Biosensors

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A Graphene Platform for Sensing Biomolecules**

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Sensitive, selective, rapid, and cost-effective analysis of biomolecules is important in clinical diagnostics and treatment. Carbon nanostructures, such as carbon nanotubes,[1] carbon nanodots,[2] and carbon nanofibers[3] have been used for this purpose. Most recently, carbon nanotubes and single-stranded DNA (ssDNA) assembly have been used by Tan and co-workers for homogeneous detection of biomolecules.[4] Graphene is a single-atom-thick and two-dimensional carbon material that has attracted great attention because of its remarkable electronic, mechanical, and thermal properties.[5] The synthesis and investigation ofthe material properties of these stiff materials have been undertaken, although little has been done to explore graphene in the analysis of biomolecules.[6] Herein we demonstrate the ability of water-soluble graphene oxide (GO) as a platform for the sensitive and selective detection of DNA and proteins.

Figure 1 shows a schematic representation of this new detection platform. As strong noncovalent binding of GO with nucleobases and aromatic compounds were reported,[7] we anticipated that GO could bind dye-labeled ssDNA and completely quench the fluorescence of the dye (step a in Figure 1). In the presence of a target, the binding between the dye-labeled DNA and target molecule will alter the conformation of dye-labeled DNA, and disturb the interaction between the dye-labeled DNA and GO. Such interactions will release the dye-labeled DNA from the GO, resulting in restoration of dye fluorescence (step b in Figure 1). This design could result in a fluorescence-enhanced detection that is sensitive and selective to the target molecule.

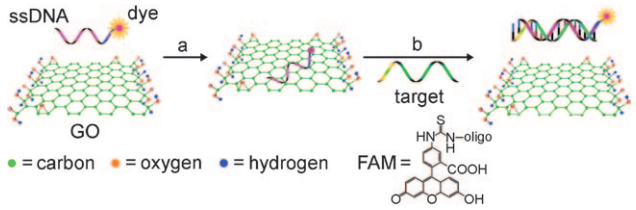

Figure 1. Schematic representation of the target-induced fluorescence change of the ssDNA-FAM-GO complex (see text for details). FAM is the fluorescein-based fluorescent dye.

To realize our design, we first examined the GO platform for the detection of DNA. Oligonucleotide sequences are as follows: the dye-labeled ssDNA sequence P1 is 5′-AGT CAG TGT GGA AAA TCT CTA GC-FAM-3′ (FAM = fluorescein-based dye), the complementary target being HIV1 (5′-GCT AGA GAT TTT CCA CAC TGA CT-3′, from the HIV-1 U5 long terminal repeat sequence), and the mismatch sequence MHIV1 is 5′-GCT AGA GAT TGT CCA CAC TGA CT-3′ (mismatch underlined). We used atomic force microscopy (AFM) to characterize the GO and P1-GO complex. Figure 2 (left) is the AFM image of GO prepared

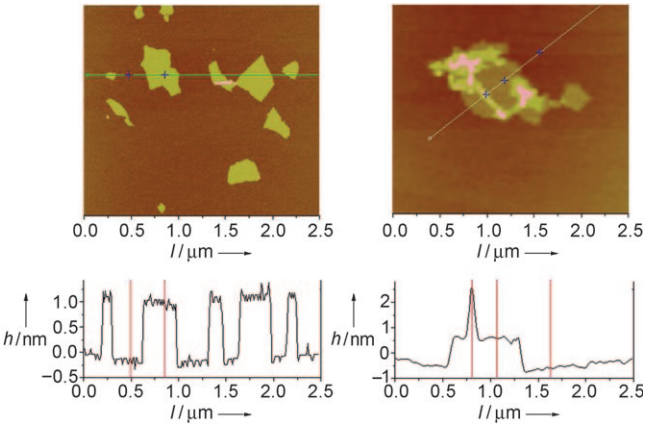

Figure 2. AFM height image of GO sheets deposited on mica substrates (left); AFM height image of DNA-GO complex (right).

according to Hummers method,[8] the thickness of GO being about 1.2 nm. Figure 2 (right) shows the typical AFM image of the P1-GO complex, where the white areas on the GO surface might be due to the presence of DNA. As anisotropy measurements are commonly used to probe molecular interactions, the fluorescence anisotropy of P1 and the P1-GO complex was measured for evidence of the adsorption of ssDNA on GO. In the absence of GO, the fluorescence anisotropy of free P1 is 0.06, whereas that for the P1-GO complex is 0.158, indicating that the ssDNA is adsorbed on GO.

