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Short communication

DNA electrochemical biosensor based on thionine-graphene nanocomposite

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ABSTRACT

A novel protocol for development of DNA electrochemical biosensor based on thionine-graphene nanocomposite modified gold electrode was presented. The thionine-graphene nanocomposite layer with highly conductive property was characterized by scanning electron microscopy, transmission electron microscopy, cyclic voltammetry and electrochemical impedance spectroscopy. An amino-substituted oligonucleotide probe was covalently grafted onto the surface of the thionine-graphene nanocomposite by the cross-linker glutaraldehyde. The hybridization reaction on the modified electrode was monitored by differential pulse voltammetry analysis using an electroactive intercalator daunomycin as the indicator. Under optimum conditions, the proposed biosensor exhibited high sensitivity and low detection limit for detecting complementary oligonucleotide. The complementary oligonucleotide could be quantified in a wide range of 1.0×10^{-12} to 1.0×10^{-7} M with a good linearity ($R^2 = 0.9976$) and a low detection limit of 1.26×10^{-13} M (S/N = 3). In addition, the biosensor was highly selective to discriminate one-base or two-base mismatched sequences.

1. Introduction

The detection of specific DNA sequence is currently an area of tremendous interest since more and more research has proved that the mutations of genes are responsible for numerous inherited human disorders (Malley and Hutcheon, 2007; Van Borsel and Tetnowski, 2007; Chamcheu et al., 2011). Besides, pathogens responsible for disease states, bacteria and viruses, are also detectable *via* their unique nucleic acid sequences. Therefore, rapid and simple determination of specific sequences of DNA in human, viral and bacterial nucleic acids at low concentration is currently in great demand (Lucarelli et al., 2004). Many techniques including fluorescence (Yang et al., 2008; Liu et al., 2010a), electrochemiluminescence (Wei et al., 2007; Wang et al., 2010), electrochemistry (Chang et al., 2007; Liu et al., 2010b), surface plasmon resonance spectroscopy (Kick et al., 2010) and quartz crystal microbalance (Fei et al., 2011; Nie et al., 2007) have been developed for the DNA detection. In these bioassay systems, DNA electrochemical biosensors have attracted broad attention due to their advantages such as low cost, fast response, small size, good selectivity and miniaturization of instruments (Jiang et al., 2008; Riccardi et al., 2006; Peng et al., 2005; Yan et al., 2001).

Graphene, a two-dimensional sheet of sp^2 conjugated atomic carbon, has stimulated intense research interest because of its large specific surface area, high thermal and electrical conductivities (Novoselov et al., 2004; Kim et al., 2008), great mechanical strength (Zhao et al., 2010), and potential low manufacturing cost (Segal, 2009). Due to its excellent conductivity and electrocatalytic activity, graphene is an ideal material for the preparation of electrochemical sensors and biosensors (Shan et al., 2009; Zhou et al., 2009; Wang et al., 2009). Bonanni and Pumera (2011) developed a graphene platform to combine the sensitivity of electrochemical impedance spectroscopy (EIS) with the high selectivity of hairpin-shaped DNA probes for the rapid detection of single nucleotide polymorphism correlated to the development of Alzheimer's disease. Hu et al. (2011) constructed a novel gold nanoparticles-ionic liquid/3,4,9,10-perylene tetracarboxylic acid/graphene sensitive platform, which was successfully used for label-free impedance sensing of DNA. The graphene-based chemo/biosensors show a considerable prospect due to their good biocompatibility for biomolecules (Chen et al., 2008).

To expand the application of graphene, non-covalent functionalization between graphene sheets and aromatic organic molecules based on π-stacking interaction has been carried out (Su et al., 2009; Xu et al., 2008), which could preserve the intrinsic properties of graphene and improve its solubility (Ghosh et al., 2010). Thionine has a planar aromatic structure that allows strong interaction with the surface of graphene sheets through synergistic non-covalent charge-transfer and π–π stacking force (Chen et al., 2011). The positive charges on thionine facilitate the solubility and

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prevent the aggregation of non-covalent functionalized graphene (Li et al., 2004). Moreover, due to a large amount of hydrophilic amino groups (—NH_2), thionine modified graphene sheets can covalently immobilize NH_2-substituted oligonucleotide probe by linker.

In this work, a DNA sensing platform based on thionine-graphene nanocomposite (Th-G) was developed. Using daunomycin as a hybridization indicator, the sensitive detection of complementary oligonucleotide was realized by monitoring differential pulse voltammetry (DPV) signal of daunomycin intercalated in double-stranded DNA (dsDNA). This sensing method provides potential applications for the ultrasensitive detection of different DNA sequences.

2. Experimental

2.1. Reagents

Graphite oxide (GO) was obtained from Nanjing XFNANO Materials Tech Co., Ltd., (Nanjing, China). Thionine, L-cysteine (Cys) and glutaraldehyde (GA, 25%) were provided by Sigma (USA). Daunomycin was purchased from Melone Pharmaceutical Co., Ltd., (Dalian, China). Hydrazine hydrate ($NH_2NH_2{\cdot}H_2O$, 80%) was purchased from Aladdin (Shanghai, China).

21-base synthetic oligonucleotide sequences related to anthrax lethal factor were provided by Sangon Biotech Co., Ltd., (Shanghai, China):

- Probe ssDNA (P_1): 5′-(NH_2-C_6) ATC AAT ATT TAA CAA TAA TCC-3′;
- Complementary ssDNA (C_1): 5′-GGA TTA TTG TTA AAT ATT GAT-3′;
- One mismatch-containing ssDNA (C_2): 5′-GGA TTA TTG TGA AAT ATT GAT-3′;
- Two mismatch-containing ssDNA (C_3): 5′-GGA TTA TGG TGA AAT ATT GAT-3′.

