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Short communication

Freely switchable impedimetric detection of target gene sequence based on synergistic effect of ERGNO/PANI nanocomposites

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ABSTRACT

An impedimetric and freely switchable DNA sensor based on electrochemically reduced graphene oxide (ERGNO) and polyaniline (PANI) film was presented, where ERGNO was prepared on PANI modified glassy carbon electrode (GCE). When the probe DNA was noncovalently assembled on the surface of electrode through π–π* stacking between the ring of nucleobases and the rich-conjugated structure of the nanocomposite, the electron transfer resistance value of $[Fe(CN)_6]^{3-/4-}$ increased. The negative ssDNA and the steric hindrance blocked the effective electron transfer channel of the $[Fe(CN)_6]^{3-/4-}$. After hybridization with the complementary DNA, the formation of helix induced dsDNA to release from the surface of conjugated nanocomposite, accompanied with the curtailment of the impedimetric value. The selectivity and sensitivity of this DNA sensing platform were characterized using electrochemical impedance spectroscopy in detail. The fabricated biosensor exhibited excellent performance for the detection of specific DNA sequence with a wide linear range (1.0×10^{-15} to 1.0×10^{-8} mol/L) and a low detection limit of 2.5×10^{-16} mol/L due to the synergistic effect of ERGNO/PANI nanocomposites. The hosphinothricin acetyltransferase gene (PAT) was also detected to show the switchable ability of ERGNO/PANI.

1. Introduction

Graphene is a single-atom thick planar sheet with sp^2 bonded conjugated atomic carbon ideally arranged in six-membered rings (Geim and Novoselov, 2007), which has attracted scientific and industrial interest because of its unique electronic, mechanical and thermal properties (Zhang et al., 2010). Among lots of fabrication methods of graphene, electrochemically reduced graphene oxide (ERGNO) could effectively remove excess of oxygen without adding toxic chemical reagents (Du et al., 2010). At the same time, graphene is considered as an ideal support for conjugated molecules via π–π* stacking interaction due to its unique conjugated structure and highly hydrophobic surface (Zhao, 2011). In particularly, graphene has been found to be ideal for immobilizing the probe DNA via π–π* interaction between its conjugated interface and DNA bases (Du et al., 2012). After hybridization, it has been proved the resulted dsDNA will release from conjugated interface. This induced the electrochemical or optical signal change of nanointerface, which could be adopted for freely switchable detection of the different gene sequences (Bonanni and Pumera, 2011).

Polyaniline (PANI) is an excellent conducting polymer with good environmental stability and biocompatibility, which has served as a promising material in biosensing fields, especially in the DNA sensors (Arora et al., 2007a, 2007b; Prabhakar et al., 2008). In addition, to extend the functions and improve the performances of PANI-based DNA sensors, PANI was often integrated with other functional materials, such as graphite oxide (Wu et al., 2005), gold nanoparticle (Tian et al., 2004), polymeric acid (Zhang et al., 2007). For example, Bo et al. (2011) developed a graphene and PANI nanolayers film with highly conductive and biocompatible nanostructure for sensitive and selective DNA detection. The probe was successfully immobilized on the PANI/graphene/GCE via phosphoramidate bonds between the amino group of the PANI and phosphate group of the oligonucleotides at 5' end. Using daunomycin as an indicator, the ssDNA/PANI/graphene/GCE showed high selectivity and sensitivity towards complementary DNA sequence.

In the DNA sensors, compared with voltammetric methods, the emerged electrochemical impedance spectroscopy (EIS) is a non-invasive method and can be performed even on insulating substrates for characterizing DNA-functionalized electrodes. Therefore, EIS can be applied to monitor the DNA immobilization and hybridization, which could induce the changes of the intrinsic properties and interfacial properties of the sensing substrates (Yang et al., 2008; Hu et al., 2010; Wang et al., 2011a).

In this work, the probe DNA was successfully immobilized on ERGNO, which was initially electrochemically reduced on PANI modified glassy carbon electrode (PANI/GCE). The impedimetric

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value increased obviously when the probe DNA was adsorbed on ERGNO/PANI/GCE due to π–π^* stacking. Besides, the results also demonstrated that ERGNO/PANI/GCE was more satisfactory for immobilizing the probe DNA compared with PANI/GCE or ERGNO/GCE. After hybridization, the escape of dsDNA from the surface of conjugated ERGNO/PANI/GCE induced impedimetric value shrinking, which can serve as a powerful tool to detect the target DNA without any complex labeling steps.

2. Experimental

2.1. Apparatus and reagents

Electrochemical measurements were carried out on a CHI 660C electrochemical workstation (Shanghai CH Instrument Company, China), which consisted of a GCE or a modified GCE, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode.

Graphite powder (spectral pure, Sinopharm Chemical Reagent Co., China); PANI suspension (1 g/L) was provided by College of Materials Science and Engineering, Qingdao University of Science and Technology (Zhang et al., 2009a). All the chemical reagents were of analytical grade and used as received without further purification.

