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Gold nanoparticles-enhanced bisphenol A electrochemical biosensor based on tyrosinase immobilized onto self-assembled monolayers-modified gold electrode

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ABSTRACT

A novel electrochemical sensor based on the immobilization of tyrosinase (tyr) onto gold nanoparticles (nano-Au) and thioctic acid amide (T-NH_2) self-assembled monolayers (SAMs)-modified gold electrode has been developed for the determination of bisphenol A (BPA). It was found that the nano-Au could significantly enhance the electrochemical response of tyr/nano-Au/T-NH_2/Au electrode to BPA, and the enhancement effect of nano-Au on the current response was also related to the enzyme. The results indicated that the biosensor could be used as a detector for BPA determination with a linear range from 3.99×10^{-7} mol/L to 2.34×10^{-4} mol/L and a detection limit of 1.33×10^{-7} mol/L. In addition, this biosensor showed good reproducibility.

1. Introduction

Bisphenol A (BPA), a type of estrogen present in the environment, can produce harmful effects on the endocrine systems of humans and wild animals [1,2]. It is the most widely used industrial compound, and also extensively used in the plastic industry, dental fillings, and the lining of food cans [3].

To prevent the noxious effects of BPA, it is very important to establish a sensitive and simple method for the determination of BPA. Various analytical methods have been reported for the determination of BPA [4,5]. By comparison, electrochemical sensors exhibit rapid response and low cost. Furthermore, they have extra advantages of high sensitivity, simple operation, the potential for miniaturization, and the possibility of *in situ* analysis [6].

In order to enhance the sensitivity of the sensor, gold nanoparticles (nano-Au) were introduced during the modification of electrochemical sensor. Nano-Au can play an important role in the adsorption of enzyme due to the large specific surface area, desirable biocompatibility and high surface free energy of nano-sized particles [7].

In the present work, we fabricated a BPA biosensor based on the immobilization of tyrosinase (tyr) on thioctic acid amide (T-NH_2) and nano-Au-modified Au electrode (tyr/nano-Au/T-NH_2/Au). Initially, the T-NH_2 was self-assembled on the Au electrode surface to form self-assembled monolayers (SAMs), and then the nano-Au was chemisorbed onto the amino groups of the SAMs. Finally, the enzyme was immobilized on the nano-Au surface of the electrode. The developed biosensor showed an enhanced ability for the highly sensitive detection of BPA.

2. Experimental

Tyrosinase (tyr) was obtained from Sigma and used without further purification. Chloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Sigma. Thioctic acid amide (T-NH_2) was obtained from Aldrich Chemical Co., Inc. Other chemicals were of analytical grade.

Electrochemical experiments were carried out with a CHI 750C electrochemical workstation (Shanghai CH Instruments, China) at room temperature. A three-compartment electrochemical cell contained a platinum wire auxiliary electrode, a Ag/AgCl (3 mol/L KCl) reference electrode and tyr/nano-Au/T-NH_2 modified gold electrode as working electrode.

The cleaned electrode was immersed in a 10 mmol/L anhydrous ethanol solution of T-NH_2 at 4 °C for *ca.* 24 h. The modified electrode was rinsed with ethanol and ultra pure water to remove physically adsorbed T-NH_2. Then the electrode was immersed into a fresh 100 mg/L $HAuCl_4$ solution, and the electrochemical deposition of nano-Au was conducted at −200 mV for 1 min [8].

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After that, 5 μL of 2.6 mg/mL tyrosinase solution was dropped onto the surface of the nano-Au/T-NH_2/Au electrode, and refrigerated at 4 °C overnight. Finally, the enzyme electrode was immersed in 0.1 mol/L PBS to wash out non-immobilized enzyme, and then stored in PBS (pH 7.0) at 4 °C for future use.

3. Results and discussion

Fig. 1 shows the cyclic voltammograms of different modified electrodes in 5 mmol/L $[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$. A pair of well-defined redox peaks, characteristic of a diffusion-limited redox process, was observed at the bare gold electrode (Fig. 1, curve a). After dipping the electrode in the T-NH_2 solution, the anodic and cathodic currents decreased (Fig. 1, curve b), as the T-NH_2 can hinder the transmission of electrons toward the electrode surface. When a nano-Au monolayer incorporated onto the modified electrode surface, the anodic and cathodic currents increased (Fig. 1, curve c). The reason may be that nanometer-sized gold colloids played an important role similar to a conducting wire or electron-conducting tunnel, which made it easier for the transfer of electrons to take place [9,10]. After tyrosinase was adsorbed into nano-Au monolayer membrane, a further decrease in both the anodic peak and cathodic peak was observed (Fig. 1, curve d). This may be contributed to the non-conductivity of enzyme film, which hinders the access of the electrons to the electrode.

