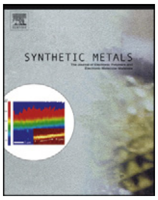
Synthetic Metals 159 (2009) 2091–2096

Contents lists available at ScienceDirect

Synthetic Metals

journal homepage: www.elsevier.com/locate/synmet



Enzymatic synthesis of conductive polyaniline using linear BSA as the template in the presence of sodium dodecyl sulfate

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ARTICLE INFO

Article history:
Received 2 June 2009
Received in revised form 10 July 2009
Accepted 22 July 2009
Available online 28 August 2009

Keywords:
Enzymatic polymerization
Conductive polyaniline
Protein template

ABSTRACT

Enzymes as biocatalysts with powerful catalytic activities and extreme specificities have been applied in various polymer syntheses, which are considered to be environmentally acceptable processes. In this study, we explored the possibility of using bovine serum albumin (BSA), an innate protein, as the template for the synthesis of conductive polyaniline (PANI) by horseradish peroxidase (HRP), and we found that the denatured BSA effectively promoted the synthesis of conductive PANI in the presence of sodium dodecyl sulfate (SDS) based on UV–vis spectrophotometer analysis. Meanwhile, parameters influencing the activity of enzyme and polymerization were investigated, and PANI was characterized by FTIR, TGA, and cyclic voltammetry. Our results demonstrated that the enzymatic synthesized PANI by using uncoiled BSA:SDS as the template presented excellent electrochemical properties through dedoping/doping procedure.

1. Introduction

During the past two decades, conductive polymers have been under intensive studies and developments [1]. Among them, polyaniline (PANI) is particularly attractive, because it has moderately high conductivity, good thermal and oxidative stability, and can be easily synthesized through chemical or electrochemical approaches [2–7]. The electrical properties of conductive PANI can be reversibly switched over the full range of conductivity from insulators to metallic conductors through an oxidation–reduction process [8–10].

Chemical polymerization of aniline is generally carried out in a strong acid condition, which is considered to be a simple and inexpensive process [11–13]. By comparison, electrochemical polymerization of aniline has several advantages over chemical polymerization since the process is clean and no toxic oxidants involved, and the synthesized polymers possess flexible electrical properties [2,4]. However, PANI synthesized by both approaches is often confronted with over-oxidation state that may result in the degradation of PANI [5,14]. Meanwhile, the lack of unique structure and low solubility in common organic and aqueous solvents restrict its industrial applications [10,15]. In contrast, enzymes have several noteworthy features such as powerful catalytic activity and notable specificity under mild reaction conditions. The advantages for enzymatic synthesis of PANI are expected as (i) the polymerization is performed under a mild reaction condition without using toxic reagents, which is an environmentally benign process; (ii) the structure and solubility of PANI can be greatly improved by optimizing the reaction conditions, which is benefit for further processing [16–19].

Peroxidases, a kind of hemeproteins, have been found to catalyze the oxidation of phenols and aromatic amines with relative mild oxidizing agents such as diluted hydrogen peroxide (H_2O_2) and O_2 [20,21]. Among them, horseradish peroxidase (HRP) that catalyzes the decomposition of H_2O_2 at the expense of aromatic proton donors is particularly interested due to its well-defined properties, good stability, and low-cost commercial availability [22,23]. The mechanism of HRP catalyzed polymerization is illustrated by Scheme 1 [24],

$$\mathrm{HRP} + \mathrm{H_2O_2} \rightarrow \textbf{Compound I} + \mathrm{H_2O}$$

$$\text{Compound I} + \mathrm{RH_2} \rightarrow \textbf{Compound II} + \mathbf{RH\cdot}$$

$$\text{Compound II} + \mathrm{RH_2} \rightarrow \mathbf{HRP} + \mathbf{RH\cdot} + \mathbf{HO}$$

The overall reaction:

$$2\mathrm{RH_2} + \mathrm{H_2O_2} \rightarrow \textbf{RH-RH} + 2\mathrm{H_2O_2}$$

Scheme 1. The mechanism of HRP catalyzed polymerization.

where compound I and compound II are two active intermediates of HRP, RH_2 is a reducing substrate, such as aniline monomer in this study, and $RH^\bullet$ is a free radical intermediate that readily undergoes self-coupling with other radicals to form oligomers or polymers.

