Development of Enzymatic Sensors Based on Extended-gate-type Organic Field-effect Transistors

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ABSTRACT

Organic field-effect transistors (OFETs) are promising platforms for flexible and disposable sensors, because of their attractive properties such as mechanical stretchability, environmental friendliness, compact integration, and solution processability. Although physical sensors utilizing OFETs have been successfully demonstrated, the development of OFET-based chemical/bio-sensors is still in its infancy. In this regard, we have designed new enzyme-modified OFETs with electron mediators for selective analyte sensing. The fabricated OFET with an extended-gate electrode can reproducibly operate under ambient conditions. Importantly, the developed OFET successfully detected analytes such as nitrate, lactate, and biogenic amines in pseudo and real biological fluids. Thus, we believe that the proposed approach to develop OFET-based enzymatic sensors will open up new avenues for the realization of practical and flexible biosensing applications.

Keywords: Organic Transistors, Organic Electronics, Enzymatic Sensors, Electron Mediators

1. Introduction

Organic field-effect transistors (OFETs) are promising devices in the field of semiconductor engineering, owing to their mechanical flexibility and stretchability, environmental friendliness, and low-cost processability. The field-effect phenomenon in organic semiconductor films was first discovered by Kudo et al. in 1984 in Japan. To date, various applications utilizing OFETs have been successfully demonstrated such as rollable display and low-cost radio-frequency identification tags. More importantly, the development of flexible and disposable sensors utilizing OFETs has attracted considerable attention, because of the aforementioned properties. Although OFET-based wearable sensors for the detection of vital signs (such as blood pressure, heartbeat, and body temperature) have been widely reported, the development of chemical/bio-sensors based on OFETs is in its early stage.

To develop chemical/bio-sensing platforms utilizing OFETs, the device should be modified with molecular recognition materials. Enzymes have high substrate specificity and catalytic activities, which play crucial roles in controlling vital organs under ambient conditions. Thus, enzymes are often employed for the construction of electrical-device-based sensors. Many types of enzymatic sensors based on electrochemical techniques have been demonstrated for sensitive and selective analyte detection. However, the development of flexible sensing systems utilizing conventional enzymatic sensors is difficult because they require relatively large equipment (such as potentiostats) for the analysis of output sensing signals. However, OFETs-based sensors can be integrated with data processing circuits on a single flexible substrate. Hence, we have attempted to develop OFET-based sensors modified with enzymes (Fig. 1). In this comprehensive paper, our recent achievements toward developing enzymatic sensors based on OFETs are summarized.

2. Basic Design of Extended-gate-type OFET-based Enzymatic Sensors

The OFETs can function as an electrical switch in an electronic circuit, because the electrical current in a semiconductor layer can be controlled by applying a gate voltage. The device is constructed with three terminals (source, drain, and gate), a dielectric layer and the organic semiconductor layer. The operation principle of OFETs generally follows that of metal–oxide–semiconductor field-effect transistors (MOSFETs). Although OFETs possess the above-mentioned advantages in comparison with MOSFETs, one of the limitations of OFETs is that organic semiconductor materials are degraded readily by exposure to moisture. Therefore, we decided to employ an extended-gate structure for the OFET-based enzymatic sensor (Fig. 2(a)). In the designed structure, the driving unit of the OFET is separated from the sensing portion (the extended-gate). This indicates that the degradation of the device by aqueous solutions can be prevented. The other limitation of the OFETs is the requirement of a high applied voltage for operation (> 10 V). The OFET-based sensor device should be operated under low applied voltage for practical use.

Figure 1. Representative illustration of the concept of OFET-based enzymatic sensors.
voltage, because of the occurrence of electrolysis-derived instability of the output signal. The operation voltage of the OFET strongly depends on the capacitance of the dielectric layer. In this regard, we utilized a tetradecylphosphonic acid (TDPA) self-assembled monolayer (SAM) as the dielectric thin-film layer for the designed OFET (Fig. 2(b)). The thickness of the TDPA-SAM is very small (approximately 1.6 nm), and consequently, its capacitance is higher than that of typical dielectric materials (e.g., insulating polymers, metal oxides). Furthermore, we used a π-conjugated polymer material (poly(2,5-bis(3-hexadecylthiophene-2-yl)thieno[3,2-b]thiophene); PBTTT) as the semiconductor of the designed OFET, owing to its high thin-film uniformity and chemical stability.

