Facile fabrication of DNA biosensors based on oxidized carbon black and graphite oxide

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Abstract

We investigated electrochemical sensors based on graphite oxide (GrO) and oxidized carbon black (CbO). GrO and CbO were synthesized by the modified Hummers method. Single-stranded DNA (ssDNA) probes were synthesized with a 5' primary amine for attachment. These ssDNA oligonucleotides were immobilized on GrO and CbO using standard 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) coupling. This formed an amide bond between DNA-amine and carboxyl groups on GrO and CbO. GrO and CbO were used instead of graphite in a carbon paste material. This significantly enhanced the sensitivity of the biosensor for the reverse-complementary DNA. We detected reverse-complimentary DNA using Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) in a ferricyanide solution. The solution was spiked with the ssDNA oligonucleotide with the reverse-complementary sequence of the immobilized probe. The change in current or impedance was measured. We present early work on optimizing the fabrication method for DNA-functionalized carbon electrodes. Working electrodes were fabgvb ricated by drop-casting the active material onto a glassy carbon electrode surface.

Keywords: Biosensors, EDC coupling, Carbon black, Graphite oxide

Introduction

Biosensors can detect diverse analytes. We want to detect nucleic acids from pathogens. If our sensor is sensitive, we can detect contamination in food or drugs. Electrochemical biosensors have been demonstrated by many groups for this purpose. Electrochemical biosensors are inexpensive, sensitive, simple, and rapid[1-3]. Label-free, electrochemical DNA detection is especially attractive as it does not require chemically modified probes[4]. However, DNA probes for electrochemical detection must be conjugated to the electrode surface. This can be accomplished by covalent attachment to carbon or by a gold-thiol link. Most electrochemical methods use gold electrodes and thiol attachment chemistry. We demonstrate an alternative with several advantages [5-6]. We show that DNA can be conjugated to specific forms of carbon by well-known EDC coupling. The disadvantages of gold-thiol chemistry include a narrow potential window and high cost. Additionally, the surface of bare gold must be pristine for good functionalization. This can lead to irreproducible results. This can be overcome with careful technique but represents a significant barrier to entry.

Carbon electrodes are an attractive alternative. Carbon has a wide potential window, high electron transfer, electrical conductivity, low background current, and affordability[7]. Graphite oxide and carbon black oxide are widely used in electrochemical biosensors due to the high conductivity at room temperature, high surface area, thermal stability, and low cost [8-11]. These materials can be drop cast onto an existing conductive surface such as a glassy carbon rod. Glassy carbon is used for its corrosion resistance and homogenous surface[12]. Different methods have been studied to anchor DNA strands. Other labs have attached DNA by adsorption, entrapment in a polymer matrix, and electrografting[13-15]. In this study, we show that DNA can be directly conjugated to conductive carbon particles. The particles can be formed into an electrode by drop-casting in Nafion. We demonstrate the effectiveness of this approach by detecting reverse-complementary DNA using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

2. Experimental process

2.1. The Materials and methods section

The DNA oligonucleotides used in this paper were synthesized by Integrated DNA technologies (IDT, Coralville, Iowa, USA). The sequences of theoligonucleotides were: Probe DNA (ssDNA): /5AmMC6/TTG AGG AGG AGG AGG AGA GGC GGG TTG AGG and complementary DNA (dsDNA): 5`-TCT CCT CCT CCT CCT CTT TTC TGA ATA AGA-3`. 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), was purchased from Sigma Aldrich Co. All other reagents were of analytical reagent grade. All of the solutions were prepared with Millipore deionized water.

