CHEMISTRY OF MATERIALS

Communication

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Detection of Biomolecules via Benign Surface Modification of Graphene

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Supporting Information

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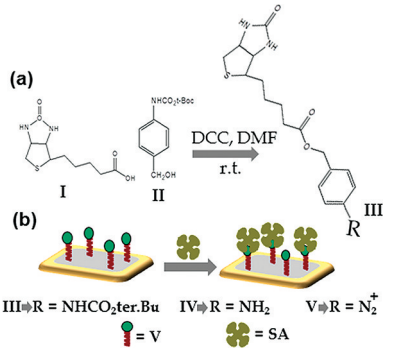
Graphene, since its isolation in 2004,[1,2] has attracted a tremendous amount of interest because of its unique chemical and physical properties.[3,4] Recent advances in large-area growth and isolation of graphene has established it as a promising candidate for several potential applications,[5−7] such as a platform for biosensing.[8] Adding functional groups in a controlled manner is a critical first step in developing biosensors that exploit graphene's unique properties. There are several reports on graphene functionalization.[9−12] Graphene oxide and exfoliated graphene are also widely used as a platform for sensing.[13−19] However, CVD graphene is a more desirable platform because of the ease of fabrication, scalability, and its inherent low cost. In addition, this method allows for transfer of graphene to any desired substrate.

The key results in this work include the modification of single layer graphene (grown via CVD) by assembly of a biotin moiety that is functionalized with a diazonium salt (compound V, Scheme 1a) and the subsequent detection of Streptavidin

Scheme 1. (a) Synthetic Scheme for Compound V; (b) Cartoon Illustrating the Biotinylated Graphene and the Selective Adsorption of Streptavidin Used in This Study



using a variety of optical techniques. Previous reports show that diazonium salts react with graphene via charge-transfer complexation, with very little covalent functionalization.[11] The compound merely shifts the Dirac point, decreasing the sheet resistance as a result of charge transfer doping. The sheet resistance decreases by a factor of 2 (from 1200 Ohm/sq to ~600 Ohm/sq) upon exposure of graphene to compound V. This mechanism is a critical advantage of this chemistry, as the optical and electronic properties are retained after modification. Compound IV was designed and synthesized (Scheme 1) where the amine end group can be diazotized and assembled on the graphene surface. Condensation of commercially available N-Boc-4-aminobenzyl alcohol (II) with Biotin(I) in the presence of dicylohexylcarbodiimide gave N-protected aminobenzyl ester of Biotin(III) in 60% yield after purification as white needles. Compound III was deprotected by stirring a solution of III in dichloromethane containing 5 equivalents of trifluoroacetic acid. Evaporation of the solvent followed by treatment with sodium bicarbonate afforded the arylamine (IV) as a light yellow powder, which was used in the next steps without further purification. The synthetic route shown here is simple and generalizable to virtually any desired sensing molecules. Characterization of the synthesized compounds is shown in the Supporting Information.

Graphene was prepared via CVD[5] using a copper foil annealed at 975 °C in 6 sccm forming gas (5% H2 in Ar) at a pressure of 500 mTorr for 10 min, followed by flowing ethylene for 10 min at the same temperature. The transfer method is described elsewhere.[20] The graphene was transferred to the desired substrate and annealed at 600 °C in vacuum for 10 min. The annealing aids in cleaning the graphene surface by removing any traces of PMMA remaining from the transfer procedure. The surface modification is achieved by the reaction of V with the graphene surface. For the surface modification, 10 mg of compound IV was suspended in 10 mL of acetonitrile mixed with 10 mg of nitrozonium tetrafluoroborate. The graphene was then functionalized with compound V by exposing a solution of V to the graphene, followed by washing with copious amounts of ethanol and water.

Compound V reacts with graphene via charge-transfer complex formation were an electron is transferred from graphene to compound V. The biotinylated graphene is then used to sense streptavidin (SA) in solution. SA was bound to the surface

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by incubating the modified graphene for 15 min, at room temperature, in solution of 1 μM SA in PBS.

