

Aptasensor for amplified IgE sensing based on fluorescence quenching by graphene oxide

Kun Hu,* Hu Yang, Jia Zhou, Shulin Zhao* and Jianniao Tian

ABSTRACT: Water-soluble graphene oxide (GO) with a two-dimensional layered nanostructure was synthesized and used as a quencher to construct a highly sensitive and selective fluorescence resonance energy transfer (FRET) aptasensor for sensing Immunoglobulin E (IgE). The fluorescein isothiocyanate (FITC)-labeled aptamer could be adsorbed stably onto the surface of GO via $\pi \rightarrow \pi$ stacking interaction, which led to the occurrence of FRET from FITC to GO, and the fluorescence of FITC-labeled aptamer was quenched by GO via energy transfer. In the presence of IgE, the fluorescence was recovered due to a higher affinity between the aptamer and IgE compared with interactions between GO and the aptamer, leading to a high signal-to-background ratio. The fluorescence intensity of the aptamer increased in proportion to the amount of IgE in the sample, so that IgE could be detected with a linear range of 60–225 pM and a detection limit of 22 pM. The assay was highly selective because the aptamer was unaffected by the presence of immunoglobulin G (IgG), human serum albumin (HSA) and bovine serum albumin (BSA). The practical application of the proposed aptasensor was successfully carried out for the determination of IgE in human serum samples. Copyright © 2012 John Wiley & Sons, Ltd.

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Keywords: graphene oxide; aptamer; IgE

Introduction

Immunoglobulin E (IgE) is found only in mammals, and is present in particularly small amounts in human blood serum. IgE and its receptors are believed to have evolved to protect mammals against parasites (1). IgE plays a key role in allergic responses, which has heightened interest and research into the mechanisms of type-1 hypersensitivity (2). IgE levels increase in patients with allergic asthma, atopic dermatitis and other immune deficiency-related diseases, such as AIDS (3,4). Therefore, it is important for clinical diagnostics that a simple and rapid method for the detection of IgE is developed that has high sensitivity and selectivity. Traditionally, the quantitative method for serum IgE determination uses two complementary antibodies specific to different epitopes of the IgE molecule. Enzyme-linked immunosorbent assays (ELISA) have suitable sensitivity with a limit of detection (LOD) for IgE of 12 ng/mL (5), but they involve time-consuming washing and incubating procedures.

Aptamers, are novel artificially selected functional DNA or RNA oligonucleotides, that are screened using an *in vitro* selection process, systematic evolution of ligands by exponential enrichment (SELEX). These DNA or RNA molecules, with unprecedented advantages such as high selectivity and affinity toward their targets, chemical stability, synthetic convenience and easy modification via functional groups have become increasingly important molecular tools for diagnostics and therapeutics. In recent years, aptamers have been used to develop biosensors for detecting different types of analytes, including small molecular compound (6), proteins (7,8), DNA (9,10), metal ions (11–13), and even whole cells (14,15). A high-affinity aptamer for IgE has been selected as a recognition molecule to detect IgE. Kenzo Maehashi and co-workers fabricated label-free protein biosensors based on aptamer-modified carbon nanotube field-effect

transistors (CNT-FETs) for the electrochemical detection of IgE with a sensitivity of up to 250 pM (16). Girault et al. reported a magnetic-beads-based immunoaffinity capillary electrophoresis (CE) method for total IgE quantification in serum using laser-induced fluorescence (LIF) detection (17). Wu et al. developed a hairpin sticky-end pairing-induced gold nanoparticle assembly for light-up colorimetric detection of IgE (18). Kennedy et al. reported a DNA aptamer against IgE-based affinity capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) (19). Chen et al. described an ultrasensitive IgE assay based on silver nanoparticle hybrid probes and metal-enhanced fluorescence with high selectivity and sensitivity (20). Yu et al. reported a hairpin aptamer-based electrochemical biosensing platform for the sensitive detection of IgE (21). Separation or washing steps are usually needed in these method, which potentially increased the cost of detection.

