Peptide-Nucleic Acid Immobilized Reaction on the Zn-polar and Oxygene-polar Surfaces of Zinc Oxide

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1. Introduction

Nanotechnology brings new possibilities for biosensors construction and for developing novel electrochemical bioassays. Microarray-based technology for analysis of gene expression and detection of gene mutations has become indispensable for both clinical and basic research. Recently, analysis of genomic mutations for drug-metabolizing enzymes (pharmacogenomics), including detection of single nucleotide polymorphisms (SNP), has received increasing attention for the prediction of the efficacy and side-effects of therapeutic drugs.

In the past research, we succeeded in detecting DNA hybridization using silicon-based Ion-Sensitive Field Effect transistor (IS-FET) [1]. Peptide nucleic acid (PNA) which is an achiral and uncharged DNA mimic was used as a probe molecule. The PNA is hybridized with negatively charged complementary DNA, resulting in the modulation of the source-drain current of the IS-FET [1, 2]. In the present work, a transparent PNA-modified IS-FET based on Zinc Oxide (ZnO) was fabricated. ZnO is a wide-bandgap (3.37eV) semiconductor and transparent to visible light. Therefore, ZnO based transparent IS-FET make it possible to monitor the DNA hybridization both electrically and visibly under an optical microscope which leads to the improvement of detection reliability. In addition, ZnO is one of the most attractive materials to use for biosensors with respect to the unique arrangement of atoms at the surface. There exist three kinds of natural surfaces of ZnO: the Zn-polar (0001) surface plane which consists of only the Zn ions, the O-polar (0001) surface plane with only the O ions, and the non-polar prismatic (10-10) plane where both Zn and O ions are coexistent. It was reported that each surface has different chemical properties to carbon oxides [4, 5] by a few groups. These reports suggest that biomolecules could be modified selectively on ZnO surfaces with different polarity.

In this study, PNA molecules were immobilized on Zn-polar and O-polar surfaces and modified surfaces were evaluated with XPS, AFM and an optical microscope. The results of these experiments suggest the capability of ZnO as a material of biosensors that enables the selective modification and detection of biomolecules.

2. Experimental procedures

The surface modification of ZnO was performed under the following steps. The ZnO single crystal with Zn-polar and O-polar was annealed at 1100 °C in air. The surfaces were next irradiated with UV light for 30 min. The surfaces were immediately immersed for 1 h at room temperature in a solution of acetone containing 1% 3’-aminopropyltriethoxysilane(APTES) and then washed with acetone. After drying, the substrates were baked at 110 °C for 30 min to silanize the surface with the aminosilane coupler. The silanized surfaces were immersed for 1 h at 37 °C in PBS(-) (0.01 M phosphate-buffered saline without Ca²⁺ and Mg²⁺, pH 7.2 - 7.4) containing 1mM N-(6-maleimido-caproyloxy)sulfosuccinimide, sodium salt(sulfo-EMCS), to introduce maleimide groups by a condensation reaction between the amino group of the silanized surfaces and the succinimide group of sulfo-EMCS. The surfaces were washed with PBS(-). Probe PNA in PBS(-) were immobilized on the sulfo-EMCS-modified surfaces by addition reaction for 1h at 37 °C between the maleimide group on the surfaces and the thiol group of the terminal cysteine of Probe PNA. The surfaces were then washed with PBS(-) and exposed for 1 h at 37 °C to an aqous solution of 1mM 6-hydroxy-1-hexanethiol to remove nonspecifically bound DNA. After immobilization of the probe PNA on the surface, probe PNA on the surfaces was hybridized for 1 h at 60 °C with 4µm of target DNA in 0.2 SSC(3mM sodium citrate containing 30mM sodium chloride solution) and then washed with the same buffer.

Scheme 1. Schematic representation of the process of covalently immobilizing the probe PNA on the gate insulator

Each immobilization step was characterized using XPS.
Measurements were made using Rigaku XPS7000 equipped with an Al Kα aluminum anode (1486.6eV).

3. Results and discussion

Figure 2(a) and (b) show the results of XPS spectra for C1s and O1s of the Zn polar and O-polar surfaces. In the spectra of C1s, the two peaks were observed in both Zn- and O-polar surfaces: the peak in lower binding energy is due to amide binding (NC=O) and the higher energy peak is due to the contaminated carbon and aliphatic chain. The intensity of amide peak increased significantly in each surfaces after the immobilization of PNA with backbone based on peptide binding. From these results, it was confirmed that PNA molecules were immobilized on each surface.

(a) Zn-polar surface

(b) O-polar surface

Figure 2. XPS spectra obtained for C1s and O1s of the (a)Zn-polar surface and (b) O-polar surface : (a) the ZnO surfaces following hydrophilic treatment by irradiation with UV light ; (β) the APTES-silanized and sulfo-EMCS-treated surfaces ; (γ) the PNA immobilized surfaces.

In O1s spectra, there were also two peaks. The lower energy peak was attributed to the ZnO crystal substrate (Zn-O) and the higher energy peak was originated from the modified molecules and hydroxyl groups. Comparing the (β) spectra between two peaks, the intensity of the peak due to the ZnO substrate were larger than the peak due to the immobilized molecules in Zn-polar spectra, while the relationship is reversed in O-polar. This result clearly shows that the concentration of immobilized molecules in O-polar surface was higher than that in Zn-polar surfaces. The same result was obtained in the optical microscope imaging. The fluorescent molecules-labeled complementary DNA were hybridized to the immobilized PNA in each surface and observed through the optical microscope. From the brightness of the image, the PNA molecules in O-polar surface were confirmed to be immobilized more stably than in Zn polar surface.

The difference of the PNA concentration between Zn-polar and O-polar surfaces was caused by the first reaction between the APTES and each surface (Figure3).

Figure 3. AFM image of O-polar surface ; (a)after annealed at 1100 β )after immersed in APTES solution and baked

When the APTES molecules bind covalently to the surfaces, condensation reaction takes place between silanol groups derived from APTES and hydroxyl groups on the surfaces. Therefore, the origin of the difference in reactivity between the Zn- and O-polar surfaces may come from the difference in the concentration of hydroxyl bases on the surfaces. Based on the surface atomic arrangement of the different surfaces, the reactivity which requires participation of the lattice oxygen would be higher in O-polar surfaces than in Zn-polar. O-polar surface is suitable for the modification of APTES and PNA, which leads to the high sensitivity of the IS-FET. Furthermore, from the viewpoint of hardness and acidity of the surfaces [6], Zn-polar face with soft acid interacts more strongly with the soft base[2] such as DNA backbone than the O-polar face, resulting in the non-specific adsorption of complementary DNA, which decreases the reliability of detecting. From these results of our study, it can be concluded that the employment of ZnO for biosensors brings the selectivity of modification and detection.

References