Several techniques were used to characterize the system described here. X-ray photoelectron spectroscopy (XPS) and Raman spectroscopy measurements are shown in Figure 1. XPS

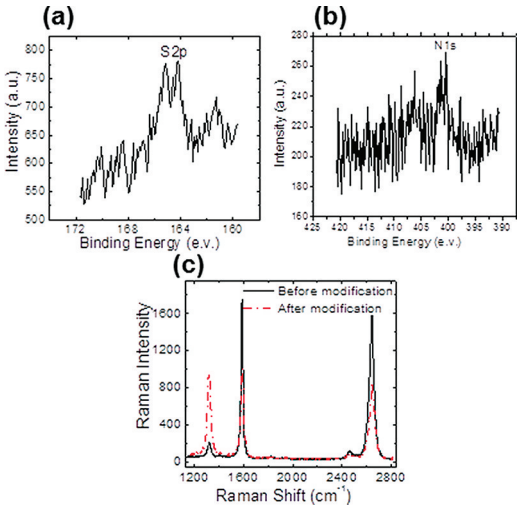

Figure 1. (a) X-ray photoelectron spectra of the biotinylated graphene showing an S2p doublet peak at 164.3 and 165.5 eV corresponding to the sulfur atom in the compound V. (b) XPS spectra showing the N1s peak at 401.3 eV corresponding to the Nitrogen atom in the Biotin. (c) Raman spectra of the graphene before and after biotinylation illustrating the increase in the D/G ratio.

was performed using a monochromatic source, Al Kα ($h\upsilon$ = 1486.6 e.v), and a photoelectron takeoff angle of 40°. The S2p photoelectron spectrum, consisting of a doublet peak at 164.3 and 165.5 eV, is shown upon functionalization of graphene with compound V. This is consistent with the presence of the sulfur atom in the biotin moiety.[21] A low intensity peak in the N1s photoelectron spectrum that is consistent with previous reports on the reaction of diazonium salts with graphene is also observed (Figure 1b).[22]

Raman spectroscopy measurements were performed using a Horiba ARAMIS Raman System with a HeNe laser source. The Raman spectra of single-layer graphene has three key characteristic peaks, the G peak, which his due to the vibrational mode of sp2 bonded carbon; a 2D peak; and the D band, which is induced by "defects" in the structure. Figure 1c contains spectra of the graphene film before and after modification with V. The key difference between the two spectra is the dramatic increase in the ratio of the D/G after modification with V. The D/G ratio increases dramatically after modification with compound V. This is in agreement with studies where donor and acceptor molecules are intercalated in few layer graphene sheets.[23] The charge transfer complex and resultant doping leads to an increase in the D/G ratio. Although no deterioration of the electrical properties is observed upon reaction with compound V, there may be some conversion of sp2 to sp3 carbon, which contributes to the D band, but it is not substantial enough to affect transport in these large area films as evidenced by the decrease in sheet resistance following modification.[11]

The surface modification was further examined via real time measurements of compound V binding to graphene utilizing surface plasmon resonance (SPR) spectroscopy. SPR is one of the most powerful techniques used for the detection of biomolecules on surfaces.[24,25] Surface plasmons are collective oscillations of the quasi free electron gas at the interface between a metal and a dielectric. Absorption of molecules on the metal surface leads to a change in the refractive index and, consequently, a shift in the dispersion of the plasmon wavevector. This shift is used to extract information about the thickness and/or the surface density of the absorbed monolayer. In this work, a single layer of graphene, of the size 1 cm × 1 cm, was transferred to a 2 nm/50 nm chromium/gold film deposited on a glass coverslip. The sample was mounted on the SPR set up in a Kretschman configuration as described previously.[26−28] Compound V was reacted with the surface, in situ, using a 300 μL volume PDMS flow cell attached to the substrate. The binding was monitored in real time by measuring the change in the reflectivity of the gold substrate as a function of time. The difference between the reflectivity signal before binding and after washing with ethanol and water indicates the degree of modification (Figure 2a). The binding of Streptavidin

