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Label-Free Biosensors Based on Aptamer-Modified Graphene Field-Effect Transistors

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Abstract: A label-free immunosensor based on an aptamer-modified graphene field-effect transistor (G-FET) is demonstrated. Immunoglobulin E (IgE) aptamers with an approximate height of 3 nm were successfully immobilized on a graphene surface, as confirmed by atomic force microscopy. The aptamer-modified G-FET showed selective electrical detection of IgE protein. From the dependence of the drain current variation on the IgE concentration, the dissociation constant was estimated to be 47 nM, indicating good affinity and the potential for G-FETs to be used in biological sensors.

After the discovery of graphene in 2004, this material having a two-dimensional hexagonal network of carbon atoms has been extensively investigated because of its high carrier mobility.[1] Recent research has shown that graphene is also a promising candidate for chemical and biosensing nanomaterials.[2] Detection of various adsorbed gases through the use of graphene technology has already been reported.[3] Furthermore, research on electrical biomolecule detection using graphene and graphene-like materials has gradually increased over the past few years. Electrochemical detection of glucose, dopamine, and serotonin[4] has been investigated. Field-effect transistors (FETs) based on reduced graphene from graphene oxide or graphene amine have been used in the detection of DNA hybridization, negatively charged bacteria, and immunoglobulin G.[5] Graphene FETs (G-FETs) with a single-layer graphene channel have been reported to detect adsorbed proteins in solution.[6] To utilize the high carrier mobility in the graphene channel, single-layer graphene is desirable.[7]

For biological sensing applications based on G-FETs, there are two important considerations. One is the Debye length, which is simply defined as the typical distance required for screening the surplus charge by the mobile carriers present in a material. The Debye length depends on the ionic strength and temperature,[8] and mobile charges in a transistor's channel are not affected by charged molecules located more than a Debye length away. Therefore, a receptor−ligand reaction must occur within the Debye length. Because a typical Debye length at room temperature is ∼5 nm in 5−10 mM buffer solution, the height of a receptor must be smaller than 5 nm. The other consideration is functionalization of the receptor without introducing defects on the single-layer graphene surface in order to enable specific detection and minimize nonspecific binding.

In this paper, we demonstrate label-free immunosensing based on an aptamer-modified G-FET. The target protein is immunoglobulin E (IgE), which is an antibody subclass found only in mammals. Although the amount of IgE in human serum is typically very low (several nM), it significantly increases in individuals with atopic dermatitis, allergic asthma, and other immune deficiency diseases.[9] To enable IgE sensing, IgE aptamers were immobilized on single-layer graphene, as confirmed by atomic force microscopy (AFM) and electrical measurements. Aptamers are artificial oligonucleotides produced in vitro. Hence, they are less expensive than antibodies but are very stable.[10] The greatest benefit of using aptamers is that they are smaller than the Debye length. The height of the IgE aptamers used in this work is ∼3 nm. As a result, protein−aptamer reactions are expected to occur inside the electrical double layer. The aptamer-modified G-FET electrically detected only IgE protein.

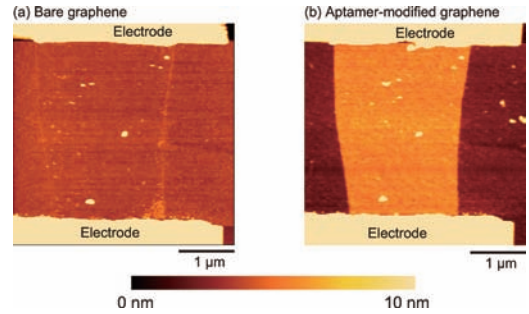

Figure 1. (a) AFM image of a G-FET with a bare graphene channel. (b) AFM image of the G-FET with an aptamer-modified graphene channel.

The fabrication of the G-FET, its functionalization with IgE aptamers, and the experimental setup are described in the Supporting Information. To confirm the immobilization of IgE aptamers on the graphene surface, AFM observations were carried out before and after functionalization (Figure 1a,b). A 0.3−0.5 nm thick graphene channel was observed, which indicates that the graphene channel is a single layer before functionalization with IgE aptamers (see Figure S3a in the Supporting Information). After functionalization, the height of the channel was found to increase to ∼3 nm (Figure S3b), indicating that IgE aptamers were immobilized only on the graphene surface. The effects of functionalizing the G-FET were also evaluated by measuring the altered electrical characteristics (Figure S4). These results show that the IgE aptamers were successfully immobilized in the graphene channel and that the G-FET can electrically detect the existence of oligonucleotides on its surface.

Next, we measured specific sensing characteristics of the aptamer-modified G-FET. Figure 2 shows the time dependence of the drain current (I_D) for an aptamer-modified G-FET at a drain voltage (V_D) of 0.1 V and a top-gate voltage (V_{TGS}) of 0.1 V in phosphate-buffered solution at pH 6.8. After 10 and 30 min, respectively, nontarget proteins, namely, bovine serum albumin (BSA, 100 nM) and streptavidin (SA, 100 nM), were added to the buffer solution, and the target human IgE protein (100 nM) was added after 50 min. To accelerate the reaction between IgE aptamers and IgE protein, the solution was stirred for several tens of seconds after the addition of each analyte. When the target protein was introduced into the graphene channel, I_D suddenly decreased. In this case, it can be considered that the positively charged IgE molecules were connected to the negatively charged IgE aptamers, resulting in a decrease in I_D. In contrast, upon addition of nontarget proteins, I_D of the aptamer-modified G-FET remained almost constant.

