Development of High Sensitive DNA Sensor by Using Probe PNA with IS-FET Electrode

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Received July 25, 2005 ; Accepted October 28, 2005

We demonstrated an ion-sensitive field-effect transistor (IS-FET) based biosensor to directly monitor the hybridization of deoxyribonucleic acid (DNA) using peptide nucleic acid (PNA) as a probe. A single strand DNA or PNA was immobilized as probes on the IS-FET. To detect the hybridization of DNA, we carried out measurements of IS-FET’s drain current hybridized by complementary target DNA. Then, the difference voltage was evaluated to be about only 10 mV by using the probe DNA, furthermore monitored as shifts about 170 mV by attempting the probe PNA. The changes in threshold voltage observed for neutral PNA-immobilized IS-FETs were more than 17 times greater than those for negatively charged DNA-immobilized IS-FETs. This approach demonstrates that the PNA-modified IS-FET-based biosensor works more effectively as a signal transducer of genetic information.

Key Words: DNA Sensor, Hybridization, IS-FET, PNA

1 Introduction

Molecular recognition based on DNA hybridization is an important reaction in discriminate between double-stranded nucleic acids and single stranded nucleic acids with high efficiency and specificity are useful tools in this post-genome sequence era.1–5) Particularly exact and rapid detections of mismatched base pairs in DNA are difficult for the diagnosis and study of genetic disease such as single nucleotide polymorphisms (SNPs). Therefore DNA chips to diagnose the disease are widely studied as addressable arrays of immobilized DNA sequences.6–8) PNA is a structural DNA analogue with a neutral N-(aminoethyl)-glycine-based pseudopeptide backbone replacing the negatively charged phosphate backbone of DNA. PNA has a strong binding affinity for complementary DNA due to the lack of repulsion between PNA and DNA.7, 8) Accordingly, PNA-immobilized IS-FETs are expected to undergo effective hybridization and enable direct detection of DNA without any modifications, such as the addition of fluorescent probe molecules. When compared with the DNA duplex, PNA strongly discriminates mismatched DNA and has a strong binding affinity for complementary DNA due to the lack of repulsion between PNA and DNA.

Therefore we have attempted development of a non-labeling type DNA chip by using an IS-FET, and modified the surface of Ta$_2$O$_5$ for PNA immobilization and we demonstrated that PNA immobilization and we demonstrated that PNA modified Ta$_2$O$_5$ plays a role in the specific molecular recognition of complementary DNA present in solution.

2 Experimental

We used a commercial IS-FET electrode made in BAS Inc., whose gate electrode consists of Ta$_2$O$_5$ thin film as a most top layer. The structure in the gate consists of SiO$_2$ (100 nm), SiN$_x$ (100 nm) and Ta$_2$O$_5$ (40 nm) thin films. By immobilizing the probe DNA to the Ta$_2$O$_5$ thin film on gate electrode, the IS-FET is utilized as a DNA sensor to detect the DNA hybridization. Since the DNA chain has negative charges, a change of source-drain current would be expected by hybridizing with complementary target DNA to impose the negative charges as shown in Fig. 1. The source-drain current measurements were 0.2 

× SSC solution at room temperature.

To detect the DNA hybridization by using this system, we attempted to immobilize the probe DNA or PNA on the Ta$_2$O$_5$ thin film. The technique was quoted by a method of B. M. Manning et. al. who carried out to immobilize a 3'-amino-modified DNA on a SiO$_2$ substrate such as a glass substrate.9) To immobilize PNA on the gate electrode, IS-FETs were immersed in 5% glutaraldehyde (Wako Pure Chemical Industries Ltd.)-phosphate buffer (pH 7.0, 1/15 M) for 2 h at 37 C and were washed with deionized water. Synthesized 15-mer PNA having ethylene glycol spacers at the Nterminal (NH$_2$-O-GGCAGTGCCCTCA

Fig. 1 A schematic system of the IS-FET measurements. It was measured by an Ag/AgCl.
CA) was purchased from Fasmac Co., Ltd. Target DNA (5'-TTGTGAGGCACGTGCC), which was complementary to the probe PNA, was purchased from Sigma Genosys Japan K.K. IS-FETs were immersed in the solution of PNA probes (5 mM) for 12 h at 37 °C. After PNA immobilization, the Schiff base was reduced by mild hydride donors, 50 mM cyanoborohydride (BH$_3$CN) for 2 h at 37 °C. IS-FETs modified with probe PNA were immersed into 0.2 × SSC / 0.1 mM EDTA hybridization buffer containing the target DNA (5 mM), which was complementary to the probe, for 12 h at 60 °C. After the hybridization reaction, IS-FETs were washed with 2 SSC / 1 mM EDTA and were immersed for 5 min in order to remove nonspecifically bound DNA.

