Korean J. Chem. Eng., **29**(12), 1666-1669 (2012)
DOI: 10.1007/s11814-012-0140-y

RAPID COMMUNICATION

Use of biologically designed gold nanowire for biosensor application

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(*Received 27 April 2012 • accepted 22 August 2012*)

Abstract−A highly sensitive tyrosinase (TYR)-based amperometric biosensor is prepared using biologically designed gold nanowires (AuNWs) for pesticide detection. The AuNWs were synthesized by dodecapeptide Midas-11 and were modified with the formation of self-assembled monolayer (SAM), followed by covalent binding with TYR. The prepared TYR-AuNWs-SPCE (screen printed carbon electrode) was compared with bare, AuNWs-, modified-AuNWs-SPCE by the measurement of cyclic voltammetry. The quantitative relationship between the inhibition percentage and the pesticide concentration at the TYR-AuNWs-SPCE was obtained by measuring the current response in various concentrations of pesticides. The reasonable detection range of parathion was determined to be 0.1 ppt through 10 ppb (R^2=0.990) with 0.087 ppt of detection limits. The higher sensitivity and wider detection range of the TYR-based biosensor was achieved by the use of biologically synthesized AuNWs.

Key words: Tyrosinase, Gold Nanowire, Peptide, Amperometric Biosensor, Pesticide

INTRODUCTION

With the growing concerns on pesticides, researchers have developed pesticide detection sensors based on electrochemical fundamentals [1-3]. Among the several electrochemical methods, the enzyme-immobilized amperometric biosensor has been considered to be the most suitable for determination of pesticides [4]. Most enzyme-immobilized amperometric biosensors are systemically simple and attractive in terms of effectiveness and selectivity. Despite those features, it has a critical limitation as a pesticide biosensor, i.e., is the sensitivity high enough to detect low-concentration pesticides [5-7]. To improve sensitivity of the enzyme-based amperometric sensor, electrode modification has been investigated by using several conductive materials such as carbon nanotube (CNT) [8,9], gold nanoparticles [10,11], and zirconia nanoparticles [12]. Particularly, gold nanocomposites have been known as a promising novel conducting material for enzyme electrode due to low electrical resistance, high chemical stability, large surface to volume ratio, and good biocompatibility [13]. Among gold nanocomposites, the advantages of nanowire structure, such as size scale, aspect ratio and better functionalization ability, lead to improved sensitivity on enzyme immobilized sensors [14,15]. Kim et al. reported that gold nanowires (AuNWs) were synthesized by peptide [16]. It is a simple method that allows one to obtain high purity AuNWs in contrast with other chemical methods which usually use toxic chemicals under harsh synthesis conditions. Also, the biologically synthesized AuNWs have micron-sized dimension and well defined morphology. In addition, the AuNWs can act as electrical connector between electrode and enzyme. For these reasons, it is expected that the use of AuNWs enhances the performance of enzyme electrode for pesticide detection at a low concentration.

The objective of this study is to investigate the use of biologically designed AuNWs for tyrosinase (TYR)-based pesticide biosensor. The AuNWs were synthesized by a dodecapeptide Midas-11 at a controlled pH of solution with an optimized concentration of gold ion. The synthesized AuNWs were attached on the screen printed carbon electrode (SPCE) surface by cross-linking, and the AuNWs attached electrode (AuNW-SPCE) was treated by 3-merchptopropionic acid (MPA) to form the self-assembled monolayer (SAM). Then, tyrosinase (TYR) was immobilized on the MPA-AuNWs-SPCE by covalent bonding. The TYR-AuNWs-SPCE was tested for the detection of parathion by amperometric measurement.

