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An amperometric biosensor and a biofuel cell of uric acid based on a chitosan/uricase–poly(furan-3-boronic acid)–Pd nanoparticles/plated Pd/multiwalled carbon nanotubes/Au electrode

Lingen Sun [a], Jiali Liu [a], Pei Zhang [a], Yue Meng [a], Chunhui Liu [a], Yixuan Ma [a], Qingji Xie [a,*], Wenhua Meng [b,*]

[a] Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, China
[b] Hunan Normal University Hospital, Changsha 410081, China

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ABSTRACT

A chitosan (CS)/uricase (UOx)–poly(furan-3-boronic acid) (PFBA)–Pd nanoparticles (PdNPs)/plated Pd (Pd_{plate})/multiwalled carbon nanotubes (MWCNTs)/Au electrode was prepared for fabricating an amperometric biosensor and a biofuel cell (BFC) of uric acid (UA). Briefly, Pd was electroplated on a MWCNTs-modified Au electrode, the UOx–PFBA–PdNPs bionanocomposite was prepared on the Pd_{plate}/MWCNTs/Au electrode through chemical oxidation of a UOx-furan-3-boronic acid adduct by Na_2PdCl_4, and then chitosan (CS) was cast-coated on the electrode. In the first-generation biosensing mode (anodic detection of enzymatically generated H_2O_2), the prepared CS/UOx–PFBA–PdNPs/Pd_{plate}/MWCNTs/Au electrode showed a linear amperometric response to UA concentration from 1.0 μM to 2.5 mM with a sensitivity of 490 μA mM^{-1} cm^{-2} and a limit of detection ($S/N = 3$) of 0.1 μM, excellent operation/storage stability, and good results of UA assay in real urine samples. The enzyme electrode also worked well in the second-generation biosensing mode involving a *p*-benzoquinone or ferrocene monocarboxylic acid mediator. Furthermore, a monopolar UA BFC was fabricated by using the enzyme electrode as the bioanode and a Pt/MWCNTs/Au electrode as the cathode, which gave an open-circuit cell voltage of 0.394 V, a short-circuit current density of 857 μA cm^{-2} and a maximum power density of 70 μW cm^{-2} at 0.22 V under simulated physiological conditions.

1. Introduction

Uric acid (UA, 2,4,6-trihydroxypurine) is an end product from purine derivatives in human metabolism. The normal level of UA is between 240 and 520 μM in serum and between 1.4 and 4.4 mM in urinary excretion [1]. An abnormal UA level in body fluids may result from several diseases, such as gout, obesity, hyperuricemia and Lesch–Nyhan syndrome [2]. As a clinically valuable diagnostic indicator, quantitative analysis of UA in body fluids has attracted wide concerns from academic and industrial communities, involving methodology researches on photometric analysis [3], high-performance liquid chromatography [4], and electroanalysis [5–7]. In general, the electroanalysis of UA can be classified into nonenzymatic and enzymatic methods. The nonenzymatic amperometric detection of UA relies on the high electroactivity of UA but it is usually susceptible to other coexisting electroactive substances such ascorbic acid (AA) and dopamine [8,9]. A more selective approach is the use of uricase (urate oxidase, UOx), which catalyzes the oxidation of UA to allantoin, H_2O_2 and CO_2. The enzymatic method generally provides the satisfactory specificity and sensitivity, quick response time, and facile operation [10]. In addition, the enzyme-based biofuel cells (BFCs) have received much attention due to their academic significance and their application potential in developing implantable devices and self-powered biosensors [11–13].

Enzyme immobilization is one of the key factors in constructing high-performance enzyme-based biosensors. Boronic acid groups can chemically react with 1,2- or 1,3-diols to generate five- or six-membered cyclic complexes at certain pH [14]. The complexation of boronic acid compounds with saccharides is attractive for biosensor development [15,16], since most proteins are glycoproteins, and the covalent modification of glycoproteins at their

* Corresponding authors. Tel./fax: +86 731 88865515.
E-mail addresses: xieqj@hunnu.edu.cn (Q. Xie), mengwh2004@163.com (W. Meng).

