PNA-DNA Hybridization Detection with One-step Electrodeposition using Nanomaterial Modified Gold Electrode

การตรวจวัดไฮบริไดเซชันของพีเอ็นเอ-ดีเอ็นเอโดยใช้วัสดุนาโนโมดิฟายด์อิเล็กโทรดทองด้วยวิธีการเกาะติดทางไฟฟ้าขั้นตอนเดียว

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Abstract

One of the important parameter that plays an important role in the performance of an electrochemical DNA biosensor is the supporting material for the immobilized probes. Using nanomaterials for electrode modification has gained a lot of attention with many advantages, such as large surface area and good electrical conductivity that can improve sensor sensitivity. The development of a direct PNA-DNA hybridization detection based on a novel pyrrolidinyl peptide nucleic acid or acpcPNA probe was studied using differential pulse voltammetric detections. Enhancement the sensitivity of DNA hybridization biosensor is presented using nanomaterial modified gold electrode. The oxidation current of an anthraquinone (AQ) tagged acpcPNA probe (acpcPNA-AQ) was measured from the electron transfer between the tagged AQ and the gold electrode based on differential pulse voltammetry (DPV). From various gold modified electrodes, polyaniline-Graphene-Ag nanocomposite coated electrode surface provided a higher sensitivity than polyaniline, polyaniline-Graphene and polyaniline-Ag due to its high surface area and good electrical conductivity of graphene and Ag nanoparticles. This DNA sensor can be used up to 33 times with 0.05 M of sodium hydroxide as the regeneration solution. Moreover, only one-step of electrodeposition of nanomaterial modification is very simple, cost-effective and fast detection of a specific DNA screening technique.

Keywords: Peptide nucleic acid, Anthaquinone, Nanomaterial, Hybridization, Electrodeposition

บทคัดย่อ

พารามิเตอร์หนึ่งที่สำคัญต่อประสิทธิภาพของดีเอ็นเอไบโอเซนเซอร์คือวัสดุสำหรับช่วยในการตรึงโพรบ วัสดุนาโนสำหรับโมดิฟายด์อิเล็กโทรดได้รับความสนใจเพราะช่วยเพิ่มพื้นที่ผิวและมีการนำไฟฟ้าที่ดีช่วยให้ความไววิเคราะห์สูงขึ้น งานวิจัยนี้พัฒนาการตรวจวัดไฮบริไดเซชันของดีเอ็นเอโดยตรงด้วยพิโรลิดินิลเพปไทด์นิวคลีอิกแอซิดโพรบหรือเอซีพีซีพีเอ็นเอโพรบด้วยดิฟเฟอเรนเชียลพัลส์โวลแทมเมตรี การเพิ่มความไววิเคราะห์โดยใช้

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วัสดุนาโนโมดิฟายด์ขั้วทองและวัดกระแสไฟฟ้าออกซิเดชันจากการส่งผ่านอิเล็กตรอนของแอนทราควิโนนซึ่งติดอยู่กับพีเอ็นเอโพรบมายังขั้ว เมื่อดัดแปลงขั้วด้วยนาโนคอมโพสิตระหว่างโพลิอะนีลีน กราฟีนและอนุภาคนาโนเงินจะให้ความไววิเคราะห์ดีกว่าการดัดแปลงขั้วด้วยโพลิอะนีลีน คอมโพสิตระหว่างโพลิอะนีลีนกับกราฟีน และคอมโพสิตระหว่างโพลิอะนีลีนกับอนุภาคนาโนเงิน เซนเซอร์นี้ใช้ซ้ำได้ถึง 33 ครั้ง ด้วยสารละลายทำลายพันธะคือโซเดียมไฮดรอกไซด์ 0.05 โมลาร์ การดัดแปลงขั้วทองด้วยวัสดุนาโนใช้วิธีเกาะติดทางไฟฟ้าขั้นตอนเดียวมีข้อดีคือเป็นวิธีที่ง่ายและรวดเร็วอีกทั้งเซนเซอร์มีความจำเพาะสูง

คำสำคัญ: เพปไทด์นิวคลีอิกแอซิด แอนทราควิโนน วัสดุนาโน ไฮบริไดเซชัน การเกาะติดทางไฟฟ้า

Introduction

The detection of specific-sequence DNA is increasingly important because of its potential applications in many areas such as clinical diagnostic of specific diseases [1], detection of gene mutation [2] and genetically modified organisms or GMOs analysis [3] and environmental monitoring [4]. Traditional methods that are currently used to detect specific DNA hybridization include Southern blot [5], dot blot [6] and fluorescence in situ hybridization (FISH) [7]. However, it is well known that these techniques are quite complex, expensive, time consuming [8]. DNA biosensor is one method that has attracted much attention for DNA hybridization detection [9]. The sensor generally composed of ss-DNA probes immobilized on a transducer surface that are able to form duplex with the target DNAs [10]. The hybridization event is then converted into a measurable signal by a transducer. Between the optical, mass sensing and electrochemical transducers, electrochemical detection offers a great promise for a highly sensitive, simple, fast detection, and cost-effective method.