Nanomaterials have been used to construct biosensors based on their optical signals with an aptamer (22–24). Graphene oxide (GO), a new inorganic nanomaterial, has attracted great deal of attention because of its remarkable electronic, mechanical and thermal properties (25), and use in synthesizing nanocomposites (26) and supermolecules (27,28). GO can bind a dye-labeled single-stranded DNA probe, and quench the fluorescence of the probe. Therefore, GO has been used as a quencher in the

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detection of proteins (29–32), DNA (33), nucleases (34,35) and metal ions (36).

In this study, we used GO as a low background signal platform for sensing IgE based on fluorescence resonance energy transfer (FRET). This simple sensing platform is outlined in Scheme 1. A fluorescein isothiocyanate (FITC)-labeled aptamer acted as the energy donor and GO acted as the energy acceptor. In the absence of IgE, the aptamer was adsorbed onto the surface of GO and the fluorescence was quenched. Upon addition, IgE binds to aptamer to form a quadruplex structure. The high affinity between IgE and the aptamer moves the aptamer far from the GO surface and results in a recovery of the fluorescence. The designed platform for sensing IgE has a low background signal, and is simple and rapid, with no separation.

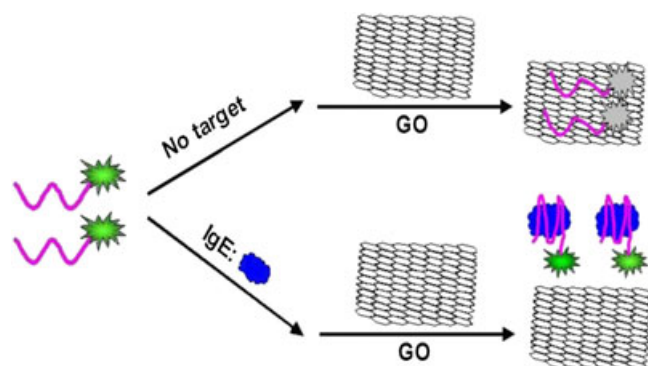
Experimental

Materials and reagents

Graphite powder was obtained from Sinopharm Chemical Reagent Co. Ltd. (Zhejiang, China). IgE, IgG, human serum albumin (HAS) and bovine serum albumin (BSA) were from Sigma Aldrich (St. Louis, MO, USA). The DNA oligonucleotide was from Sangon Biotech Co. Ltd. (Shanghai, China) and were purified using high-performance liquid chromatography (HPLC). The aptamer sequence of IgE was 5'(FITC)-GGGGCACGTT-TATCCGTCCTCCTAGTGGCGTGCCCC-3'. The solution was prepared by diluting an appropriate volume of the commercial sample in buffer (10.0 mM sodium phosphate buffer containing 1.0 mM MgCl₂, 3.0 mM KCl, pH 7.4) and was stored at 4°C. All other chemicals were of analytical grade. Water was purified using a Milli-Q plus 185 from Millipore (Bedford, MA, USA), and was used throughout the study.

Apparatus

UV–visible spectra of the GO were measured with a TU-1901 UV–visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd. Beijing, China). X-Ray diffraction (XRD) spectra were measured with a Rigaku X-ray diffractor equipped with a monochromated beam of Cu–K α radiation ($\lambda = 1.5418$ Å, 40 kV and 100 mA) from powder samples placed on a silicon substrate with a scanning speed of 3°/min and a step of 0.02° in the range 3° to 60° (2 θ) (Rigaku D/max 2500 V/pc, Tokyo, Japan). Fluorescence spectra were measured with a time-resolved fluorescence spectrometer (HORIBA JOBIN JYON, Paris, France). The fluorescence was monitored at 517 nm with an excitation of 491 nm at room temperature.



Scheme 1. Schematic illustration of strategy of sensing of IgE based on a GO FRET aptasensor.