For enzymatic polymerization of aniline in an aqueous solution, the major consideration has been that a great number of

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doi:10.1016/j.synthmet.2009.07.040

oligomers are normally produced, possibly due to the fast polymerization. To overcome this problem, it has been proposed to employ water-soluble polyanionic templates such as sulfonated polystyrene (SPS) and poly(vinylphosphonic acid) (PVP) as templates to align the aniline monomers and to promote more ordered para-directed polymerization, consequently improving the solubility of PANI for further processing [25–28]. Others have reported to use poly(acrylic acid) (PAA), an acid polyelectrolyte, as template for chemical and enzymatic polymerization of aniline [9,29]. Recently, we have interestingly found that the electrochemical synthesized PANI film in the presence of bovine serum albumin (BSA) had stable microstructure, therefore the constructed PANI/Pt electrode exhibited enhanced sensitivity and excellent stability. In particular, the lysine-rich BSA provided PANI with plenty of primary amino groups for further modifications with biomolecules, such as proteins [30].

In this study, we employed uncoiled BSA as the template for the enzymatic polymerization of aniline. We also investigated the effect of SDS, an anionic surfactant, on the polymerization in the presence of BSA. The optimum condition for synthesis of conductive PANI was established, and its structure and electrochemical properties were characterized.

2. Experimental

2.1. Chemicals

Hydrogen peroxide (35%, v/v), sodium dodecyl sulfate (SDS), and aniline monomer were obtained from Merck (KGaA Darmstadt, Germany). Horseradish peroxidase (HRP) and bovine serum albumin (BSA), as well as 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

Chemical synthesis of polyaniline is usually conducted under strong acidic condition with pH below 1 in order to produce conductive form of PANI [12,31], however, pH below 3 leaded to the dramatic decreasing of HRP activity (data not shown). Our preliminary results indicated that the citrate buffer with pH 4 was preferable for better HRP activity, which is normally recommended for enzymatic polymerization of aniline [32]. Before polymerization reaction, BSA (MW: 67 kDa) was denatured in boiling water for 5 min in the presence of SDS. The BSA:SDS mixture was then mixed with aniline solution by vigorous agitation. 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. The concentration of H_2O_2 was controlled to be below 5 mM 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 dedoped 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.

The morphology of PANI powder was visualized by an ABT-150S SEM (TOPCON Corp., Tokyo, Japan). 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 various PANI pellets and their protein contents. The composition and chemical structure of PANI were evaluated by a DA 8.3 FTIR spectrophotometer (Bomem Inc., Québec City, Canada) and a Genesys 2 UV–vis spectrophotometer (Rochester, NY, USA).

2.3. Fabrication of the CPE and PANI–CPE

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, a platinum wire was buried into the packed paste. On the other hand, the PANI–CPE 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 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 1 M HCl solution by cyclic voltammetry with a scan rate of 50 mV/s.

3. Results and discussion

3.1. The influences of BSA on the polymerization of aniline

It has been reported that the PANI synthesized in the presence of anionic templates increased its polymer chain length, conductivity, and solubility as well [25–27]. In this study, we have utilized denatured protein molecular, such as BSA, as the template for aniline polymerization. As shown by curve (b) in Fig. 1, the addition of BSA dramatically enhances the visible absorption peak near 504 nm, which is possibly due to the induction of oligomer synthesis accompanied by the transitions between quinoid and benzenoid rings (purple color). Spectrums near 330 nm indicated the π–π* transition of the conjugated benzenoid rings. A weak absorption peak near 450 nm is observed with pH 6 (curve (c) in Fig. 1), indicating the formation of multiple branched structures [13,25,33,34]. In contrast, two weak absorption peaks near 560 and 700 nm are noticed with pH 3 (curve (a) in Fig. 1), which indicating the excitonic transition of the quinoid and benzenoid rings, respectively [25].

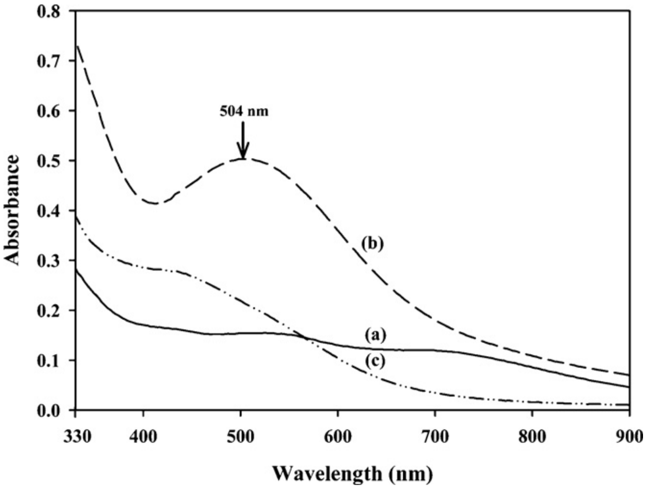

Fig. 1. UV–vis spectra of PANI obtained by the polymerization of 4 mM aniline in 0.1 M citric acid/phosphate buffer containing 0.2 mg/ml BSA with pH (a) 3.0, (b) 4.3, and (c) 6.0.