Figure 3 displays the basic electrical properties of the developed OFET. The fabricated device exhibits switching properties without hysteresis behavior (the on/off drain current ratio: \( > 10^3 \)). The estimated field-effect mobility was approximately \( 10^{-2} \) cm²/Vs. Notably, these excellent properties of the OFET are successfully demonstrated by applying low voltage (< 3 V), suggesting that the fabricated OFET is a suitable platform for the transducer of the enzymatic sensor.

The introduction of an electron mediator into the sensor device is indispensable to develop the enzymatic sensor, because the direct conduction of electrons from the enzyme to the electrode and/or the semiconductor layer is generally difficult. Accordingly, the sensing portion of the OFET is modified with an analyte-dependent enzyme and an electron mediator material (i.e., a mixture of a peroxidase and a redox polymer, or a redox-reactive monolayer) (Fig. 1). The results of the OFET-based enzymatic sensor obtained for various analytes are described in the following sections.

3. OFET-based Enzymatic Sensors Modified with an Osmium-redox Polymer

3.1 Real-time monitoring of lactate

Lactate is a glycolytic product, that acts as an energy source in muscle cells. As the lactate concentration in body fluids (i.e., blood and perspiration) is correlated with muscle loading, monitoring of lactate levels is important for exercise management. Although the sensitive and selective detection of lactate in blood by using biosensors was reported, the development of wearable-device-based sensors for real-time lactate detection is in its early stage.

To achieve non-invasive lactate monitoring, the development of an integrated and flexible sensor platform for lactate detection in sweat is desired. Accordingly, we demonstrated continuous lactate detection based on the OFET-based enzymatic sensor.

To develop the OFET-based enzymatic biosensors, we employed a mixture of horseradish peroxidase (HRP) and an osmium (Os)-redox polymer as the electron mediator for a lactate oxidase. The enzymatic reaction at the analyte-selective oxidase can be electrically mediated via the HRP/Os-redox polymer mixture. Therefore, the extended-gate electrode was modified with the lactate oxidase and HRP/Os-redox polymer bilayer for the construction of the lactate-sensing-portion of the OFET (Fig. 4). The electron relay in the complex membrane is induced by the existence of lactate in the aqueous solution, because of the conversion of lactic acid to pyruvic acid by the oxidase.
First, lactate was continuously detected by using the fabricated OFET device. Stepwise changes in the lactate concentration (0–1000 nM) caused continuous changes in the drain current ($I_{DS}$) in the OFET, suggesting that the fabricated OFET could be utilized for the real-time monitoring of the lactate levels in the aqueous solution (Fig. 5(a)). The observed $I_{DS}$ changes suggest that the transfer characteristics of the fabricated OFET are also influenced by the addition of lactate. Therefore, an electrical parameter such as the threshold voltage ($V_{TH}$) of the OFET can be utilized as the sensing signal for lactate detection.

Subsequently, we measured the transfer curves of the OFET upon titration with a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution including various analytes (which generally exist in human sweat). Figure 5(b) shows the $V_{TH}$ changes upon adding the analytes (lactate, MgCl$_2$, CaCl$_2$, NaCl, p-cresol, urea, and glucose). The $V_{TH}$ values were estimated from all the transfer characteristics. Consequently, the analyte-induced $V_{TH}$ change except for the lactate addition was very weak. These results indicate that the electrical response of the OFET is solely derived from the enzymatic redox reactions at the extended-gate electrode.

3.2 Selective detection of biogenic amines

Biogenic amines (e.g., histamine, putrescine) are generated by the amination of aldehydes and ketones or by the decarboxylation of amino acids. They are known not only as modulators of physiological functions in human body but also as freshness markers of perishables.30 For example, histamine acts as a neurotransmitter and plays a significant role in the immune response to a pathogen.31 Excessive ingestion of histamine in rotten foods causes allergy-like symptoms such as urticaria, dyspnea, and consciousness disorder.32 In addition, the detection of putrescine levels in perishables is important because putrescine combined with a food additive nitrite (or nitrate) could produce carcinogenic nitrosamines.32 Therefore, the development of an easy-to-use monitoring system for the levels of biogenic amines is imperative for various situations such as diagnosis tests, pharmaceutical chemistry, and the quality control of foods. However, biogenic amines are determined using instrumental analysis techniques (e.g., high-performance liquid chromatography,30 fluorescent-probe-based assays33). Hence, we decided to develop an easy-to-use biogenic amine sensor utilizing an OFET with a diamine-oxidase modified electrode.34