3 Results and Discussion

We confirmed the DNA immobilization on the Ta$_2$O$_5$ thin film by XPS measurement. To carry out the measurement, the Ta$_2$O$_5$ thin film was fabricated on a Si substrate by vapor deposition, and the probe DNA was immobilized by the method on the substrate. Figure 2 shows XPS spectra of the DNA immobilization before and after on the Ta$_2$O$_5$ thin film. In the case of Si$_2p$ as shown in Figure 2 (a), the weak intensity was obtained nevertheless the silane coupling agent was not immobilized. This will be the reason why the Si$_2p$ substrate was detected, and the peak intensity became stronger by operating the DNA immobilization. This result shows the modification by the silane coupling agent on the Ta$_2$O$_5$ surface. To investigate the DNA immobilization, spectra of a P element were measured as shown in Figure 2 (b). The P$_{2p}$ spectra were not observed for the non-immobilized DNA substrate at all, while was obtained a spectrum attributed to the P$_{2p}$ by the DNA immobilization. Then, a rate of Ta, O and P elements was Ta (25.7%), O (64.2%) and P (10.1%). By using these values, the amount of the immobilized DNA was estimated. A total of Ta and O atoms per cm$^2$ in this surface is $1.5 \times 10^{-8}$ atoms as $1.5 \times 10^{-8}$ cm at which size of the Ta and O atoms is approximately. Since an applied 21 mer DNA has 21 P elements per one DNA molecule, the amount of the immobilized DNA is estimated at

$$\frac{1}{1.5 \times 10^{-8}} \times \frac{10.1}{25.7 + 64.2} \times \frac{1}{21} = 3.6 \times 10^5 \text{[molecules/cm$^2$]}$$

(1)

These results would support that the amino-modified DNA is immobilized by the amino-silane agent via the cross linker on the Ta$_2$O$_5$ thin film.

Detecting the DNA as a non-labeling sensor by using the IS-FET, $I_{\text{drain}}$-$V_G$ properties was measured for a bare gate electrode, the gate electrode immobilized with the probe DNA and the gate electrode hybridized with a target DNA. As the DNA chain has negative charges, the change of source-drain current would be expected by hybridizing with complementary target DNA to impose negative charges. The sequences of the probe and target DNA are shown by the following sentences. Probe DNA: 5'-GCAGTACATGTGACGACGTCG-3' target DNA: 3'-CGTCATCGTACACTGCTCACG-5'

$I_{\text{drain}}$-$V_G$ property indicated decreases of the current with following bare, immobilized DNA and hybridized DNA as shown in Figure 3 (a). These results suggest that the immobilized probe DNA on the gate makes $I_{\text{drain}}$ current decrease to impose bias to the gate by the negative charges in DNA, furthermore the current decrease similar.
larity by hybridizing the target DNA. Especially a difference between the single strand DNA and the hybridized double strand DNA was about 10 mV under the constant $I_{\text{drain}}$ current. $\sqrt{I_{\text{drain}}}$ vs. $V_G$ curves is indicated in order to evaluate a threshold voltage $V_T$ [V] as shown in Fig. 3 (b), since an intercept of straight line for the curve gives the threshold voltage. The threshold voltages of the IS-FET immobilized with the probe DNA and hybridized DNA was about $-1.708$ V and $-1.698$ V, respectively. Hence a difference of each threshold voltage $\Delta V_T$ evaluated about 10 mV. When the measurement was carried out by the use of non-complemental target DNA, the $I_{\text{drain}}$ current did not change. This result suggests that the probe DNA does not hybridize with the non-complemental target DNA, but hybridize with the complemenal target DNA.

