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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.

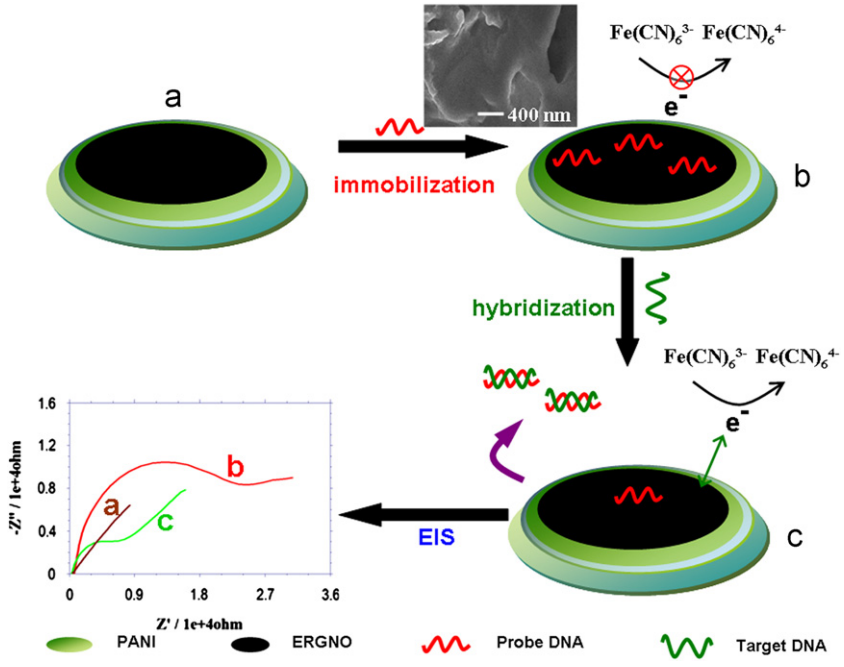
2.4. Probe DNA immobilization and hybridization

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.

The reported result for every electrode in this assay was the mean value of three parallel measurements.

3. Results and discussion

3.1. Sensing route of impedimetric detection

The immobilization and hybridization of the specific gene on the modified electrode are shown in Scheme 1. In the Nyquist diagram, the semicircle diameter is equal to electron transfer resistance (noted as R_{et}), which relates to the conductivity of the electrode/electrolyte interface. From Nyquist diagram, it can be seen that ERGNO/PANI/GCE (curve a) reveals nearly no arc at the high frequency section, which ascribes to the good electrical conductivity of PANI and ERGNO. After DNA immobilization (curve b, *the inset:* SEM image of ssDNA/ERGNO/PANI/GCE), the R_{et} value increases obviously as compared with curve a. The electron transfer of the electrode/electrolyte interface is prevented by the electronegative phosphate skeletons of probe DNA, which enlarges the R_{et} value of the modified electrode. After the probe DNA hybridized with the target DNA (curve c), the R_{et} value reduces clearly. After hybridization, the strong and stable binding between ssDNA and cDNA induces the resulted dsDNA releasing from electrode surface, as the interaction between ssDNA and ERGNO is weaker than the binding between ssDNA and cDNA (Bonanni and Pumera, 2011).

3.2. Electrochemical characterization of DNA modified electrode

Fig. 1A displays the Nyquist plots of ssDNA/GCE (a), ssDNA/PANI/GCE (b), ssDNA/ERGNO/GCE (c), and ssDNA/ERGNO/PANI/GCE (d) using $[Fe(CN)_6]^{3-/4-}$ as the indicator. As compared with curve a, the R_{et} value of the curve b increases due to the positively charged PANI adsorb negatively charged ssDNA by electrostatic interaction. At the same time, the rich π structure of PANI emerges to efficiently immobilize the probe DNA via π–π* interaction between conjugated interface and DNA bases (Liu et al., 2011). Similarly, the R_{et} value on the ssDNA/ERGNO/GCE (curve c) increases more obviously, which should be ascribed to same strong noncovalent interaction between ERGNO and DNA base (Bonanni and Pumera, 2011). Among them, the ssDNA/ERGNO/PANI/GCE (curve d) presents a maximal signal as compared with the signals obtained at the other three electrodes. This maybe ascribe to the synergistic effect of ERGNO/PANI film, which could effectively increase the loading of the probe DNA. The result indicates that the ERGNO/PANI/GCE could provide an ideal platform for the DNA immobilization.

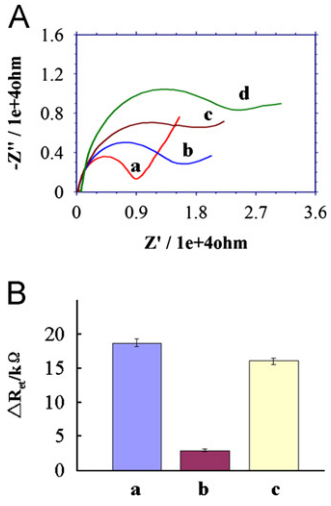


Fig. 1. (A) Nyquist plots of 1.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol/L NaCl at (a) ssDNA/GCE; (b) ssDNA/PANI/GCE; (c) ssDNA/ERGNO/GCE; (d) ssDNA/ERGNO/PANI/GCE. (B) Histogram for comparison of signal change induced by hybridization: hybridized with cDNA (a), ncDNA (b), and single-base mismatched DNA (c). *Note:* $\Delta R_{et} = R_{ssDNA} - R_{dsDNA}$.

