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Simple and label-free electrochemical assay for signal-on DNA hybridization directly at undecorated graphene oxide

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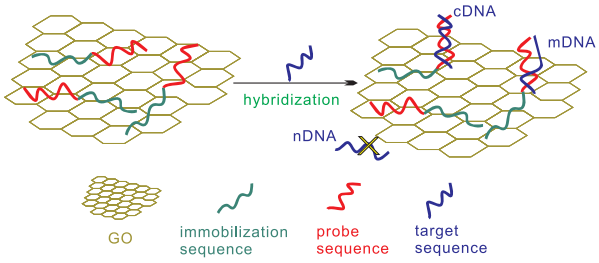
HIGHLIGHTS

- A strategy developed for DNA detection with no need to decorate GO or label DNA.
- Specially designed ssDNA consists of immobilization part and probe part.
- Hybridization leads to 'lying' ssDNA to 'standing' dsDNA.
- Conformational and negative charge changes induce signal-on impedimetric result.
- Potential applications in DNA nanostructure frameworks and nanoelectronics.

GRAPHICAL ABSTRACT



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ABSTRACT

Exploring graphene oxide (GO), DNA hybridization detection usually relies on either GO decoration or DNA sequences labeling. The former endows GO with desired chemical, optical, and biological properties. The latter adopts labeled molecules to indicate hybridization. In the present work, we propose a simple, label-free DNA assay using undecorated GO directly as the sensing platform. GO is anchored on diazonium functionalized electrode through electrostatic attraction, hydrogen bonding or epoxy ring-opening. The π–π stacking interaction between hexagonal cells of GO and DNA base rings facilitates DNA immobilization. The adsorbed DNA sequence is specially designed with two parts, including immobilization sequence and probe sequence. In the absence of target, the two sequences lie nearly flat on GO platform. In the presence of target, probe hybridizes with it to form double helix DNA, which 'stands' on GO. While the immobilization sequence part remains 'lying' on GO surface. Hence, DNA hybridization induces GO interfacial property changes, including negative charge and conformational transition from 'lying' ssDNA to 'standing' dsDNA. These changes are monitored by electrochemical impedance spectroscopy and adopted as the analytical signal. This strategy eliminates the requirement for GO decoration or DNA labeling, representing a comparatively simple and effective way. Finally, the principle is applied to the detection of conserved sequence of the human immunodeficiency virus 1 pol gene fragment. The dynamic detection range is from 1.0×10^{-12} to 1.0×10^{-6} M with detection limit of 1.1×10^{-13} M with 3σ. And the sequences with double- or four-base mismatched are readily distinguishable. In addition, this strategy may hold great promise for potential applications from DNA biosensing to nanostructure framework construction based on the versatile DNA self-assembly.

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1. Introduction

Nucleic acid detection is highly significant in a number of areas related to human health such as diagnosis of infectious diseases, genetic mutations and clinical medicines. The detection needs a hybridization event between probe sequence and target sequence based on the nucleobases matching. The process is transduced to either fluorescent or electrochemical signals through opto- or electroactive tags that are either covalently or noncovalently (*via* intercalation) attached to nucleic acids [1]. The label-free manners record conformational or interfacial property changes of recognition layer induced by hybridization. Various nanomaterials have been employed as signal transducer, on which nucleic acids are immobilized. These substrates possess high surface area and good conductivity, providing more binding sites and surface-enhanced charge transfer for enhanced selectivity and sensitivity [2]. For instance, GaN nanowire [2], carbon nanotubes [3–6], quantum dots [7], conducting polymers [8,9], gold nanoparticles and wire [10,11], etc. have been explored in the detection system with a wide range of techniques, including electrochemistry or electronics, chemiluminescence, photoluminescence, fluorescence, etc.

Since its discovery in 2004 [12], graphene has led to an explosion of interest. It is a single-atom-thick and two-dimensional sp^2 carbon networking material with remarkable electronic, mechanical and thermal properties [13,14]. It finds potential applications in nanoelectronics, sensors, nanocomposites, batteries, supercapacitors and hydrogen storage [15]. Besides, the high specific surface area of 2630 $m^2\ g^{-1}$ enables it to afford an ultrahigh loading capacity for biomolecules and drugs [16,17]. Particularly, graphene oxide (GO), a precursor for graphene, holds favorable features of electronics, π–π stacking interaction, and fluorescence quenching ability [18–20]. Facile synthesis, high water dispersibility, tunable surface functionalization, and good biocompatibility contribute to its biological applications [15,21].

