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Surface Modification with Aryldiazonium Salt Chemistry of Extended-Au Gate Field-Effect Transistor for Ultra-Sensitive Detection of Low-Molecular-Weight Biomarker

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Abstract

In this study, a novel approach towards a highly sensitive and selective detection of low-molecular-weight biomolecules was proposed. An extended-Au gate field-effect transistor biosensor showed a high detection sensitivity to various biomolecules; therefore, the sensor surface was chemically modified by multilayer thin film to not only detect selectively target small molecules at the Au gate but also prevent non-specific signals based on impurities. As a result, the modified sensor detected the small biomolecules such as dopamine in nM order, which approached onto the Au gate electrode, but the large size of biomolecules such as paromomycin was hardly detected because it did not pass through the modified film. The result demonstrated the possibility of building a novel sensing platform for the potentiometric biosensors.

1. Introduction

Selective molecular sensing is of great importance in clinical applications. Due to the background noises caused by the impurities in physiological samples, realization of selective detection of biomarkers, especially of small biomolecules, remains a great challenge. In this regard, field-effect transistor biosensors (FET biosensors) may meet the requirements of the detection of low-molecular-weight biomarker, thanks to its ability to sensitively detect the changes in surface charges [1]. In particular, the preliminary study revealed that the extended-Au gate FET biosensors (EG-Au-FET) enables an ultra-sensitive detection of various biomarkers based on the electrochemical and catalytic reactions between biomolecules and the Au gate surface. Hence, if the sensor surface is designed so that only a small biomolecule as target reaches the Au sensing surface, selective detection of such small molecules can be realized. Moreover, by suppressing the impurities to enter the area under Debye length, the area where electrical signal can be detected by FET biosensors [2], the S/N ratio can be significantly improved. In this study, the Au electrode was modified by the multilayered thin film of aromatic compounds using aryldiazonium salt chemistry to differentiate the molecules reaching the Au sensing surfaces depending on the sizes of molecules, as illustrated in Fig. 1.

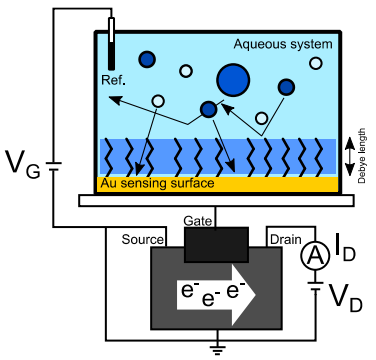

Fig. 1. Conceptual illustration of the EG-Au-FET biosensors to differentiate the molecules reaching Au sensing surface by the size.

Aryldiazonium salt chemistry is one of the promising method for universal surface functionalization [3]. As reactive aryl radicals formed by the electrochemical reduction of aryldiazonium salt directly react with the substrate, a stable multilayer thin film can be grafted on various substrates such as Au. Furthermore, the layer growth can proceed to as high as 10 nm, thus the layer thickness can be designed around Debye length. For the fundamental investigation, dopamine (MW: 153.2), a well-known neurotransmitter and paromomycin (MW: 713.7), an aminoglycoside antibiotic, were utilized as model biomolecules with different sizes. The response of the multilayer grafted EG-Au-FET biosensors to the addition of model biomolecules was studied.

2. Experimental

Grafting multilayer thin film on Au electrode

Multilayer thin film of phenethyl ethanol was grafted on the Au electrode using diazonium salt chemistry. First, 100 mL of 0.5 M HCl was stirred in a 300 mL beaker in ice-cold water. 28 mg 2-(4-aminophenyl)ethanol was added and stirred for 5 min. Then, 1 mL of 200 mM $NaNO_2$ in distilled water was added dropwise to the mixture, and stirred for 15 min. A cleaned Au electrode was immersed in the solution, and cyclic potential was applied from 0.4 V to -0.3 V (vs Ag/AgCl) at 50 mV/s using potentiostat (PS-14, Toho Technical Research) coupled with a function generator (AFG3102C, Tektronix). After 5 cycles, the substrate was washed thoroughly with methanol and water. The schematic is summarized in Fig. 2. The layer thickness was determined using atomic force microscopy (Keysight 5500, Toyo Corporation) by taking the difference between the thickness of bare Au and modified Au electrodes. Wettability of the surface was investigated using contact angle measurement

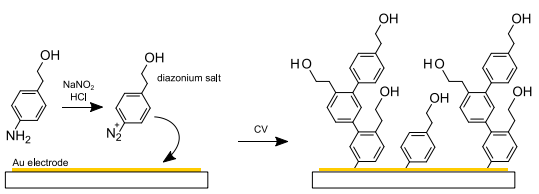

Fig. 2. Schematic of surface modification using aryldiazonium salt chemistry

(CA-W, Kyowa Interface Science).

FET real-time measurement

Multilayer modified Au electrode was connected to the extended gate of the n-channel junction-type FET (K246-Y9A, Toshiba), and the gate surface potential was monitored at constant I_{DS} = 700 µA and V_G = 0 V in real-time manner using FET real-time monitoring system (Optogenesys). In the operation, sensing surface was separated using polycarbonate ring, and initially filled with 300 µL 10 mM standard phosphate buffer (10 mM). After the stabilization of surface potential, the analyte was continuously injected to the solution using syringe pump.

