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A bimetallic nanocomposite modified genosensor for recognition and determination of thalassemia gene

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ABSTRACT

The main roles of DNA in the cells are to maintain and properly express genetic information. It is important to have analytical methods capable of fast and sensitive detection of DNA damage. DNA hybridization sensors are well suited for diagnostics and other purposes, including determination of bacteria and viruses. Beta thalassemias (βth) are due to mutations in the β-globin gene. In this study, an electrochemical biosensor which detects the sequences related to the β-globin gene issued from real samples amplified by polymerase chain reaction (PCR) is described for the first time. The biosensor relies on the immobilization of 20-mer single stranded oligonucleotide (probe) related to βth sequence on the carbon paste electrode (CPE) modified by 15% silver (Ag) and platinum (Pt) nanoparticles to prepare the bimetallic nanocomposite electrode and hybridization of this oligonucleotide with its complementary sequence (target). The extent of hybridization between the probe and target sequences was shown by using linear sweep voltammetry (LSV) with methylene blue (MB) as hybridization indicator. The selectivity of sensor was investigated using PCR samples containing non-complementary oligonucleotides. The detection limit of biosensor was calculated about 470.0 pg/μL.

1. Introduction

The easy fabrication of DNA surfaces which are reproducible, stable and selective to complementary DNA sequences is crucial in the development of emerging biotechnologies such as DNA chips and simple diagnostic devices for detecting a few sequences of target DNA [1]. Biosensors are small devices employing biochemical molecular recognition properties as basis for selective analysis. Due to their specificity, speed, portability and low cost, biosensors offer exciting opportunities for numerous decentralized clinical applications [2].

Recently our research group has published several articles regarding DNA biosensors modified by mono nanoparticles such as gold, platinum or silver [3–6]. However, the use of binary nanoparticles for improving the electrochemical sensing devices is an extremely promising [7]. As the addition of the second metal lead to variation in particle size, shape, surface morphology, chemical and physical properties. Multilayer of conductive nanoparticles give rise to porous, high surface area electrode, where the local microenvironment of metallic nanoparticles can be controlled by crosslinking elements and causes the specific and selective interactions with substrates [8,9].

In addition to direct electrochemistry of DNA (using intrinsic DNA electroactivity and surface activity), indirect approaches have been introduced in DNA electroanalysis and DNA biosensor technology [10]. To improve the sensitivity of electrochemical assays and to achieve better, more reliable analysis, there is a great demand for labels with higher specific activity. The most commonly used labels for electrochemical sensors to date have been enzymes and small electroactive indicator molecules [11]. A number of formats for electrochemical detection are described in the literature, including hybridization with probes conjugated to a redox active label [12–16]. Methylene blue (MB), an organic dye which belongs to the phenothiazine family, has been widely used in DNA hybridization detection as an electrochemical indicator [17–24].

Meric et al. described the use of MB as hybridization indicator for electrochemical detection of Hepatits B and TT virus in the surface of carbon paste electrode [25]. Pournaghi-Azar et al. used pencil graphite electrode and MB as electroactive label for the sense

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of strand of interleukine-2 gene [26]. Raoof et al. described development of an electrochemical biosensor based on peptide nucleic acid (PNA) probe for detection of target DNA sequence and single nucleotide mutation in p53 tumor suppressor gene corresponding oligonucleotide using methylene blue (MB) as an electrochemical indicator [27]. Chen et al. utilized Muts protein and MB marker for mutation recognition in β-thalassemia gene on to the gold electrode [28].

Hemoglobin (Hb) is the essential oxygen transporting protein in animal kingdom. In human, the major component of red cells in postnatal life consists of 2α- and β-globin chain (HbA). Globins are encoded by 1β and 2α genes located on the short arm of chromosome 11 and 16, respectively. Thalassemia is recessively inherited disorders characterized by a quantitative reduction of the α- or β-globin genes expression leading to anemia [29]. B-Thalassemia is monogenic diseases which are transmitted most frequently in an autosome recessive way [30]. The disease appears generally during the first year of life. It manifests with severe anemia (Hb < 7 g/dL) requiring regular blood transfusion [31].

Beta thalassemias are due to mutations in the β-globin gene. The DNA biosensor showed here was proved to detect β-globin gene efficiently on the modified carbon paste electrode (CPE) with silver and platinum nanoparticles modified carbon paste electrode (MCPE) by indirect method. We used MB as electroactive indicator and polymerase chain reaction (PCR) products of real samples. No literature has been recorded using similar procedure for detection of the thalassemia gene PCR products originating from real blood samples.