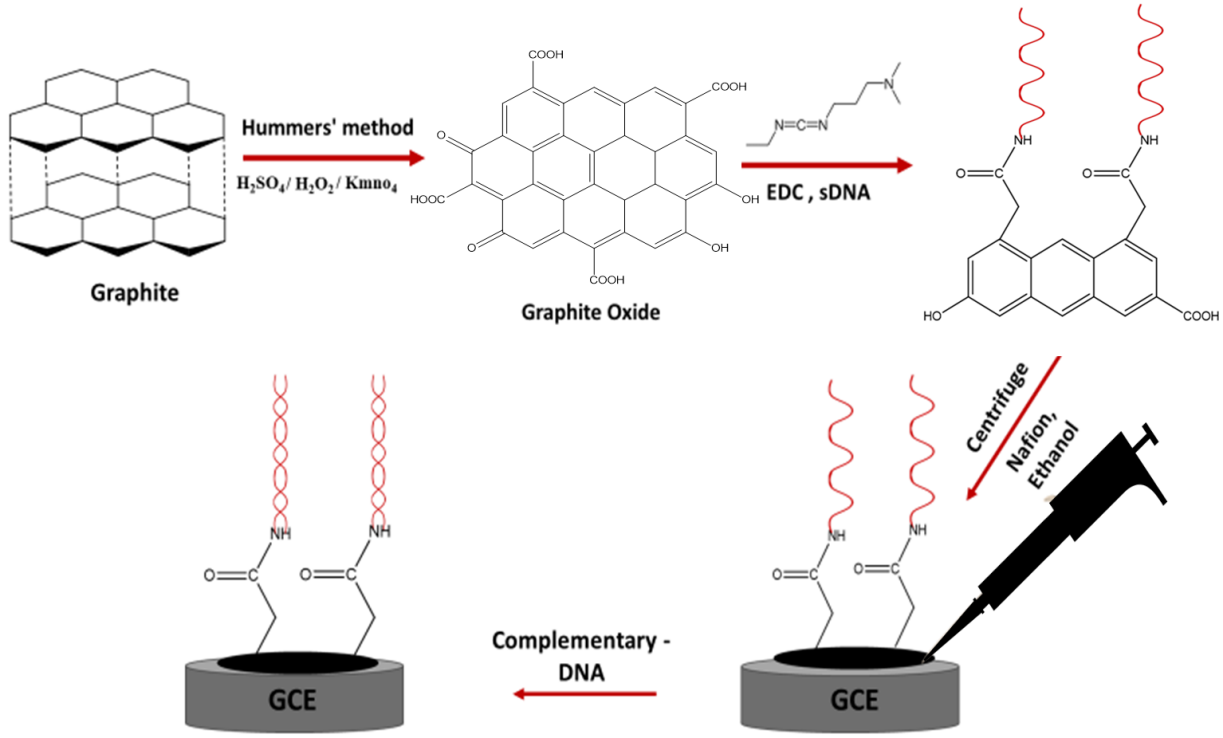
2.2 Synthesis of graphite oxide and carbon black oxide

GrO and CbO were prepared from graphite powder and carbon black powder, respectively, by following the Hammer's method. Firstly, 0.5 g of $NaNO_3$ and 3 g of graphite powder were dissolved in 25 mL concentrated 98-99% sulfuric acid. Then the mixture was stirred for 3 h in an ice bath. 3 g of $KMnO_4$ as a potent oxidizing agent was slowly added to the mixture at room temperature. The mixture was stirred for 12 h. The oxidation reaction was interrupted by the addition of 20 ml 30 % H_2O_2 solution. Immediately after the addition of 30 % hydrogen peroxide, the color of the mixture changes to pale yellow. Subsequently, 120 mL of purified water was added to the solution. The resultant product was repeatedly centrifuged with H2O2 to adjust the pH 4. The final product was placed in an oven (60 ºC) for 12 h. Synthesis of CbO proceeded in the same manner[16-17].

2.3 Covalently immobilization of DNA

Single standard DNA was covalently attached to the carboxyl group of CbO and GrO surface through EDC coupling. The GCE electrode was polished with 0.3 mm γ-Al_2O_3., then sonicated in deionized water about 30 s and then rinsed with deionized water. For immobilization of the oligonucleotide, 5 mg oxidized graphite and carbon black were suspended in a 1 M MES (pH 4.5) containing 0.1 M EDC. Amine-modified ssDNA was added to the suspension (1 μM final conentration) and vortexed for 2 hr at room temperature. The suspension was then washed with PBS buffer (pH 7.4) by centrifugation and resuspension. Finally, the 50

μL of ethanol, 25μL 5% Nafion, and 50μL DI water were added. The mixture was sonicated for 1 h. Then, a droplet of 2.5 μL of the nafion-carbon-DNA suspension was applied to the surface of the GCE. For hybridization, 4μL of complementary oligonucleotide was mixed in 200 μL of 20 mM NaCl in PBS buffer solution (contains 137 mM sodium chloride ,2.7 mM potassium chloride, and 10 mM Phosphate Buffer pH: 7.4) and applied to the ssDNA modified GrO/CbO. After 20 minutes, the surfaces was extensively washed for 20 minutes. Finally, the electrode was dried at room temperature at least for 3 hours. Scheme 1 shows the conjugation of a ssDNA onto the GrO surface and hybridization with a complementary target.