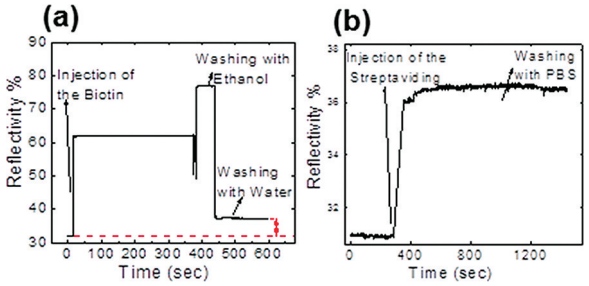

Figure 2. Surface plasmon resonance spectra showing real time binding of V with graphene. The difference between the baseline and the signal after washing indicates the binding of compound V. (b) SPR showing real-time binding of streptavidin to the biotinylated graphene. Streptavidin remains on the surface even after washing with PBS buffer.

to the modified surface was also monitored by SPR. The biotinylated graphene substrate was exposed to a 1 μM SA solution. The real time measurements of SA binding are shown in Figure 2b. Upon washing with PBS, there is no change in the reflectivity signal due to the high affinity between Biotin and the SA. Reflectivity scans were performed after each binding step. Fitting of the reflectivity scans gave a SA surface density of 1.184 ng/mm^2. Scanning electron microscopy (SEM) imaging was used to determine the concentration dependence on SA binding. This was done by modifying the graphene substrate with the same concentration of compound V (40 mg/10 mL). The biotinylated graphene was then exposed to differing concentrations of Streptavidin-coated magnetic beads (Mitenyi Biotecestimated concentration 1012 beads/mL) for 15 min. Figure 3a is a plot of the number of beads as a function of their concentration in solution, clearly indicating a concentration dependence. The reaction begins to saturate at concentration above 10%.

One of the most critical issues in developing viable biosensors is preventing nonspecific binding of the biomolecules. Any nonspecific binding can contribute to the detected signal and give false positives. In our system, the graphene surface exhibited no detectable nonspecific binding when the unmodified graphene is exposed to streptavidin. This was shown by incubating an unmodified and a biotinylated graphene layers in a solution of the SA-modified magnetic beads. The comparison is

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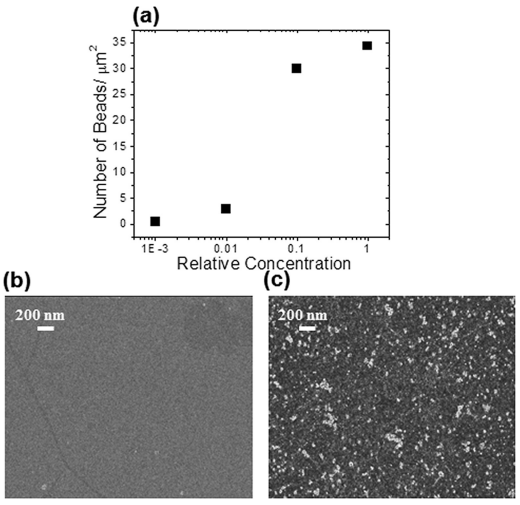

Figure 3. (a) Plot of the concentration of SA beads in solution versus the number of beads adsorbed to the surface of the biotinylated graphene as determined by SEM imaging. The plot shows a clear concentration dependence. (b) SEM image of bare graphene after incubation with the SA-beads, followed by washing with water. This illustrates a lack of nonspecific binding of Streptavidin. (c) SEM image of the biotinylated graphene after incubation with the SA beads followed by washing with water.

shown in Figure 3b where the unmodified graphene shows no adsorption while the biotinylated graphene is clearly modified with the beads (Figure 3c). This indicates that graphene may not need to be passivation when used as a biosensor. Although one should not generalize to all biomolecules, this could provide a distinct advantage over traditional platforms such as gold films where nonspecific adsorption is difficult to prevent.

In summary, a compound was designed and synthesized to biotinylate graphene using a diazonium salt functionality. The surface modification was verified using XPS, Raman spectroscopy, SPR spectroscopy and SEM imaging. Importantly, the chemistry is benign and does not destroy graphene's unique electrical and optical properties. The biotinylated graphene was shown to effectively sense Streptavidin in solution and the adsorption was measured in real-time using surface plasmon resonance spectroscopy. One important observation is that the unmodified graphene surface does not allow nonspecific binding as demonstrated in our system. This provides a distinct advantage of using graphene as a sensing platform, as compared to traditional substrates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org/

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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