Figure 3 shows the fluorescence emission spectra of P1 at different conditions. The fluorescence spectrum of P1 (in Tris-HCl buffer) and in the absence of GO shows strong fluorescence emission owing to the presence of the fluorescein-based dye. However, in the presence of GO, up to 97% quenching of the fluorescence emission was observed

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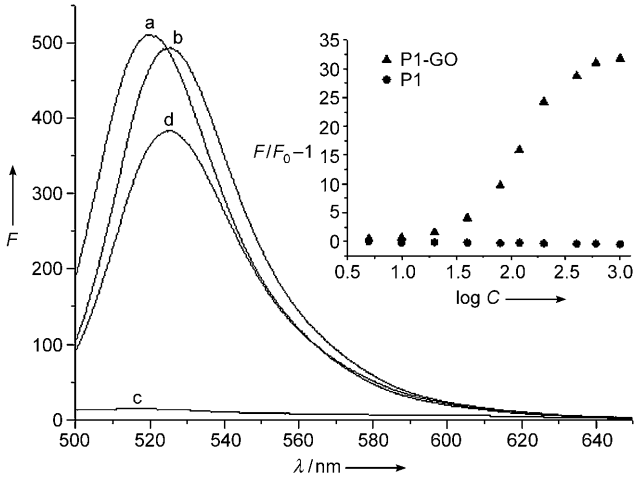

Figure 3. Fluorescence emission spectra (excitation at 480 nm) of P1 (50 nM) at different conditions: a) P1 in Tris-HCl buffer; b) P1 + (300 nM) HIV1; c) P1 + GO; and d) P1 + GO + (300 nM) HIV1. Inset: fluorescence intensity ratio of P1 (●) and P1-GO (▲) with (F/F_0-1) plotted against the logarithm of the concentration (C) of HIV1. Excitation: 480 nm, emission: 525 nm.

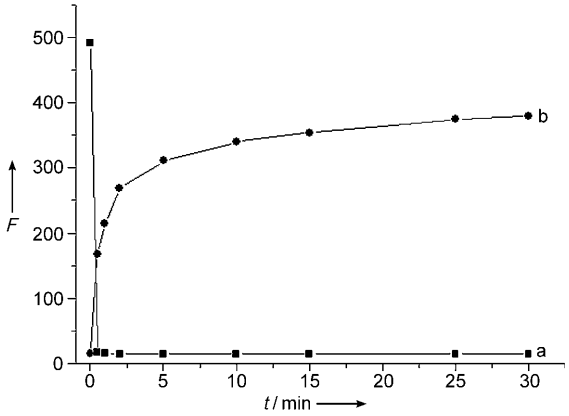

Figure 4. a) Fluorescence quenching of P1 (50 nM) in Tris-HCl buffer by GO as a function of time, and b) Fluorescence restoration of P1-GO in Tris-HCl buffer by HIV1 (300 nM) as a function of time. Excitation: 480 nm, and emission: 525 nm.

(Figure 3, curve c). This observation indicates strong adsorption of the ssDNA strand on GO and high fluorescence quenching efficiency of GO. Meanwhile, the P1-GO complex had significant fluorescence enhancement upon addition of complementary target HIV1 (Figure 3, curve d). The fluorescence of the free P1 was, however, scarcely influenced by the addition of HIV1 (Figure 3, curve b). The inset in Figure 3 illustrates the fluorescence intensity changes (F/F_0-1) of P1 and P1-GO upon addition of different concentrations of HIV1, where F_0 and F are fluorescence intensities at 525 nm in the absence and the presence of HIV1, respectively. In the case of P1 only, no significant variation in the fluorescence intensity of P1 was found in the target concentration range. However, for the P1-GO complex, a dramatic increase in the fluorescence intensity was observed.

The kinetic behaviors of P1 and GO, as well as of the P1-GO complex with HIV1, were studied by monitoring the fluorescence intensity as a function of time. Figure 4a shows the fluorescence quenching of P1 in the presence of GO as a function of incubation time. ssDNA adsorption on the surface of GO is very fast at room temperature. It reaches equilibrium in one minute. However, the formation and release of the dsDNA (double-stranded DNA) from GO is relatively slow (Figure 4b). The selectivity of the sensing platform described herein has been determined by examining the fluorescence responses of P1-GO toward HIV1 and the single-base mismatch target (MHIV1). The F/F_0 value obtained upon addition of 150 nM of MHIV1 is about 47.1% of the value obtained upon addition of 150 nM of HIV1 on P1-GO. This DNA-sequence signal specificity is lower than that of molecular beacons, but higher than linear DNA probes, which cannot discriminate single-base mismatch targets.

To illustrate the generality of this method, we analyzed the platform for the detection of protein (human thrombin). A fluorescein-based dye has been linked to the human thrombin aptamer (5′-TCT CTC AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-FAM-3′), where the stronger binding affinity of DNA with GO should enable high quenching efficiency. In our experiment, up to 96% quenching was observed. Figure 5 shows the fluorescence emission spectra of