One of the important parameters that plays an important role in the performance of an electrochemical affinity DNA biosensor is the supporting material for the immobilized probes. Using nanomaterials for electrode modification has gained a lot of attention with many advantages, such as large surface area and good electrical conductivity that can improve sensor sensitivity [11]. Electrochemical DNA sensor designed with nanomaterials has been shown to have low limit of detection, high signal and high sensitivity [12]. In recent years, graphene has received attention due to its provided excellent electrical conductivity that can improve the sensitivity. Therefore, an electrochemical DNA affinity biosensor incorporating with nanomaterials is interesting since the materials will help enhance the signal, sensitivity and provide the low limit of detection. A simple, fast detection and low cost electrochemical DNA biosensor for the detection of DNA hybridization based on nanomaterials modified gold electrode was investigated. Moreover, various surface modifications were studied by incorporated graphene and/or silver nanoparticles in a polyaniline layer that was electrodeposited on the electrode.

Materials and Methods

Materials

The anthraquinone (AQ) tagged lysine-modified acpcPNA (Ac-TTT TTT TTT-LysNH_2), acpcPNA-AQ. The acpcPNA-AQ probes were purified by reverse phase HPLC (to 90% purity) and its identity was verified by

MALDI-TOF mass spectrometry. Synthetic target DNA (5′-AAA AAA AAA-3′) used was synthesized and purified by Bioservice Unit, National Science and Technology Development Agency and BioDesign Co., Ltd., Thailand. The blocking thiol of 11 carbon length with the –OH terminating head group, 11-mercapto-1-undecanol (11-MUL) was purchased from Aldrich (Steinheim, Germany). Graphene nanosheets (4-5 layers, thickness of 8 nm, surface area 600-750 m^2g^{-1}, particle diameter 2 μm) were obtained from Cheap Tubes Inc (Brattleboro, USA). All buffers were prepared with deionized water treated with a reverse osmosis-deionizing system (Pentair, Inc., USA). Before using, buffers were filtered through a nylon membrane filter with pore size 0.2 μm, 47 mm diameter (Vertical®, Spain) and degassed, respectively. Other chemicals were analytical reagent grade and were used as received.

Methods

Immobilization of acpcPNA-AQ probe

Gold rod electrodes (99.99% purity) with a diameter of 3.0 mm were cleaned by dipping in piranha solution (conc. H_2SO_4 : 30% H_2O_2 equal to 3:1 %v/v) with sonication for 30 min followed by rinsing with distilled water. Then, they were polished using alumina slurry (5, 1, 0.3 μm of the particle size), on a smooth polishing cloth until a mirror-like surface was obtained and subsequently washed with distilled water. The electrodes were placed in a plasma cleaner (Model PDC-32G, Harrick, New York, USA) to remove organic and inorganic molecules adsorbed on the electrodes surface [13].

The modified gold electrodes were prepared within four types, i.e., polyaniline (PANI), polyaniline composited with graphene (PANI-Graphene), polyaniline composited with silver nanoparticles (PANI-Ag) and polyaniline composited with graphene and silver nanoparticles (PANI-Graphene-Ag).

For a PANI modified surface, a PANI film was electrodeposited onto a gold electrode in 0.50 M H_2SO_4 with 0.10 M aniline aqueous solution, mixed with 0.25 M polyacrylic acid (PAA) to get a better stability with improved polymer properties [14]. The electrodeposition was performed by cyclic voltammetry for 10 scans using the potential range from -0.2 to 1.2 V vs. Ag/AgCl with a scan rate of 50 mVs^{-1}. The PANI coated gold electrode was cleaned by rinsing with distilled water and treated with 5.0 % (v/v) glutaraldehyde in 10 mM phosphate buffer pH 7.00 at room temperature for 20 min to activate the aldehyde groups. Then 20 μL of 5.0 μM of acpcPNA-AQ probe [15] was placed on the modified electrode for 24 h in the refrigerator (4 °C). Finally, the immobilized acpcPNA-AQ electrode was immersed in 1.0 mM of 11-mercaptoundecanol (11-MUL) solutions for 1 h to block any remaining pinholes, hence preventing any non-specific binding on the electrode surface. For other nanocomposited modified electrode, PANI-Graphene, PANI-Ag and PANI-Graphene-Ag, either 2.0 mg mL^{-1} graphene or 0.01 M $AgNO_3$ [16] or both were added into the electrodepositing solution accordingly, followed by an immobilization and a blocking steps.

