High-sensitive Label-free Biosensors Based on Carbon Nanotube Field-effect Transistors Modified with Aptamers

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ABSTRACT

We have fabricated label-free protein biosensors based on aptamer-modified carbon nanotube field-effect transistors (CNTFETs) to detect immunoglobulin E (IgE). Since aptamers are artificial oligonucleotides, the aptamers are smaller in size than the Debye length. Therefore, the biosensors are expected to detect IgE with high sensitivity. After the 5'- amino modified aptamers were covalently immobilized on the CNT channels, the electrical properties of the CNTFETs were monitored in real time. The introduction of target IgE at various concentrations caused a sharp decrease in the source-drain current and gradual saturation at lower values. The amount of the net source-drain current before and after IgE introduction increased as a function of IgE concentration. Therefore, IgE at 250 pM could be effectively detected. Aptamer-modified CNTFETs are a promising candidate for label-free protein biosensors.

Keywords: label-free protein biosensors, carbon nanotube field-effect transistors, aptamers, immunoglobulin E, high sensitivity

1 INTRODUCTION

Label-free electrical monitoring of biorecognition events provides a promising platform, which is simpler, less expensive and requires less energy. Rapid testing of different proteins is required in various applications, including clinical diagnostics, environmental testing, food analysis, bioterrorism detection technologies, etc [1]. Carbon nanotube field-effect transistors (CNTFETs) are one of the most promising candidates for the development of high-sensitive biosensors [2-7], because CNTs have one-dimensional nanostructures and thus a high aspect ratio. However, in the case of antibody-modified FET biosensors based on antigen-antibody reactions, since antibodies are much larger in size than the Debye length, the antigen-antibody reactions occur outside the electrical double layer in the buffer solutions and thus most of target-protein charges are canceled. For this reason, it has always been considered to be a difficult task to high sensitively detect proteins with such FET biosenosors.

In this study, we have detected immunoglobulin E (IgE) using CNTFETs, in which CNT channels were modified with aptamers. Aptamers are artificial oligonucleotides that can be generated to recognize amino acids, drugs, and proteins with high specificity [8]. The aptamers can be engineered in vitro easily, and therefore are relatively inexpensive. Moreover, the aptamers are smaller in size than the Debye length, as shown in Fig. 1. As a result, formations of aptamers and target proteins occur inside the electrical double layers in buffer solution, and the aptamers can be immobilized in dense on the CNT channels. They have also demonstrated stronger and more selective affinity for their target proteins than the corresponding antibodies. Therefore, aptamer-modified CNTFET biosensors are expected to detect target proteins with high sensitivity and high selectivity. IgE is an antibody subclass, found only in mammals. IgE is capable of triggering the most powerful immune reactions. Most of our knowledge of IgE has come from research into the mechanisms of type 1 hypersensitivity. In this paper, we have fabricated the aptamer-modified CNTFET biosensors, and the electrical properties of the CNTFETs have been measured in real time after introduction of IgE at various concentrations.

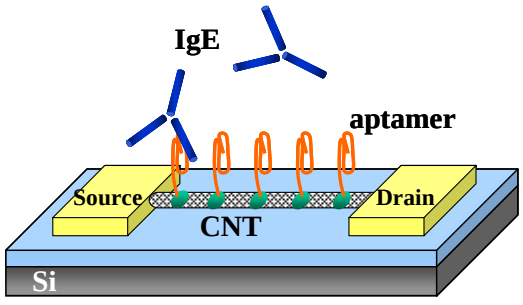

Fig. 1. Schematic structure of protein biosensors based on an aptamer-modified CNTFET.

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2 EXPERIMENTAL

The CNTs used in these studies were grown using a thermal chemical vapor deposition (CVD) approach described previously [9]. The CNTs were synthesized by ethanol CVD using the patterned Co chemical catalyst, which were formed by the conventional photolithography and lift-off process technology, on heavily doped p^+-Si substrates capped with 100 nm thick SiO_2. The spacing between the source and drain electrodes was about 4 μm. The source and drain contacts (Ti/Au) were formed onto the patterned chemical catalyst after the growth of the CNTs. The fabricated samples were *p*-type CNTFETs at room temperature in air.

