* double bonds between EDOT units. In addition, the major absorption peak in the wavelength range of 280–400 nm with pH 2 demonstrates the π–π^* transitions due to the formation of very short oligomers of EDOT, including dimers and trimers. Obviously, the enzymatic synthesis of PEDOT is very sensitive to pH. On the other hand, we have previously reported that the optimum pH for HRP catalyzed polymerization of aniline (ANI) was pH 4 [8], suggesting some differences involved in the synthesis of PEDOT and PANI.

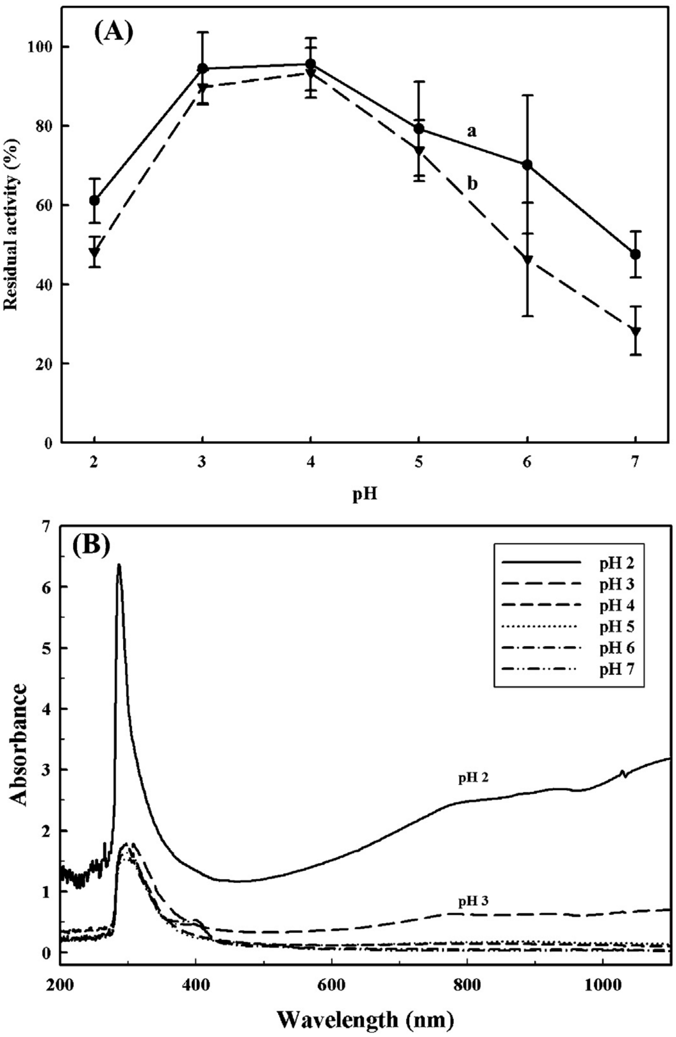

Fig. 1. (A) The effect of pH on the activity of HRP, where lines (a) and (b) indicate the HRP was incubated in 1 M phosphate–citrate buffer with different pH under room temperature for 30 and 60 min, respectively; (B) the effect of pH on the HRP catalyzed polymerization of EDOT.

The molecular docking was then performed by using CDOCKER protocol in Discovery Studio 2.5.5 with CHARMm-based molecular mechanics force field. As shown in Fig. 2, there is no significant difference for EDOT or aniline to interact with the key residues of HRP at the entrance of its hydrophobic pocket. Both have a distance of ~4 Å to the heme group located in the active domain of HRP and