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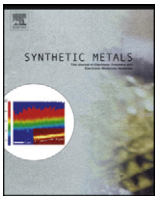

Enzymatic synthesis of conductive polyaniline in the presence of ionic liquid

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ABSTRACT

Enzyme catalyzed aniline polymerization has shown notable advantages, such as benign process and good solvent solubility for polyaniline. In this study, we further explored the possibility of using anionic surfactant sodium dodecylbenzenesulfonate (SDBS) as the template for the synthesis of conductive polyaniline by horseradish peroxidise (HRP), particularly in the presence of imidazolium-based ionic liquid [bmim]OTF. We found that the use of [bmim]OTF offered the protection for HRP against the inactivation by SDBS. Meanwhile, [bmim]OTF ensured the synthesis of conductive PANI in the presence of SDBS based on UV–vis spectrophotometer analysis. The conductive PANI was further characterized by FTIR, TGA, and cyclic voltammetry. Our results demonstrated that the enzymatic synthesized PANI in the presence of both SDBS and [bmim]OTF possessed excellent electrochemical properties.

1. Introduction

Polyaniline (PANI) is an attractive conductive polymer due to its moderately high conductivity, good thermal and oxidative stability, and being easily synthesized through chemical or electrochemical approaches [1–6]. Its electrochemical properties can also be regulated reversibly over the full range of conductivity from insulators to metallic conductors through the doping and dedoping processes [7–9].

In comparison with traditional chemical and electrochemical approaches, enzyme catalyzed polymerization of aniline has been demonstrated with some prospective features such as (i) the polymerization can be performed under a mild reaction condition without using toxic reagents, which is considered as an environmentally benign process; (ii) the physical structure and the solubility of product PANI can be greatly improved by optimizing the reaction conditions, which is benefit for further processing [10–13]. Horseradish peroxidase (HRP) is commonly recommended for the polymerization of aniline and its catalytic mechanism has been previously illustrated [14].

Similar to using acid polyelectrolyte sulfonated polystyrene (SPS) as template for aniline polymerization [15,16], the anionic surfactants, such as sodium dodecylbenzenesulfonate (SDBS) and sodium dodecyl sulfate (SDS), can also serve as ionic templates by forming micelles that create ideal local environments with higher charge density, lower pH and hydrophobic regions to promote the synthesis of conducting polyaniline [15–17]. However, ionic surfactants are usually considered as severe degradation agents for enzyme activities. With the concentrations over their critical micellar concentrations (CMC), ionic surfactants may result in the completely loss of enzyme activities [16,18]. To overcome the problems, we have previously proposed to use the linear bovine serum albumin (BSA) to neutralize the negative effect of SDS on HRP activity and to successfully synthesize the polyaniline with fair electrochemical properties [18].

Recently, room temperature ionic liquids (ILs) are founded to be able to improve enzyme activity and stability, in which make them good candidates as alternative solvents or co-solvents for biocatalysis [19]. Meanwhile, ionic liquids have been noticed to reduce the CMC of certain surfactants [20], therefore may reduce the applied concentrations of ionic surfactants that are necessary for the synthesis of conductive polyaniline.

In this study, we employed the 1-butyl-3-methylimidazolium trifluoromethansulfonate ([bmim]OTf), a double amphiphilic ionic liquid, as a co-solvent for the enzymatic polymerization of aniline in the presence of SDBS. We have investigated the effects of SDBS and [bmim]OTf on the activity of HRP, as well as the synthesis of conductive polyaniline. In addition, the structure and the electrochemical properties of synthesized polyaniline were examined by different characterizations.

2. Experimental

2.1. Chemicals

Hydrogen peroxide (35%, v/v), aniline monomer, and 1-butyl-3-methylimidazolium trifluoromethansulfonate ([bmim]OTf) were obtained from Merck (KGaA Darmstadt, Germany), sodium

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dodecylbenzenesulfonate (SDBS) was made by ACROS Organics (New Jersey, USA). Horseradish peroxidase (HRP), graphite powder, and paraffin oil were purchased from Sigma–Aldrich Corp. (St. Louis, USA). All other reagents that used for preparing buffers and standard solutions were purchased from various commercial sources and were of analytical grade. 1-Step^{TM} ABTS for HRP activity was purchased from PIERCE Chemical Co. (Rockford, IL, USA) and was performed according to manufactory's procedure.

2.2. Polymerization

Our previous results indicated that the citrate buffer with pH 4 was preferable for better HRP activity, which was normally recommended for enzymatic polymerization of aniline [18,21]. Before initiating polymerization, a mixture containing buffer solution, SDBS, aniline, or ionic liquid [bmim]OTf was prepared by vigorous agitation under 4 °C. To process the polymerization, a certain amount of HRP was added with gentle mixing. H_2O_2, served as a mild oxidant agent to trigger the enzymatic polymerization of aniline, was then added dropwisely with a 3 min time interval until complete in order to eliminate its effect on HRP activity. The polymerization processed for another 4 h and PANI product was collected by centrifugation. The pellet was washed with distilled water and PANI powder was obtained by lypholization for about 12 h. Alternatively, PANI was de-doped with 10 ml of 1 M NH_4OH followed by being doped with 10 ml of 1 M HCl before lypholization. The dark green PANI powder was then subjected to various characterizations.