Scheme 1. The immobilization of a ssDNA onto the GrO surface and hybridization with a complementary target

2.4 Electrochemical detection

A conventional three-electrode system (Pine WaveDriver Galvanostat, Pine insturments, Durham, NC, USA) was used for electrochemical characterization with a modified GrO/CbO coated glassy carbon as working electrode, saturated Ag/AgCl (3.5 M KCl) as a reference electrode and a Pt as a counter electrode in 0.1 M KCl solution containing 6 mM of $K_3Fe(CN)_6$. All measurements were performed at room temperature. Cyclic voltammetry and electrochemical impedance spectroscopy of the redox probe were performed to detect reverse-complimentary DNA. Cyclic voltammetry measurements were carried out between -0.4 V and 0.6 V. Scan rate of these measurements are 100 mV s^{-1}. EIS was performed to monitor the whole procedure in the modification of the electrodes. Impedance measurements were carried out between 1 mHz and 1 Hz applying AC amplitude of 25 mV. The electrolyte for impedance measurement was 1 M KCl containing 6 mM potassium ferricyanide.

3. Result and Discussion:

Figure 1 presents the performance of CbO and GrO electrodes conjugated to DNA. Single standard DNA (ssDNA) was immobilized on the surface through the formation of covalent amide bonds between the amino groups of the oligonucleotides and carboxyl groups on the GrO and CbO. In Figure 1.B, the anodic and cathodic current at 180 mV are the oxidation and reduction of iron cyanide in solution. The electron transfer (and therefore, the current) is reduced when the electrode is conjugated to DNA (Figure 1A.II). The current is further reduced when complementary DNA is added (Figure 1.A.III). Figure 1.C shows the performance of the CbO electrode conjugated to DNA. Conjugation CbO to DNA also caused a decrease in charge transfer and a reduced current. We attribure the reductions in current to the electrostatic repulsion between DNA and ferricyanide. This increases after addition of complementary DNA.

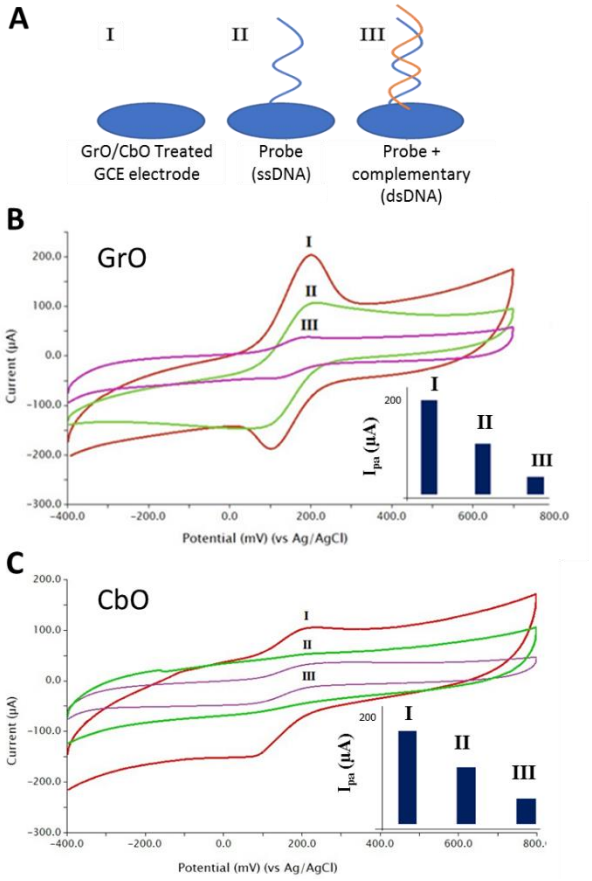


Fig. 1. DNA conjugation detected with Cyclic Voltammetry. (A) A schematic shows (I) the no-DNA control, (II) ssDNA-coated electrode and and (III) dsDNA-coated electrode. (B) Cyclic voltammograms of the GrO-modified electroeds recorded in 10 mM potassium ferricyanide [$K_3Fe(CN)_6$] in 1 M potassium nitrate at a scan rate of 0.1 Vs^{-1}, in the potential range between -0.4 to +0.7 V A. for the (I) GrO electrode, (II) ssDNA-GrO electrode, and (III) dsDNA-GrO electrode. (C) Equivalent cyclic voltammograms for (I) CbO electrode, (II) ssDNA-CbO electrode, and (III) dsDNA-CbO electrode. Insets show the baseline-corrected peak anodic current for each sample.

