Electrochemical Biosensor consisted of conducting polymer layer on gold nanodots patterned Indium Tin Oxide electrode for rapid and simultaneous determination of purine bases

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A R T I C L E   I N F O

Article history:
Received 21 September 2013
Received in revised form 18 December 2013
Accepted 20 December 2013
Available online 15 January 2014

Keywords:
Biosensor
Adenine and Guanine
Differential pulse voltammetry
Conducting polymer nanostructures
Human serum

A B S T R A C T

The present work focused on the development of new simple method for fabrication of conducting poly(4-aminophenol) nanostructures layered on gold nanodots patterned indium tin oxide (ITO) electrode based on the self assembly of the monomer. This was followed by electrochemical polymerization of 4-aminophenol molecules. In addition, we studied the electrochemical catalytic activity of the modified electrode towards a mixture of two protein bases (Adenine and Guanine). The modified gold nanodots ITO electrode was fabricated based on thermal evaporation of pure gold metal onto ITO surface through polystyrene monolayer. Then, a monolayer of 4-aminophenol was self-assembly immobilized onto the gold nanodots array/ITO electrode. This was followed by electrochemical polymerization process based on cyclic voltammetry technique. The electrochemical behavior of the adenine and guanine mixture at the modified electrode was investigated based on differential pulse voltammetry technique. The results indicated that the polymer nanostructures modified gold nanodots/ITO electrode exhibited an excellent electrocatalytic activity towards the oxidation of adenine and guanine with a detection limit of 500 and 250 nM, respectively. Moreover, our finding demonstrated a linear relationship between the concentration of both adenine and guanine and their oxidation current peaks (R = 0.9953 and 0.9935, respectively). Finally, the modified electrode was successfully used to detect adenine and guanine in human serum sample. Therefore, we proposed that this biosensor could have high sensitivity for simultaneous determination of adenine and guanine in the related physiology process.

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1. Introduction

Adenine (A) and guanine (G) are two of the purine bases that play a vital role as essential building blocks of nucleic acids. These two bases are fundamental compounds in different biological systems, contribute in several processes such as energy transduction, metabolic co-factors and cell signaling. Abnormal change of A and G concentration is considered as indication for the deficiency of the immunity system. Thus, there is great demand for the monitoring of the variation of A and G concentration in living organisms.

Several techniques have been developed to determine the concentration of these purine bases in DNA such as HPLC and isotope dilution mass spectrometry techniques [1]. Although these techniques showed high sensitivity and selectivity, they are time-consuming and require complicated instruments. Therefore, they are unsuitable for real time determination field.

Recently, electrochemical methods for detection of purine bases (A and G) have attracted attention due to their advantages including: fast response speed, cheap instrument, good sensitivity and selectivity [2–4]. However, the direct oxidation of A and G at the traditional bare electrodes has shown poor response with slow electron transfer, in addition to higher over potential of their oxidation that leading to a low selectivity and sensitivity [5]. Hence, numerous chemically modified electrodes such as carbon ionic liquid electrode and fullerene−C60−modified glassy carbon electrode [6,7] had been developed to widen the potential range in which oxidation of A and G occurs. While, these modified electrodes has shown improvement in oxidation signals; however, most of them has elicited complicated preparation process, high background current and leakage of modified electrodes. It is still a challenging to develop stable electrode modified material with high sensitivity and selectivity.

Different metal and semiconductor nanoparticles (that exhibit unique optical, electrochemical and catalytic properties) have been...
widely used to improve the electrode’s surface area as well as its sensitivity. Therefore, it enhanced their applications in electrochemical sensors and biosensors [8–13]. Furthermore, there is growing interest in the use of gold (Au) nanoporous modified electrode due to the wide range of applications that require inertness, conductivity and/or large surface area [14]. Also, nickel oxide or zinc oxide nanoparticles modified electrodes exhibited high electrocatalytic activity towards different biological materials [15,16].

