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Measurement of Enzyme Molecules and Their Reactions Using Graphene-FET Equipped with Microwells

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Abstract

Graphene is an attractive material for the FET-based electrical biosensor, due to its high carrier mobility, high responsivity to charged biomolecules and chemical stability in aqueous solutions. However, the electrical biosensing has an issue of the Debye screening. In this study, target biomolecules, urease, was detected not by their own surface charges, but by their small reaction products, ammonia. This novel scheme is hardly affected by the Debye screening, since the reaction products are smaller than the Debye length. Moreover, the detection required only around 50000 urease molecules by encapsulation of the sample solution in the microwell equipped on the graphene-FETs.

1. Introduction

Graphene has the highest carrier mobility of the known materials in theory. Also, its transfer characteristics keenly respond to the binding of dopant atoms or molecules on its surface. These unique electrical characteristics are useful for highly sensitive sensors such as gas sensors [1]. Moreover, graphene is chemically stable in aqueous solution. It is different than silicon, which is now commonly used as the device material for the semiconductor devices including electrical sensors. This enables graphene and analyte molecules to bind directly in aqueous solution. All these attractive characteristics provide graphene field-effect transistor (graphene-FET) with a strong potential for the application to highly sensitive biosensor.

However, the electrical biosensing using graphene-FET has an issue of Debye screening, that is, graphene's electrical characteristics are not affected by the surface charges of target biomolecules outside the Debye length from graphene surface. The Debye length is several nanometers or less in physiological conditions and often smaller than the targets. This issue diminishes the stability and reproducibility of the biosensing using graphene-FET.

The authors focused on the small molecules of reaction products from target biomolecules. The indirect detection for the biomolecules using their products is able to minimize the effect of Debye screening, since the product molecules are smaller than the Debye length.

In this study, graphene-FETs detected urease, an enzyme which produces ammonia from urea. Enzymatic reaction and its kinetics were successfully measured as a hole current change of graphene-FETs. Further, the assay solution was encapsulated in the microwell on the graphene-FET. The amount of the detected biomolecules was highly reduced owing to the accumulation of the products in the microwell.

2. Experimental Procedure

Fabrication of graphene-FET device with microwell

Figure 1(a) and (b) show the graphene-FET device used in this study. The graphene-FETs were fabricated using graphene exfoliated from the kish graphite and transferred on silicon wafer. The graphene on the wafer was then connected by gold source/drain electrodes with nickel base through electron beam lithography, electron beam deposition and lift-off process. Next, Al_2O_3 layer was deposited on the graphene-FETs by atomic-layer deposition. Fluoropolymer (Cytop, Asahi Glass Co., Ltd.) was spin-coated on the device and etched using oxygen plasma after photolithography. The microwell was completed after resist removal and alkaline etching of Al_2O_3.

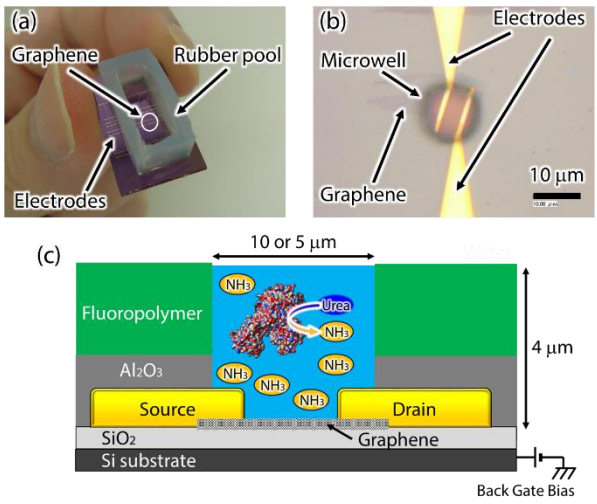

Fig. 1 Graphene-FET device equipped with microwell. (a) Photograph of the device chip. (b) Microscopic image of the microwell on the graphene-FET. (c) Schematics of the device. After encapsulation, reaction products were accumulated in the microwell.

Measurement of the reaction solution

Before the measurement, a silicone rubber pool was placed on the graphene-FETs to store the aqueous solution. The microwell was sealed only in the experiment in Fig. 4, and in other experiments the microwell was opened to 200 μL solution in the rubber pool. The urease solution consists of 1 μM urease, 400 mM urea and 100 mM sodium phosphate (pH 7.8 at the initial state). The ammonia solution consists of ammonia and pure water. Hole currents of the graphene-FETs were monitored under 100 mV drain voltages using a semiconductor parameter analyzer.

