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Electrochemical detection of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone using a cytochrome P450 2E1 decorated biosensor

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ABSTRACT

In this work, a novel electrochemical biosensor for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) was developed based on cytochrome P450 2E1 (CYP2E1). The biosensor was fabricated by immobilization of CYP2E1 with poly (diallyldimethylammonium chloride) (PDDA) - poly (sodium-*p*-styrenesulfonate) (PSS) electrolyte film on a screen-printed carbon electrode (SPCE) electrodeposited with gold nanoparticles (AuNPs). The procedures of electrode modification were characterized by using scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS). With the good conductivity of AuNPs as well as the immobilized CYP2E1, the electrochemical performances of the proposed biosensor for the detection of NNK showed a wide linear range of 0–386 μM with a sensitivity of 79 $\mu A\,cm^{-2}\,mM^{-1}$ and a low detection limit of 7.71 μM. Additionally, excellent selectivity and reproducibility were also obtained. The fabricated NNK electrochemical biosensor was successfully employed in urine sample determination, exhibiting great potential in the real sample analysis.

1. Introduction

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), as one kind of tobacco-specific nitrosamines, is derived from nitrosation reaction of nicotine and was classified by the International Agency for Research on Cancer (IARC) as Group I carcinogen [1,2]. NNK in cigarette smoke is a major tobacco carcinogen and is considered to be involved in the human tobacco-induced cancers, especially lung cancer [3,4]. Due to the significant role of NNK in the evaluation of potential health risks caused by tobacco [5], it is greatly desired to have a rapid and effective analytical method for the determination of NNK in vitro and in vivo.

In recent years, several methods have been extensively utilized to analyze NNK in cigarette tobacco, including gas chromatography-thermal energy analyzer (GC-TEA) [6,7], gas chromatography–mass spectrometry (GC–MS) [8,9], gas chromatography-tandem mass spectrometry (GC–MS/MS) [10,11], and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [12,13]. However, most of these methods require complicated sample pretreatment, expensive instruments and special operators. Electrochemical method is an alternative analytical technique for NNK detection with the features of simple operation, rapid analysis, high sensitivity, and low cost. Recently, the strategies for the electrochemical detection of NNK have been developed by using hemin-functionalized redox electrodes for the electrocatalytic reduction of NNK [14], and cytochrome P450s coupled with cytochrome P450 reductase (CPR) microsomes-modified electrodes catalytic NNK oxidation [15], which pave the way for the NNK electrochemical sensing. However, the performances of the electrochemical biosensors in the quantitative determination of NNK have been no systematically evaluated. And the application of electrochemical NNK biosensors in real sample analysis need to be further investigated.

There has been much attention paid on the research of electrochemistry of P450 family ever since the pioneering works by Scheller's and Archakov's groups [16–20]. Cytochrome P450 2E1 (CYP2E1) is one of the superfamily of heme-thiolate monooxygenases and expressed in the human liver [21]. CYP2E1 is the major hemeprotein involved in the oxidation of various N-nitrosamines and one of the prominent contributors to the metabolism of NNK [22,23]. On the basis of the found direct electron transfer between the enzymatic heme iron of CYP2E1 and the electrode [24,25], it is promising to immobilize CYP2E1 on the electrodes to construct an electrochemical biosensor for NNK determination. In order to obtain the direct electrochemistry of CYP2E1 and the high catalytic activity toward NNK, a further immobilization strategy for enzyme is also needed.

In this paper, we proposed a high performance NNK electrochemical biosensor based on CYP2E1 immobilized on the gold nanoparticles

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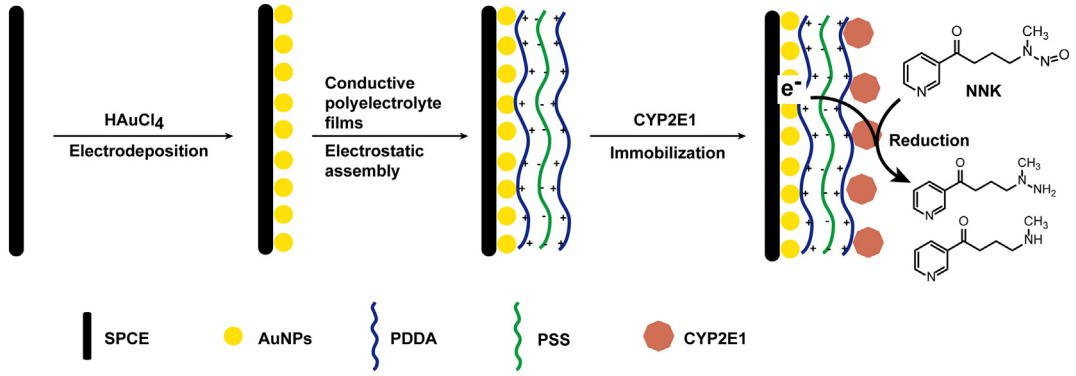

Scheme 1. Illustration for the stepwise fabrication of the NNK electrochemical biosensor.