Recently, we have developed Au nanoporous layers modified electrode to change the mass transport processes from planar diffusion to thin layer character [17,18]. That could shift the potential under which the target redox active species from that required to electrolyse the interfering species that facilitate the differentiation between species that oxidize or reduce at similar potentials under planar diffusion conditions [19,20]. Moreover, conducting polymers have a wide variety of applications such as sensors, cathode material of a lithium secondary battery, electrocatalysts and microelectronic devices [21,22] due to their good electrochemical properties. In addition, conducting polymers coating metal nanostructures have been of great interest due to the ability of them to generate additional electro-catalytic sites as well as the probable electronic interactions between metal nanostructures and groups in the polymer [23,24]. Recently, modified Au nano–particles based on self–assembly is promising for the construction of nano-devices and nano-biosensors [25].

Self-assembly of 4–Aminothiophenol (ATP) onto bulk Au electrode has been used for selective determination of dopamine and ascorbic acid [26]. However, the bulk electrodes bear disadvantages; slow electron transfer, low sensitivity, and poor selectivity [27]. In the recent years, ATP has attracted significant attention in producing assembly layer onto nanoparticles via covalent or electrostatic interactions [28]. The difference in reactivity between the thiol and amine ends of ATP has been effectively utilized to design molecular assemblies leading to unique morphologies and chemical treatments [29].

On the contrary, previous studies have reported fabrication of conducting polymer coating Au nanostructures based on dispersive Au nanoparticles into thin film such as PATP (poly(4–aminothiophenol)) over the surface of different electrodes [30–33]. Up to our knowledge, the present work is the first to report fabrication of a newly polymer nanostructures modified electrode based on thermal evaporation of pure gold metal onto ITO surface through monolayer of polystyrene. These Au nanodots acted as template for uniform immobilization of ATP molecules based on self-assembly of a monolayer of ATP onto Au nanodots/ITO electrode. Due to the selectivity interaction between ATP molecules and Au surface, ATP molecules could be attached solely to the surface of Au nanodots. Thus, ATP will be converted into PATP based on electro-chemical polymerization of the ATP film. The electro-chemical oxidation behavior of A and G was investigated by PATP/Au nanodots/ITO electrode. In summary, the performance of the fabricated electrode such as; sensitivity, linear range and selectivity, has been evaluated and discussed. Also, the applicability of this modified electrode has been demonstrated by determining G and A bases in real samples.

2. Experimental

2.1. Materials

ATP, A, G, human serum (HS) and phosphate-buffered saline (PBS) (pH 7.4, 10 mM) were purchased from Sigma (St. Louis, MO, USA). The other chemicals used in this study were obtained commercially as reagent grade. All solutions were prepared with de-ionized water (DIW).

2.2. Instruments

Electrochemical polymerization of ATP into PATP and all electrochemical experiments were performed using a potentiostat (CHI–660, CH Instruments, USA) controlled by general–purpose electrochemical system software. A homemade three–electrode system consisted of a nano–PATP/Au nanodots/ITO substrate as a working electrode, a platinum wire as the auxiliary electrode and Ag/AgCl as the reference electrode. In addition, the synthesis of nano–PATP/Au nanodots/ITO was characterized by Fourier transform infrared spectrophotometer (FT–IR) using Nicolet is10 FT–IR spectrometer (Thermo Scientific). The morphologies of Au nanodots/ITO as well as nano–PATP/Au nanodots/ITO were analyzed by a scanning electron microscope (SEM) (ISI DS–130 C, Akashi Co., Tokyo, Japan).

