Chinese Chemical Letters 26 (2015) 160–166

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Application of diazo-thiourea and gold nano-particles in the design of a highly sensitive and selective DNA biosensor

Mohammad Hossein Mashhadizadeh, Rasoul Pourtaghavi Talemi *

Faculty of Chemistry, Kharazmi (Tarbiat Moallem) University, Tehran, Iran

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 12 August 2014
Accepted 29 August 2014
Available online 16 September 2014

Keywords:
DNA biosensor
Gold nano-particles
Thiourea
Methylene blue

ABSTRACT

An effective procedure for constructing a DNA biosensor is developed based on covalent immobilization of NH_2 labeled, single strand DNA (NH_2-ssDNA) onto a self-assembled diazo-thiourea and gold nano-particles modified Au electrode (diazo-thiourea/GNM/Au). Gold nano-particles expand the electrode surface area and increase the amount of immobilized thiourea and single stranded DNA (ssDNA) onto the electrode surface. Diazo-thiourea film provides a surface with high conductibility for electron transfer and a bed for the covalent coupling of NH_2-ssDNA onto the electrode surface. The immobilization and hybridization of the probe DNA on the modified electrode is studied by differential pulse voltammetry (DPV) using methylene blue (MB) as a well-known electrochemical hybridization indicator. The linear range for the determination of complementary target ssDNA is from $9.5(\pm0.1)\times10^{-13}$ mol/L to $1.2(\pm0.2)\times10^{-9}$ mol/L with a detection limit of $1.2(\pm0.1)\times10^{-13}$ mol/L.

1. Introduction

DNA biosensors are characterized by obvious advantages, including high sensitivity, avoidance of potential interferences from the sample solution turbidity, compatibility with micro-fabrication, and comparatively low cost, thus they have been widely employed in the biosensing field [1,2].

Molecular self-assembly has become a popular surface derivatization procedure due to its simplicity, versatility, and establishment of a high level of order on a molecular scale [3–5]. Self-assembled monolayers (SAMs) have been used in electroanalytical chemistry for modification of the electrodes to develop sensors [6] and biosensors [7,8].

Despite the fact that label-less approaches for the detection of DNA hybridization based on impedimetric measurements [9] represent the majority of the works reported in this area, the introduction of labels to amplify the recognition event has only recently been reported. Enzymes, such as alkaline phosphatase and horseradish peroxidase [10,11], an electroactive tag like methylene blue [12], anthraquinone [13], liposomes [14], and redox molecules have been investigated as labels. The use of redox molecules is also particularly interesting [15–17]. Methylene Blue (MB) is an organic dye that has been extensively reported as an electrochemical reporter in biosensing applications [18]. MB binds specifically to guanine bases [19] and after hybridization, however, the current signal of the biosensor decreases since less MB can bind to dsDNA.

Thiourea, $SC(NH_2)_2$, is an organo-sulfur compound with a structure similar to the urea. Thiourea has been used for toning silver-gelatin photographic prints [20]. On the other hand, diazonium organic salts have been extensively used for the surface modification of a wide range of metal and semiconductor materials. The diazonium group of functionalized electrodes is of special interest owing to its ability to further react with phenolic, imidazole, or amino groups to form covalent diazo bonds for the achievement of different types of surface modification. For example, Radi *et al.* [21], reported an effective protocol for covalent immobilization of horseradish peroxidase on a gold electrode surface by a diazotization-coupling reaction.

However to our knowledge, there is no report of using diazo-thiourea in the construction of a DNA biosensor. Therefore, an objective of this work was the design of an effective strategy for constructing an electrochemical DNA biosensor with less expensive compounds, such as thiourea and gold nanoparticles. Firstly, Au electrode was electro-deposited with gold nano-particles which greatly increased the effective surface of the electrode. Then, the gold nano-particle modified Au electrode was self-assembled with

* Corresponding author.
E-mail addresses: rasoulpourtaghavi@gmail.com, rasoulpourtaghavi@khu.ac.ir (R.P. Talemi).

http://dx.doi.org/10.1016/j.cclet.2014.09.004

thiourea through Au–S bonding. Diazo-thiourea was formed through diazotization on the electrode surface for the covalent immobilization of probe ssDNA and finally DNA hybridization was detected by the DPV method.

2. Experimental

Chemicals and materials: Potassium nitrate, sulfuric acid, hydrogen peroxide, mercaptohexanol, hydrogen tetrachloroaurate ($HAuCl_4{\cdot}3H_2O$), $[Ru(NH_3)_6]^{3+}$, $K_3Fe(CN)_6$, and $K_4Fe(CN)_6$ were purchased from the Merck Company (Germany). Five 15-base oligonucleotides were purchased from the Aminsan Company (Tehran, Iran). The base sequences were as follows:

Probe ssDNA (S_1): 5′-NH_2-TTATGCCGCAATGCA-3′.
Complementary target ssDNA (S_2): 5′-TGCATTGCGGCATAA-3′.
Three-base mismatched ssDNA (S_3): 5′-TGAATTACGGCTTAA-3′.
One-base mismatched ssDNA (S_4): 5′-TGCATTGAGGCATAA-3′.
Non-complementary ssDNA (S_5); 5′-AATACGGCGTTACGT-3′.

All oligonucleotide stock solutions were prepared in phosphate buffer solution (PBS, pH 7.0).

