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Notes

An Extended-gate Type Organic FET Based Biosensor for Detecting Biogenic Amines in Aqueous Solution

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This study is the first to report on the detection of biogenic amines in an aqueous solution using an organic field-effect transistor (OFET) device with an extended gate electrode modified with a layer of diamine oxidase and a horseradish peroxidase osmium-redox polymer. The limit of detection (LOD) for histamine was estimated to be 1.2 μM. These results reveal that extended-gate type OFET devices are highly suitable enzyme-based biosensors for the detection of biogenic amine levels.

Keywords Organic field-effect transistor, histamine, putrescine, osmium polymer, horseradish peroxidase, diamine oxidase

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Introduction

Biogenic amines, such as histamine and putrescine, are produced by bacteria through the decarboxylation of amino acids or by the amination of aldehydes and ketones. Histamine causes an inflammatory response, plays a significant role in the immune response to a pathogen, acts as a neurotransmitter, and regulates physiological functions in the gastrointestinal tract.[1] Putrescine has been studied as a marker in fresh foods that have spoiled, and as carcinogenic nitrosamines when handled by salting or smoking, or preserved with food additives such as nitrite salts to prevent botulism.[2] Therefore, the detection of biogenic amines is considered very meaningful in medicinal and pharmaceutical chemistry, as well as food science. To date, the detection of biogenic amines has relied on high-performance liquid chromatography (HPLC),[3] electrochemical sensors[4–7] or fluorescent probes.[8–11] Although these analytical methods are very accurate and reliable, the development of low-cost and easy-to-use sensor devices for the monitoring of biogenic amine concentrations has become necessary. Accordingly, our research has focused on employing sensors based on electronic devices that possess an organic semiconductor as an active layer, known as organic field-effect transistors (OFETs).[12] Because OFETs are solution-processable, stretchable, flexible and low in manufacturing costs, we believe that the OFET-based sensors are an emerging platform for biosensor devices. Through this study, we have succeeded in developing bio/chemical sensors based on an extended-gate type OFET that accurately and reproducibly detect biomolecules in aqueous media.[13–17] We herein report on an enzyme-based OFET biosensor that possesses an extended-gate electrode on a flexible plastic film substrate for the detection of the biogenic amines histamine and putrescine. A layer of diamine oxidase and a horseradish peroxidase osmium-redox polymer[18] was coated onto the extended-gate electrode, enabling the detection of amine levels in aqueous media (Fig. 1). To the best of our knowledge, this is the first report on a biogenic amine sensing device based on an OFET biosensor with an extended gate.

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Experimental

Reagents and chemicals

Each of the reagents and solvents used in this study were commercially available and used as supplied. Tetradecylphosphonic acid, histamine dihydrochloride, spermine tetrahydrochloride, spermidine, diamine oxidase from porcine kidney, and glutaraldehyde solution were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Tyramine hydrochloride, cadaverine and putrescine were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Supplies of 1,2-dichlorobenzene, dihydrogenphosphate dihydrate, and disodium hydrogenphoshate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Cytop® (CTL-809M), polyethylene naphthalate (PEN) film, poly{2,5-bis(3-hexadecylthiophene-2-yl)thieno[3,2-*b*]-thiophene} (pBTTT-C_{16}), gold, aluminum, FC-43 fluorinert, Teflon® AF1600, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and osmium polymer were purchased from Asahi Glass Co., Ltd. (Tokyo, Japan), Teijin DuPont Films (Tokyo, Japan), Merck KGaA (Darmstadt, Germay), Tanaka Kikinzoku Kogyo (Tokyo, Japan), Furuuchi Chemical Co. (Tokyo, Japan), 3M Co. (St. Paul, MN), Dupont (Wilmington, DE), and Dojindo Laboratories (Kumamoto, Japan), and Bioanalytical Systems (West Lafayette, IN), respectively. Phosphate buffer and HEPES buffer solutions were prepared using Milli-Q water (18 MΩ cm at 25°C).