Results and discussion

Characterization of single layer GO

GO was synthesized from graphite powder using a modified version of the method described in Hummers and Offman (37). Samples were characterized by XRD and UV–vis. The sharp peak centered at 10.2 (2 θ) of the XRD spectrum belongs to GO (Fig. S1) (37). The UV–vis spectrum of GO shows two characteristic peaks (Fig. 1). The maximum absorption of GO at 230 nm corresponds to a $\pi \rightarrow \pi^*$ transition of aromatic C–C bonds (38,39), and a minor peak at 303 nm is due to $n \rightarrow \pi^*$ transitions of C=O bonds (40).

Optimization of aptasensor

Figure 2 shows the fluorescence quenching of the aptasensor at different GO concentrations. The fluorescence intensity of the aptasensor decreased with increases in the GO concentration. When the concentration of GO was > 0.1 mg/mL, > 80% fluorescence was quenched. At this GO concentration, the fluorescence intensity of the aptasensor reduced to 18% of the original intensity within 1 min, and showed good stability in solution (Fig. 3a). This indicates that the proximity of the aptasensor to GO is critical for efficient energy transfer from FITC to GO. As shown in Fig. S2, there

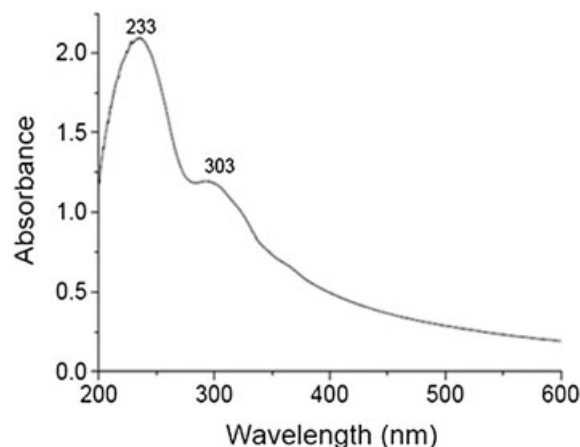


Figure 1. UV–Vis absorption spectrum of GO.

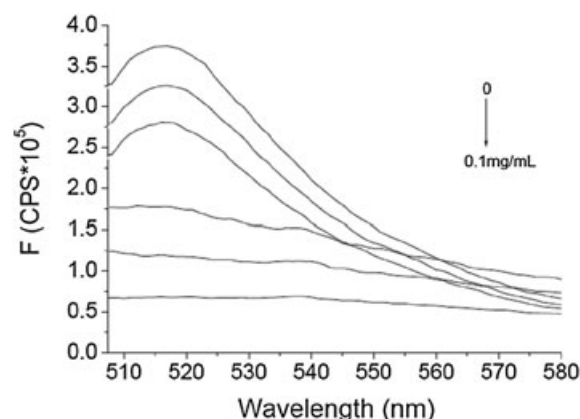


Figure 2. Fluorescence spectra of aptasensor (10.0 nM) in the presence of different concentrations of GO. $\lambda_{\text{ex}} = 491$ nm, $\lambda_{\text{em}} = 517$ nm.

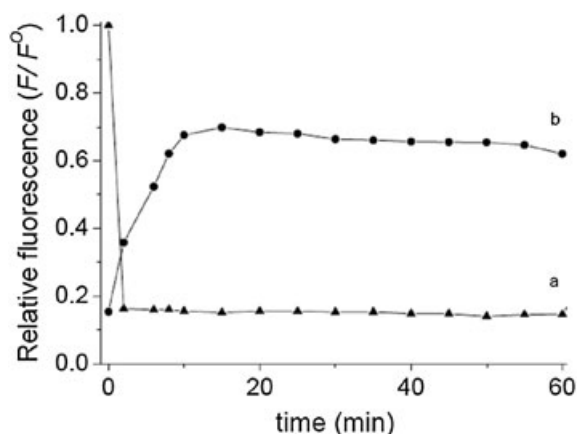


Figure 3. Fluorescence stability of FITC-labeled aptamer in the presence of GO (0.1 mg/mL) (a) without and (b) with IgE (200.0 pM). The aptamer concentration was 10.0 nM, where F and F_0 correspond to the fluorescence intensity with and without IgE, respectively.

was broad absorption across the visible spectrum of GO, which overlaps with the fluorescence emission spectrum of FITC at around 517 nm. Spectral overlapping resulted in fluorescence quenching following an energy transfer mechanism (26,27).