Figure 6(a) shows the transfer characteristics of the OFET upon titration with a HEPES-buffer solution of histamine. The transfer curves were negatively shifted with the increase in the histamine concentration (0–40 µM), indicating that the interfacial potential shift on the extended-gate electrode was caused by the oxidation-state change of osmium ion (Fig. 4).35 Notably, we obtained a linear relationship between the low histamine levels (0–10 µM) and the observed changes in $V_{TH}$ (Fig. 6(a)). The limit of detection36 was estimated to be 1.2 µM, indicating that the sensitivity of the fabricated OFET-based sensor is comparable to that of the reported electrochemical sensors. To investigate the selectivity of the fabricated sensor device, we carried out titration experiments for various biogenic amines (putrescine, cadaverine, tyramine, spermine, and spermidine) (Fig. 6(b)). While the OFET exhibited almost no responses to cadaverine, tyramine, spermine, and spermidine, it exhibited significant responses to histamine and putrescine. The almost similar response to histamine and putrescine might be derived from the cross-reactivity of the diamine oxidase with both the compounds. Although the selectivity of the OFET-based
enzymatic sensor can be improved by employing a more specific enzyme such as a histamine oxidase, the detection of the total amount of diamines in perishables is still valuable for monitoring food freshness.37

4. OFET-based Enzymatic Sensor for Selective Detection of Nitrate in Saliva

The determination of nitrate ion (NO$_3^-$) is important from the standpoint of environmental assessment, because the contamination of drinking water by NO$_3^-$ could be caused by various wastes (e.g., chemical fertilizers, livestock wastes, food wastes).38 In addition, serious diseases such as infant methemoglobinemia or bladder/gastric cancers could be induced by the over-ingestion of NO$_3^-$19,20. Furthermore, psychological stress can be quantified by measuring the nitrate level, because the NO$_3^-$ concentration in saliva might be reflected in psychological activity.41 Although many research groups have demonstrated nitrate detection based on colorimetric42 or fluorometric spectroscopy,43 ion chromatography,44 or ion-selective electrodes,45 the development of an on-site quantitative assay for NO$_3^-$ is not fully established. Therefore, we developed a nitrate reductase-modified OFET for the on-site detection of nitrate.46

To prepare the nitrate-sensing portion, we decided to functionalize the extended-gate electrode with a bipyridinium-based SAM (BP-SAM).47 BP derivatives have reversible redox reactivity, which indicates that the BP-SAM can play the role of an electron-transfer mediator for the nitrate reductase on the electrode (Fig. 7(a)). The fixation process of BP and the nitrate reductase is as follows: the gold electrode modified with 2-aminoethanethiol was immersed in a HEPES-buffer solution containing N-methyl-N'-(carboxyethyl)-4,4'-bipyridinium, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt). Subsequently, the fabricated electrode was covered with the nitrate reductase and glutaraldehyde. Glutaraldehyde was utilized as an immobilizer for the enzyme on the BP-SAM.

