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A highly sensitive choline biosensor based on bamboo-like multiwall carbon nanotubes/ionic liquid/Prussian blue nanocomposite

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ABSTRACT

A highly sensitive amperometric choline biosensor was fabricated using bamboo-like multi-walled carbon nanotubes (BCNTs)/ionic liquid (IL)/Prussian blue (PB) nanocomposite modified glassy carbon (GC) electrode. The PB nanoparticles were first electrodeposited on the surface of a BCNTs/IL/GC electrode. The Ni^{2+} ions were then electrochemically incorporated into the PB structure to improve its stability in mild alkaline media. Choline oxidase was next immobilized on the modified electrode using a cross-linking method. Amperometric measurements of choline were performed at −0.05 V vs. Ag/AgCl in 0.05 M phosphate buffer, pH 7.4. The choline biosensor exhibited a high sensitivity of 345.4 μA mM^{-1} cm^{-2} with a detection limit of 4.5×10^{-7} M. The amperometric response was linear from 4.5×10^{-7} to 1.0×10^{-4} M. The proposed biosensor displayed a short response time of 2 s, low K_m value of 9.7×10^{-5} M, good storage stability and high selectivity.

1. Introduction

Choline is a vital nutrient [1], which is required for three main physiological purposes: (1) it plays an important role in the synthesis of some essential phospholipids that provide structure to cell membranes and facilitate transmembrane signaling [2]; (2) choline is a major source of methyl groups via its metabolite, betaine [3]; (3) it is a precursor of the neurotransmitter acetylcholine, which is involved in memory and muscle control [4]. Therefore, the quantitative determination of choline is important in clinical analysis, especially in early diagnosis of brain disorders such as Alzheimer's and Parkinson's diseases [5]. Among the various methods available for choline detection, amperometric choline oxidase (ChOx)-based biosensors have attracted considerable attention because of their simplicity, reliability, rapid response, high sensitivity and low cost [6]. In these biosensors, choline concentration can be obtained by amperometric detection of hydrogen peroxide (H_2O_2), a side product of the enzymatic reaction (reaction 1) [7]:

$$\text{Choline} + 2O_2 + H_2O \xrightarrow{\text{ChOx}} \text{betaine} + 2H_2O_2 \quad (1)$$

However, a great drawback of this approach is the high overpotential required for H_2O_2 oxidation, at which many other electroactive substances in real samples (i.e., ascorbic acid, uric acid, etc.) are electrochemically oxidized, resulting in interfering signals [8]. In order to solve this problem, several approaches have been employed to lower the oxidation potential of H_2O_2, such as using horseradish peroxidase [9] or redox mediators [10].

Prussian blue (PB) is an electron transfer mediator, considered as an "artificial peroxidase" due to its electrocatalytic activity towards H_2O_2 reduction [11]. In addition to its low cost, ease of preparation and high sensitivity, PB allows the low-potential detection of H_2O_2 and thus greatly enhances the biosensor selectivity [12]. So, it is intensively used in amperometric oxidase-based biosensors [13]. However, the poor stability of PB-based biosensors at physiological pH seriously restrains its applications [14]. To improve the alkaline pH stability of PB, some progress has been made such as using an outer polymer film like Nafion® [15], or PB hybrid

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composites [14,16]. Recently, it has been demonstrated that the incorporation of Ni^{2+} ions into the structure of electrochemically deposited PB films could significantly improve its stability in alkaline media [13].

Carbon nanotubes (CNTs) are widely used in electrochemical biosensors due to their unique physical and chemical properties [10]. They are considered to be suitable mediators for PB-modified electrodes because of their ability to adsorb PB nanoparticles [17] and high electrical conductivity [18]. PB/CNTs hybrids exhibit enhanced electrocatalytic activity toward H_2O_2 reduction and improved stability [14]. In addition, these nanocomposites form a highly porous three-dimensional network with a large surface area, providing an opportunity for loading additional amounts of enzymes [19].

Ionic liquids (ILs) are a class of organic salts consisting of large organic cations and weakly coordinating anions, with melting points around or below room temperature [20]. They have many attractive features such as high conductivity, wide electrochemical window, good stability and biocompatibility [20,21]. It is proven that ILs not only promote PB growth on the electrode, but also enhance the electrocatalytic activity of PB-modified electrodes to H_2O_2 [16,22] and improve its electrochemical stability [16]. Furthermore, it is found that CNTs can form gelatinous composite materials, called bucky gels (BG), upon being ground with ILs [23]. Compared to electrodes based on either CNTs or IL, BG-modified electrodes display better analytical performance [23]. Recently, BG-modified electrodes have been proposed as suitable platforms for fabricating PB-based biosensors, because of the synergistic effects of CNTs and IL on the electrocatalytic properties of PB [24]. On the other hand, ILs could provide a biocompatible microenvironment for enzymes through several factors including hydrogen bonding, hydrophilicity and ion kosmotropicity [25]. It has been found that the best electrocatalytic activity of ChOx could be obtained on CNTs/IL composites including a hydrophilic IL, i.e. 1-allyl-3-methylimidazolium bromide ([AMI]Br) [25].