3.3. Selectivity, sensitivity and switchable ability of DNA hybridization recognition

The ssDNA/ERGNO/PANI was hybridized with different DNA sequences to test selectivity of the impedance assay of DNA hybridization. The ΔR_{et} value ($\Delta R_{et} = R_{ssDNA} - R_{dsDNA}$) obtained from cDNA hybridization (Fig. 1B, column (a)) shows the highest difference (Hu et al., 2012) compared with that from the hybridization of ncDNA (Fig. 1B, column (b)) and single-base mismatched sequence (Fig. 1B, column (c)). The results showed that our sensing platform can distinguish the cDNA, ncDNA, and single-base mismatched sequence. Therefore, this DNA biosensor shows good selectivity for the DNA hybridization detection.

The probe DNA modified electrode was adopted to detect quantitatively the different concentrations of the complementary PML/RARA sequences and the related Bode plots are shown in Fig. 2A. The difference of log Z value (namely $\Delta\log Z$) at 10^4 Hz between ssDNA/ERGNO/PANI/GCE (curve a) and hybridization-inducing ERGNO/PANI/GCE (hybridized with target DNA) was adopted as the measurement signal (Wang et al., 2011a). The plot of the $\Delta\log Z$ versus the logarithm of the PML/RARA fusion gene target sequence concentration is shown in Fig. 2B. It can be seen that the range of detection for the PML/RARA fusion gene was from 1.0×10^{-15} to 1.0×10^{-8} mol/L with a good linear relationship (the correlation coefficient 0.9959). The limit of detection was 2.5×10^{-16} mol/L using 3σ, where σ was the

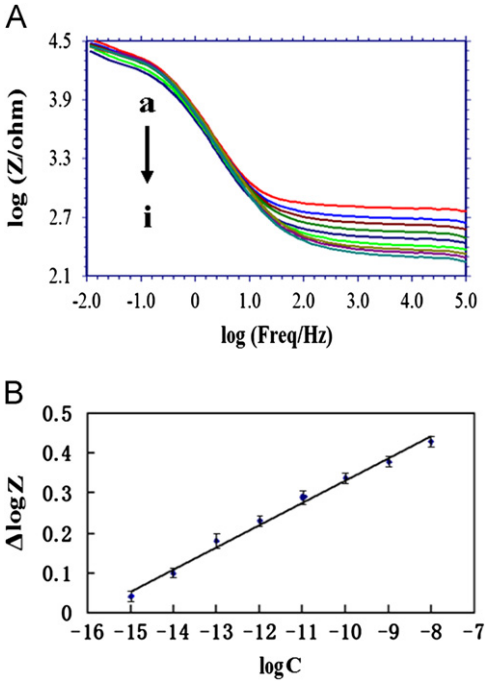


Fig. 2. (A) Bode plots recorded at the ssDNA/ERGNO/PANI/GCE (a) and after hybridization with its complementary PML/RARA fusion gene sequence of different concentrations: from (b) to (i) corresponding to from 1.0×10^{-15} to 1.0×10^{-8} mol/L, respectively; Experimental conditions were the same as in Fig. 1 (B) The plot of $\Delta\log Z$ against the logarithm of the PML/RARA fusion gene fragment concentrations. Each point is the mean of three measurements, and the error bars correspond to the standard deviation.

standard deviation of the blank solution with 11 parallel measurements (Xu et al., 2004; Wang et al., 2011a; Hu et al., 2012).

To illustrate the switchable ability of this constructed DNA sensor (Bonanni and Pumera, 2011; Liu et al., 2011; Zhang et al., 2009b), the hosphinothricin acetyltransferase gene (PAT), which is one of the important screening detection genes for the transgenic plants (the sequence information was illustrated in Yang et al. (2009)), was also detected through ERGNO/PANI film via EIS as an example (shown in Supplied materials, Fig. S1). The successful immobilization induced the increase of R_{et} value (Fig. S1, curve a) compared with that of ERGNO/PANI/GCE (Scheme 1, curve a in Nyquist diagram). The hybridization brought shrinking of R_{et} value (Fig. S1, curve b). This result proved the prepared DNA sensing platform could freely detect the different target genes just like the optical sensors reported in previous works (Liu et al., 2011; Wang et al., 2011b).

4. Conclusions

In summary, an impedimetric DNA sensor was fabricated in this paper, which integrated the individual advantage of ERGNO and PANI for the freely switchable detection of DNA sequences. A freely switchable detection mode for the different DNA target sequences was realized due to the hybridization product dsDNA releasing from the sensing surface. The fabricated biosensor exhibited excellent performance for the detection of PML/RARA 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 by EIS. The PAT gene sequence can also be detected by this impedimetric DNA sensor.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/0.1016/j.bios.2012.11.007.

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