Surface morphology characterization

The surface morphology of the four nanomaterial modified electrodes were observed using scanning electron microscopy (SEM) and energy dispersion X-ray spectroscopy (EDX). Both SEM images and EDX spectra were characterized with a JSM 5800 Quanta from JEOL, Japan, operated at an accelerating voltage of 20 kV.

Electrochemical measurement

The PNA-DNA hybridization measurement was studied based on three electrode system, i.e., a modified gold electrode working, a Ag/AgCl reference electrode and a Pt counter electrode, connected to a μ-Autolab PGSTAT Type III potentiostat/galvanostat (Metrohm Applikon, Utrecht, The Natherlands) controlled by GPES 4.9 software (Eco Chemie, Herisau, Switzerland). The hybridization response was the decrease of the oxidation peak of the electrochemical indicator AQ (tagged to the PNA probe) detected by differential pulse voltammetry (DPV). The DPV was operated from -1.2 to -0.3 V, against the Ag/AgCl reference electrode, with a scan rate of 50 mVs^{-1}, a step width of 100 ms, a step potential of 5.0 mV, the pulse width and pulse amplitude were 60 mV. The DPV was performed in a batch system contain with 100 mM sodium phosphate buffer pH 7.00 with 100 mM potassium chloride. In this technique two potential pulses of amplitude E_1 and E_2 and length t_1 and t_2, respectively are first applied with $t_1 >> t_2$ and $\Delta E = E_2 - E_1$. The potential is scanned in the negative ($\Delta E < 0$) or positive direction ($\Delta E > 0$) in such a way that a delay between each pair of pulses is introduced in order for the equilibrium to be re-established. In this potentiostatic technique the difference current responses I_{DPV} or $\Delta I = I_2(t_1 + t_2) - I_1(t_1)$ is plotted versus E, referred to as differential pulse voltammogram [17].

Results

Electrochemical characterization of the immobilization step

Figure 1 shows an example of the electrochemical behavior of the PANI-Graphene-Ag coated on the gold electrode surface studied by cyclic voltammetry using 5.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl between -0.3 to 0.7 V at a scan rate of 0.1 Vs^{-1} *vs* a Ag/AgCl reference electrode. The clean gold surface showed a voltammogram with oxidation and reduction peaks (Figure 1 (a) and inset). Both peaks increase when PANI-Graphene-Ag nanocomposite was deposited onto the electrode surface (Figure 1 (b)) indicated that the PANI-Graphene-Ag helped to increase the electrical conductivity. When 5.0 % (v/v) glutaraldehyde in 10 mM sodium phosphate buffer pH 7.00 was used to activate the covalent bonding between the amine group of the PNA-AQ probe and the free amine group of PANI at room temperature for 20 min, the redox peaks of the electrode decrease (Figure 1 (c)). The response was further reduced when PNA-AQ probes were immobilized (Figure 1 (d)). The modified electrode surface was then react with ethanolamine pH 8.50 to occupy all the remained aldehyde groups of glutaraldehyde that were not bound to the probes. Finally, this modified electrode was rinsed with 100 mM phosphate buffer pH 7.00 and then immersed in 1.0 mM 11-MUL solution for 60 min cover any pinholes on the electrode surface. The cyclic voltammogram showed complete blockage of the redox species (Figure 1 (e))

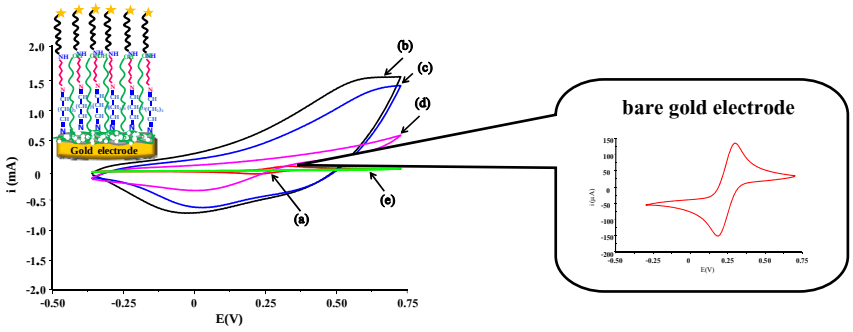

Figure 1 Cyclic voltammograms behavior obtained in 5.0 mM $K_3[Fe(CN)]_6$ with 0.10 M KCl solution from the immobilization PNA-AQ probe; bare gold electrode (a) then electrodeposited with PANI-Graphene-Ag nanocomposite (b) and crosslinked with glutaraldehyde (c) for immobilization with PNA-AQ probe (d) and finally blocked with 11-MUL (e).