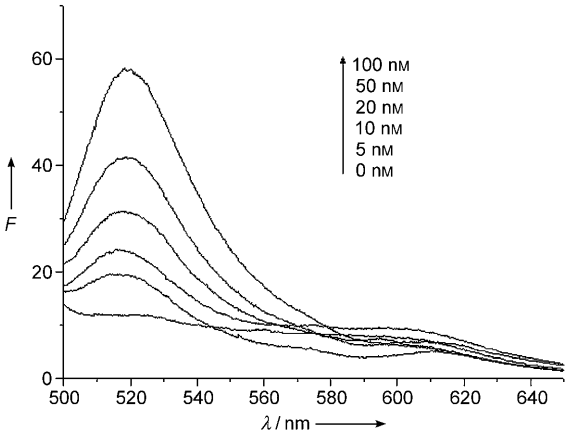

Figure 5. Fluorescence spectra of the fluorescein-based dye-labeled aptamer-GO (dye-labeled aptamer 50 nM) in the presence of different concentrations of human thrombin. Excitation: 480 nm.

the fluorescein-based dye-labeled aptamer-GO complex in the presence of different concentrations of human thrombin. This was illustrated by the fluorescence, which increased approximately fivefold upon addition of 100 nM human thrombin. The limit of human thrombin detection, based on three times the signal-to-noise level, was estimated to be about 2.0 nM, which is approximately tenfold lower than that of the regular dye-quencher pair-labeled aptamers[9] and close to that of the aptamer-carbon nanotube complex.[4b]

Aptamers have high binding specificity, and our results showed that the fluorescein-based dye-labeled aptamer-GO complex maintained this advantage. As shown in Figure 6, when tested with human serum albumin (HSA), bovine serum albumin (BSA), human IgG, and bovine thrombin each at a

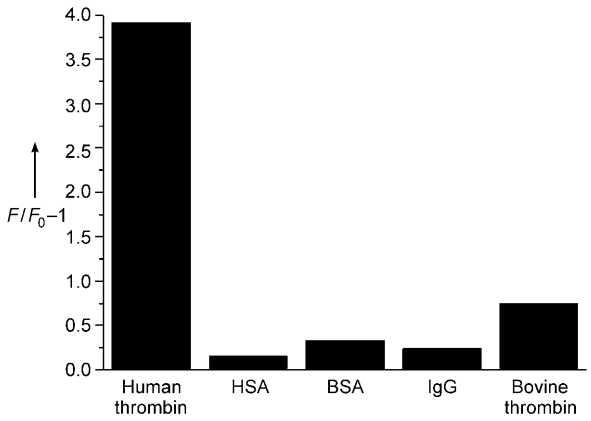

Figure 6. Fluorescence intensity changes (F/F_0-1) of the fluorescein-based dye-labeled aptamer-GO toward different proteins. Excitation: 480 nm, and emission: 520 nm.

concentration of 100 nM, the fluorescein-based dye-labeled aptamer-GO exhibited a much lower fluorescence response to these proteins compared to that of fluorescein-based dye-labeled aptamer-GO. However, significant fluorescence enhancement occurred after the introduction of 100 nM human thrombin. This result clearly demonstrates that fluorescein-based dye-labeled aptamer-GO could be used as a sensitive and selective platform for target protein detection, without the interference of other proteins.

In summary, we have demonstrated that GO, like carbon nanotubes, can be used as a platform for fast, sensitive, and selective detection of biomolecules. However, compared to carbon nanotubes, the low cost and large production scale of GO makes it a promising material for devising biosensors.

Experimental Section

Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers method.[8] Oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. All proteins were purchased from Sigma. Stock solutions of the samples were prepared by directly dissolving the proteins in NaCl (0.01 mol L^{-1}) and stored in a refrigerator at −20 °C. Standard protein solutions were prepared by serial dilution of stock solutions with Tris-HCl buffer (pH 7.4, 0.05 mol L^{-1}).

Characterization of the platform: Fluorescence profiles were obtained with a Hitachi F-4600 fluorometer (Hitachi Co. Ltd., Japan). Atomic force microscopy (AFM) images were recorded using a Nanoscope IIIa multimode atomic force microscope (Veeco Instruments, USA) in tapping mode to simultaneously collect height and phase data. A droplet of graphene oxide or the P1-GO complex dispersion (about 0.01 mg mL^{-1}) was cast onto a freshly cleaved mica surface, followed by drying at room temperature.

Fluorescence response of P1-GO towards HIV1: P1 was prepared as 50 nM in 20 mM Tris-HCl buffer (pH 7.4, containing 100 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$) and mixed with GO for 5 min prior to the addition of HIV1. The final HIV1 concentration in samples ranged from 1 nM to 1 μM. After allowing this mixture to hybridize for about 30 min at room temperature, the fluorescence of the mixture was detected.

Fluorescence response of fluorescein-based dye-labeled aptamer-GO towards thrombin: Fluorescein-based dye-labeled aptamer (50 nM) was prepared in Tris-HCl buffer (20 mM, pH 7.4, containing 100 mM NaCl, 5 mM KCl, and 5 mM $MgCl_2$) and mixed with GO for 5 min prior to the addition of thrombin. The final thrombin concentration in samples ranged from 5 nM to 100 nM. After allowing this mixture to bind for about 30 min at room temperature, the fluorescence of the mixture was detected.

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