The oxidation of BPA was a pH-dependent reaction. The effect of solution pH on the current response of the biosensor was studied by cyclic voltametry in 0.1 mol/L PBS in pH values range of 6.0–9.0. Fig. 2 shows the dependence of the anodic current on the solution pH. It can be seen that the largest anodic current is obtained at a pH 7.0. The anodic current increased with the increasing of the solution pH when the solution pH was lower than 7.0. However, the anodic current decreased when the solution pH was higher than 7.0. Hence, the solution pH 7.0 was used in the following experiments, which was in accordance with the optimum pH range of 5–8 reported for free tyrosinase [11].

The relationship between the oxidation peak potential (E_{pa}) and pH is shown in Fig. 2 (inset). The linear shift of E_{pa} toward the negative potential with an increasing pH indicated that protons were directly involved in the oxidation of BPA. It obeyed the following equation: E_{pa} (V) = −0.0536 pH + 1.169 (R^2 = 0.9973). The slope of the plot of E_{pa} *vs.* pH is −53.6, which is approximately close to the theoretical value of −57.6 at 291.15 K, indicating that

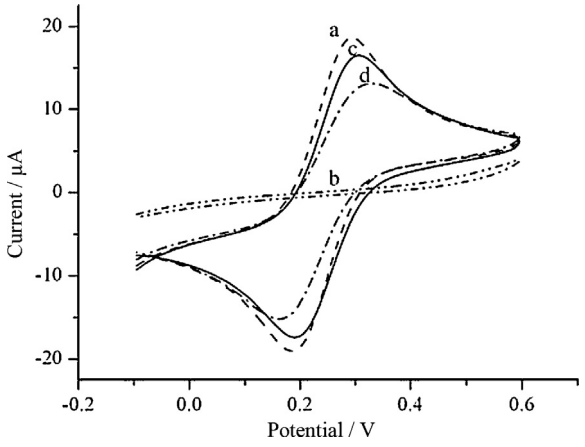

Fig. 1. Cyclic voltammograms obtained at bare Au electrode (a), T-NH_2/Au (b), nano-Au/T-NH_2/Au (c), and tyr/nano-Au/T-NH_2/Au (d) in 5 mmol/L $[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$ (pH 7.0) with a scan rate of 50 mV/s.

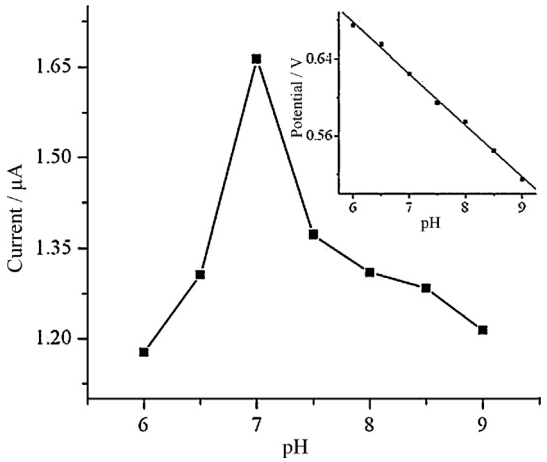

Fig. 2. Effects of pH value on current and potential (inset) response to 10^{-5} mol/L BPA. Scan rate, 50 mV/s.

the electron transfer was accompanied by an equal number of proton in electrode reaction [12].

It is well known that tyrosinase is a copper protein, which catalyzes the oxidation of BPA to *o*-quinones. Fig. 3 shows cyclic voltammograms in 1.0×10^{-5} mol/L BPA at different modified electrodes. At the bare Au electrode (Fig. 3, curve a), the oxidation peak is observed with a low peak current, suggesting that the response of BPA at Au electrode is poor. No redox peak was observed at T-NH_2/Au electrode (Fig. 3A, curve b), indicating that the direct oxidation of BPA at the T-NH_2/Au electrode is very difficult. Compared with the T-NH_2/Au electrode, a small oxidation peak occurred at the tyr/T-NH_2/Au (Fig. 3A, curve c) electrode. This is attributed to a very weak oxidation behavior of *o*-quinine liberated from the biocatalytic reaction, which indicated that the T-NH_2 film could not effectively form an electron-transfer channel, and obstructed the electron-transfer of the enzymatic reaction. When nano-Au was immobilized on the T-NH_2/Au surface, the oxidation peak current (Fig. 3A, curve d) of BPA was higher than that at T-NH_2/Au. Nano-Au has a small dimensional effect, the quantum size effect as well as the large surface area, which contributed to the significant improvement of the electrocatalytic activity of the modified electrode. However, an obvious oxidation peak was observed at a tyr/nano-Au/T-NH_2/Au electrode (Fig. 3A, curve e), indicating that tyr/nano-Au/T-NH_2/Au had obvious catalytic activity toward the BPA oxidation compared with the nano-Au/T-NH_2/Au and tyr/T-NH_2/Au electrode. The reasons can be attributed to the synergetic activity of tyrosinase and nano-Au, which lead to the larger electroactive surface of the modified electrode for the determination of BPA. The increase only in oxidation current revealed that the oxidation reaction of BPA is totally irreversible, which is in agreement with previous reports [12,13]. The anodic peak currents increase linearly with BPA concentration from 3.99×10^{-7} mol/L to 2.34×10^{-4} mol/L with a detection limit of 1.33×10^{-7} mol/L, with a correlation coefficient of 0.998. The detection limit was found to be 1.33×10^{-7} mol/L at S/N of 3.