As we functionalized the electrode using multi-step reactions, the formation of the BP-SAM on the extended-gate gold electrode was characterized in a step-by-step manner using contact angle goniometry (CAG) and photoemission yield spectroscopy (PYS) in air. The CAG results showed that the wettability of the 2-aminoethanethiol-modified gold was higher than that of an untreated gold electrode. This change is attributed to the hydrophobicity of 2-aminoethanethiol (Fig. 7(b)). The PYS measurement showed a shallower work function on the 2-aminoethanethiol-modified gold than that on the untreated gold (Fig. 7(c)). This result indicated that the electron-donating group covered the gold electrode.48 More importantly, a higher contact angle and a deeper work function were obtained after the BP-SAM treatment. This is presumed to be because the positively charged BP unit was attached on the gold electrode. To investigate the attachment of the BP-SAM on the gold surface, we carried out X-ray photoelectron spectroscopy (XPS). The XPS results demonstrated the presence of nitrogen, sulfur, and oxygen elements derived from the BP derivative (Fig. 7(d)). Judging from these results, the SAM-based electron mediator was successfully introduced on the extended-gate gold electrode. We thereafter covered the electrode with the nitrate reductase using the aforementioned process, and performed a titration experiment for NO$_3^-$ in a HEPES-buffer solution in the presence of sodium dithionite, which serves as an electron donor to the nitrate reductase. Consequently, a distinct negative shift of the transfer characteristics was obtained with the increase in the nitrate concentration (Fig. 8(a)). In contrast, almost no electrical response of the OFET was observed upon the addition of NO$_3^-$ in the absence of sodium dithionite (Fig. 8(b)). These results suggested that the electrical response of the OFET-based nitrate sensor is most probably due to the electron-relay on the extended-gate electrode. The surface potential of the extended-gate could be derived from the changes in the valence of the BP with the occurrence of the enzyme reaction (BP$^{2+}$ $\rightarrow$ BP$^+$), which results in the change in the channel conductance within the OFET.47 Hence, the electrical characteristics of the OFET modified with the BP-SAM were indirectly affected by the enzyme reaction on the electrode.

The selectivity of the fabricated OFET sensor was evaluated by measuring representative anions (Cl$^-$, SCN$^-$, HPO$_4^{2-}$, and HCO$_3^-$). On the basis of the relationship between the anion concentration and the observed changes in $I_{TH}$ for each anion (Fig. 8(c)), we confirmed that the fabricated sensor device can selectively detect nitrate in aqueous media. The selective response to nitrate can be explained by the specific reactivity of the nitrate reductase. The above-mentioned results encouraged us to implement the nitrate detection in a real sample (= diluted human saliva). Consequently, the OFET electrically responded to the nitrate ion in the diluted human saliva (Fig. 8(d)). Notably, the recovery for the nitrate addition was estimated to be 97.4±1.8%, indicating that the accuracy of the OFET-based assay for nitrate is comparable to that of a conventional colorimetric assay39 (100.4±5.2%). As men-

Figure 7. (a) Electron relay on the extended-gate electrode modified with a nitrate reductase and a BP-SAM. (b) Water contact angle measurements on the gold electrodes. (c) PYS results of the gold electrodes. (d) XPS results of C1s, O1s, N1s and S2p regions of the BP-SAM-treated gold electrode.
FETs or ion-selective electrodes, the OFET-based enzymatic sensors process of the designed OFET is much simpler than that of inorganic detection of target analytes in aqueous media. As the fabrication devices on skin can be achieved by using the OFET. Hence, we believe that the combination of OFETs and enzymatic electrodes can be employed as disposable sensors. Importantly, the sensing portion of the OFET was constructed on a flexible plastic substrate, which indicates that non-invasive health monitoring with wearable devices on skin can be achieved by using the OFET. Hence, we believe that the combination of OFETs and enzymatic electrodes will be an attractive platform for the easy-to-use and on-site detection of analytes at various situations in the near future.