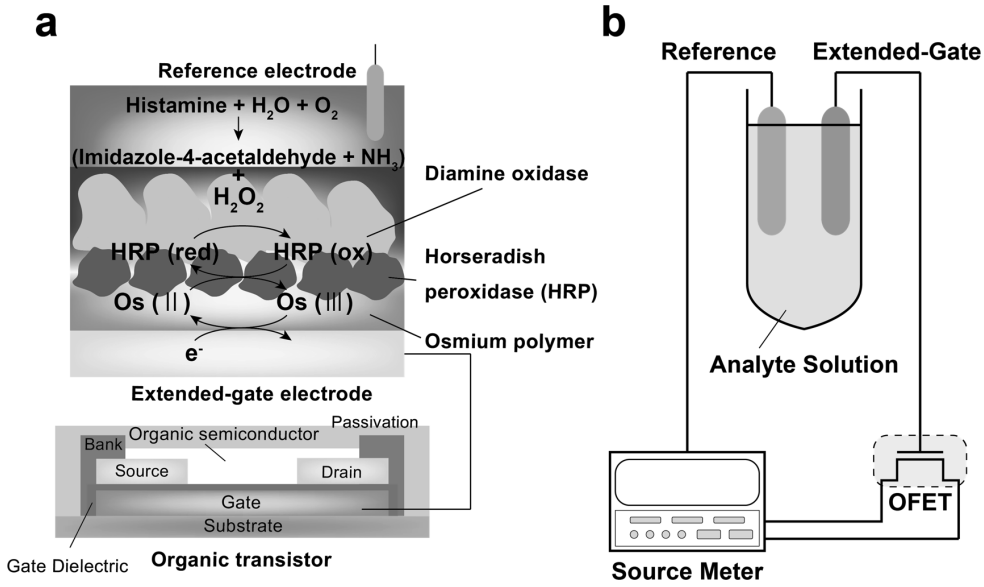

Fig. 1 Schematic illustrations of (a) the extended-gate type OFET biosensor for detecting biogenic amines and (b) the measurement system.

Apparatus

Metal electrodes were deposited using vacuum evaporation equipment supplied by Cryovac Co. (Osaka, Japan). The oxygen plasma treatment was performed using a PC-300 plasma cleaning system from Samco Inc. (Kyoto, Japan). The UV ozone treatment was carried out with a UV253H UV ozone cleaning system from Filgen Inc. (Aichi, Japan). The bank layers were prepared using an IMAGEMASTER 350 dispensing equipment from Musashi Engineering Inc. (Tokyo, Japan). The pH values of solutions were measured using a D-51 pH meter (Horiba Ltd., Kyoto, Japan). The Ag/AgCl reference electrode was purchased from BAS Inc. (Tokyo, Japan). The electrical characteristics for all OFET devices used were measured using a Model 2636B source meter (Keithley Instruments, Cleveland, OH).

Fabrication of the OFET-based biosensor

The aluminum (Al) gate electrode was deposited onto a glass substrate *via* thermal evaporation (30 nm thick). The gate dielectric layer consisted of an aluminum-oxide layer (5 nm thick) and a tetradecylphosphonic acid (1.7 nm thick) self-aligned monolayer (SAM).[19,20] The aluminum-oxide layer was formed by treating the Al gate electrode with an oxygen plasma, using a plasma power of 300 W and treatment duration of 50 min. The SAM was formed by immersing the substrate in a 2-propanol solution of tetradecylphosphonic acid at room temperature. Au source-drain electrodes (30 nm thick) were deposited onto the gate dielectric layer using thermal evaporation and patterned using a shadow-mask. The channel width and length of the OFET device were 1010 and 18 µm, respectively. For the preparation of the bank layers, a 1 wt% solution of an amorphous fluoropolymer in FC-43 was applied using the dispenser equipment. Subsequently, a semiconducting polymer, pBTTT-C_{16}[21] was drop-casted from a 0.03 wt% solution of 1,2-dichlorobenzene and followed by an anneal at 150°C for 30 min in a nitrogen atmosphere. To passivate the device, Cytop® (CTL-809M) was spin-coated onto the device, followed by a baking step at 100°C for 10 min (100 nm thick).

An extended-gate electrode consisting of gold or Au (50 nm thick) was prepared on a PEN film substrate (125 µm thick) using thermal evaporation, such that the sensing area for the extended-gate electrode was 15 mm². The fabricated electrode was coated with a horseradish peroxidase osmium-redox polymer and allowed to stand for 24 h at 8°C. The electrode was subsequently covered with the mixture of diamine oxidase from porcine kidney (> 0.05 unit/mg) in Dulbecco's phosphate-buffered saline and glutaraldehyde (2 vol%). To immobilize enzymes on the covered electrode, it was allowed to stand for 30 min at room temperature. Finally, the electrode was rinsed with a HEPES buffer solution (100 mM, pH 7.4).