The reproducibility of the current response of the enzyme electrode was investigated in 1.5 μmol/L BPA using six different electrodes prepared independently. The relative standard deviation (RSD) was 4.6%, revealing that this method had good reproducibility. The analytical performance of the resulting biosensor was compared with those reported for other recently modified biosensor [13–16] found in Table 1. It can be deduced that the developed tyr/nano-Au/T-NH_2/Au biosensor allows a wide range of linearity, and good reproducibility.

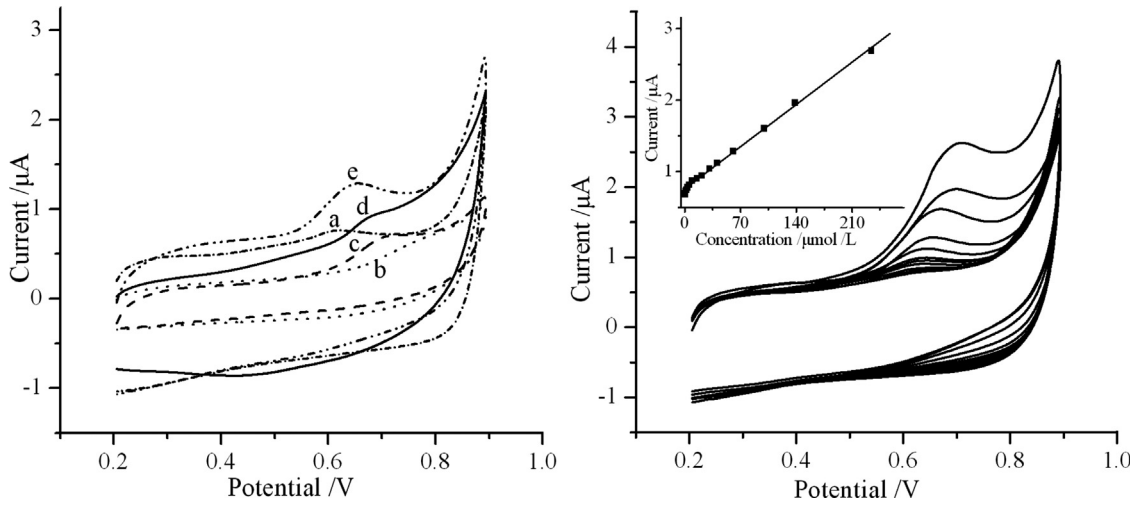

Fig. 3. (A) Cyclic voltammograms at bare Au electrode (a), T-NH_2/Au electrode (b), tyr/T-NH_2/Au electrode (c), nano-Au/T-NH_2/Au electrode (d), and tyr/nano-Au/T-NH_2/Au electrode (e) in 0.1 mol/L PBS (pH 7.0) containing 1.0×10^{-5} mol/L BPA. (B) Cyclic voltammograms obtained at tyr/nano-Au/T–NH_2/Au electrode upon successive addition of BPA into 0.1 mol/L PBS (pH 7.0). Inset: calibration curve of the biosensor.

Table 1
Analytical characteristics of different modified biosensors for BPA.

Electrode	Linear range (μmol/L)	*r*	LOD (μmol/L)	RSD (%)	Ref.
Tyr/thionine/CP	0.15–45	–	0.15	7	[13]
Tyr/MWNTs-CoPc-SF/GCE	0.05–3.0	0.9979	0.03	4.8	[14]
MCM-41/CP	0.22–8.8	0.997	0.038	6.4	[15]
SWCNT/ssDNA/Au	0.5–3.8	0.998	0.011	–	[16]
Tyr/nano-Au/T-NH_2/Au	0.399–234	0.998	0.133	4.6	This work

CP, carbon paste; MWNTs, multiwalled carbon nanotubes; CoPc, cobalt phthalocyanine; SF, silk fibroin; MCM-41, kind of mesoporous silica molecular sieves; ssDNA, single-stranded.

4. Conclusions

In this study, we developed a novel biosensor for the determination of BPA based on tyrosinase immobilized onto nano-Au/T-NH_2/Au modified electrode. The introduction of nano-Au obviously enhanced the electrochemical response of BPA at the tyr/nano-Au/T-NH_2/Au electrode. The anodic peak current increased linearly with the concentration of BPA. This methodology has several attractive advantages, such as the lack of a need for a redox agent in the working cell, simple preparation process, the wider linear range, and good reproducibility.

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