MATERIALS AND METHODS

1. Chemicals

Hydrogen tetrachloroaurate (III) ($HAuCl_4$), metallic gold powder, sodium phosphate monobasic, sodium phosphate dibasic, tyrosinase (from mushrooms, EC 1.14.18.1, 50,000 units/mg), 3-mercaptopropionic acid (MPA), ethanol, *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), acetic acid (0.01 M, pH 4.5), catechol, Nafion® perfluorinated ion exchange resin (5 wt% in lower aliphatic alcohols/H_2O

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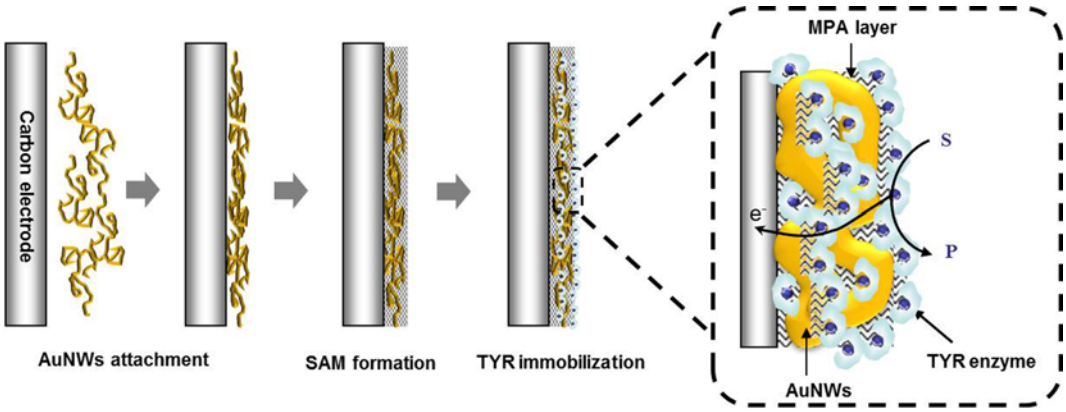

Fig. 1. Schematic diagram of the preparation of TYR-AuNWs-SPCE.

mix contains 15-20% water), and parathion (parathion-ethyl) were supplied by Sigma-Aldrich (St. Louis, MO, USA). All the chemicals used in this study were analytical reagent grade. Aqueous solutions were prepared with Milli-Q water (18 MΩ cm^{-1}) as needed.

2. Synthesis of Gold Nanowires (AuNWs) by Midas Peptides

Kim et al. described the details of the synthesis of gold nanowires by peptide and materials relevant to the synthesis [16]. The preparation procedure of gold nanowires by peptide for the current study is summarized below. Gold binding and synthesizing peptide (i.e., Midas-2) comprising 12 amino acids (TGTSVLIATPYV) was obtained by combinatorial peptide phage display techniques. Midas-11 peptide (TGTSVLIATPGV) was derived by substituting 11th position of amino acid of Midas-2 with glycine. After adjusting pH to 5.7, the Midas-11 peptide solution was added to 0.5 mM $HAuCl_4$ and incubated for three days at 37 °C. The synthesized AuNWs were collected by centrifugation (5415D Centrifuge, Fisher Scientific, Pittsburgh, PA) at 9,300 ×g for 10 min and then washed twice with autoclaved deionized water. Finally, the diameter and morphology of synthesized AuNWs were analyzed by transmission electron microscopy (HR-TEM, JEOL JEM 2100, Japan).

3. Preparation and Characterization of TYR-AuNWs-SPCE

A tyrosinase-based gold nanowire (AuNWs) electrode was prepared with a screen printed carbon electrode (SPCE) (AC1.W4.R1, WE: Carbon (7105), RE: Ag/AgCl (60/40%), CE: PtAu (15/85%), BAS, West Lafayette, IN, USA). The carbon working electrode (diameter=1 mm) was modified with the synthesized AuNWs by a drop casting method. First, 0.5 ml of the synthesized AuNWs was dried at 40 °C and then re-dispersed in 10 μL of Nafion by ultrasonication for 5 min. One microliter of the mixture of AuNWs and Nafion solution was casted on the surface of a carbon working electrode by pipetting, and then dried under air for 10 min at room temperature. The prepared AuNWs-SPCE was washed three times with distilled water and dried under N_2 (g). Then, 50 μL of 20 mM of MPA solution (in ethanol/distilled water, 75 : 25, v/v) was dropped on the surface of AuNWs-SPCE and incubated for 2 h at room temperature under darkness. The MPA treated AuNWs-SPCE was washed several times with ethanol and distilled water, and dried under N_2 (g). Next, the AuNWs-SPCE was treated with 50 μL of a fresh mixture of NHS (0.05 M) and EDC (0.2 M) for 10 min. Subsequently, 20 μL of TYR solution (3,900 U mL^{-1}) was added and kept for 12 h at 4 °C. The prepared TYR-AuNWs-SPCE was thoroughly washed with 0.1 M PBS (pH 7.0) before use. A schematic diagram of the preparation procedure of the TYR-AuNWs-SPCE is shown in Fig. 1. To characterize the prepared TYR-AuNWs-SPCE, cyclic voltammetric measurements were performed with an AUTOLAB PGSTAT 30 electrochemical system with GPES software (Eco Chemie, Utrecht, The Netherlands) in a microcon cell (volume= 1.5 mL).

