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Sialylglycan-modified Field Effect Transistor for Detection of Charged Lectin under Physiological Conditions

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Determination of protein charges under physiological conditions by field effect transistors (FETs) is challenging because of the screening effect by solution counter ions. We showed that sugar-chain-modified FETs can detect proteins under physiological conditions. Protein charges are detectable on the gate interface by applying sugar chains as receptors: these sugar chains are short and highly flexible. Threshold voltage shifts caused by adsorption of SSA lectin from *Sambucus sieboldiana* onto sialylglycan-modified FET showed a linear relationship with SSA concentrations under physiological conditions.

Field effect transistors (FETs) are promising tools for biomolecular detection. Intrinsic charges of biomolecules adsorbed on the gate surface can be directly detected as electrical signals: this method is label-free and cost-effective and enables rapid detection. Therefore, biomolecular detection using FETs has been widely investigated.[1–8] Regarding clinical application of FET biosensors, it is desirable to detect biomolecules under physiological conditions because bioactivity under such conditions is high and real-time monitoring of biomolecules is possible. However, FET-based detection of proteins under physiological conditions poses great difficulties because of the high screening effect by solution counter ions. Under physiological conditions, a certain length scale, termed the Debye length, in which target biomolecules cause field effect to the FET gate is ca. 1 nm. Hence, target biomolecules cannot exist within the Debye length because the size of the antibody (10–12 nm), which is a common receptor, is greater than the Debye length.[5–8] To overcome this problem, application of small receptor molecules such as an antibody fragment prepared by an enzyme[6] is considered to be effective because adsorption of biomolecules on the gate interface is critical for biomolecular detection under physiological conditions. However, in the proposed method, complicated procedures and excessive amounts of reagents are necessary to obtain the desired molecules. Here, we propose an FET biosensor modified with sugar chains as receptors. Sugar chains (lengths ranging from a few Å to a few nm) are shorter than antibodies and play crucial roles in various bioactivities through specific sugar–protein interaction.[9] Using the interaction, protein detection by sugar chain microarrays or sugar-chain-modified electrodes has been reported.[10–15] In addition, sugar chains are highly flexible because of their molecular frame. Therefore, target biomolecules readily approach the gate interface when sugar chains are utilized as receptors of FET biosensors, and this enables biomolecular detection under physiological conditions. In this study, we demonstrated detection of SSA lectin from *Sambucus sieboldiana* by using sialylglycan-modified FET (Figure 1a).

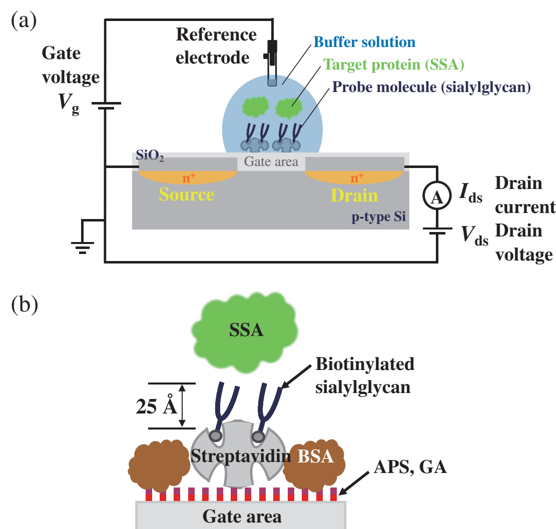

Figure 1. (a) Schematic illustration of SSA lectin from *Sambucus sieboldiana* detection by sialylglycan-modified field effect transistor (FET). (b) An image showing modification of the FET gate surface with sialylglycan.

The sialylglycan-modified FET was fabricated as shown in Figure 1b. An FET gate surface (n-type FET; size, 10 µm (length) × 1000 µm (width), Toppan Printing Co., Ltd.) was exposed to O_2 plasma at 200 W for 1 min (PR301; Yamato Scientific Co., Ltd.) to introduce a hydroxy group on the surface. After exposure to the plasma, the gate surface was immersed in a 1% [v/w] solution of 3-aminopropyltriethoxysilane (Sigma-Aldrich Inc.) in toluene at 60 °C for 7 min under argon atmosphere (purity, 99.99%). The surface that was modified with aminopropylsilyl (APS) monolayer was ultrasonically cleaned in a mixture of methanol and toluene (1:1) for 15 min and rinsed with ethanol, and residual solvents were removed with a stream of N_2 gas. After modification of the APS monolayer, the gate surface was annealed at 160 °C for 2 h under argon atmosphere. Subsequently, in preparation for crosslinking with protein, the amino-terminated gate surface was soaked in a solution of 2.5% glutaraldehyde (GA; Kanto Chemical Co., Inc.) in phosphate buffer (PB; ionic strength, 160 mM; pH, 6.0) at room temperature for 30 min. Thereafter, streptavidin (1 mg mL^{-1}, Funakoshi Co., Ltd.) was immobilized on the gate surface at room temperature for 1 h. To block nonspecific adsorption of proteins onto residual GA sites, bovine serum albumin (BSA, 3% [w/v]; Jackson ImmunoResearch Laboratories Inc.) was applied on the gate surface. After constitution of the protein layer, biotinylated sialylglycan, which was prepared by *endo-β-N*-acetyl glucosaminidase from *Mucorhiemalis*