Aptasensor for sensing of IgE

In order to confirm that the fluorescence recovery was induced by IgE, and several control experiments were performed. Figure 4 shows the fluorescence emission spectra of the aptasensor under different conditions. As can be seen, the fluorescence of

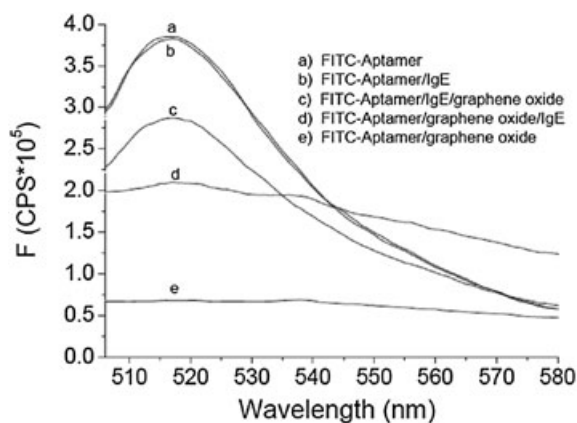


Figure 4. Fluorescence emission spectra under different conditions: (a) FITC-aptamer, (b) FITC-aptamer in the presence IgE, (c) aptamer-IgE in the presence of GO, (d) aptasensor-GO in the presence of IgE, and (e) aptamer in the presence of GO. The concentrations of aptamer, IgE and GO were 10.0 nM, 180.0 pM and 0.1 mg/mL, respectively.

aptasensor had no changes with and without IgE in the absence of GO (traces a and b). The fluorescence of the aptasensor was quenched rapidly in the presence of GO (trace e). When IgE was added to the GO-aptasensor complex, the fluorescence of the aptasensor was recovered (traces c and d) and showed good stability (Fig. 3b). These control experiments indicated that the fluorescence of the GO-aptasensor complex recovered in the presence of IgE was due to the high affinity of the aptamer for IgE. This can be used to recognize IgE.

Figure 5 shows the fluorescence recovery of the aptasensor in the presence of different concentrations of IgE. With increasing concentrations of IgE, the fluorescence intensity of the aptasensor increased linearly over the concentration range 60–225 pM. The regression equation of the calibration curve for IgE was $y = 0.0073x + 0.9158$, with a correlation coefficient of 0.9980. Where y is the fluorescence intensity and x is the concentration of IgE (pM). The detection limit (signal/noise = 3) was estimated to be 22 pM. Inter- and intraday assay repeatability was investigated by analyzing a standard solution of IgE at 100 pM. The results showed that interday RSD was 2.7% ($n = 9$), and intraday relative standard deviation (RSD) was 4.3% ($n = 9$) over 5 days. Table 1 summarizes different nanomaterial platforms that implement IgE detection based on nanoparticles and aptamer or antibody. By contrast, GO makes the current method simple, and more time- and cost-efficient in comparison with other nanomaterials.