In the field of DNA biosensing, GO takes up an important position. Researches elucidate that single-stranded DNA (ssDNA) could be stably adsorbed on GO sheet, with nucleobases lying nearly flat on the surface [22]. This behavior was mainly based on π–π stacking interaction between the rings in ssDNA nucleobases and the hexagonal cells of GO. This interaction was also the root cause of ssDNA binding more strongly than double-stranded DNA (dsDNA) to the sheet [23]. According to this interaction mechanism and GO's quenching ability, a type of fluorescent DNA bioassay has been constructed [20,24–26]. Though it is sensitive and selective, this strategy needs oligonucleotides to be fluorophore labeled. Without label, electrochemical impedance spectroscopy (EIS) is often adopted to monitor GO interfacial property changes upon DNA immobilization and hybridization [27–29]. For example, coupling graphene, hairpin-shaped DNA has been successfully used for single nucleotide polymorphism detection [30,31]. After hybridization, dsDNA detached from graphene surface, generating an impedimetric signal drop. To keep double helix on the platform and gain signal-on result, post-isolational modifications for graphene are usually required to introduce desired functional groups, thereby increasing the number of manipulations. In our previous work, perylene derivatives have been adopted to decorate graphene [19,32,33]. Epitaxial graphene with carboxyl group is also prepared [34], however the fabrication procedure is complicated.

Herein, excluding DNA label and GO decoration, a simple, signal-on impedimetric DNA bioassay is proposed. GO platform was first modified on diazonium functionalized glassy carbon electrode (GCE) through electrostatic attraction, hydrogen bonding or epoxy ring-opening. The adsorbed ssDNA, consisting of immobilization sequence part (polyA) and probe sequence part, laid on GO surface by π–π stacking interaction. In the presence of target, probe hybridized with it and 'standing' dsDNA was formed. While, the immobilization sequence part still laid on GO (Scheme 1). Hence, DNA hybridization induced GO interfacial property changes. These changes were monitored by EIS and the signal was used to indicate hybridization action.

2. Experimental details

2.1. Chemical reagents

Graphite powders (320 mesh) and methylene blue (MB, 98.5%) were of spectroscopically pure and obtained from Shanghai Chemicals, China. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Sigma–Aldrich, USA. Sodium dodecylsulfate (SDS) was purchased from Shanghai Reagent Company, China. *p*-Phenylenediamine (H_2N-Ph-NH_2, 97%) was obtained from Alfa-Aesar. Other reagents were analytical grade and used as received. All aqueous solutions were prepared with ultra-pure water (>18 MΩ) from a Milli-Q Plus system (Millipore).

The 38-base synthetic sequence (ssDNA), its target (conserved sequence of the human immunodeficiency virus 1 (HIV-1) pol gene fragment, cDNA), double-base mismatched, four-base mismatched and non-complementary DNA were all synthesized in Sangon Biotechnology Inc. (Shanghai, China). Their base sequences were as follows: ssDNA: 5′-$(A)_{18}$ GCT TGC CAA TGA TCT GTC CA-3′; cDNA: 5′-TGG ACA GAT CAT TGG CAA GC-3′; double-base mismatched DNA: 5′-TGG ACA AAT CAT CGG CAA GC-3′; four-base mismatched DNA: 5′-TGT ACA AAT CAT CGG CAG GC-3′; non-complementary DNA: 5′-CAT CTC ATG GCC GAT TCG TG-3′. All oligonucleotide stock solutions (1.0×10^{-4} M) were prepared by using Tris–HCl solution (pH 7.0), which were stored at 4 °C. More diluted solutions were obtained by diluting an aliquot of the stock solution with ultra-pure water. The hybridization solution was diluted with 2× SSC (pH 7.0), which consisted of NaCl (0.30 M) and sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$; 0.030 M).

2.2. Instruments

Fourier transform infrared (FTIR) spectrum was recorded using a Bruker Tensor 27 Spectrometer. Transmission electron microscopy (TEM) was conducted using a JEOL 2000 transmission electron microscope operating at 200 kV. X-ray photoelectron spectrum (XPS) was carried out on an ESCALAB MK II X-ray photoelectron spectrometer. UV–vis absorption spectra were recorded using a Hitachi U-3900 spectrophotometer. Zeta potentials were measured by dynamic light scattering (Malvern Nano-ZS, U.K.). EIS measurements were carried out with Solartron 1255B frequency response analyzer (Solartron Inc., UK). Cyclic voltammetry (CV) was performed using a conventional three-electrode cell with a platinum wire as the auxiliary electrode and an Ag|AgCl (saturated KCl) as reference in a CHI 660 Electrochemical Workstation (CHI). Working electrodes were bare or modified GCEs (d = 3 mm). Before use, GCEs were carefully polished to a mirror finish with 1.0, 0.3 and 0.05 μm alumina slurries, successively.

2.3. GO fabrication and modification on diazonium functionalized GCE

GO was prepared by oxidizing natural graphite powder based on a modified Hummers' method [35,36]. As-prepared GO was suspended in ultra-pure water to obtain a brown dispersion, which was then subjected to dialysis to completely remove residual salts and acids for 4 days. The resulting purified GO was collected by centrifugation and air-dried. GO powders were dispersed in ultra-pure water to create a 0.05 wt% dispersion. The dispersion was exfoliated through ultrasonication for 1 h, where the bulk GO powders were transformed into GO nanoplatelets. Then 2% Na_2CO_3 was added