For the other modified gold electrode (PANI, PANI-Graphene and PANI-Ag nanocomposites) the cyclic voltammograms followed the same trend during the modification steps.

Surface Morphology

Scanning Electron Microscopy (SEM)

Figure 2 shows the SEM micrographs of the four types of the nanomaterials modified gold electrode surface. The PANI Film has a fibrous network structure having a diameter of 40 - 70 nm (Figure 2 (a)) (measured from an SEM image using electronic digital caliper (Kovet, Japan)). As for the PANI-Graphene nanocomposite (Figure 2 (b)), the nanofiber structure of PANI was embedded with graphene. For PANI-Ag nanocomposite, silver nanoparticles with the particle size 50 - 90 nm were decorated on the surface of the PANI nanofibers (Figure 2 (c)). Figure 2 (d) shows the morphology of PANI-Graphene-Ag nanocomposite, graphene sheets were seen embedded within the PANI nanofiber with the silver nanoparticles decorated on the PANI nanofibers.

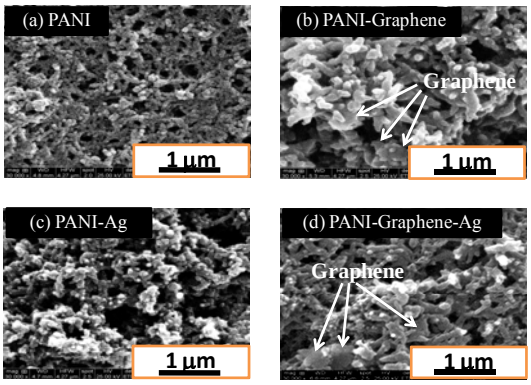

Figure 2 SEM images of the gold electrode surface modified with PANI (a), PANI-Graphene (b), PANI-Ag (c) and PANI-Graphene-Ag (d) nanocomposites.

Energy Dispersion X-ray Spectroscopy (EDX)

Characterization the Ag composite with PANI and PANI-Graphenr. Both PANI-Ag and PANI-Graphene-Ag nanocomposites were confirmed with EDX spectra (Figure 3 (a and b)), which revealed the peak of silver (Ag) and confirmed the presence of silver element. That the result is, Ag nanoparticles were successfully decorated on PANI in the preparation of PANI-Ag and PANI-Graphene-Ag nanofiber composites.

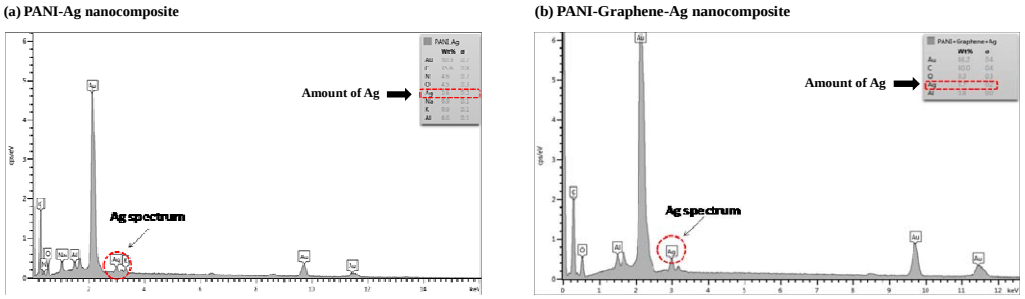

Figure 3 EDX spectra of gold electrode surface modified with PANI-Ag (a) and PANI-Graphene-Ag (b) nanocomposites.

Reproducibility of PANI modified electrode

The reproducibility of four PANI modified electrode was investigated using complementary target DNA in the concentration of 1.0×10^{-10} - 1.0×10^{-7} M. From the result, the sensitivities of four modified electrodes were found in the same range, indicating a good reproducibility.

Optimization the concentration of silver nitrate

In electrodeposition process, Ag^{+} can be change to Ag^{0} that is the silver nanoparticle with the unique electrical property. The concentration of silver nitrate was optimized with the concentration 0.01, 0.10, 0.20, 0.40, 0.60 and 0.80 M. PNA-DNA hybridization was investigated using complementary target DNA in the concentration of 1.0×10^{-10} - 1.0×10^{-7} M. From the result, silver nitrate 0.20 M provided the highest sensitivity (Figure 4).