Selectivity of aptasensor

IgG, HSA and BSA were used under the same conditions to investigate the selectivity of the current detection system at a

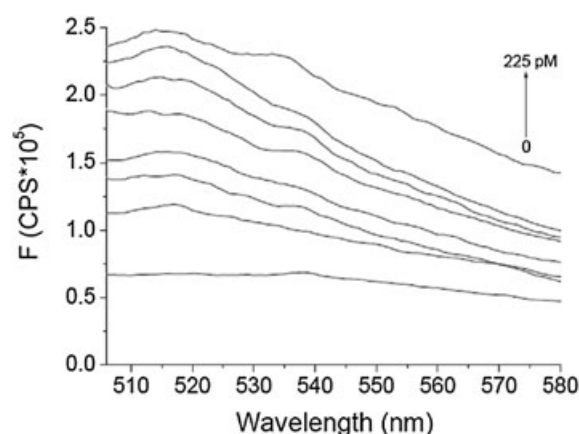


Figure 5. Fluorescence spectra of the GO aptasensor via different concentrations of IgE (0, 60.0, 90.0, 120.0, 150.0, 180.0, 210.0, 225.0 pM). The concentrations of aptamer and GO were 10.0 nM and 0.1 mg/mL, respectively.

Table 1. Comparison of sensitivity for different IgE assay method

Analytical method	Nanomaterials	Linear range	LOD	Ref.
Electrochemical	Carbon nanotube	12.6 pM–20 nM	250 pM	16
CE-LIF	Magnetic beads	12.6 pM–2.6 nM	12.6 pM	17
Colormetric	Gold nanoparticles	10 nM–1 pM	189 oM	18
Fluorescence	Silver nanoparticle	0.49–1000 ng/mL	40 pg/mL	20
FRET	Graphene oxide	60–225 pM	22 pM	This study

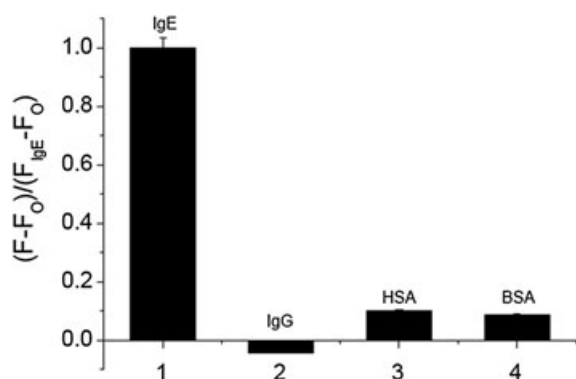


Figure 6. Selectivity of the IgE analysis using the method shown in Scheme 1. The IgE concentration was 200.0 pM and all other interference proteins were 2.0 nM. F and F_0 are the fluorescence intensity with and without the presence of IgG, HSA and BSA, respectively. F_{IgE} is the fluorescence intensity in the presence of IgE.

Table 2. Assay results of IgE in human serum samples using the GO FRET aptasensor

Sample	Content ± SD (pM)	Added IgE (pM)	Found IgE ± SD (pM)	Recovery (%)
1	731.8 ± 1.2	100.0	92.1 ± 3.1	92.1
		500.0	415.0 ± 3.7	83.0
		1000.0	812.3 ± 4.5	81.5
2	526.3 ± 0.9	100.0	93.5 ± 2.2	93.5
		300.0	270.3 ± 2.3	90.1
		500.0	441.1 ± 2.5	88.2
3	656.7 ± 1.8	100.0	91.5 ± 3.2	91.5
		500.0	427.5 ± 3.9	85.5
		1000.0	820.1 ± 4.3	82.0

concentration of 2.0 nM. Figure 6 shows that the fluorescence changes induced by other nontarget proteins were very low compared with the fluorescence intensity with 200.0 pM of target protein. The results demonstrated that the fluorescence recovery was specifically triggered by aptasensor/target binding and that the aptasensor showed good selectivity toward the target.

In order to verify the applicability of the proposed aptasensor for real sample analysis, the aptasensor was used to quantify IgE in three healthy human serum samples and the results showed good recovery in ranged of (81.2–93.5%) (Table 2).

