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Precipitated and chemically-crosslinked laccase over polyaniline nanofiber for high performance phenol sensing

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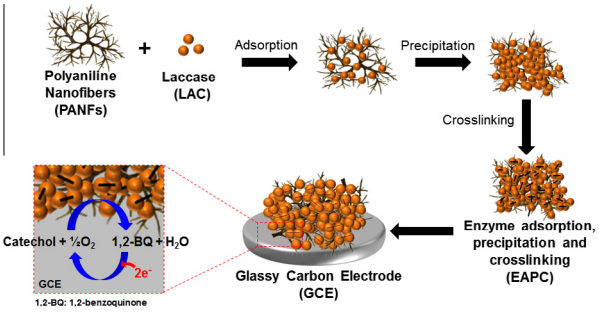
HIGHLIGHTS

- Laccase (LAC) based amperometric biosensor for detection of phenolic compounds.
- LAC was immobilized on the matrix of polyaniline nanofibers (PANFs).
- Enzyme adsorption, precipitation, and crosslinking (EAPC) was applied to LAC on PANFs.
- EAPC-LAC on PANFs offers high enzyme loading and stability.
- EAPC-LAC shows a great promise for highly sensitive and stable phenol biosensors.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study aims at fabricating a laccase (LAC) based amperometric biosensor for detection of phenolic compounds. LAC was immobilized into the porous matrix of polyaniline nanofibers (PANFs) in a three-step process, consisting of enzyme adsorption, precipitation, and crosslinking (EAPC). Immobilized LAC on PANF in the form of EAPC was highly active and stable when compared to control samples of 'enzyme adsorption (EA)' and 'enzyme adsorption and crosslinking (EAC)' samples. For example, the activity of EAPC was 19.7 and 15.1 times higher than those of EA and EAC per unit weight of PANF, respectively. After 6 days at room temperature, EAPC maintained 100% of its initial activity, while EA and EAC retained only 7.7% and 11% of their initial activities, respectively. When the samples were subjected to the heat treatment at 60 °C over 3 h, EAPC maintained 74% of its initial activity, while EA and EAC retained around 1% of their initial activities, respectively. To demonstrate the feasible application of EAPC in biosensors, the enzyme electrodes were prepared and used for detection of phenolic compounds, which are environmentally hazardous chemicals. The sensitivities of biosensors with EA, EAC, and EAPC were 20.3 ± 5.9, 26.6 ± 5.4 and 518 ± 11 μA mM^{-1} cm^{-2}, respectively. At 50 °C for 5 h, EAPC electrode maintained 80% of its initial sensitivity, while EA and EAC electrode showed 0% and 19% of their initial sensitivities, respectively. Thus, LAC-based biosensor using EAPC protocol with PANFs showed a great promise for developing a highly sensitive and stable biosensor for detection of phenolic compounds.

1. Introduction

Phenolic compounds, which are easily found in nature, have been considered as environmentally hazardous materials (Bruce et al., 2001). They are present in wastewater streams of the oil,

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paint, paper, polymer and pharmaceutical industries. When phenolic compounds are transferred into the food chain by wastewater streams, they can lead to dangerous and toxic effect on living organisms because phenolic compounds have poor biodegradability and high toxicity on ecological system as well as human body (Philippat et al., 2013). Thus, quick and accurate determination of phenolic compounds from the environments is of great importance (Ahmaruzzaman, 2008). Electrochemical enzyme biosensors can provide a simple and fast way for the determination of phenols (Karim and Fakhruddin, 2012; Rao et al., 2014). However, the use of such kind of enzyme biosensors have two major limitations when employed for in-situ monitoring of phenolic compounds in various environmental conditions, especially using them continuously over a long operation time. These two major limitations that hamper their successful uses for the practical applications are a low sensitivity and a short operation time, both of which corresponded to low loading and poor stability of enzymes, respectively. Low sensitivity and short operation time of enzyme biosensors can be improved via the enzyme immobilization in/on nanostructured materials by increasing enzyme loading and preventing the enzyme denaturation.

Nanobiocatalytic approaches using conductive nanomaterials have generated successful results that can potentially lead to the development of high performance enzyme electrodes with high loading and stability of enzymes for the various electrochemical biosensor applications (Kim et al., 2008; Kwon et al., 2010; Kim et al., 2011a). One of the successful strategies in nanobiocatalysis is the enzyme adsorption, precipitation and crosslinking (EAPC) method on polyaniline nanofibers (PANFs). Conductive PANFs can be synthesized via an easy and inexpensive process when compared to other nanostructured materials such as nanoparticles, electrospun nanofibers, mesoporous materials and carbon nanotubes. We previously reported biofuel cells using glucose oxidase immobilized on PANFs, showing a reasonably high power output with an exceptionally high long-term stability (Kim et al., 2011b).