Results and Discussion

Electric characteristics of the fabricated OFET device

To detect biogenic amines in aqueous media, the OFET device must be operated at low voltages and should be stable enough for repeated use. The fabricated OFET devices were therefore subjected to repeated measurements of their electrical characteristics. The gate voltage (V_{GS}) was swept from 0 to –3 V while the drain-source voltage (V_{DS}) was kept at –3 V. The field-effect mobility in the saturation region was estimated to be 0.01 cm²/Vs and the on/off current ratio was ~30, values that are adequate for biosensor applications. More importantly, no hysteresis was observed in the transfer curve (Fig. S1, Supporting Information). Judging from these results, we concluded that the fabricated OFET device operated stably and reliably enough for use in biogenic amine sensing.

Changes in OFET output current with increasing histamine concentration

Next, we investigated the ability of the fabricated OFET biosensor to electrically detect histamine in an aqueous solution. Figure 2 shows the time course change of the current (I_{DS}) of the OFET biosensor for stepwise changes in the histamine concentration in a HEPES buffer solution (0.5 mL) at pH 7.4 at room temperature. To avoid the initial bias-stress effects on the OFET, we operated the device ($V_{DS} = V_{GS} = –2.5$ V) for 1 h

before adding histamine. As a result, we observed a clear response to the addition of histamine, which implies that the OFET biosensor was effective in monitoring the enzymatic reaction. Continuous changes in I_{DS} were observed by the stepwise addition of histamine, indicating that the OFET sensor can be used for real-time monitoring.

Changes in OFET transfer characteristics with the addition of histamine

Changes in I_{DS} correspond to the field effect modulation of the electrical conductivity in the fabricated OFET device channel region, which is affected by the addition of histamine. Figure 3(a) exhibits the electrical transfer characteristics of the OFET upon titration with a HEPES-buffer solution (0.5 mL) of histamine. Interestingly, the histamine titration showed a negative shift of the transfer curve with increasing histamine concentration, while no significant change in the gate current was observed. This shift of the transfer curve could be attributed to a change of the hole concentration in the OFET conduction channel,[22-24] which is derived from the interfacial potential shift on the extended-gate electrode by the oxidation-state change of osmium ion. In addition, the stability of the gate current suggests that no electrochemical reaction (*i.e.*, the electrolysis of water) was caused by applying the gate voltage.[25,26] Taken together, the enzymatic redox chain reaction on the extended-gate electrode brought about the observed changes in the OFET transfer characteristics. The relationship between the histamine concentration and changes in the threshold voltage (V_{TH}) are summarized in Fig. 3(b). We obtained a linear relationship in the region of the low concentration (0 – 10 µM), and the limit of detection (LOD)[27] was estimated to be 1.2 µM, which is comparable or higher to the sensitivity of other electrochemical sensors reported in the literature.[28,29]

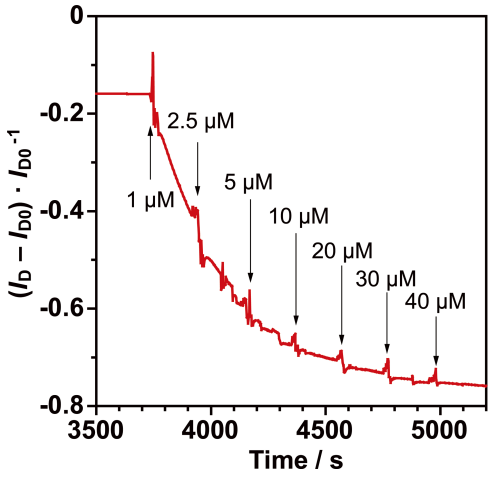

Fig. 2 Time course change of the drain current (I_{DS}) with increasing concentration of histamine. $V_{DS} = V_{GS} = -2.5$ V.