A Q50 TGA instrument (TA Instruments Inc., Delaware, USA) under the nitrogen atmosphere with a heating rate of 10 °C/min was employed to characterize the thermal decomposition of PANI pellets and their contents. The composition and chemical structure of PANI were also evaluated by a DA 8.3 FTIR spectrophotometer (Bomem Inc., Québec City, Canada) and a Genesys 2 UV-vis spectrophotometer (Rochester, NY, USA). The conductivities of the PANI pellets in 10 mm thickness were measured by a Quatek/CHI-5601Y four-point sheet resistance test system (Quatek Inc., Taipei, Taiwan).

2.3. Fabrication of the CPE and PANI–CPE electrodes

Carbon paste electrode (CPE) was prepared by mixing paraffin oil with graphite powder. The paste was further blended by an agitate mortar and packed into a plastic holder. To fabricate a CPE electrode, a platinum wire was buried into the packed paste. On the other hand, the PANI–CPE electrode was prepared by mixing certain weight ratio of graphite power and doped PANI power with paraffin oil. A portion of CPE was then replaced by the PANI–CPE paste. The electrochemical characterization was proformed in a miniature electrochemical cell by using the constructed CPE or PANI–CPE electrode as the working electrode, a platinum wire as the auxiliary electrode, and a Ag/AgCl 3 M NaCl electrode as the reference electrode. A PC-controlled CHI621B electrochemical analyzer (CH Instruments, Austin, USA) was used to quantify the fabricated electrodes in 10 mM of potassium ferricyanide ($K_3[Fe(CN)]_6$) in 50 mM phosphate buffer (pH 7.4) by cyclic voltammetry with a scan rate of 20 mV/s.

3. Results and discussion

3.1. Ionic liquid rescues the activity of HRP in the presence of SDBS

For chemical synthesis of dispersible PANI, the stable micelles of SDBS acted as not only the template to attract the anilinium cations to form PANI but also the doping agent to increase the conductivity of PANI [22]. However, it is not appropriate to apply SDBS with a concentration above its critical micelle concentration (CMC), which is about 1.6 mM, in an enzyme-catalyzed reaction. As shown in Fig. 1 (line a), SDBS diminished the HRP activity significantly, where HRP retained less than 20 percentage of its original activity with 0.4 mM of SDBS that was far below its CMC, indicating SDBS easily caused the dramatic structure alternation of HRP due to the coulombic attractive interactions and hydrogen bonds between HRP residues and the anionic sulfonate headgroup. In addition, the sodium ions from SDBS may create an environment to have a salt-out effect on HRP. This negative effect became severer with less enzyme applied (line b in Fig. 1) or by increasing the incubation time (data not shown). For a chemical polymerization of aniline, SDBS with a concentration below CMC failed to form stable micelles and resulted in the formation of PANI with low conductivity and poor solubility [16,23].

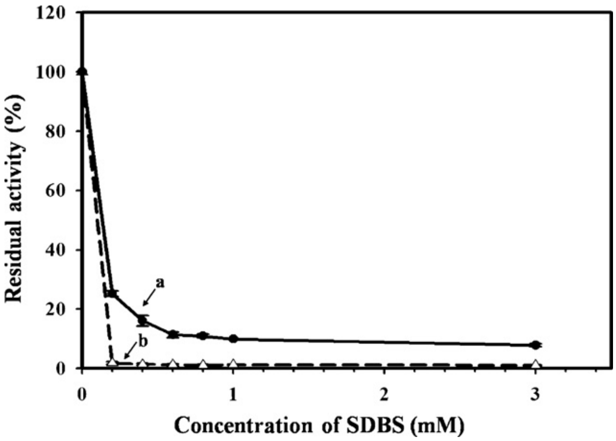

Fig. 1. The effect of SDBS on the activity of HRP. HRP was incubated with SDBS in 0.1 M citrate–phosphate buffer (pH 4) under 4 °C for 15 min before activity analysis, where the concentration of HRP was (a) 0.5 μg/ml and (b) 0.025 μg/ml.

It has been reported that the ionic liquid can serve as an alternative surfactant and may reduce the CMC of other surfactants [20]. In this study, we investigated the contribution of ionic liquid [bmim]OTf to maintain the activity of HRP for the synthesis of conductive polyaniline. As shown in Fig. 2, the addition of ionic liquid moderated the negative effect of SDBS on HRP activity. In the

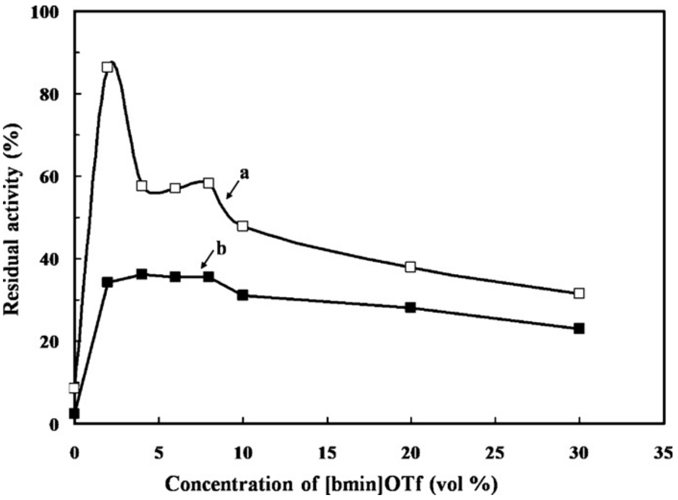

Fig. 2. The rescue of HRP activity by [bmim]OTf. HRP with a concentration of 0.025 μg/ml was incubated in 0.1 M citrate-phosphate buffer (pH 4) containing [bmim]OTf and SDBS for 15 min before activity analysis, where the concentration of SDBS was (a) 0.2 mM and (b) 1 mM.