An experimental setup for detection of IgE using CNTFETs was carried out as follows. First, the CNT channels were incubated into 1-pyrenebutanoic acid succinimidyl ester in dry dimethylformamide solutions (linkers) for 1 hour in a 5 mM solution before the IgE aptamer immobilization. Next, in order to covalently immobilize IgE aptamers on the CNT channels, the devices were submerged into the 5'- amino modified aptamers in a 12 μg/ml solution overnight. Then, unreacted linkers were blocked by ethanolamine (100 μM solution, 30 min). Afterwards the aptamer-modified channels in the CNT-FETs were immersed into 10 mM phosphate buffer solutions. A Ag/AgCl reference electrode (Bioanalytical Systems, West LaFayette, IN) was used as a gate electrode to minimize the environmental effects. Finally, the electrical properties of the CNTFETs were measured in real time by Semiconductor Parameter Analyzer 4156C or B1500A (Agilent Technologies, Inc.) after introduction of IgE at various concentrations.

3 RESULTS AND DISCUSSION

The electrical properties of the CNTFETs were measured in real time at room temperature. Before modification of linkers, the fabricated samples still showed *p*-type characteristics in 10 mM buffer solutions.

First, the drain current-drain bias characteristics of the CNTFET was measured before and after modification of IgE aptamers on the CNT channel. The current increased after the IgE-aptamer modification. The increase in conductance for the *p*-type CNTFET devices comes from an increase in negative charge density on the CNT channel. This result is consistent with the fact that aptamers are negatively charged oligonucleotides. Therefore, the result indicates that IgE aptamers were successfully modified on CNT channels. Spontaneously, it is confirmed that no current to a reference electrode were measured.

Figure 2 shows time dependence of source-drain current of CNTFETs at the source-drain bias of 0.2 V and at the gate bias of 0 V after the introduction of target IgE at 20 nM onto the IgE aptamer-modified CNTFET while monitoring in real time. Adding the target IgE caused a sharp decrease in the source-drain current and then gradual saturation at lower values. This result indicates that the biosensor could detect IgE molecules at 20 nM. There could be two possibilities to explain this behavior. One is the screening effect for some negative charges of the aptamers by IgE molecules when the reactions of IgE and aptamer occur. The other is the increase in Schottky barrier height between metal electrodes and CNT channel, which is due to adsorption of IgE molecules on CNT channel at the CNT-metal contacts.

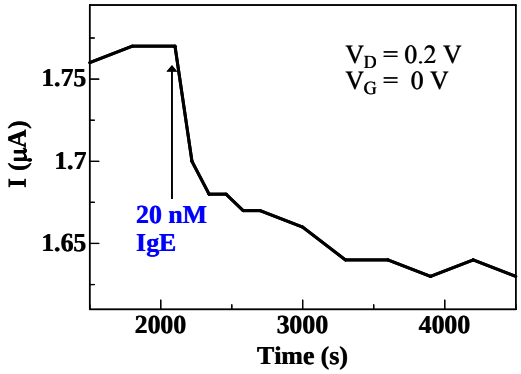

Fig. 2. Time dependence of source-drain current of the CNTFET at the source-drain bias of 0.2 V and at the gate bias of 0 V after the introduction of target IgE at 20 nM onto the IgE aptamer-modified CNTFET. Arrows indicate the point of adding IgE.

Electrical properties of the IgE-aptamer-modified CNTFETs were carried out in order to investigate the stability and selectivity of the biosensors. Figure 3 shows time dependence of source-drain current of the CNTFET at the source-drain bias of 0.2 V and at the gate bias of 0 V. No conductance change was observed after additional 10 mM buffer solution was introduced into the nanosensor device, indicating that the device have high stability. Next,

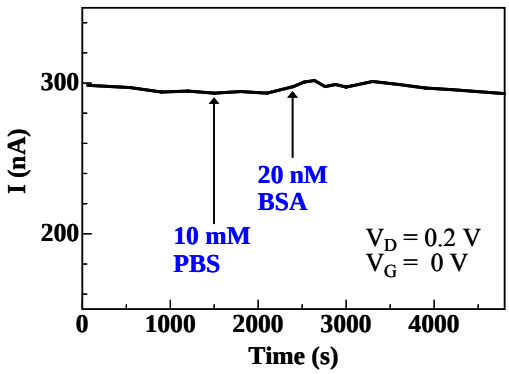

Fig. 3. Time dependence of source-drain current of the CNTFET at the source-drain bias of 0.2 V and at the gate bias of 0 V after introduction of additional buffer solution and nontarget BSA onto the IgE aptamer-modified CNTFET. Arrows indicate the point of adding buffer solution and nontarget BSA.