(AuNPs) electrodeposited screen-printed carbon electrodes (SPCE). Since AuNPs as a kind of promising nanomaterials to enhance the electron transfer capability of the modified electrodes for their excellent electronic and electrochemical properties [26,27], as well as the large effective surface area, high chemical stability, and good biocompatibility [28,29], the substrate electrodes were prepared by electrodeposition of AuNPs on the home-made SPCE (AuNPs-SPCE). Then, CYP2E1 was immobilized onto AuNPs-SPCE with a polyelectrolyte film composed of poly (diallyldimethylammonium chloride) (PDDA) and poly(sodium-*p*-styrenesulfonate) (PSS) via electrostatic assembly [30,31]. The analytical performances of the fabricated electrode were discussed in terms of sensitivity, stability, selectivity and reproducibility. In addition, the biosensor was demonstrated its capability toward NNK detecting in urine samples.

2. Experimental section

2.1. Materials and reagents

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*-nitrosonornicotine (NNN) were purchased from Toronto Research Chemicals Inc. Due to its low solubility in water, a 1 mg mL^{-1} standard solution of NNK was prepared by dissolving 10 mg NNK in methanol. Recombinant human CYP2E1 derived from *Escherichia coli* was purchased from Shanghai QF Biosciences Co., Ltd. Gold (III) chloride trihydrate ($HAuCl_4{\cdot}4H_2O$) and poly (diallyldimethylammonium chloride) (PDDA) were obtained from Aladdin Chemistry Co., Ltd. Poly (sodium-*p*-styrenesulfonate) (PSS) was purchased from Energy Chemical. PDDA and PSS solutions were prepared with ultrapure water, at concentrations of 1 mg mL^{-1} and 3 mg mL^{-1}, respectively. Nicotine was from Shanghai Jingke Chemical Technology Co., Ltd. All other chemical reagents were in analytical grade and used without further purification. All aqueous solutions were prepared with ultrapure water (18.2 MΩ cm) obtained from Laboratory Water Purification System.

2.2. Fabrication of the modified electrodes

SPCEs were home-produced according to previous described procedures [32,33]. Before use, SPCEs were activated in 0.5 M H_2SO_4 solution by cyclic voltammetry from 1.5 V to 2.0 V for 20 cycles. After that, AuNPs were electrochemical deposited on the electrode surface by immersing the pretreated SPCEs in 1% $HAuCl_4$ solution under an applied potential of −0.2 V for 30 s. The obtained electrode was washed with ultrapure water and labeled as AuNPs-SPCE. Then 6 μL PDDA solution and 6 μL PSS solution were assembled on AuNPs-SPCE by alternating drop-casting, to construct a polyelectrolyte film modified electrode by combining PDDA with PSS on electrode, named as PDDA/PSS/PDDA/AuNPs-SPCE. Finally, 3 μL CYP2E1 solution (2 mg mL^{-1}) was immobilized onto the electrode surface and incubated overnight at 4 °C, the resulting electrode, labeled as CYP2E1/PDDA/PSS/PDDA/AuNPs-SPCE, was washed carefully with ultrapure water. All the proposed electrodes were stored at 4 °C before use.

2.3. Electrochemical measurements

All electrochemical measurements were performed on a CHI1040B electrochemical workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode system was applied with a prepared modified electrode as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl (3 M KCl) electrode as the reference electrode. The electrochemical behavior of the fabricated electrodes was investigated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and chronoamperometry (I-t). All electrochemical experiments were performed at room temperature.

3. Results and discussion

3.1. Characterization of the modified electrodes

To construct the NNK electrochemical biosensor, AuNPs were firstly electrochemical deposited on the home-made SPCEs as the substrate electrodes, leading to a significantly increase in the surface area for the polyelectrolyte films adsorption. Then, a polyelectrolyte film composed of PDDA and PSS was adsorbed on AuNPs-SPCE via electrostatic assembly in order to obtain a conductive and biocompatible film for the following CYP2E1 immobilization. The stepwise fabrication of the proposed CYP2E1/PDDA/PSS/PDDA/AuNPs-SPCE for NNK electrochemical sensing is illustrated in Scheme 1.

The surface morphologies of the stepwise fabrication of modified electrodes were characterized by scanning electron microscopy (SEM, JSM-6360LV, JEOL). In Fig. 1, SEM images of bare SPCE (A), AuNPs-SPCE (B), PDDA/PSS/PDDA/AuNPs-SPCE (C) and CYP2E1/PDDA/PSS/PDDA/AuNPs-SPCE (D) are respectively presented. First, Fig. 1A depicts the surface of bare SPCE, which is composed of a great deal of irregular graphite-based particles. While Fig. 1B shows uniformly distributed AuNPs by electrochemical deposition on the SPCE surface. The particle size of AuNPs was about 100 nm. As shown in Fig. 1C, the electrode surface was covered by smooth films after being decorated with PDDA/PSS/PDDA polymer film. Furthermore, the SEM image in Fig. 1D presents some laminar structures randomly distributed on the electrode, indicating that the CYP2E1 was attached onto the surface of PDDA/PSS/PDDA/AuNPs-SPCE. These modification procedures were also confirmed by Energy Dispersive Spectrometer (EDS) as shown in Fig. S1. The change in the elemental composition of the electrode surface conformed well with the modified AuNPs, PDDA/PSS, and CYP2E1.

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) of the fabricated electrodes were performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl to observe the electron transfer efficiency of the as-prepared electrodes and the results are shown in