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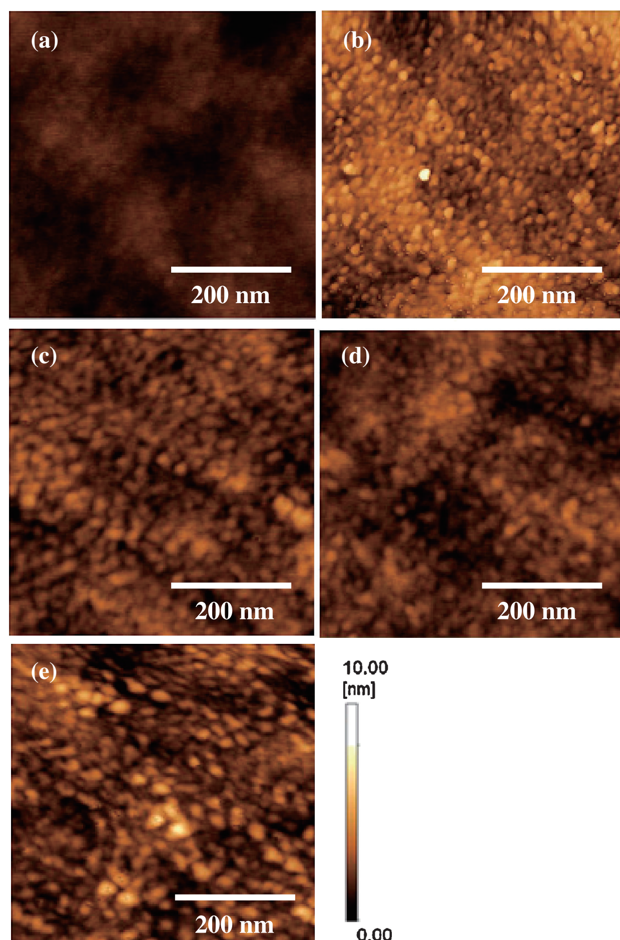

Figure 2. Surface morphologies of the FET gate surface after (a) introduction of glutaraldehyde (GA), roughness (R_a) value: 0.372 nm; (b) immobilization of streptavidin, R_a value: 0.801 nm; (c) blocking with bovine serum albumin (BSA), R_a value: 0.561 nm; (d) modification with sialylglycan, R_a value: 0.560 nm; and (e) addition of SSA, R_a value: 0.789 nm.

(Tokyo Chemical Industry Co., Ltd.),[13] was applied and kept at room temperature for 1 h: this resulted in the generation of a sialylglycan-modified gate surface. Gate voltage (V_g)–drain current (I_d) characteristics were measured with a digital source meter (Keithley 2400) before and after addition of SSA (Seikagaku Biobusiness Co.) to the sialylglycan-modified FET. The measurements were performed in PB by sweeping the V_g from −2 to 3 V with a drain voltage of 1 V. Threshold voltage shifts (ΔV_g) were evaluated from the V_g–I_d measurements. Morphological analyses of the FET gate surface were performed with a scanning probe microscope (SPM; tapping mode, SPM-9600; Shimadzu Co., Ltd.).