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glycosyl groups may intrinsically less degrade their bioactivity than that directly at their peptide chains. In addition, various conducting and nonconducting polymers have been widely used for enzyme immobilization [17,18], but the use of boronic acid group-bearing polymers to immobilize enzymes has been less studied to date [19]. We believe that the exploitation of new boronic acid group-bearing polymers and their bionanocomposites for high-performance biosensing remains an intriguing and important research theme.

It is well known that carbon nanomaterials, such as carbon nanotubes and graphene, have become star nanomaterials in so many research fields including electroanalytical chemistry, being mainly due to their excellent electron conductance, large surface area, high chemical stability, high mechanical strength, and acceptable biocompatibility [20–22]. The nanocomposites of carbon nanomaterials and noble metal nanoparticles (NPs), such as Pd [23], Au [15], Pt [24,25], and bimetallic NPs (e.g. AuPd [26], PtAu [27], PdPt [28]), have also been used as chemical and electrochemical catalysts for synthesis and analysis applications. Of particular interest, the Pd-based materials are proven to possess high catalytic activity toward many chemical reactions, at least 50-fold higher abundance than Pt on the earth [29], and more efficient oxidative activity of common Pd salts in comparison with their Pt counterparts. Hence, Pd has been widely studied for catalysis and catalysis-based analytical applications [30].

Herein, a new amperometric enzyme electrode and its biofuel cell (BFC) were fabricated based on a uricase (UOx)–poly(furan-3-boronic acid) (PFBA)–Pd nanoparticles (PdNPs) bionanocomposite. The bionanocomposite was synthesized on a Pd-electroplated and multiwalled carbon nanotubes (MWCNTs)-modified Au electrode, and then an outer-layer chitosan (CS) film was coated on the electrode. The thus-prepared CS/UOx–PFBA–PdNPs/Pd_{plate}/MWCNTs/Au electrode exhibited excellent UA-analysis performance both in the first- and second-generation biosensing modes. A high-performance UA/O_2 BFC was also constructed using this enzyme electrode as the bioanode and a Pt/MWCNTs/Au electrode as the cathode.

2. Experimental

2.1. Apparatus and chemicals

All electrochemical experiments were conducted on a CHI660C electrochemical workstation (CH Instrument Co.) and a conventional three-electrode electrolytic cell was used. An Au disk electrode with 3.0 mm diameter (area = 0.071 cm^2) served as the working electrode, a KCl-saturated calomel electrode (SCE) as the reference electrode, and a carbon rod as the counter electrode. All potentials reported here are cited versus SCE. UV–vis absorption spectra were collected on a UV2450 UV–vis spectrophotometer (Shimadzu Co., Japan). Scanning electron microscopy (SEM) studies were performed on a Hitachi S4800 scanning electron microscope with a field emission electron gun. Transmission electron microscopy (TEM) images were collected on a JEM-2100F transmission electron microscope (Japan). The BFC experiments were conducted on an Autolab PGSTA 30 electrochemical workstation (Eco Chemie BV, The Netherlands) with the GPES 4.9 software.

UOx (EC 1.7.3.3; from *Candida* sp.; activity $\approx$ 4.7 U mg^{-1}), and furan-3-boronic acid (FBA) were purchased from Sigma. $PdCl_2$ was purchased from Tianjin Chemical Reagents Station (Tianjin, China). MWCNTs material was purchased from Chengdu Organic Chemicals Co., Ltd. (Chengdu, China), and purified in concentrated nitric acid before use [31]. CS, glucose, AA, UA and urea were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *p*-Benzoquinone (BQ) and ferrocene monocarboxylic acid (FcMA) were purchased from Suzhou Time-Chem Technologies Co., Ltd. (Suzhou, China). All other chemicals were of analytical grade or better quality and used as received. Phosphate buffer solution (PBS) consisting of 0.10 M KH_2PO_4–K_2HPO_4 + 0.1 M K_2SO_4 (pH 7.0) served as the supporting electrolyte. 0.30 wt% CS solution was prepared in 0.10 M acetate buffer solution (pH 5.4). The standard urate solution was prepared daily by dissolving UA in 0.1 M NaOH, neutralizing to pH 8.0 by HCl and then diluting it with the pH 7.0 PBS. The palladium precursor solution (Na_2PdCl_4) was prepared by adding 14.2 mg of $PdCl_2$ and 9.4 mg of NaCl and diluting it to 4 mL with H_2O [32]. Milli-Q ultrapure water (Millipore, $\geqslant$18 MΩ cm) was used throughout. All experiments were performed at room temperature around 25 °C.