Selectivity for biogenic amines

We also evaluated the selectivity of the fabricated OFET biosensor in the detection of biogenic amines. In the titration experiments, histamine, putrescine, cadaverine, tyramine, spermine and spermidine were included. The titration results are summarized in Figs. 4 and S2 (Supporting Information), showing that the addition of cadaverine, tyramine, spermine and spermidine resulted in virtually no response. Weak fluctuation in response is probably due to physisorption on the extended-gate electrode. Unfortunately, histamine and putrescine are difficult to distinguish from each other, which is presumed to be because diamine oxidase can react not only with histamine but also putrescine. Although the discrimination of histamine and putrescine could be achieved by employing histamine oxidase[4] with the OFET device, monitoring the combined amount of diamines is still useful for freshness sensing in foods.[30]

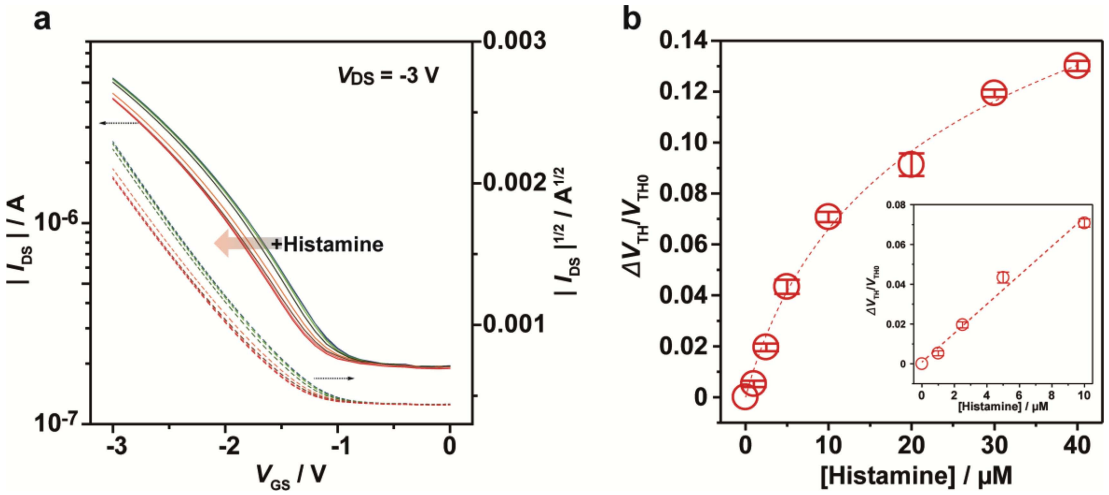

Fig. 3 (a) Transfer characteristics (I_{DS} – V_{GS}) of the OFET sensor upon titration with histamine in a HEPES-buffer solution (100 mM) at pH 7.4 at room temperature. $V_{DS} = -3$ V. [Histamine] = 0 – 40 µM. (b) Changes in threshold voltage (V_{TH}) of the OFET device from histamine at various concentrations in a HEPES-buffer solution (100 mM) at pH 7.4 at room temperature. The measurements were carried out after immersion of the extended-gate electrode into the HEPES-buffer solution for 10 min. A functionalized extended-gate was repeatedly used for the titration. The inset shows the lower end of the titration.

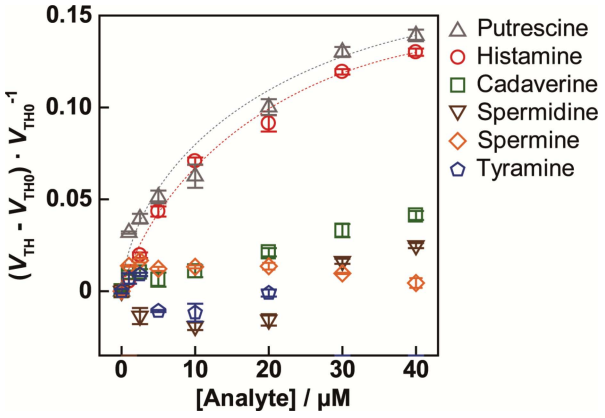

Fig. 4 Changes in the threshold voltage (V_{TH}) for the OFET device by the addition of various biogenic amines in a HEPES-buffer solution (100 mM) at pH 7.4 at room temperature. [Analyte] = 0 – 40 μM.

Conclusions

We are the first to report on the sensing of biogenic amine levels in aqueous media using an OFET-based biosensor with an enzyme-functionalized extended-gate electrode on a flexible plastic film. The fabricated OFET device could be operated at low voltages and exhibited response to histamine and putrescine. We believe that these results will dictate the criteria for detecting biogenic amines using OFET-based biosensors in the future. Additional research on OFET biosensors for use in biogenic amine detection is being carried out in our laboratory.

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Supporting Information

The electric characteristics of the fabricated OFET are shown in Fig. S1. The titration results of putrescine, cadaverine, tyramine, spermine and spermidine are shown in Fig. S2. These materials are available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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