Here, the amount of the immobilized DNA on the gate was calculated by using the $\Delta V_T$ value. The gate size is $L=30 \mu m$, $W=350 \mu m$, and the permittivity: $\varepsilon$ and thickness: $d$ of $Ta_2O_5$, $Si_3N_4$, and $SiO_2$ thin films in the gate electrode is $\varepsilon=25$, 6, 4, and $d=40$, 100, 100 nm, respectively. Therefore each capacitance $C$ [F] is given by $C = \varepsilon \cdot \varepsilon_0 \cdot \varepsilon \cdot L \cdot W / d$, where is $C_{Ta_2O_5} = 58 \times 10^{-12}$, $C_{Si_3N_4} = 5.6 \times 10^{-12}$, and a sum of these capacitances is about $2.1 \times 10^{-12}$ [F]. Here, since the difference of threshold voltage $\Delta V_T$ between the ss-DNA and ds-DNA is 10 mV, an increased quantity of electricity per area by the hybridization; $\Delta Q_{\text{DNA}}$ [C/cm$^2$] is

$$\Delta Q = \frac{C \Delta V_T}{LW} = 2.1 \times 10^{-10} \text{[C/cm$^2$]}$$

by using a $Q=CV$ equation. Now, the quantity of electricity par one DNA molecule is $e \cdot [C] \times [21] \text{[mer]} = 1.6 \times 10^{-19} \times 21 = 34 \times 10^{-19}$ [C /molecule] by using elementary charge ($e = 1.6 \times 10^{-19}$ [C]) since the 21 mer origonucleotides consist of 21 phosphor bases, which have 21 negative charges. Hence the amount of the immobilized DNA per area are estimated at

$$\frac{2.1 \times 10^{-10}}{34 \times 10^{-19}} = 6.3 \times 10^8 \text{[molecules/cm$^2$]}.$$  

The amount of immobilized DNA was estimated at $3.6 \times 10^8$ by XPS, and different at about 100 times from the value measured by the IS-FET. This result suggests that a non-specific adsorption by the target DNA on the IS-FET would contains in the value. At present, the method might have an assignment and need a correction for the obtained value.

In the case of using PNA as the probe, Fig. 4 shows representative DNA hybridization measurements. DNA hybridization induces an exponential decrease in the drain current for up to 1.5 h. In contrast, the control experiment showed no changes in the drain current. We were thus able to demonstrate a real-time decrease in the drain current during DNA hybridization.

The threshold voltages before and after hybridization were $-3.50 \text{V and } -3.33 \text{ V}$, respectively in Fig. 5. Based on these results, large positive shifts in the threshold voltage were observed as high as 170 mV. This indicates that the threshold voltage is sensitive to the charge present in the gate region due to polyanionic DNA hybridization. In contrast, the threshold voltage of DNA-immobilized IS-FETs shifted approximately 10 mV as the positive direction. We consequently found that the sensitivity of PNA-modified IS-FETs was more than 17 times than that of DNA-modified IS-FETs. One of the reasons for this is that the surface charge of the DNA modified IS-FET is already negatively charged and the surface charge only doubles at most by hybridization, while the surface charge of the DNA modified IS-FET is expected to change makedly from neutral to negative. Therefore PNA-immobilized IS-FETs will be effective for direct detection of DNA. Similarly, we have calculated the surface charge density $Q$, which is approximately $6.1 \times 10^{-10} \text{ C/cm}^2$ and the number of hybridized DNA molecules at least approximately $2.6 \times 10^8$ molecules/cm$^2$. Compared with results of Fig. 3, the number of hybridized DNA molecules has increased due to the improvement in the reaction process for immobilization. Furthermore the density of double-stranded PNA/DNA molecules is higher than that of double-stranded DNA molecules. It is likely that the difference between PNA and DNA is due to the stronger affinity of PNA for DNA. These results demonstrate the possibility of using an IS-FET based on PNA as a DNA sensor and we expect potential applications in medical diagnostics and molecular biology.

4 Conclusion

Using an ISFET based on PNA, we observed through
the I-V characteristics that the hybridization of surface-immobilized PNA with complementary DNA induces an apposite shift in the threshold voltage. These variations correspond to changes in the surface potential at or near the gate insulator/electrolyte interface induced by complementary DNA recognition.

References