We interpret the greater reduction in current on GrO (as compared to CbO) to two causes: lower resistance and more efficient functionalization. GrO shows higher efficiency of charge transfer from GrO to ferricyanide. The current for unmodified GrO is much higer than unmodified CbO. High performance of charge transfer on GrO is due to the regular structure of graphite (which reduces resistance). GrO also shows better functionalization.

GrO consists of graphene oxide sheets and displays oxygen-containing functional groups on the surface (alcohols, quinones, and carboxylic acids). At consequence, GrO has a high potential to create a covalent bond between the oxygen and amine [18-20].

Nyquist plots of the EIS spectra of CbO/GrO electrodes in a 1M KCl aqueous solution containing 6 mM $[K_3Fe(CN)_6]$ are shown in Figure 2. The bare GrO/CbO electrodes had a small AC impedance, indicating that the electron transfer between the electrode and electrolyte was fast (Figure 1B/1C). The AC impedance increased after the GrO/CbO were conjugated to the DNA. This suggested more resistance to electron transfer between the electrode and the ferricyanide. The resistance is further increased when complementary DNA is added. EIS corroborated the CV results and indicates that the GrO has lower resistance to electron transfer compared to the CbO electrode.

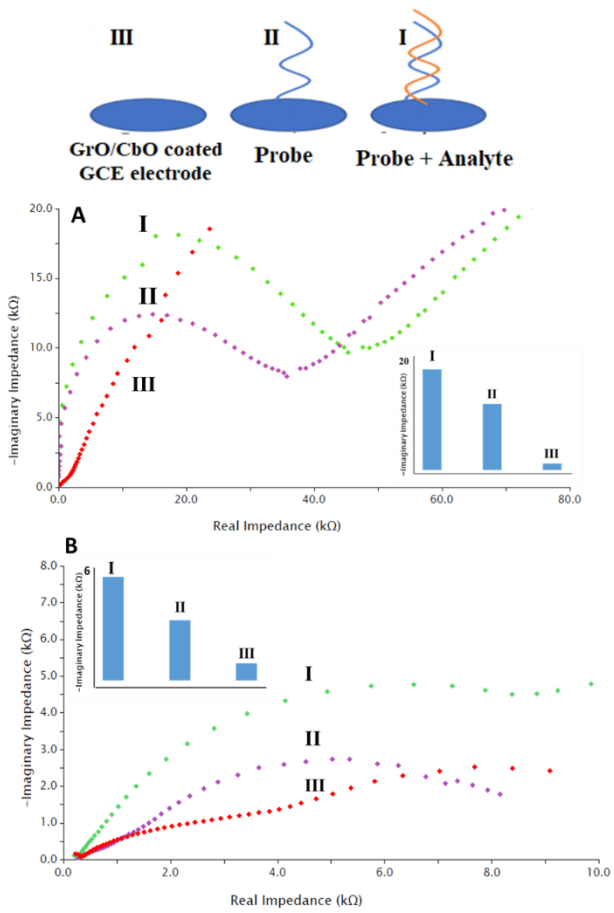


Figure 2. DNA conjugation detected with Electrochemical Impedance Spectroscopy (EIS). (A) A schematic shows (I) the no-DNA control, (II) ssDNA-coated electrode and and (III) dsDNA-coated electrode. (B) Nyquest plots of the EIS spectra recorded in 10 mM potassium ferricyanide $[K_3Fe(CN)_6]$ in 1 M potassium nitrate at frequency between 1 MHz and 1 Hz and AC amplitude is 25 Mv for (I) GrO electrode, (II) ssDNA-GrO electrode, and (III) dsDNA-GrO electrode. (C) Equivalent Nyquest plots for the EIS spectrum of (I) CbO electrode, (II) ssDNA-CbO electrode, and (III) dsDNA-CbO electrode.

6. Conclusion:

This study has introduced a method for conjugating amine-modified DNA to carbon black oxide and graphene oxide. We showed that graphene oxide and carbon black oxide bind DNA and this prevents charge

transfer to ferricyanide. This phenomenon allowed to detect reverse-complementary DNA. Reduced peak current was attributed to the complementary DNA. This is a label-free biosensor: it requiring no indicators or labels on the DNA. We show that graphite oxide is superior in performance to carbon black oxide. We attribute the higher performance to the ordered structure and lower resistance in graphite oxide compared to the more disordered, paracrystalline structure of carbon black [21]. This conjugation method is simple and rapid for generating DNA sensors.

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