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Direct detection of enzyme-catalyzed products by FET sensor with ferrocene-modified electrode

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ABSTRACT

An FET-based biosensor with a ferrocene-modified gold electrode detects the enzyme-produced electrons by using mediators that transfer the electrons from the enzyme to the sensor. Since an extended-gate FET sensor with a light-shielding mask can be operated without a light-shielding box, a small portable instrument will soon be realised. However, when the FET sensor detected enzyme-catalyzed products with the mediators under light conditions, measurements fluctuated due to photo-reduction of the mediators, resulting in decreased sensitivity. To improve sensitivity by reducing the fluctuation, we developed a procedure for directly detecting enzyme-catalyzed products without using the mediators. The key technique used in this procedure was a measurement technique using our developed potential-keeping method, in which the modified electrode of the FET sensor was oxidised by ferricyanide solution to make its surface the same high potential every time, and this high potential was kept until measurement because of the high input impedance of the FET structure. After this method was applied, the interfacial potential of the gold electrode decreased depending on the amount of enzyme-catalyzed products due to the ferrocene molecules immobilised on the gold electrode directly reacting with the products. The results obtained in light conditions indicated that model compounds of the products were detected from 10 μM to 10 mM with the Nernstian response of 59.2 mV/decade. Also, this method was applied to pesticide detection by using the enzyme inhibition by pesticide, and 5 ppb of diazinon was successfully detected by using only a sensor chip.

1. Introduction

Electrochemical enzyme-based biosensors that utilise enzymes as recognition elements have been widely studied. These biosensors are normally based on an enzyme-catalyzed reaction that produces electrons or protons. Ion-sensitive field-effect transistor (ISFET)-based biosensors, which are categorised in enzyme-based biosensors, detect the protons produced by enzyme-catalyzed reactions. The initial concept of the ISFET-based enzyme sensor was proposed by Janata and Moss (1976). Since the first results of using an ISFET-based biosensor for measuring penicillin were reported (Caras and Janata, 1980), it has been developed for detecting various kind of analytes such as glucose, urea, and so on (Dzyadevych et al., 2006). To take advantage of their high sensitivity and ease of integration into analysis systems, the ISFET-based sensors are commonly studied in the field of clinical medicine and in biotechnological and environmental applications (Schöning and Poghossian, 2002). However, since pH change caused by proton production is suppressed by a buffer solution, the sensitivity tends to lower because of its high buffer capacity (Caras and Janata, 1980; Miyahara and Moriizumi, 1985). Even though a solution with high ionic strength and high buffer capacity is suitable for the enzyme activity, it is difficult to operate ISFET-based enzyme sensors in such a solution.

Recently, another type of FET sensor, an FET-based biosensor with a ferrocene-modified gold electrode, has been reported (Ishige et al., 2009). This sensor detects not protons but electrons by using mediators that transfer the produced electrons to the ferrocene molecules on the gold electrode of the FET sensor. Thus, the sensitivity of the FET-based biosensor with a ferrocene-modified gold electrode is hardly affected by pH and buffer capacity. Therefore, the FET-based biosensor with the ferrocene-modified gold electrode can be easily operated while keeping its high sensitivity in suitable buffer conditions for the enzyme activity, which is high ionic strength and high buffer capacity. By applying the FET-based biosensor using ferricyanide and ferrocyanide as mediators to detect enzyme-catalyzed reaction, serum cholesterol was detected with the Nernstian slope of 57 mV/decade, which is 97% of theoretical value.

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Meanwhile, since the FET-based biosensor is light sensitive, it must be operated in dark conditions such as in a light-shielding box, which spoils the advantage of its smallness. We also developed an extended-gate FET sensor that can be operated without a light-shielding box by masking the FET structure within a sensor chip (Kamahori et al., 2007). By applying the extended-gate FET sensor to the enzyme-based biosensor, a small portable instrument will soon be realised. However, when the extended-gate FET sensor with the light-shielding mask detected enzyme-catalyzed products with the mediators under light conditions, measurements fluctuated, resulting in a sensitivity decreasing and its applications being more limited. This study found that fluctuation was caused by not only light sensitivity of the FET structure but also photo-reduction of mediators. To improve sensitivity by reducing the fluctuation, we devised a new procedure for directly detecting enzyme-catalyzed products without using mediators. In this procedure, a measurement technique using the potential-keeping method played an important role in the FET-based biosensor with a ferrocene-modified electrode. In this method, the modified electrode of the FET sensor was oxidised by ferricyanide solution to keep its surface at high potential because of high input impedance of the FET structure. Thus, the extended-gate FET sensor with the ferrocene-modified electrode can directly detect the enzyme-catalyzed products without mediators under light conditions. In addition, this procedure was applied to detect pesticide using the enzyme inhibition by pesticide.

