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Fragment-Modified Graphene FET for Highly Sensitive Detection of Antigen-Antibody Reaction

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Abstract

For high-sensitive and specific protein sensing using graphene field-effect transistors (G-FETs), the antigen-binding fragment (Fab), which is a component of conventional antibody, was functionalized onto the graphene surface. Since the height of the Fab is approximately 3 nm, the antigen-antibody reaction is expected to occur inside the electrical-double layer in the buffer solution. After functionalization of Fab onto the G-FET, the transfer characteristics shifted in the positive gate-voltage direction, indicating that the Fab was successfully modified onto the graphene surface. And then, plots of the conductance change and the target proteins concentration were fitted by the Langmuir adsorption isotherm. These results indicate that the Fab-modified G-FETs have high potentials for high sensitive biological sensors using antigen-antibody reactions.

Key words:specific protein sensing, graphene field-effect transistors,antigen-binding fragment,electrical-double layer,biological sensors

Introduction

Graphene is a two-dimensional material and its unique electrical characteristics show high potential for sensing applications [1-6]. Label-free biosensors based on graphene field-effect transistors (G-FETs) with high sensitivity have been reported in recent years [7-11]. In the general FET-based biosensing measurements, the formation of the electrical-double layer is very important concept [12-14]. The mobile charges in the transistor's channel no longer feel the charged molecules placed more than the Debye length away. Since the Debye length is basically very small (~5 nm), it is difficult for FETs to detect the antigen using whole antibody (~15 nm). Therefore, we used antigen-binding fragment (Fab: ~3 nm) as shown in Fig. 1 [15]. In this study, Fab-modified G-FETs were fabricated to detect the specific proteins with high sensitivity.

Experimental Procedure

G-FETs were fabricated on a thermally oxidized 280-nm-thick SiO_2 layer on the degenerately doped Si substrates. Monolayer graphene flakes were only used for the channel in this work to obtain a high sensitivity, and they were prepared using bulk graphite and a clear adhesive tape. After the layer number was confirmed by Raman spectroscopy [16,17], the source and drain gold electrodes were formed by conventional electron-beam lithography, vacuum evaporation, and the lift-off method. An optical image of a G-FET is shown in Fig. 2.In order to perform sensing measurements, The silicone rubber container was put on the graphene channel to prevent solution leakage. And an Ag/AgCl reference electrode was used for applying top-gate voltage. Figure 3 shows an experimental setup of the G-FET in a solution.

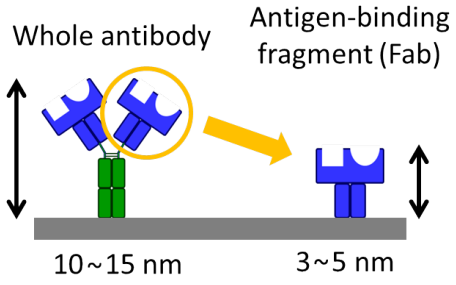

Fig. 1. Schematic of a whole antibody and an antigen-binding fragment.

The following is a fabrication process of Fab-modified G-FETs. First, the G-FETs were immersed in 1-pyrenebutanoic acid succinimidyl ester, which was used as linkers [18-20]. Then, for the covalent immobilization of the Fab on

the graphene surface, the devices were immersed in Fab in phosphate-buffered saline (PBS). In this work, heat shock proteins (HSP) were used as a target protein [21,22].

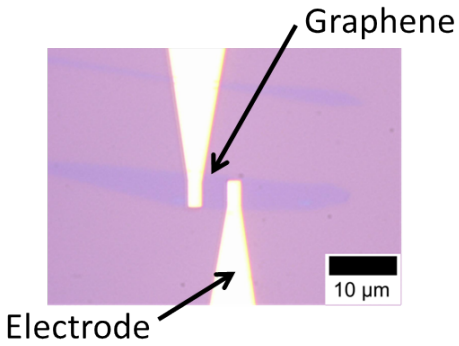

Fig. 2. Optical microscopy image of a G-FET.

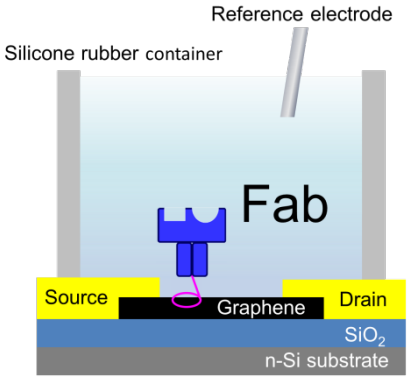

Fig. 3. Schematic image of a measurement system.

Results and Discussion

The conductance (C) versus top-gate voltage (V_{TG}) characteristics for the G-FET with bare graphene channel and Fab-modified graphene channel in PBS are shown in Fig. 4. The C-V_{TG} curves showed an ambipolar characteristic, indicating that the Fermi level in graphene was changed by the V_{TG}. After functionalization of Fab, transfer characteristics were shifted in the positive direction, indicating that the Fab was successfully functionalized on the graphene channel. This positive shift can be considered owing to the Fermi level shift. Thus, it is considered that Fab has negative charge in PBS at pH 7.4. Furthermore, the C-V_{TG} characteristics have little change in slope, indicating that the Fab-fabrication process was carried out without introducing defects on the graphene surface.