Apparatus: Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) measurements were performed with an EN50081-2 electrochemical workstation (PalmSense, Netherlands). Electrochemical impedance spectroscopy (EIS) measurements were acquired on an Autolab 302 electrochemical workstation. All electrochemical experiments were performed with a conventional three-electrode system comprising a gold working electrode (unmodified or modified), an Ag/AgCl (KCl, 3.5 mol/L) reference electrode, and a platinum counter electrode (Azar Electrode, Iran). The amplitude of the alternating voltage was 10 mV. Scanning electron microscopy (SEM) was performed with a JEOL-JSM 6700F scanning electron microscope (JEOL Ltd., Japan).

Sensor fabrication: Initially, an Au electrode was cleaned [22] and then the gold nano-particles modified Au electrode (GNM/Au) was prepared based on a previously reported procedure [24]. Next, the GNM/Au was rinsed with doubly distilled water (DDW) and dried in air before further modification. For assembly of thiourea, the GNM/Au electrode was immersed into a 5 mmol/L thiourea solution for 12 h at r.t. Then, the obtained electrode was completely cleaned with ethanol and water to remove unassembled thiol components and denoted as thiourea/GNM/Au. Following the thiourea self-assembling, the sensor surface was washed with DDW and subsequently was back-filled by dropping 50 μL of a 0.01 mol/L aqueous solution of mercaptohexanol onto the electrode surface which was then incubated for 30 min at room temperature. The sensor was then thoroughly washed with DDW, leaving an organized mixed SAM of chemisorbed thiourea and MCH (this step was not shown in the brief Fig. 1). Afterwards, the thiourea/GNM/Au electrode was treated using the optimum procedure for diazotization [23]. In brief, the thiourea/GNM/Au electrode was transferred to a 0.1 mol/L HCl solution at 2–4 °C, and 100 mg $NaNO_2$ was slowly added to a total concentration of about 0.05 mol/L, which is a slight excess to ensure the completeness of the reaction. After 30 min incubation, the electrode was removed and immediately was rinsed with ice water and the as-prepared electrode was denoted as diazo-thiourea/GNM/Au. Finally, 4 μL of 1.0×10^{-6} mol/L probe NH_2-ssDNA (S_1) was dropped onto the surface of the diazo-thiourea/GNM/Au electrode which was maintained at r.t. for 1 h. The resulting modified electrode was denoted as S_1/diazo-thiourea/GNM/Au.

Hybridization of modified electrode: The S_1/diazo-thiourea/GNM/Au electrode was immersed in 0.02 mol/L PBS (pH 7.0) containing different concentrations of target (S_2) or mismatch (S_3 or S_4) ssDNA with shaking for 30 min at 37 °C. After hybridization, the obtained electrodes were washed with the same PBS buffer and water to remove non-specifically bound DNA. The electrodes thus obtained were denoted as S_1–S_2/diazo-thiourea/GNM/Au, S_1–S_3/diazo-thiourea/GNM/Au, and S_1–S_4/diazo-thiourea/GNM/Au, respectively.

Indicator binding: MB was accumulated onto the surface of modified electrode by immersing it in the stirred PBS buffer (0.02 mol/L, pH 7.0) containing 20 μmol/L MB and 20 mmol/L NaCl for 5 min without applying any potential. Then, the electrode was rinsed with the same PBS buffer.

Voltammetric detection: The fabricated biosensor was transferred to an electrochemical cell, including 10 mL of 0.02 mol/L PBS

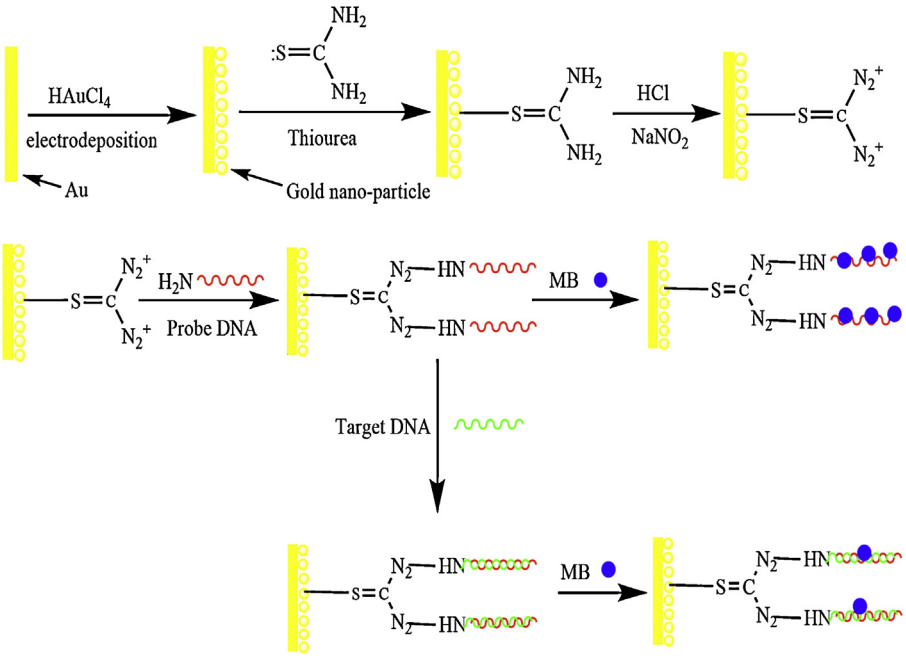

Fig. 1. The steps involved in the fabrication of the probe DNA-modified electrode for detection of a target sequence.