4. Amperometric Detection of Pesticide

Detection of a pesticide determination was carried out by comparing the current response of the TYR-AuNWs-SPCE to a substrate (i.e., catechol) in the absence and presence of pesticide with a picoammeter (model 6487, KIETHLEY, USA). The current responses were measured at a constant potential (−0.1 V vs. NHE) using 1 mM catechol in PBS (0.1 M, pH 7) solution. Then, various amounts of pesticides were injected to the electrolyte, and the electrode was incubated for 30 s for enzyme inhibition by pesticide. Subsequently, the current response was recorded under the same condition. This procedure was reported for the measurement of activity change of TYR before and after the inhibition by pesticide. The inhibition percentage of was calculated by Eq. (1):

$$I\ (\%) = \frac{I_1 - I_2}{I_1} \times 100 \quad (1)$$

where, I (%) is the inhibition percentage, I_1 and I_2 are the current response of the TYR-AuNWs-SPCE before and after the addition of pesticide (e.g. parathion and carbaryl). Calibration curves were plotted in the range of 0.01 ppt to 1 ppm. After the inhibition tests, the electrode was washed three times with PBS.

RESULTS AND DISCUSSION

1. Detection Mechanism of Pesticide

The principle of pesticide detection using an enzyme-based biosensor is the inhibition of enzyme activity by pesticide [17]. As shown in Fig. 2, the immobilized TYR enzyme on the AuNWs-SPCE electrode oxidizes catechol generating quinone in the presence of dissolved oxygen. The enzymatic reaction product, quinone, is reduced at the electrode surface by accepting 2 e^-, corresponding to the electrochemical reduction current. The TYR activity can be identified indirectly by the current response. When an inhibitor such as pesticide exists, the enzymatic reaction (i.e., the oxidation of catechol

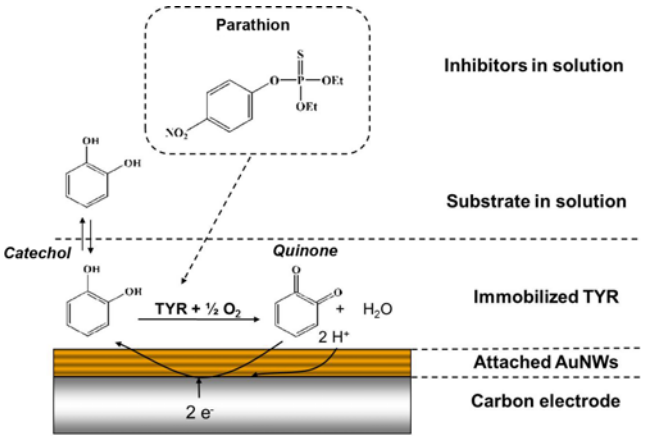

Fig. 2. The detection mechanism of pesticide using TYR-AuNWs-SPCE.

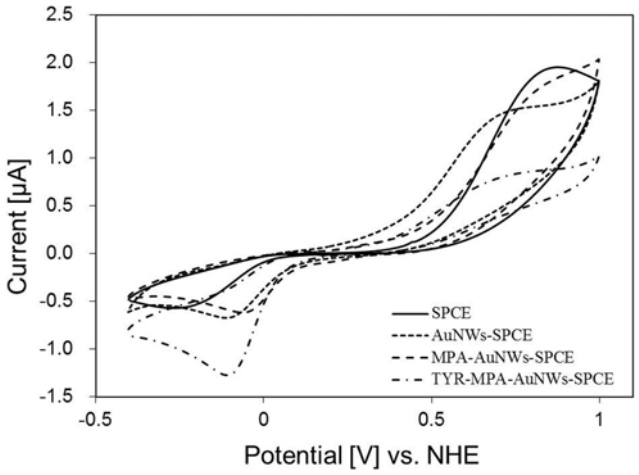

Fig. 4. The comparison of bare SPCE, AuNWs-SPCE, MPA-AuNWs-SPCE and TYR-MPA-AuNWs-SPCE for 1 mM of catechol in O_2-saturated PBS (0.1 M, pH 7) at 50 mV s^{-1}.