2.3. Fabrication of Au nanodots/ITO substrate

Au nanodots modified ITO substrates have been fabricated by using monolayer of polystyrene (PS) as an evaporation mask based on the thermal deposition of Au layer onto ITO substrate. The PS masks that has been prepared by the following method; 7 μL of PS particles with a diameter of 500 nm (10 wt % aqueous solution) mixed with a surfactant (Triton–X and methanol 1:400 v/v) in a ratio of 1:1 was applied onto the ITO substrate. Different spin speeds (500, 1000 and 1500 rpm) for 1 min were used. The substrate was then left to dry in the spin coater with a covered lid to maintain a consistent drying ambient and evaporation rate [34]. Then, an Au layer (50 nm) was thermally evaporated through the deposited PS nano–sphere mask. Fifty nm thickness of the Au layer has been selected to fill the spaces between the PS nano-spheres and to obtain the triangle nanostructures. This resulted in a uniform enhancement of the electro-chemical activity. In addition, evaporation of thicker Au layer will not change the morphology of the resulted triangle nano-structures. After dissolution of the PS mask in chloroform, an array of ordered triangle Au nanostructures was left onto the ITO substrate.

2.4. Fabrication of PATP/Au nanodots/ITO electrode

A monolayer of 4–aminothiophenol was self-assembled on Au nanodots/ITO substrates by immersing the substrates in 2 mM of 4–aminothiophenol ethanol solution for 2 hrs, then the fabricated ATP–modified substrates were rinsed thoroughly by DIW and ethanol successively, then dried under N2 gas. Fabrication of nano–PATP/Au nanodots/ITO was achieved by applying cyclic voltammetry (CV) technique to ATP self–assembled monolayer in 0.5 M HClO4 aqueous solution from 0.0 V to 1.0 V for 15 cycles at scan rate of 50 mV/sec.

3. Results and Discussion

3.1. Characterization of PATP/Au nanodots/ITO electrode

Herein, the uniform distribution of Au nanodots over ITO has been used as an effective template to develop nano–PATP/Au nanodots/ITO substrates, because of the selectivity interaction between ATP molecules and Au surface. ATP molecules could be attached only on the surface of Au nanodots by covalent bonds between thiols ends of ATP molecules and Au nanodots (Scheme 1). Different spin speeds were used to achieve a uniform monolayer over large area of the substrate. Fig. 1a showed that multilayer film was obtained by applying spin speed of 500 rpm; while, spin speed of 1500 rpm resulted in non-compacted monolayer film (Fig. 1b). Whilst, a closed- compacted monolayer film (Fig. 1c) was obtained with spin speed of 1000 rpm speed.
The morphologies of Au nanodots/ITO and PATP/Au nanodots/ITO electrodes were characterized by SEM images. Fig. 2a demonstrated that the Au nanostructures exhibit triangle shapes of lateral dimensions about 80 nm in diameter. The modification of these triangle Au nanodots with ATP was followed by electrochemical polymerization of ATP into PATP, resulted in coating of the Au nanodots with a thin film of the PATP and the triangle nature was changed to irregular structures (Fig. 2b). In addition, the CV behavior of ATP/Au nanodots/ITO electrode in 0.5 M HClO₄ solution demonstrated an anodic current peak at 0.683 V and cathodic current peak at 0.385 V in the first cycle scan as shown in Fig. 2c. Interestingly, we noticed that the anodic peak was decreased after the first cycle scan with negative shift from 0.683 V to 0.67 V. In the meantime, another anodic current peak at 0.46 V appeared then increased gradually with the number of potential cycles (Fig. 2d). These changes signified the formation of the electro-active monolayer PATP film. Furthermore, synthesis of PATP/Au nanodots/ITO was also confirmed by using FT-IR (Fig. 3) in the range between 500-3500 cm⁻¹ that showed a characteristic peaks at 832 cm⁻¹ (C-H deformation vib. out of plane), 1166 cm⁻¹ (the characteristic bonds of electron delocalization), 1498 cm⁻¹ (characteristic for bond of benzenoid/amine) and 1590 cm⁻¹ (characteristic for bonds of quinoid/imine), which demonstrated the successful polymerization of ATP to form PATP/Au nanodots/ITO substrate.