3. Results and Discussion

Response of EG-Au-FET biosensors to various biomolecules

First, the detection sensitivity of EG-Au-FET biosensor was investigated for various biomolecules using continuous injection of analyte solution, and the results are summarized in Fig. 3. The results revealed that the Au electrode was sensitive to various biomolecules such as dopamine-related biomolecules, uric acid, glucose, and bovine-serum albumin (BSA). Moreover, for some of the biomolecules, the detection sensitivity was as low as in pM or nM order. Interestingly, the detection mechanism was not likely to depend only on the changes in surface charges, since the surface potential shifted in negative direction of all the molecules tested regardless of the intrinsic positive/negative charges of the molecules. One possible mechanism is the catalytic oxidation of biomolecules on the Au sensing surface. For instance, dopamine-related biomolecules are known to be easily oxidized on the Au surfaces [4].

Response of aryl-multilayer grafted EG-Au-FET biosensor

Regarding the chemically-modified Au gate, the modified surface was characterized using contact angle measurement and AFM measurement. The contact angle deceased from 105° to 70° by after the modification. Thus, the surface became considerably hydrophilic because of hydroxyl group on the aryl-compound. Moreover, the thickness of the film was determined to be 7 nm. The Debye length is approximately 3 nm in 10 mM phosphate buffer solution, so that the film was successfully grafted around the designed thickness.

Moreover, the effect of the multilayer modification on the detection sensitivity was investigated by continuously adding dopamine and paromomycin. As shown in Fig. 4A, dopamine was sensitively detected by both bare Au electrode and modified Au electrode sensors. On the other hand, the signal for the addition of paromomycin was suppressed for the modified sensor (Fig. 4B). Dopamine was small enough to penetrate through the grafted layer, whereas paromomycin did not reach the sensing layer (Fig. 4C). Thus, the biomolecules were successfully differentiated by the size.

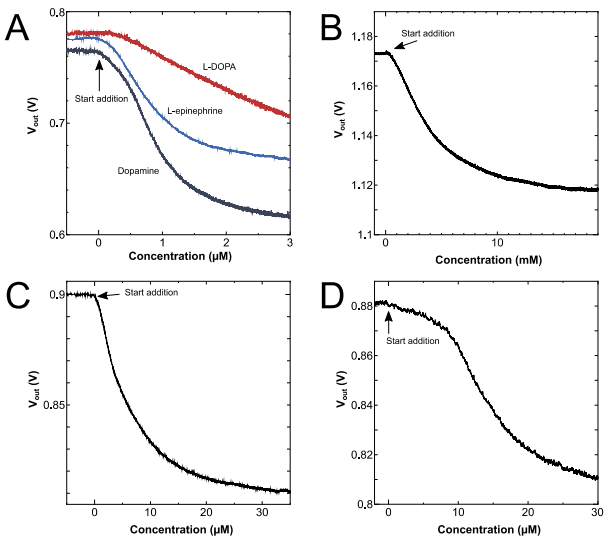

Fig. 3. Real-time change in surface potential for the addition of various biomolecules. **(A)** Dopamine, L-DOPA, and L-epinephrine. Each of 0.03 mM injected at 5 µL/min. **(B)** Glucose. 50 mM injected at 5 µL/min. **(C)** Uric acid, 0.1 mM injected at 5 µL/min. **(D)** Bovine-serum albumin, 1mg/mL injected at 5 µL/min. The *x*-axis is converted to the

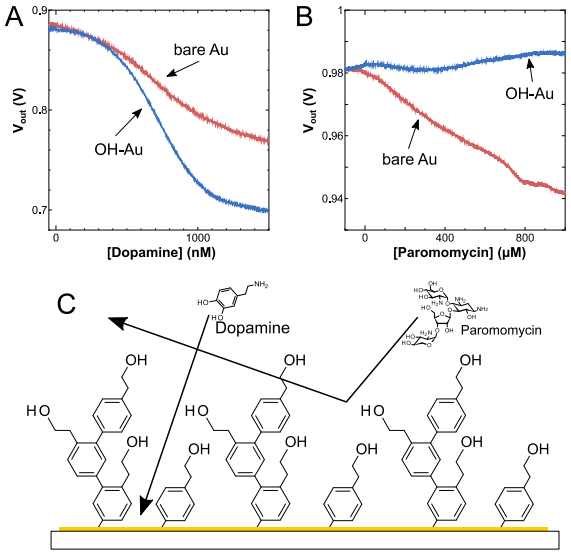

Fig 4. Change in surface potential for the continuous injection of analyte solution into Au (red line) and OH-Au (blue line). Injection started at 0. **(A)** Addition of dopamine, 0.003 mM at 5µL/min. **(B)** Addition of paromomycin, 2 mM at 5 µL/min. **(C)** Illustration of the effect of sensor modification.

4. Conclusions

In this study, the surface of ultra-sensitive EG-Au-FET biosensor was chemically modified by the aryl-multilayer thin film so that only the small biomolecules reached the sensing surface and then were selectively detected. Resultantly, we have shown the possibility of this approach to be used in the selective sensing of small biomolecules. By supplying the polymeric filter on the film further, more selective detection between small molecules may be realized.

References

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