To confirm the antigen-antibody reaction, the target HSP concentration dependence was measured and the dissociation constant (K_D) between Fabs and HSPs was estimated. Figure 5 shows the time dependence of C at the drain voltage (V_D)= 0.1 V and V_{TG}= -0.05 V. Every ten min, the HSPs at various concentrations were introduced onto the Fab-modified G-FETs. The concentrations of HSPs were 0.01, 0.1, 0.8, 7, 10, 60, and 100 nM. The stepwise decrease in C was clearly observed after the injections of the HSPs at various concentrations. The C for each HSP concentration was maintained at an approximately constant value. In this device, since the carriers were holes at V_{TG}= -0.05 V, the decreased C indicates that the Fab-modified G-FETs detected the positive charges of the HSPs in the electrical double layer. This concentration dependence reveals that the G-FET detects HSPs at concentrations as low as 100 pM, and that dynamic range of HSP detection is several hundreds of pM to hundreds of nM, indicating the high sensitivity of Fab-modified G-FETs [23].

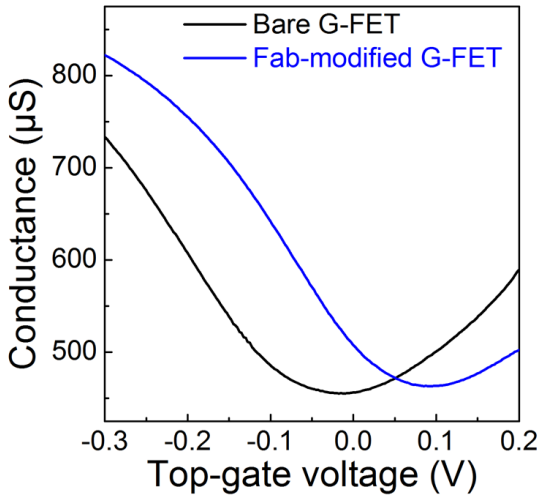

Fig. 4. Transfer characteristics of the G-FET for as fabricated (black line) and after modification of Fab (blue line).

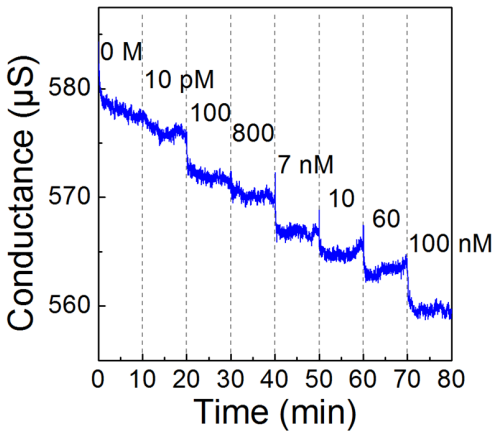

Fig. 5. Time course of I_D for the Fab-modified G-FET at V_D of 0.1 V and V_{TG} of -0.05 V. At 10 min intervals, various concentrations of HSPs were introduced on the Fab-modified G-FET.

Then, Figure 6 shows the net change in the conductance (ΔC) plotted as a function of HSP concentration (C_{HSP}). The data indicates that the reaction between Fab and HSP in the graphene channel follows the Langmuir adsorption isotherm [8,24]. The curve was fitted by the data points. From Fig. 6, K_D was estimated to be 2.3 nM. These sensing characteristics show that the Fab-modified G-FETs detects the antigen-antibody reaction on the surface, and highly sensitive biological sensors can be realized with G-FETs using Fab.

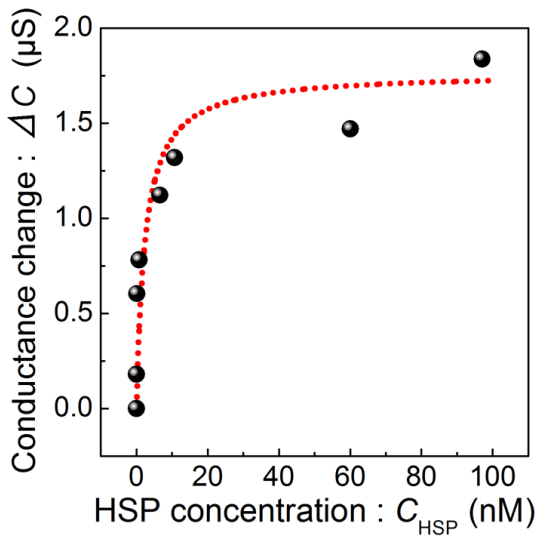

Fig. 6. Conductance change versus HSP concentration. The red dashed line shows a fitting curve to the Langmuir adsorption isotherm.

Conclusions

We have fabricated Fab-modified G-FETs for biological sensors to utilize the antigen-antibody reaction on the graphene channel surface. The I_D-V_{TG} curve shifted in the positive direction without changes in the transconductances of these G-FETs following the functionalization of Fabs, indicating that Fabs were successfully immobilized without the introduction of defects on the graphene surface. The dissociation constant was estimated to be 2.3 nM on the basis of the HSP concentration dependence. The results show that we detect HSPs with high sensitivity without the need for the labeling process. The use of Fabs has several advantages in biosensors based on G–FETs. Fabs are found in all of the antibodies; therefore, it is expected that Fab-modified G-FETs can improve disease diagnosis. Therefore, Fab-modified G-FETs are promising devices for highly sensitive biological sensors.

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