3.2. Electrochemical behavior of PATP/Au nanodots/ITO electrode

Fig. 4 illustrated the CV behavior of Au nanodots/ITO, ATP/Au nanodots/ITO, PATP/bulk Au/ITO and PATP/Au nanodots/ITO electrodes in 5 mM Fe(CN)₆³⁻/⁴⁻. Au nanodots/ITO electrode showed a couple of well-defined redox peaks at 0.28 and 0.17 V. While, these redox peaks disappeared completely at ATP/Au nanodots/ITO electrode, which could be attributed to the negatively charged of the ATP monolayer that blocking the diffusion of Fe(CN)₆³⁻/⁴⁻ ions from solution to the electrode surface [35]. Nevertheless, after electro-polymerization of ATP to PATP, the redox peak current

![Scheme 1. Preparation of PATP nanostructures patterns gold modified Indium Tin Oxide.](image-url)
increased significantly at PATP/Au nanodots/ITO compared with ATP/Au nanodots/ITO, indicating that PATP can effectively increase the electron transfer rate of Fe(CN)₆³⁻/⁴⁻. Moreover, the findings demonstrated that PATP/Au nanodots/ITO electrode has shown significantly higher redox peak current compared with PATP coated bulk Au/ITO.

3.3. Electrochemical oxidation behavior of adenine and guanine

The oxidation mechanism of G and A on the surface of three different modified electrodes was investigated by DPV (Fig. 5). The DPV behavior of the mixture of A (80 μM) and G (80 μM) in PBS solution at the bare Au/ITO electrode (Fig. 5a) displayed a broad
Fig. 5. DPVs of (a) mixture of 80 μmol l⁻¹ of A and G at different electrodes in PBS (pH 7.4) at bare Au nanodots/ITO (---), ATP/Au nanodots/ITO (---), and PATP nanostructures/Au nanodots/ITO (●●●●) electrodes within potential range from 0.2 V to 1.6 V, (b) mixture of 80 μmol l⁻¹ of A and G at PATP nanostructures/Au nanodots/ITO electrode within potential range from 1.6 V to 0.2 V, (c) 80 μmol l⁻¹ of A in different pH values at PATP nanostructures/Au nanodots/ITO electrode within potential range from 1.6 V to 0.2 V, (d) mixture of 80 μmol l⁻¹ of A and G in PBS (pH 7.4) (--) or in HS (——) at PATP nanostructures/Au nanodots/ITO electrode within potential range from 1.6 V to 0.2 V, E vs (Ag/AgCl)/V.

overlapped peak with two small oxidation peaks at 810 and 900 mV, so it was difficult to distinguish independent oxidation potential peaks of A and G. In contrast, the DPV for a mixture of 80 μM of A and G at ATP/Au nanodots/ITO (Fig. 5a) produced two anodic peaks at 830 and 1210 mV for G and A, respectively. While, the DPV response for the same bases mixture at PATP/Au nanodots/ITO electrode showed that the oxidation peaks potential was shifted negatively for G (780 mV) and positively for A (1320 mV) as shown in (Fig. 5a). These results revealed that the peak to peak separation of A and G at PATP/Au nanodots/ITO (540 mV) was larger than that at ATP/Au nanodots/ITO (380 mV). The large potential to peak separation can effectively increase the selectivity of the simultaneous determination of A and G. This denoted that the uniform distribution of PATP layer over Au nanodots could enhance the electrostatic accumulation of the positively charged G and A bases into PAPT nanostructures. Thus, the fabricated modified electrode facilitated the electron transfer of G and A, resulted in increase of the oxidation signals.