Conclusions

We have developed a mix-and-read format aptasensor-based assay for the detection of IgE with GO, a highly efficient quencher, using FRET. GO, enhances the signal-to-background ratio thus providing the basis of a GO aptasensor for sensing IgE. The fluorescence intensity of the aptasensor was quenched due to energy transfer from the FITC-labeled aptamer to GO. The fluorescence of the aptasensor recovered in the presence of IgE because of the specific affinity between the aptamer and IgE, which attracted the aptamer away from the GO surface. Nonspecific targets had no obvious influence on the assay system, which was therefore suitable for the detection of IgE at physiological levels with high sensitivity and selectivity.

Supporting Information

Supporting information can be found in the online version of this article.

Acknowledgments

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Supporting Information

Aptasensor for Amplified IgE Sensing Based on Fluorescence

Quenching by Graphene Oxide

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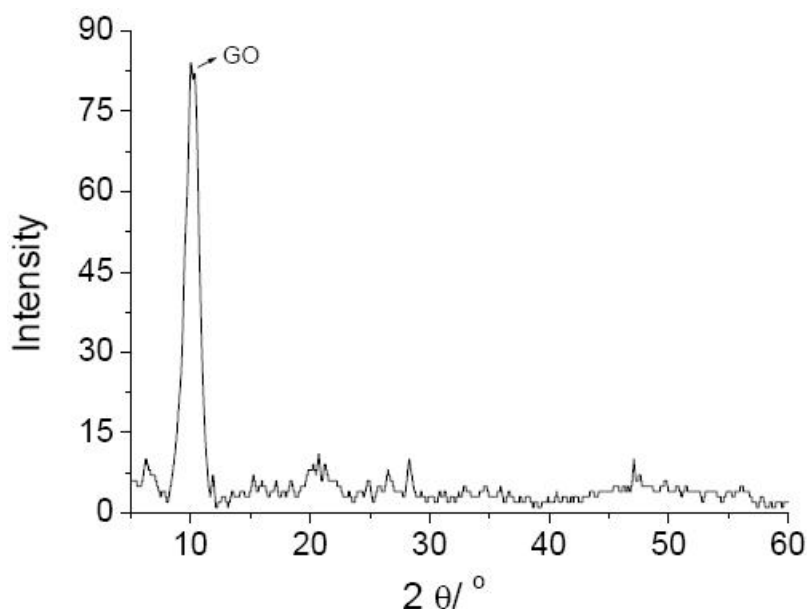


Figure S1. XRD spectrum of GO

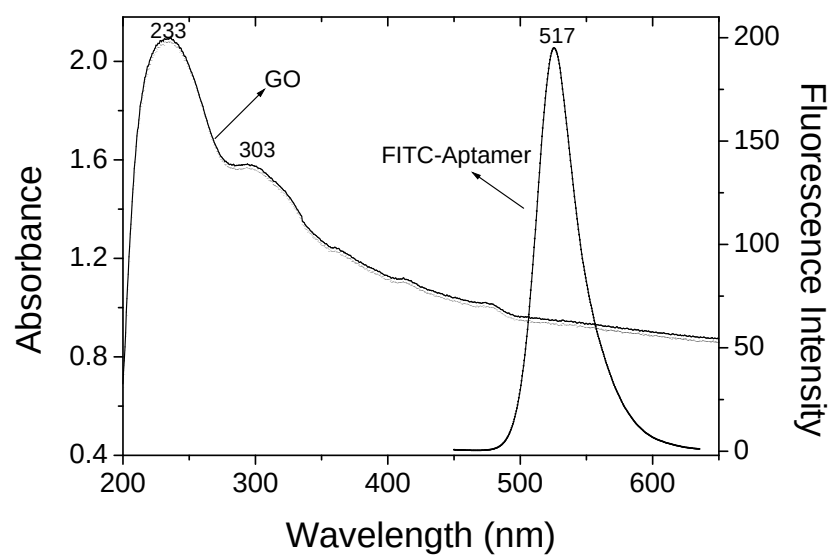


Figure S2. UV–Vis spectra of graphene oxide (GO) spectrally overlap with the fluorescence emission spectrum of FITC-labeled aptamer.