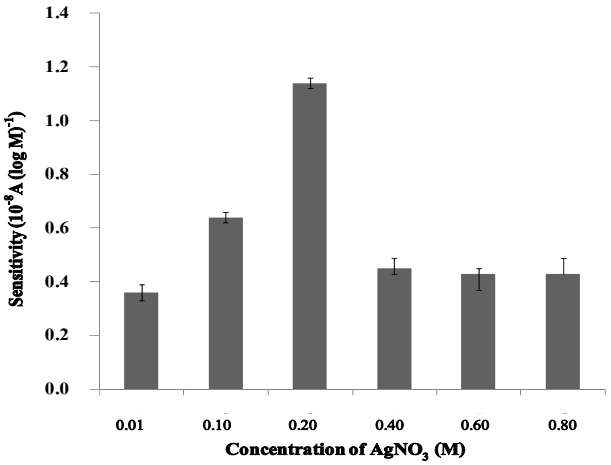

Figure 4 Comparison the sensitivities of PNA-DNA hybridization.

Hybridization study with target DNAs

Oxidation peak current from the electron transfer of AQ (acpcPNA-AQ probe) to the electrode surface was measured using DPV. In the absence of the synthetic target DNA, single-stranded acpcPNA-AQ probe can be closed to the electrode thus the electron transfer between the AQ and the electrode can easily occur, resulting in a high current. After PNA-DNA hybridization, the formation of the duplexes between the probes and the target DNAs made the probe structure more rigid. Therefore, the AQ at the end of the acpcPNA probe moved further away from the electrode surface resulting in the decrease of the response.

For an example of the DPV responses that obtained from four concentrations of the target DNA (1.0×10^{-10} and 1.0×10^{-7} M) is shown in Figure 5. Without the target DNA the flexible acpcPNA-AQ probes provided the highest current (Figure 5 (a)). Upon hybridization between the probe and target DNA, the more rigid PNA-AQ/DNA duplex stands moved the tagged AQ away from the gold electrode surface resulting in the increased of the electron transfer distance between the AQ and the electrode surface which reduced the current (Figure 5 (b)-(e)).

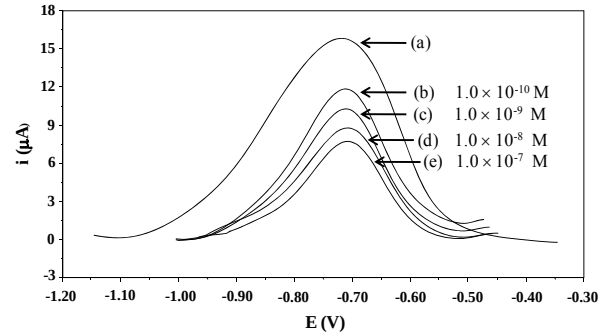

Figure 5 Voltammograms of DNA biosensor using acpcPNA-AQ probe (a) and the duplexes (b) – (e).

Sensitivities of the modified electrodes

The sensitivities (slopes of the calibration curve) of the four nanomaterial modified electrodes based on the hybridization with complementary target DNA (1.0×10^{-10} - 1.0×10^{-7} M). The highest sensitivity was obtained from the PANI-Graphene-Ag nanocomposite coated gold electrode surface, higher than PANI-Graphene, PANI-Ag and PANI by 6.7, 4.7 and 2.5 times, respectively. These results showed that the excellent electrical conductivity of PANI, graphene and silver nanoparticles in the nanocomposite modified gold electrode have contributed to the enhancement of the DNA biosensor sensitivity.

Discussion

A novel nanocomposite of polyaniline, graphene and silver nanoparticle (PANI-Graphene-Ag) has been successfully prepared and used for the modification of gold electrode based DNA biosensor using one-step of electrodeposition. Graphene and silver nanoparticle can substantially improve the electrical property and surface area of the modified electrode, leading to enhanced sensitivity of the DNA biosensor. To test the applicability of this sensor, PANI-Graphene-Ag modified gold electrode was used for the hybridization with complementary DNA that provided the highest sensitivity.

Conclusions

DNA affinity biosensor based on the acpcPNA-AQ probes immobilized on the nanomaterials modified gold electrode was successfully fabricated. The large surface area and good electrical conductivity of the nanomaterials provided an enhanced signal and sensitivity. Moreover, the one-step electrodeposition modified gold electrode surface is simple and rapid.

Acknowledgements

For the successful of this work would be thanks Yala Rajabhat University (YRU); and Prof. Dr. Tirayut Vilaivan for providing the acpcPNA probes.

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