On the other hand, Fig. 5b displayed the DPV behaviors of G and A at PATP/Au nanodots/ITO electrode by applying the potential in the reverse direction (from 1.6 V to 0.2 V) to study the reversibility of these oxidation processes. These results demonstrated that the oxidation processes of G and A are irreversible processes. In addition, the results of the oxidation process of A at different pH values (Fig. 5c) demonstrated the ability of the modified PATP/Au nanodots/ITO electrode to detect A in the acidic electrolyte (4.5 pH) and up to the weak alkaline electrolyte (7.4 pH). However, electoactivity for the modified PATP/Au nanodots/ITO electrode toward oxidation of A could not be observed at alkaline electrolyte (9 pH). This electrochemical activity of PATP/Au nanodots/ITO at weak alkaline electrolyte could be explained by the presence of acidic groups on the ITO surface which appeared to act as acidic counter-ions [21,36]. Moreover, Fig. 5d showed the DPV behaviors of the mixture of G and A in HS at PATP/Au nanodots/ITO electrode, which demonstrated that G and A displayed the same behaviors for mixture of G and A in PBS. In addition, different concentration of A in HS was determined and the recovery percentages were investigated (Table 1). These results indicated that PATP/Au nanodots/ITO

<table>
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<th>Table 1</th>
<th>Determination of A in human serum solution.</th>
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<tr>
<td>Samples</td>
<td>Added (μM/L)</td>
</tr>
<tr>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
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<tr>
<td>5</td>
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Thus, electrode responds well for the recovery of A in real samples with high sensitivity without interferences.

3.4. Simultaneous determinations of guanine and adenine

Fig. 6a illustrated the DPV curves for various concentration of A (50 nM–100 μM) in PBS containing 20 μM of G, which demonstrated that the IpA was proportional to the concentration of A in the range of 500 nM–100 μM (Fig. 6a inset) with no significant change for the oxidation current peak of G. In addition, DPVs for a mixture of increasing concentration of G (250 nM–90 μM) with a fixed concentration of A (50 μM) were presented (Fig. 6b). The anodic peak current response to G concentration showed to increase linearly within range from 250 nM to 90 μM (Fig. 6b inset), with no significant change in the oxidation current peak of A. These results demonstrate that both G and A showed linear concentrations in the range from 500 nM to 100 μM at PATP/Au nanodots/ITO electrode by using DPV technique, with an experimental limit of detection down to 500 nM for A and 250 nM for G. This detection limit was lower than that was reported for graphene–Nafion composite film modified glassy carbon electrode or [37,38].

Therefore, the modified PATP/Au nanodots/ITO electrode could be applied for simultaneous determination of A and G. In addition, the anodic peak current corresponding to the oxidation of A and G in the presence of 1% human serum exhibited almost the same anodic peak current for the same mixture in PBS solution. These results indicated that PATP/Au nanodots/ITO electrode could be suitable for the determination of G and A in real samples without interferences.

3.5. Stability and reproducibility of PATP/Au nanodots/ITO

The efficiency of the modified PATP/Au nanodots/ITO electrode for the detection of A was tested in phosphate buffer (pH 7.4) for twenty days (Fig. 7). In the first 5 days, the current response showed 2% decrease of its initial response. Over the next 5 days, the current response decreased by about 3% and in the following 10 days, the decrease was 5%. The total decrease over the time period was 10%. Thus, PATP/Au nanodots/ITO retains 90% of its original activity after 20 days and this continued to exhibit excellent response to A.

4. Conclusions

In a nutshell, the current study reported an easy and rapid method for fabrication of PATP/Au nanodots/ITO electrode for simultaneous monitoring of the electro-chemical oxidation behavior of A and G purine bases. The results demonstrated that the peak-to-peak separation of A and G obtained at the fabricated electrode was a little larger than ATP/Au nanodots/ITO electrode. In addition, the modified electrode was successfully used to determine A and G concentration in human serum as a real sample, presenting no interference with detection limits of 500 nM and 250 nM for A and G, respectively. Therefore, this modified electrode is expected to be a promising technique for real sample biosensor applications.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government
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