The aim of this study was to fabricate a sensitive choline biosensor based on the synergistic beneficial roles of PB, CNTs and IL. To the best of our knowledge, there has been no report of using the PB/CNTs/IL nanocomposite for fabricating biosensors. In this study, PB was first electrodeposited on the BG-modified electrodes including [AMI] Br. Then, Ni^{2+} ions were incorporated into the PB structure to improve its pH stability in mild alkaline medium. A cross-linking method was subsequently utilized to immobilize ChOx using Nafion® and glutaraldehyde (GA). Some of the experimental conditions, related to the preparation of the modified electrode, were optimized and the analytical characteristics of the developed biosensor were evaluated under optimized conditions.

2. Experimental

2.1. Reagents

ChOx (E.C. 1.1.3.17) from Alcaligenes species (11 U mg^{-1}), choline chloride, Nafion® (5 wt.% ethanol solution) and [AMI]Br (purity >97%) were purchased from Sigma (St. Louis, MO, USA). Bamboo-like multiwall CNTs (BCNTs, purity >95%, diameter 30 ± 10 nm, length 1–5 μm) were obtained from NanoLab Inc. (Brighton, MA, USA). Nitric acid (HNO_3, 65%), hydrochloric acid (HCl, 37%), GA (50%, v/v), potassium phosphate (KH_2PO_4, K_2HPO_4), nickel dichloride ($NiCl_2$), potassium chloride (KCl), N,N-dimethylformamide (DMF), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), potassium ferricyanide ($K_3[Fe(CN)_6]$), bovine serum albumin (BSA) and a 30% H_2O_2 were all of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Apparatus and measurements

The electrochemical experiments were performed with an Autolab Potentiostat-Galvanostat 302N (Metrohm-Autolab B.V., Utrecht, The Netherlands), controlled by a NOVA 1.7 software. All electrochemical measurements were carried out at room temperature in a conventional three-electrode cell. A silver/silver chloride (Ag/AgCl) electrode (3 M KCl solution, Metrohm-Autolab B.V., Utrecht, The Netherlands) and a platinum wire were used as reference and auxiliary electrodes, respectively; A bare or modified glassy carbon (GC) electrode (Metrohm-Autolab B.V., Utrecht, The Netherlands), with a diameter of 2 mm, was used as the working electrode. Raman spectra were obtained using a confocal Raman spectrometer (Senterra R200-L, Bruker Optics, Ettlingen, Germany). The Fourier transform infrared (FTIR) spectrum of functionalized BCNTs was recorded on a FTIR spectrophotometer (Tensor 27, Bruker, Germany) at a resolution of 4 cm^{-1}, using a KBr disk. Scanning electron microscopic (SEM) images were observed through a field emission scanning electron microscope (FE-SEM, model S-4160, Hitachi, Tokyo, Japan). Energy dispersive X-ray spectroscopy (EDX, Tescan, VEGA-3 LMU VPSEM, Czech Republic) was used to evaluate chemical composition of the modified electrodes after incorporation of Ni^{2+}.

2.3. Functionalization of BCNTs

BCNTs were functionalized according to a slightly modified reported procedure [24,26]. BCNTs (10 mg) were sonicated in 30 mL of 35% HNO_3 at 40 °C for 6 h. After centrifuging the resultant suspension at 20,000 rpm, the excess top nitric acid was removed and BCNTs were soaked in deionized water. Then, the nanotubes were filtered through a 0.2 μm polytetrafluoroethylene filter (PTFE, Sartorius AG, Germany) and rinsed with deionized water until a neutral pH value was achieved. The obtained wet cake was dried under an IR lamp. Two mg of pristine or functionalized BCNTs and 300 mg KBr powder were then grinded in an agate mortar, with an agate pestle and the ground mixtures were transferred into the cylinder bore and pressed. The resultant KBr discs were analyzed with a FTIR spectrophotometer.

2.4. Preparation of BG

BG nanocomposite was prepared according to the procedure described by Du et al. [27]. Briefly, 2 mg of functionalized BCNTs powder and 1 mL of [AMI]Br were mixed and ground in an agate mortar for about 1 h. The suspension was then centrifuged for 30 min at 18,000 rpm. By removing the top transparent liquid (pure IL), the gel phase was isolated. The resultant BG was dispersed in 1 mL DMF with the aid of sonication.

2.5. Fabrication of the modified electrodes

2.5.1. BG/GC electrodes

Before each experiment, the GC electrodes were polished with 1.0, 0.3 and 0.05 μm alumina slurry, respectively. Then, the electrodes were thoroughly rinsed with double-distilled water and allowed to dry at room temperature. The BG/GC electrodes were obtained by casting 2 μl of the BG suspension (see Section 2.4) on the surface of GC electrodes and dried in air.

2.5.2. PB/BG/GC electrodes

PB nanoparticles were electrochemically deposited on the surface of BG/GC electrodes by continuous potential cycling of the modified electrodes (30 cycles) between 0.0 and 1.0 V at a scan rate of 0.1 V s^{-1}. The background electrolyte solution for PB