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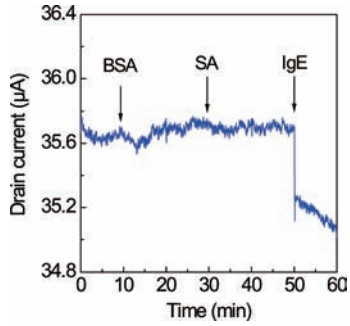

Figure 2. Time course of I_D for an aptamer-modified G-FET. At 10, 30, and 50 min, respectively, BSA and SA (nontarget proteins) and IgE (the target protein) were injected into the aptamer-modified graphene channel.

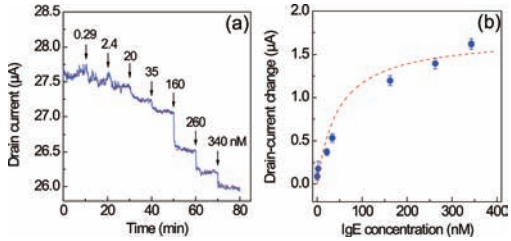

Figure 3. (a) Time course of I_D for an aptamer-modified G-FET. At 10 min intervals, various concentrations of IgE were injected. (b) Change in drain current vs IgE concentration. The red dashed curve shows a fit to the Langmuir adsorption isotherm with $K_D = 47$ nM.

Nonspecific sensing of these proteins by bare G-FETs is shown in Figure S5. All of the proteins could be electrically detected using a G-FET with a bare graphene channel. Therefore, the results indicate that the nonspecific binding of nontarget proteins was successfully suppressed in the case of the aptamer-modified G-FET.

Lastly, using aptamer-modified G-FETs, we estimated the dissociation constant (K_D) for the IgE aptamer and IgE protein by monitoring I_D at various IgE concentrations. The target IgE protein at concentrations of 0.29, 2.4, 20, 35, 160, 260, and 340 nM was introduced into an aptamer-modified G-FET while I_D was monitored in real time (Figure 3a). I_D decreased stepwise after injection of the target IgE at each concentration. Figure 3b shows the net change in drain current (ΔI_D) plotted as a function of IgE concentration. ΔI_D sharply increased with increasing IgE concentration from 0.29 to 160 nM and then gradually became saturated above 160 nM. The data indicate that the reaction between IgE aptamer and IgE protein in the graphene channel follows the Langmuir adsorption isotherm, which is given by

$$\Delta I_D = \frac{\Delta I_{D,max} C_{IgE}}{K_D + C_{IgE}}$$

where $\Delta I_{D,max}$ and C_{IgE} are the saturated net change in drain current and the concentration of IgE protein, respectively. From the fitted curve shown in Figure 3b (dashed line), $\Delta I_{D,max}$ and K_D were estimated to be 1.8 μA and 47 nM, respectively, indicating a high affinity between the IgE aptamer and IgE protein.

Aptamer-based immunosensors have been intensively investigated in recent years. Liss et al.[11] developed aptamer-based quartz crystal microbalance biosensors with K_D values of several nanomolar. Mendonsa and Bowser[12] reported aptamer−IgE reactions using capillary electrophoresis, and their average K_D was 29 nM. Katilius et al.[13] investigated a fluorescent signaling aptamer and fitted the IgE concentration dependence with $K_D = 46$ nM. Aptamer-modified immunosensors using carbon nanotube FETs for which $K_D = 1.9$ nM were reported by Maehashi and co-workers.[14] Turgeon et al.[15] studied aptamer equilibria using gradient micro-free-flow electrophoresis with $K_D = 48$ nM. Our result ($K_D = 47$ nM) is comparable to these previous results. Thus, the present G-FET has sufficiently high sensitivity to estimate dissociation constants for receptor and ligand reactions.

In conclusion, we have investigated an aptamer-modified G-FET as a label-free immunosensor. IgE aptamers were immobilized in a single-layer graphene channel. AFM observations confirmed the presence of structures with a height of ~3 nm, corresponding to the height of the IgE aptamers. The drain current was increased by functionalization, indicating that IgE aptamers were successfully immobilized without the formation of defects on the graphene surface. The aptamer-modified G-FET electrically detected IgE protein, whereas other proteins were not detected; these findings indicate that nonspecific binding of nontarget proteins was successfully suppressed. The dissociation constant was estimated to be 47 nM on the basis of the IgE concentration dependence. These results clearly show that G-FETs are promising devices for use as label-free biological sensors that can electrically detect biomolecules.

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Supporting Information Available: Procedures for fabrication and functionalization of the IgE aptamer-modified G-FET and details of AFM observation and sensing characteristics. This material is available free of charge via the Internet at http://pubs.acs.org.

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