2.2. Procedures

The Au electrodes were pretreated thoroughly according to a reported procedure with minor modifications [33]. Briefly, the bare Au electrode was carefully polished in 0.5 and 0.05 μm alumina suspensions in sequence. After being thoroughly rinsed with water, the polished electrode was ultrasonically treated sequentially in water, ethanol, and water, each for 5 min, to remove residual alumina powder. Then, the Au electrode was treated with Piranha solution (H_2SO_4:H_2O_2, 3:1 in *v*/*v*) for 15 s. Afterward, the Au electrode was subjected to electrochemical rinsing in 0.50 M aqueous H_2SO_4, until the cyclic voltammogram became reproducible. The Au electrode was then characterized by cyclic voltammetry (CV) in 2 mM $K_4Fe(CN)_6$ + 0.1 M K_2SO_4, and the peak-to-peak separation was observed to be within 75 mV, confirming that the Au electrode was well cleaned for subsequent experiments.

The biosensors were prepared as illustrated in Scheme 1 and explained in detail as follows. Firstly, 1 mg MWCNTs were dispersed in 1 mL dimethyl formamide (DMF) and sonicated for 15 min, then 5.5 μL of the MWCNTs dispersion was cast on a bare Au electrode (MWCNTs/Au) and air-dried. A Pd_{plate}/MWCNTs/Au electrode was fabricated by electroplating Pd onto the MWCNTs/Au electrode at −0.2 V versus SCE for 500 s in 5.0 mM $PdCl_2$ + 0.1 M H_2SO_4 aqueous solution. Secondly, a UOx–FBA adduct was prepared by dissolving 4.0 mg UOx and 4.0 mg FBA in 1.00 mL PBS and shaking the solution for 30 min to achieve reaction equilibrium. 2.5 μL UOx–FBA mixture and 2.0 μL of 20 mM Na_2PdCl_4 were then cast on the Pd_{plate}/MWCNTs/Au electrode to allow reaction at room temperature for 30 min. Finally, 1.5 μL of 0.30 wt% CS solution was cast-dried to prevent enzyme leaching and strengthen the stability of the enzyme film (CS/UOx–PFBA–PdNPs/Pd_{plate}/MWCNTs/Au). Before use, the enzyme electrodes were treated by CV from −0.5 V to 0.8 V in pH 7.0 PBS to ensure reduction of possibly residual Na_2PdCl_4 and electropolymerization of possibly residual FBA monomer. Similarly, a CS/PFBA–PdNPs/Pd_{plate}/MWCNTs/Au electrode was prepared in the absence of UOx, and a CS/UOx–PFBA–PdNPs/Au electrode was prepared in the absence of Pd_{plate}/MWCNTs. When not in use, the prepared enzyme electrodes were stored in PBS at 4 °C (refrigerator).

In the first-generation biosensing mode, the enzyme electrodes were tested by potentiostating them at 0.55 V under solution-stirred conditions to detect oxidation of enzymatically generated H_2O_2. The response current was marked with a change in the value between the steady state current after substrate addition and the initial background current without substrate. Urine samples used in this study were obtained from healthy people. Prior to analysis, we diluted an urine sample 10 times using the supporting electrolyte (pH 7.0 PBS) without further pretreatments [34]. In the second-generation biosensing mode, CV in a quiescent solution was used for the mediator FcMA, and chronoamperometry at 0.35 V in a stirred solution was used for the mediator BQ.