by TYR) is retarded and less amount of quinone is generated; correspondingly, the reduction current response decreases. By comparing the current response in the absence and the presence of pesticide, the inhibition percentage of TYR activity by pesticide is determined, where the concentration of pesticide can be estimated from the current ratio.

2. Characterization of TYR-AuNWs-SPCE

Two-dimensionally structured AuNWs were synthesized by Midas-11 peptide. As shown in Fig. 3, the diameter of AuNWs was in the range of 20 to 30 nm. It was expected that the use of the AuNWs synthesized by high purity method enhanced the performance of enzyme-based biosensor due to its biocompatibility, good conductivity, and large surface area. The large surface area of the AuNWs improved the current response and acted as conductive mediator for the effective electrical contact of enzyme in the electrode.

Cyclic voltammetry was conducted to characterize the electrodes in 1 mM catechol in O_2-saturated PBS (0.1 M, pH 7). The voltammograms of bare, AuNWs-, and TYR-MPA-AuNWs-SPCE are compared in Fig. 4. At the bare SPCE, the redox peak of catechol was observed at 0.9 and −0.3 V vs. NHE, respectively. The redox peak currents at the AuNWs-SPCE increased compared to the bare SPCE, while the peak separation ΔEp decreased by the enhanced sensitivity attributed to the presence of AuNWs. When the AuNWs-SPCE was modified with the MPA, there were no significant changes in the peak current, but ΔEp increased due to an insulating property of the MPA layer [17,18]. After the TYR immobilization, lower anodic peak current (I_{pa}) and higher cathodic peak (I_{pc}) were observed. The change of redox peak currents is caused by the enzymatic oxidation of catechol. As catechol is consumed by enzymatic reaction as well as by electrochemical oxidation, the amount of catechol which is oxidized by electrochemical reaction decreased, resulting in the decreased I_{pa}. Quinone, the oxidation product of catechol, is also reduced by both of the reactions (i.e., enzymatic and electrochemical reaction). More importantly, some of the reduced product (i.e., catechol) is re-oxidized by tyrosinase through the enzymatic catalysis. Consequently, the significant increase in I_{pc} was observed at the TYR-AuNWs-MPA-SPCE.

3. Amperometric Analyses for Pesticides Detection

To realize the feasibility of *in situ* detection and fast screening of a pesticide, an amperometric measurement of the TYR-AuNWs-SPCE was conducted using a portable power supply (picoammeter). The current response of the electrode to a substrate (i.e., 1 mM catechol) before and after addition of a pesticide was recorded at –0.1 V vs. NHE. The incubation time for TYR inhibition by exposure to pesticide was 30 s. Prior to every measurement, the electrode was immersed into the PBS (0.1 M, pH 7) for 1 min to restore

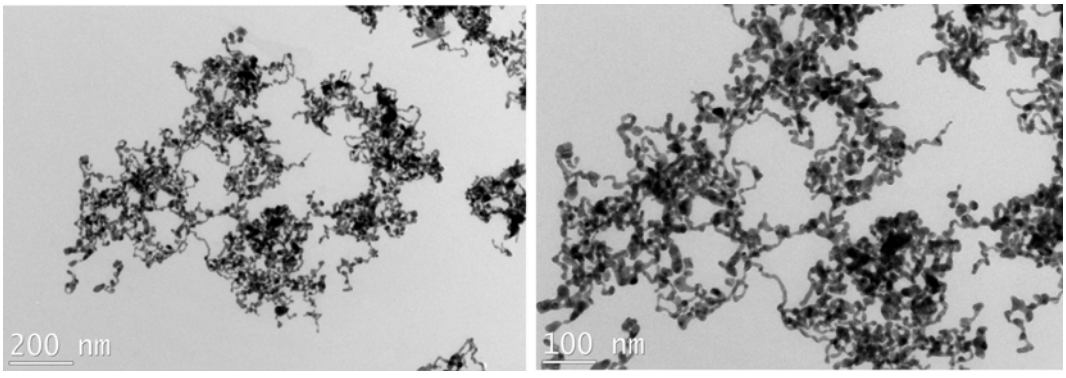

Fig. 3. TEM images of AuNWs used in this study. The AuNWs were synthesized by Midas-11 peptide.