The surface morphologies of the FET gate surface after introduction of GA, immobilization of streptavidin, blocking with BSA, modification with sialylglycan, and addition of SSA are shown in Figure 2. The roughness (R_a) values of the surface in each of the above-mentioned modification state were 0.372, 0.801, 0.561, 0.560, and 0.789 nm, respectively. These differences in the R_a values indicated changes of the gate surface in each modification state. First, immobilization of streptavidin on the GA surface was confirmed by an increase in the R_a value (Figure 2b). Second, decrease in the R_a value after addition of BSA indicated that the part of the GA surface where streptavidin was not immobilized was covered with BSA, and this resulted in a mixed protein layer of streptavidin and BSA (Figure 2c). In contrast, the R_a value calculated after addition of sialylglycan was almost the same as that calculated after BSA blocking (Figure 2d). This result could be attributed to the fact that sialylglycan (length, approximately 25 Å and width, 2.5 Å) is too short to be analyzed by SPM. Finally, increase in the R_a value after addition of SSA indicated adsorption of SSA on the sialylglycan-modified surface (Figure 2e). In addition, fluorescence studies revealed that the amount of fluorescein isothiocyanate (FITC)-conjugated SSA adsorbed on the sialylglycan-modified SiO_2 substrate increased with increase in the concentration of FITC-conjugated SSA (data not shown). From the results of the morphological analyses and fluorescense observation, it was confirmed that sialylglycan was modified successfully on the FET gate surface as a receptor.

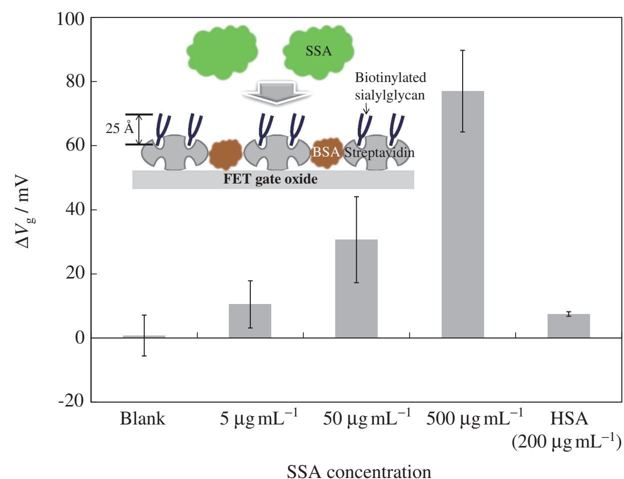

Figure 3. Relationship between SSA concentrations and the magnitude of threshold voltage shifts (ΔV_g) caused by SSA adsorption. Blank indicates addition of SSA-free PB. Human serum albumin (HSA) was added as a negative control.

Specific binding of SSA onto the sialylglycan-modified FET gate surface is detected as a shift in the gate voltage, ΔV_g. The ΔV_g shifted in positive direction by 86 mV after addition of 500 μg mL^{-1} SSA (Supporting Information, Figure S1).[16] SSA possesses negative charges in PB (pH, 6.0) because the isoelectric point of SSA, calculated from the amino acid sequence, is 4.8. The adsorption of SSA molecules converted the surface charge to negative, which caused the ΔV_g. Figure 3 shows the relationship between SSA concentrations and ΔV_g values caused by application of SSA to the sialylglycan-modified FET under physiological conditions. As shown in Figure 3, the ΔV_g values were proportional to the SSA concentrations ranging from 5 to 500 μg mL^{-1}, and the adsorption may saturate at that concentration. This shows that ΔV_g is related to the concentration of SSA. Taken together, the results show that the ΔV_g values were derived from the amount of SSA adsorbed on the FET gate surface. Thus, SSA was detected by sialylglycan-modified FET under physiological conditions. In general, it is difficult to detect proteins by FET

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under physiological conditions because of the short Debye length (ca. 1 nm).[5–8] In our model, sialylglycan is shorter than antibody, which is a common receptor, and might be more flexible than antibody which has a three-dimensional structure. Therefore, SSA might readily approach the gate interface and exist within the Debye length formed from the insulated protein layer. To confirm the specificity of the sialylglycan-modified FET, ΔV_g was evaluated after application of human serum albumin (HSA) to the sialylglycan-modified FET as a negative control (Figure 3). The ΔV_g value under 10 mV indicated that nonspecific adsorption of HSA on the sialylglycan-modified gate hardly occurred, and this suggests that the sialylglycan-modified FET was highly specific for SSA. Thus, we achieved protein detection under physiological conditions by using sugar-chain-modified FET without preparation of antibody fragments or dilution of the buffer solution.

In this study, we showed that sugar chains utilized as a receptor were useful for FET-based protein detection under physiological conditions. It is expected that more sensitive detection will be accomplished by utilizing shorter sugar chains. Furthermore, if more medically important sugar chains become available through advancement of glycomics, the strategy of using sugar chain receptors will greatly contribute to FET-based biosensor detecting various biomolecules, such as disease markers, viral proteins, and cells.

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16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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