3. Results and Discussions

Ammonia detection

At first, the changes in hole current of graphene-FET was monitored under series of ammonia concentration. Figure 2 shows linear decrease with increasing ammonia concentration on log scale, while it was reported that the pH increase (i.e. proton decrease) induces hole carrier on graphene [2]. The mechanism of the hole carrier reduction in this experiment is still unclear, but ammonia and/or ammonium ion are considered to induce electron carrier on graphene, as previously observed in gas phase [1]. In this experiment, the proton concentration was much smaller than ammonia concentration. Hence ammonia had the dominant effect on the graphene-FET characteristics.

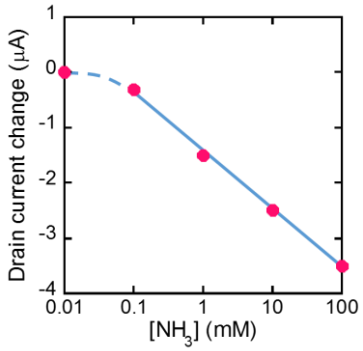

Fig. 2 Changes in hole currents of graphene-FET on ammonia concentration. Drain current at 0.01 mM was subtracted from all data points. The back-gate voltage was 0 V.

Detection of urease reaction

Next, urea was introduced to urease solution on graphene-FET. In this experiment, graphene surface was equilibrated by 1 μM urease before the introduction of electrically neutral urea, 400 mM at final concentration. Also, surface charges of urease did not change in the experiment, since the isoelectric point of urease was around 5.0. Therefore, the hole current decrease in Fig. 3 shows that the ammonia product from urease was monitored by graphene-FET in real time.

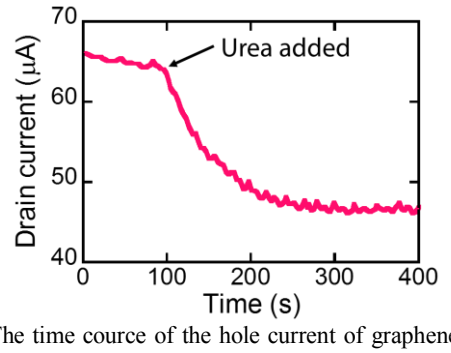

Fig. 3 The time cource of the hole current of graphene-FET under urease reaction. The back-gate voltage was 20 V.

Detection of urease reaction in microwell

Finally, urease reaction solution was encapsulated in the microwell and the urease reaction was monitored by graphene-FET. After the encapsulation, the hole current was exponentially decayed over time, as shown Fig. 4. It indicates that enzymatic reaction in femtoliter volume was properly monitored by graphene-FET. Also, according to the results in Fig. 2 and Fig. 4(b), it is indicated that the enzymatic reaction rate was almost constant for 200 s. It is consistent with the estimation from the Michaelis constant of urease, catalytic rate constant of urease and microwell volume (10.5 mM, 1100s^{-1} and 80 fL, respectively). In this experiment, the amount of detected urease molecules was as little as 50000 molecules. Urease is a biomarker of the infection of *H. pylori*, which causes stomach cancer. This highly sensitive urease sensor will be applied to the detection for the pathogen.

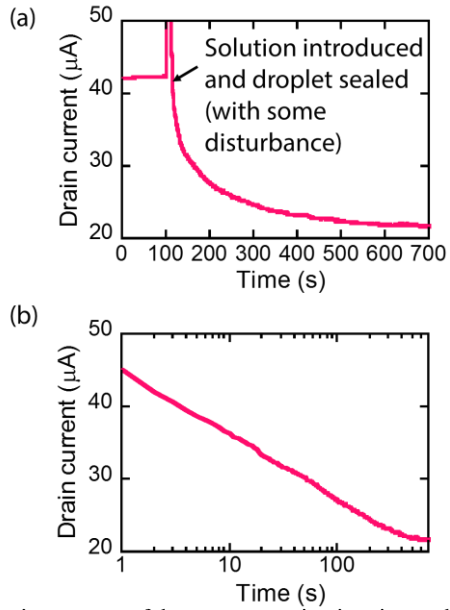

Fig. 4 The time course of the urease reaction in microwell monitored by graphene-FET in (a) linear and (b) log scale. In (b), the reaction solution was introduced at t=0 s. The back-gate voltage was 20 V.

4. Conclusions

The graphene-FETs successfully measured urease molecules and their reaction kinetics in real time. The detection required only 50000 urease molecules using graphene-FET equipped with microwell.

Acknowledgements

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References

[1] F. Schedin *et.al.*, Nat. Mater. **6** (2007) 652.

[2] Y. Ohno *et.al.*, Nano Lett. **9** (2009) 3318.