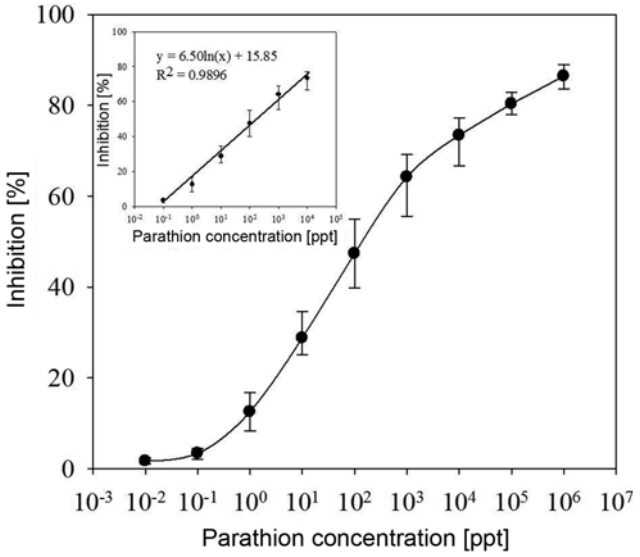

Fig. 5. Inhibition effect of the TYR-AuNWs-SPCE with the addition of pesticides parathion: 1 mM catechol in PBS (0.1 M, pH 7), applied potential=−0.1 V vs. NHE, incubation time= 30 s. These data were based on triplicate measurements.

the enzyme activity, and the electrode restored to the nearly initial activity was then used to measure the current response at each concentration of parathion. A quantitative relationship between the inhibition percentage (I) and the pesticide concentration is presented in Fig. 5. A reasonable relationship is observed in the range of 0.1 ppt to 10 ppb for parathion. The dynamic range of these pesticides is 5 orders of magnitude with the LOD of 0.087 ppt for parathion. The lower detection limits and wider detection ranges for pesticide in the TYR-AuNWs-SPCE developed in this study are significant improvements over previous reports on TYR-based biosensors. For example, Wang et al. reported an interdigited platinum planar electrode for carbaryl detection with a dynamic range of 60 ppb to 3 ppm (LOD=40 ppb) (Wang et al., 2006); Shim et al. reported that 0.01-10 ppb of carbaryl (LOD=8 ppt) and 0.01-1 ppb of parathion (LOD=5 ppt) was detected at a glassy carbon (GC) electrode assembled with the TYR-immobilized membrane [19]. In addition, other studies using CNT [20,21] and AuNW [22] have shown that their working ranges are ppb level, indicating the TYR-AuNWs-SPCE in this study has a higher sensitivity for the detection of pesticides. Recently, Viswanathan et al. developed an ssDNA-modified-SWCNT based AChE biosensor for parathion detection [23]. Even though the electrochemical biosensor has demonstrated a similar detection performance (dynamic range=2.6 ppt-0.26 ppm, LOD=0.26 ppt) with the sensors, it was a voltammetric measurement and needed a longer incubation time (i.e., 15 min). Considering analysis time and simplicity, an amperometric enzyme sensor developed in this study may be a more convenient device.

CONCLUSIONS

We have developed a highly sensitive TYR-based biosensor using the AuNWs synthesized by peptide. The use of AuNWs resulted in increased sensitivity and also allowed the electrode to be used in a wider detection range. The TYR-AuNWs-SPCE enabled to detect parathion up to 0.087 ppt (LOD value). Enhanced performance of the TYR-based biosensor was achieved by the use of AuNWs synthesized by Midas-11 peptide. This AuNWs based TYR electrode has a strong potential to be a screening tool and a monitoring and/or alarming system for pesticide-free water environment.

ACKNOWLEDGEMENT

This work was supported by the Research Center for Biomolecular Nanotechnology and the WCU (R31-2008-000-10026-0) program at GIST.

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