2. Materials and methods

2.1. Chemicals and reagents

The following chemicals and reagents were used in the experiments: ethanol, potassium chloride, potassium dihydrogenphosphate, disodium hydrogenphosphate, sodium chloride, sodium hydrogencarbonate, sodium hydroxide, potassium hexacyanoferrate (III), potassium hexacyanoferrate (II), diazinon, acetylthiocholine, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), eserine sulfate from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); 11-ferrocenyl-1-undecanethiol (11-FUT) from Dojindo (Kumamoto, Japan); and acetylcholine esterase (AChE) from Liofilchem (Abruzzo, Italy).

All reagent solutions were prepared with deionised water (resistively of 18.2 MΩ cm) from an ultra-pure water system (model WD500, Yamato Scientific Co., Ltd., Tokyo, Japan).

2.2. Fabrication of extended-gate FET sensors

Fig. 1 shows a diagram of the extended-gate FET sensor chip (5 mm × 7.5 mm). The extended-gate FET sensor consists of three parts: the gold electrode, the FET structure that transduces the change in the interfacial potential on the gold electrode into a change in the drain current, and the light-shielding mask that protects the FET structure from light irradiation. The sensor chip was fabricated using a semiconductor process. The FET had an n-channel depletion structure (formed by As^+ ion implantation at 120 keV with a dose of 6.0×10^{11} molecules/cm^2) with p-type source and drain areas. The width and length of the channel region (W/L) were 2400 μm and 5 μm, respectively, and the gate insulator of the FET consisted of a 17.5-nm SiO_2 layer, formed by a wet oxidation method, under a 1.3-μm Si_3N_4 layer formed by plasma-enhanced chemical vapor deposition. The light-shielding mask was an 800-nm thick aluminium layer. The gold electrode (400 μm × 400 μm) was fabricated by sputter depositing an adhesion layer of nickel–tungsten alloy and then a gold layer (100 nm, 99.99% purity) on aluminium wire that was connected to the gate area of the FET. The fundamental characteristics of an extended-gate FET sensor were described previously (Ishige et al., 2006).

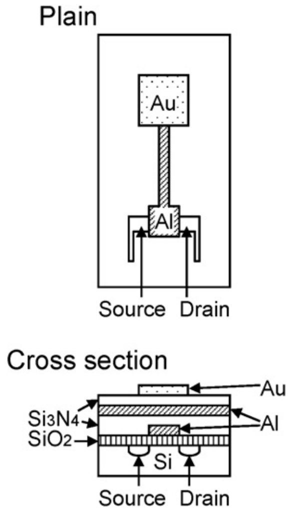

Fig. 1. Diagram of the extended-gate FET sensor chip (5 mm × 7.5 mm), which consists of three parts: a gold electrode, an FET structure, and a light-shielding mask. The FET is connected to the gold electrode (400 μm × 400 μm) by an aluminum wire under a 1.3-μm Si_3N_4 layer.

The alkanethiol modification procedure for the gold electrode on extended-gate FET sensor chips was as follows. The sensor chips were kept in an alkanethiol solution for 1 h. The concentration of ferrocenyl-alkanethiol (11-FUT) solution was 5 mM in ethanol. After alkanethiol modification, the sensor chips were rinsed with ethanol and deionised water twice to remove unreacted alkanethiol molecules. The sensor chips were kept in a 0.1-M sodium sulfate solution at room temperature until use.

2.3. Measurement procedure

The FET sensor is light sensitive because of its photo-induced carrier. To investigate light sensitivity of the extended-gate FET sensor with or without a light-shielding mask, gate bias (V_G)–drain current (I_D) curves as characteristics of FET sensors were measured by a semiconductor parameter analyzer (4155C, Agilent, Tokyo, Japan) in 0.1-M sodium sulfate solution with an Ag/AgCl reference electrode containing saturated KCl (RE-1C, BAS, Tokyo, Japan). FET sensors used in measurements were either with or without a light-shielding mask, and the conditions were light (under a fluorescent lamp, FPL36EX-W DK, Hitachi, Tokyo, Japan) or dark (in a light-shielding box). The range of the wavelength of the fluorescent lamp is 400–720 nm.

The interfacial potential of the ferrocene-modified gold electrode on the FET sensor was measured as follows. The drain current of the FET sensor was measured by the semiconductor parameter analyzer with the Ag/AgCl reference electrode to which a 0.1-V DC voltage with a superimposed sine wave (1 MHz, 0.2 V_{p-p}) was applied by a function generator (1940 Multifunction Synthesizer, NF circuit design block, Kanagawa, Japan). A measurement technique using a superimposed high-frequency voltage (Kamahori et al., 2007) was applied to improve the stability of the interfacial potential on the gold electrode of the FET sensor. The measured drain current was converted into the interfacial potential on the basis of the V_G–I_D curve that was measured by the semiconductor parameter analyzer by applying V_G directly to the gold electrode of the extended-gate FET sensor in dry conditions.

To investigate the effect of light on the redox reaction detection system using the FET sensor, interfacial potential of the ferrocene-modified gold electrode on the FET sensor was measured in ferricyanide and ferrocyanide solution used as redox