2. Experimental

2.1. Reagents and materials

MB was analytical grade and was purchased from Merck. A 20-mer oligonucleotide corresponding to sense strand of human β-thalassemia gene (IVSII-1) was used as the probe and its complementary (CIVSII-1) oligonucleotide corresponding to antisense strand of human β-thalassemia gene was used as target DNA. Both probe and target DNA were supplied as lyophilized powder by Bioneer Company, South Korea with the following sequences:

Probe DNA (IVSII-1): 5′-ACTTCAGGATGAGTCTATGG-3′

Complementary DNA (CIVSII-1): 5′-CCATAGACTCATCCTGAAGT-3′

The PCR amplified DNAs (0.45 ng/mL) obtained from real blood samples were donated by Genetic Laboratory of Amirkola Children Hospital and were prepared as reported elsewhere [33,34]. Two sets of PCR products were used in this study. One corresponding to the β-globin gene and complementary to the oligonucleotides described above and another non-complementary sequence (A and B) amplified by PCR. Their sequences are as follows:

A (Fc): 5′-TGG GGA TGG AGA ACT-3′

B (α2 IVS-I):5′-CAC TCT TCT GGT CCC CAC AGACT-3′

All oligonucleotides stock solution (100 μM) were prepared with TE buffer solution (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.00) and kept frozen. More diluted solutions of oligonucleotides were prepared using 0.50 M acetate buffer solution (pH 4.80) containing 20 mM NaCl. The stock solution of MB (1.0 mM) was prepared using distilled and sterilized water. H_2PtCl_6 and $AgNO_3$ were purchased from Merck Company. Other chemicals were of analytical grade. The distilled and sterilized water was used in all solution preparations. Each measurement consists of the immobilization cycle carried on a fresh MCPE surface. All the experimental procedures were performed at room temperature in an electrochemical cell.

2.2. Instrumentation

Electrochemical experiments were performed by AUTOLAB PGSTAT 30 electrochemical analysis system; GPES 4.9 and FRA 4.9 software package (Eco Chemie, Netherlands). All electrochemical measurements were carried out using conventional three-electrode cell.

MCPE or CPE was used as working electrodes (surface area of 0.03 cm^2) and Pt wire served as auxiliary electrode. All potentials are reported against an Ag|AgCl|KCl(3 M) reference electrode. All parameters in procedure were optimized before and published elsewhere [32]. Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) were taken using Tescan Vega II XMU and Nanosurf easyscan 2, alternatively.

2.3. Procedure

2.3.1. Synthesis of nanoparticles

Platinum nanoparticles (NPt) were prepared according to the literature [35,36]. Briefly, 60 mL of a 2.0 mM aqueous H_2PtCl_6 solution were mixed with 3 mL of 50 mM aqueous sodium citrate solution, then under vigorous stirring, 7 mL 120 mM aqueous $NaBH_4$ solution were added drop wise.

Silver nanoparticles (NAg) were prepared by a chemical reduction method reported in literature [37]. In this method, $AgNO_3$ (17.0 mg) was dissolved in 100 mL water in a 250 mL tri-neck flask. The solution was heated to boiling with a hemisphere heating mantle under vigorous magnetic stirring. After boiling for 2 min, an aqueous solution of sodium citrate (35 mM, 10 mL) was rapidly added to the flask. The solution gradually turned yellow within a few minutes, indicating the formation of Ag nanoparticles. The solution was kept boiling for an additional 6 min. After that, the heating mantle was removed, and the solution was allowed to cool.

For taking AFM picture, the sample was prepared by putting a drop of stock solution of nanoparticles on the surface of a lam. After drying the drop, the lam was scanned by Easyscan 2 Flex. The particle size was determined using Nanosurf easy Scan 2 software and most of the particles size was between 10 to 20 nm.

2.3.2. Preparation of MCPE

CPE was prepared according to the literature [38]. For fabrication of silver nanoparticles modified carbon paste electrode, sufficient Ag nanoparticles were added to carbon paste to obtain modified CPE containing different percentages of Ag nanoparticles (Ag-MCPE). A portion of the resulting paste was packed into the bottom of a glass tube. The electrical connection was implemented via copper wire fitted into the glass tube. The surface of the resulting electrode was smooth on a weighing paper and rinsed carefully with distilled water. The procedure for preparation of Pt-MCPE and Ag/Pt-MCPE has been just similar to the preparation of Ag-MCPE.

2.3.3. Electrochemical activation of the working electrode

The electrochemical pretreatment of the surface of electrode was performed on the optimized potential of 1.80 V vs. SCE for 5 min in 0.50 M acetate buffer solution (pH 4.80) containing 20 mM of NaCl without stirring.

2.3.4. Immobilization of the probe on the working electrode

After activation, for immobilization of the probe on the electrode, it was immersed in 0.50 M acetate buffer solution (pH 4.8) containing 1.0 μM probe and 20 mM of NaCl. Afterward, the 0.5 V vs. Ag|AgCl|KCl(3 M) was applied on the electrode for 5 min in the stirred solution (200 rpm) at room temperature. Then the electrode was rinsed with sterilized and deionized water.