0.1 M phosphate buffer solution with 0.1 M KCl and 0.05 M NaCl (0.1 M PBS, pH 7.4) was used as the supporting electrolyte. All chemicals used in the experiments were of analytical grade. Milli-Q water (18.25 MΩ cm) was used throughout the experiments.

2.2. Instruments

Transmission electron microscopy (TEM) images were obtained by a Hitachi H-600 (Japan, at an acceleration voltage of 100 kV) and scanning electron microscopy (SEM) images were carried out on an XL30 ESEM-FEG (Netherlands, FEI Company, at an acceleration voltage of 15.0 kV).

Electrochemical measurements were performed using a CHI 660D electrochemical workstation (Shanghai CH Instrument Co., China). A conventional three-electrode system was used with a saturated calomel electrode (SCE) as the reference electrode, a platinum sheet as the counter electrode, and a modified gold electrode (AuE, 1.5 mm in diameter) as the working electrode. EIS measurements were tested by an Autolab PGSTST 30 analyzer (Metrohm Autolab B.V., Switzerland) using $[Fe(CN)_6]^{3-/4-}$ as the electrochemical probe. 5 mV amplitude of sine voltage signal was applied to the three-electrode system under open circuit potential, and the frequency varied from 0.1 Hz to 100 kHz.

2.3. Preparation of graphene and Th-G nanocomposite

Graphene was prepared according to the literature (Hummers and Offeman, 1958; Kovtyukhova et al., 1999). Briefly, the procedure was as following: the GO dispersion (5 mg dispersed in 50 mL water) was firstly exfoliated by sonicating under ambient conditions for 40 min. Then, $NH_2NH_2{\cdot}H_2O$ (1% v/v) was added into the GO dispersion. The resulting mixture was heated to 100 °C and kept stirring for 24 h. Subsequently, the solution was filtered, and the filtrate was discharged. Finally, black hydrophobic powder graphene was obtained by drying in vacuum at 60 °C, and stored at ambient conditions.

Th-G suspension was prepared as follows: 0.1 mg/mL of GO dispersion (5 mg of GO nanosheets into 50 mL water) was obtained by ultrasonication for 40 min at room temperature. After centrifugation at 3000 rpm for 5 min, the undissolved substance was discarded and the brown supernatant was kept for further use. Then, 10 mL of 2 mM of thionine aqueous was added into the above solution, and stirred vigorously for at least 12 h. After that, 200 μL $NH_2NH_2{\cdot}H_2O$ (80%) was dropped into the resulting dispersion. After being vigorously shaken for 10 min, the mixture was heated to 95 °C under reflux for 1 h. Finally, the stable dark dispersion was centrifuged and washed three times. The dark centrifugate was dissolved in 2 mL of distilled water.

2.4. Fabrication of DNA biosensors

The preparation of the DNA electrochemical biosensor is illustrated in Fig. 1. The bare AuE was carefully polished with 1.0, 0.3 and 0.05 μm alumina slurries, followed by successive sonication in Milli-Q water and ethanol for several minutes, respectively. Prior to use, the AuE was immersed in Piranha's solution for 10 min (a hot mixed solution of 30% H_2O_2 and concentrated H_2SO_4, volume ratio 3:7. Attention: strong hazardous chemicals), followed by rinsing with water and absolute ethanol. Then, the AuE was continuously scanned within a potential range of −0.35 V to +1.70 V in freshly prepared deoxygenated 0.5 M H_2SO_4 until a voltammogram characteristic of the cleaned AuE was established.

The freshly pretreated AuE was rinsed with water, dried with N_2 flow and then immersed in 20 mM Cys solution (0.1 M PBS, pH 7.4) for 24 h. A Cys-modified gold electrode (Cys/AuE) was obtained after thoroughly washing with water. The Cys/AuE was immersed into 0.5% GA solution for 2 h to obtain the GA modified gold electrode (GA/Cys/AuE). In this procedure, Cys was firstly self-assembled on the gold electrode, and then GA was covalently attached on the Cys/AuE. Then, 5 μL prepared Th-G suspension was dropped on the surface of the GA/Cys/AuE and dried in air to form Th-G nanocomposite modified electrode (Th-G/GA/Cys/AuE). The Th-G/GA/Cys/AuE was then washed with water and immersed into 0.5% GA solution for 2 h to obtain the GA modified electrode (GA/Th-G/GA/Cys/AuE). Finally, the GA/Th-G/GA/Cys/AuE was incubated in 20 μM P_1 for another 2 h to covalently immobilize the probe ssDNA, and thus the P_1/GA/Th-G/GA/Cys/AuE was obtained after washing with 0.1 M PBS (pH 7.4).

2.5. Hybridization and electrochemical measurements of the biosensor

The hybridization procedure was performed by immersing P_1/GA/Th-G/GA/Cys/AuE into 100 μL analyte solution (C_1, C_2 or C_3) with desired concentration for 40 min at 35 °C, and then the hybridized electrode was rinsed with 0.1 M PBS (pH 7.4) to remove the non-specifically adsorbed DNA. The obtained electrodes after hybridization with above different sequences were denoted as C_1-P_1/GA/Th-G/GA/Cys/AuE, C_2-P_1/GA/Th-G/GA/Cys/AuE and C_3-P_1/GA/Th-G/GA/Cys/AuE, respectively.

The hybridized electrode was placed in 10 μM daunomycin (0.1 M PBS, pH 7.4) for 15 min. The electrode was rinsed with 0.1 M PBS (pH 7.4) for several times to remove the physically adsorbed molecules. Then hybridized electrode was used for electrochemical measurements.