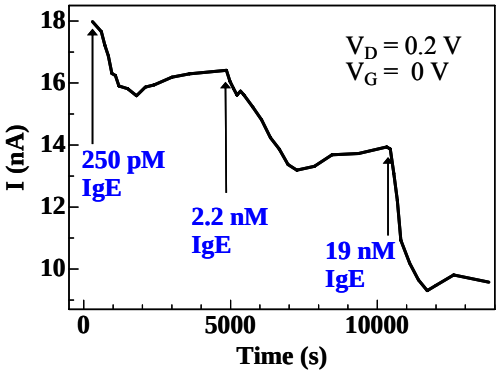

Fig. 4. Time dependence of source-drain current of the CNTFET at the source-drain bias of 0.2 V and at the gate bias of 0 V after the introduction of target IgE at various concentrations onto the IgE aptamer-modified CNTFET. Arrows indicate the point of adding IgE.

nontarget bovine serum albumin (BSA) of 20 nM was introduced into the IgE aptamer-modified CNTFET sensor, revealing that the source-drain current did not change, as shown in Fig. 3. The result indicates that nonspecific binding of BSA was successfully suppressed in the biosensors. Therefore, aptamer-modified CNTFET biosensors can detect target proteins with high sensitivity and high selectivity.

Figure 4 shows time dependence of source-drain current of CNTFETs at the source-drain bias of 0.2 V and at the gate bias of 0 V after the introduction of target IgE at various concentrations onto the IgE aptamer-modified CNTFET while monitoring in real time. For every concentration, the source-drain current sharply decreased after adding the target IgE and then was gradually saturated at lower values. Furthermore, according to the results in Fig. 4, the amount of the net source-drain current before and after IgE introduction for every concentration is plotted in

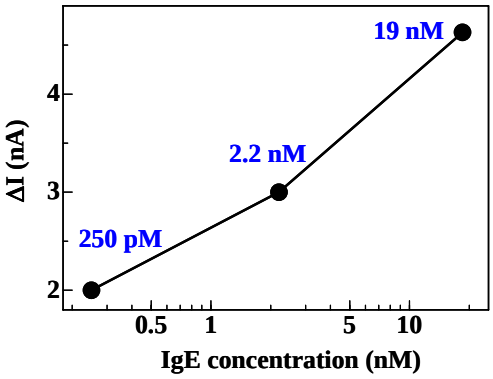

Fig. 5. IgE concentration dependence of the net source-drain current before and after IgE introduction onto the CNTFETs.

Fig. 5. The results reveal that the amount of the net source-drain current increased as a function of IgE concentration of 0.25, 2.2 and 19 nM. The results indicate that IgE at 250 pM could be effectively detected using the aptamer-modified CNTFET. Therefore, the biosensors can detect IgE molecules with high sensitivity.

Recently, label-free biosensors have been fabricated by other groups to detect IgE [10, 11]. One is aptamer-based quarts crystal biosensors, the other electrochemical biosensors with aptamer-based array electrodes. They have also detected IgE with high sensitivity, indicating that aptamer-modified devices are useful to detect proteins such as IgE.

4 CONCLUSIONS

We have detected IgE using CNTFETs, in which CNT channels were modified with aptamers. Aptamers, which are artificial oligonucleotides, are smaller in size than the Debye length and, have selective affinity for target protein. Therefore, the aptamer-modified CNTFET biosensors are expected to detect IgE with high sensitivity. After the 5'-amino modified aptamers were covalently immobilized on the CNT channels, the electrical properties of the CNTFETs were monitored in real time. The source-drain current sharply decreased, and gradually saturated at lower values after the introduction of target IgE at various concentrations onto the aptamer-modified CNTFET while monitoring in real time. The amount of the net source-drain current before and after IgE introduction increased as a function of IgE concentration. Therefore, IgE at 250 pM could be effectively detected.

If we use CNTFETs with higher performance, sensitivity to detect target protein will be improved. Our aptamer-based CNTFET biochip is a promising candidate for the development of an integrated, high-throughput, multiplexed real-time biosensor for medical, forensic and environmental diagnostics.

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