The 18-base oligonucleotides probe (probe DNA), its complementary sequence DNA (cDNA, target DNA, namely an 18-base fragment of PML/RARA fusion gene sequence, originated from promyelocytic leukemia (PML)/retinoic acid receptor alpha (RARA) fusion gene sequence), single-base mismatched DNA, and non-complementary sequence DNA (ncDNA) were synthesized by Shanghai Sangon Biotechnology Limited Company. Their base sequences and stock solutions were the same as that reported by Zhang et al. (2012).

2.2. Preparation of graphite oxide (GO) and GNO

GO was prepared from graphite powder according to a modified Hummers' method (Du et al., 2010). The resulting GO was suspended in water to obtain a homogeneous dispersion and then was sonicated for 30 min to get GNO suspension.

2.3. Fabrication of modified electrode

Before use, PANI suspension was sonicated for 5 min, and then, 10 μL PANI suspension (1 g/L) was dropped on the surface of GCE and dried at room temperature. The obtained electrode (PANI/GCE) was coated with GNO by dripping 10 μL homogeneous suspension of GNO (0.1 g/L) to get GNO/PANI/GCE. The ERGNO/PANI/GCE was prepared by immersing the GNO/PANI/GCE into a phosphate buffer solution (PBS, pH 7.0), followed by electrochemical reduction of GNO at a constant potential of −1.0 V for 500 s (Du et al., 2012). As a comparison, the ERGNO/GCE was fabricated by the same method just without PANI existence.

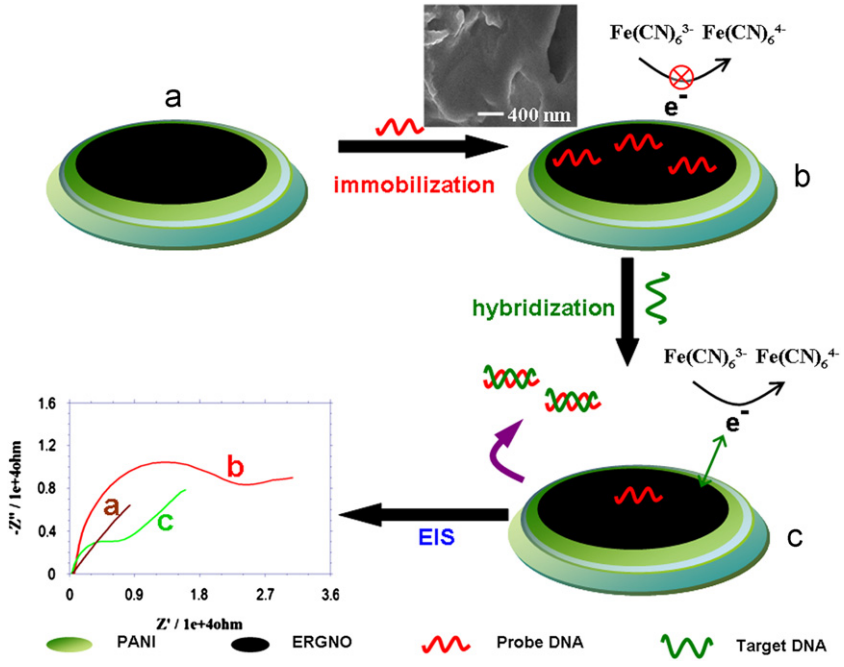
2.4. Probe DNA immobilization and hybridization

The ERGNO/PANI/GCE was immersed in a 5.0 mL Tris-HCl buffer (pH 7.0) solution containing 10^{-6} mol/L probe DNA for 30 min. Then, the electrode was washed with 0.2% sodium dodecyl sulfonate (SDS) solution and ultrapure water for 5 min in turn to remove the unbound probe DNA. The resulted electrode was noted as ssDNA/ERGNO/PANI/GCE.

Hybridization was performed by immersing the probe modified electrode in 2× sodium saline citrate (SSC) buffer solution containing a certain of target DNA (Zhang et al., 2012) for 20 min. The modified electrode was also washed with 0.2% SDS solution for 5 min and denoted as dsDNA/ERGNO/PAN/GCE. The hybridization reactions of the probe DNA with ncDNA and single-base mismatched DNA were also carried out in the same process.

2.5. Electrochemical measurements

EIS measurements were performed in 0.1 mol/L NaCl solution containing 1.0 mmol/L $K_3[Fe(CN)_6]$ and 1.0 mmol/L $K_4[Fe(CN)_6]$. The AC voltage amplitude was 5 mV, the applied potential was 0.172 V vs. SCE, and the frequencies ranged from 10 kHz to 0.01 Hz.



Scheme 1. Schematic representation of the immobilization and hybridization of DNA on the ERGNO/PANI/GCE. *The inset*: SEM image of ssDNA/ERGNO/PANI/GCE.