In the present work, we immobilized laccase (LAC), which can oxidize phenol compounds (Solomon et al., 1996; Rao et al., 2014), in a form of EAPC on PANFs and evaluated its electrochemical sensing performance for detection of catechol as one of phenolic compounds. To our knowledge, the fabrication of such LAC-based enzyme electrode in the form of EAPC using PANFs as the support for the electrochemical phenol sensing has never been reported. For comparative studies, we also prepared the enzyme electrodes using the enzyme adsorption (EA) and the enzyme adsorption and crosslinking (EAC) methods. By comparing a performance stability of EA, EAC and EAPC, it would be possible to understand the effects of enzyme precipitation and enzyme crosslinking processes on the fabrication of LAC-based enzyme electrode and its electrochemical performances toward the phenol sensing.

2. Materials and methods

2.1. Laccase immobilization over PANF in the form of EA, EC and EAPC

LAC from *Trametes versicolor* was purchased from Sigma (St. Louis, MO, USA). Immobilized LAC samples in the forms of EA, EAC, and EAPC were prepared by following the protocols (Kim et al., 2011b). In details, the PANF were synthesized by mixing aniline and ammonium persulfate as initiator (Huang, 2006; Li et al., 2009). First, aniline monomer solution was dissolved in 1 M HCl (volume ratio of 8.5:1.5) and 0.1 M ammonium persulfate solution was prepared in 1 M HCl. Both aniline and ammonium persulfate solutions in HCl were mixed and shaken at 200 rpm and room temperature for 24 h. After the polymerization reaction, PANFs were centrifuged down, washed using DI water excessively for 3 times, suspended in DI water, and stored at 4 °C until use.

Immobilization of LAC over PANF in forms of EA, EAC and EAPC was previously reported (Kim et al., 2014) and modified protocol was carried out. To prepare the immobilized LAC in the form of EA, 2 mg of PANF were incubated in the 10 mg/ml of LAC solution in 100 mM sodium phosphate buffer (pH 6.5) under shaking (150 rpm) condition for 2 h. For EAC, the glutaraldehyde (GA) as the chemical crosslinking agent was introduced to the EA sample with a final concentration of 0.5% (w/v) under shaking (50 rpm) condition at 4 °C for 17 h. EAPC was prepared by introducing the ammonium sulfate solution with a final concentration of 50% (w/v) into the buffer solution containing both EA sample and free LAC. In the presence of the ammonium sulfate salt, the free LAC (i.e., LAC that is not adsorbed over PANF surface) was precipitated out to form the enzyme aggregates. Following this enzyme precipitation step, the GA solution with a final concentration of 0.5% (w/v) was added into the mixture to chemically crosslink the individual LAC molecule within the precipitated LAC aggregates and these aggregates over the surface of EA sample (See Fig. 1). To cap unreacted aldehyde groups, the samples were shaken at 200 rpm in 100 mM Tris–HCl buffer (pH 7.4) for 30 min, and the samples were excessively washed under shaking (200 rpm) for 3 times with the 100 mM sodium phosphate buffer (pH 6.5). EA, EAC, and EAPC were stored in 100 mM sodium phosphate buffer (pH 6.5) with the final concentration of 2 mg/ml at 4 °C until use.

2.2. Activity and stability measurement of LAC-immobilized PANF

The activities of the samples were measured by following the protocol found in the previous paper (Ride, 1980). The LAC activity was measured via triplicate experiments based on the oxidation of syringaldazine, which is a well-known substrate for the conventional laccase assay. To measure the activity of immobilized LAC on PANF, 100 μL of each LAC-immobilized PANF sample solution was mixed with 800 μL of 100 mM sodium phosphate buffer (pH 6.5), and the mixtures were incubated at 30 °C for 10 min. To this mixture, 100 μL of 0.216 mM syringaldazine in methanol was added. The absorbance at 530 nm was measured using a spectrophotometer (UV-1800, Shimadzu), and the activity of the LAC-catalyzed reaction was calculated from the slope of the time-dependent increase of absorbance at 530 nm. The stabilities of LAC-immobilized samples were checked by measuring their time-dependent residual activities after incubating the samples at different temperatures (40 and 60 °C) in the buffer.

2.3. Scanning electron microscopy (SEM) analysis

PANF, EA, EAC and EAPC were analyzed by using a scanning electron microscopy (SEM, Hitachi S-4300, Hitachi, Tokyo, Japan). For the SEM analysis, all the samples were washed by deionized water and freeze-dried for 24 h. After platinum (Pt) coating, the samples were analyzed at an accelerating voltage of 15 kV. By checking twenty randomly-chosen spots in each SEM image, the average thicknesses of PANF, EA, EAC and EAPC were determined.

2.4. Preparing enzyme electrodes of EA, EAC and EAPC

Nafion® solution (5 wt%) was mixed with 0.5 mg/ml of LAC-immobilized PANF sample, and the mixture was stirred at 4 °C for 1 h. The suspension (20 μL) of the mixture was applied over glassy carbon electrodes (GCE, 3 mm diameter, CH Instruments, Austin, TX, USA), and dried under ambient conditions for 1 h. The electrodes were stored in the 100 mM sodium phosphate buffer (pH 6.5) at 4 °C until use.

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