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References

1. H. Sirringhaus, Adv. Mater., 26, 1319 (2014).
2. K. Kudo, M. Yamashina, and T. Morizumi, Jpn. J. Appl. Phys., 33, 130 (1994).
3. G. Gelinck, P. Heremans, K. Nomoto, and T. D. Anthopoulos, Adv. Mater., 22, 3778 (2010).
4. V. Subramanian, J. M. J. Fréchet, P. C. Chang, D. C. Huang, J. B. Lee, S. E. Molesa, A. R. Murphy, D. R. Redinger, and S. K. Volkman, Proc. IEEE, 93, 1330 (2005).
5. G. Schwartz, B. C.-K. Tee, J. Mei, A. L. Appleton, D. H. Kim, H. Wang, and Z. Bas, Nat. Commun., 4, 1859 (2013).
6. D.-L. Kim, T. Q. Trung, B.-U. Hwang, J.-S. Kim, S. Jeon, J. Bae, J.-J. Park, and N.-E. Lee, Sci. Rep., 7, 12705 (2017).
7. T. Yokota, Y. Inose, Y. Terakawa, J. Reeder, M. Kaltenbrunner, T. Ware, K. Yang, K. Mahnichi, T. Murakawa, M. Sekino, W. Voit, T. Sekitani, and T. Someya, Proc. Natl. Acad. Sci. U.S.A., 112, 14533 (2015).
8. N. J. Ronkainen, H. B. Halsall, and W. R. Heineman, Chem. Soc. Rev., 39, 1747 (2010).
9. A. J. Bandodkhar, W. Jia, and J. Wang, Electroanal., 27, 562 (2015).
10. K. Fukuda, T. Minami, T. Minami, M. Watanabe, T. Fukuda, D. Kumaki, and S. Tokito, Adv. Electron. Mater., 1, 1400052 (2015).
11. R. Shiwaku, H. Matsu, K. Nagamine, M. Uematsu, T. Mano, Y. Manayanma, A. Nomura, K. Tsuchiyi, K. Hayasaka, Y. Takeda, T. Fukuda, D. Kumaki, and S. Tokito, Sci. Rep., 8, 6368 (2018).
12. D. Kumaki, T. Umeda, and S. Tokito, Appl. Phys. Lett., 92, 093909 (2008).
13. T. Minami, T. Minami, R. Kurita, O. Niwa, S. Wakida, K. Fukuda, D. Kumaki, and S. Tokito, Appl. Phys. Lett., 104, 243701 (2014).
14. Y. Sasuki, T. Minami, T. Minami, and S. Tokito, Electrochem., 85, 775 (2017).
15. H. Klauk, U. Zechieschlag, J. Pfitzner, and M. Halik, Nature, 445, 745 (2007).
16. K. Fukuda, T. Hamamoto, T. Yokota, T. Sekitani, U. Zechieschlag, H. Klauk, and T. Someya, Appl. Phys. Lett., 95, 203101 (2009).
17. I. McCulloch, M. Heeney, C. Bailey, K. Genevicius, I. Macdonald, M. Shkunov, D. Spartrowe, S. Tierney, R. Wagner, W. Zhang, M. L. Chabiny, R. J. Kline, M. D. McGeehee, and M. F. Toney, Nat. Mater., 5, 528 (2006).
18. F. Hirschhaeuser, U. G. A. Satller, and W. Mueller-Klieser, Cancer Res., 71, 6921 (2011).
19. L. V. Billat, Sports Med., 22, 157 (1996).
20. W. Jia, A. J. Bandodkhar, G. Valdés-Ramírez, J. R. Windmiller, Z. Yang, J. Wang, Anal. Chem., 85, 6553 (2013).
21. D. Khodagholy, V. F. Curto, K. J. Fraser, M. Gur, N. V. Zaryanov, V. N. Nikitina, E. V. Karpova, E. E. Karyakina, and A. A. Bao, Adv. Mater., 22, 123 (2014).
22. Y. Wang, H. Xiu, J. M. Zhang, and G. Li, Sensors, 8, 2043 (2008).
23. L. Rassaei, W. Olthuis, S. Tsujimura, E. J. R. Sudhölter, and A. van den Berg, Sensors, 12, 8749 (2012).
24. L. V. Billat, S. J. Vanhove, P. Bogaerts, M. Weckx, C. Ooms, J. Vanhooren, and K. Van Deun, Rev. Sci. Instrum., 83, 093904 (2012).
25. N. V. Zaryanov, V. N. Nikitina, E. V. Karpova, E. E. Karyakina, and A. A. Bao, Anal. Chem., 89, 11198 (2017).
26. T. Minami, T. Sato, T. Minami, K. Fukuda, D. Kumaki, and S. Tokito, Biosens. Bioelectron., 74, 45 (2015).
27. H. Kang, R. Liu, H. Sun, J. Zhen, Q. Li, and Y. Huang, J. Phys. Chem. B, 116, 55 (2012).
28. S. Timur, Y. Yigzaw, and L. Gorton, Sens. Actuators, B, 113, 684 (2006).
29. R. Kurita, K. Hayashi, T. Horiiuchi, O. Niwa, K. Maeyama, and K. Tanizawa, Lab Chip, 2, 34 (2002).
30. S. Sentellas, Ō. Núñez, and J. Saurina, J. Agric. Food Chem., 64, 7767 (2016).
31. M. V. White, J. Allergy Clin. Immunol., 86, 599 (1990).
32. I. Al Bulushi, S. Poole, H. C. Deeth, and G. A. Dykes, Crit. Rev. Food Sci. Nutr., 49, 369 (2009).
33. T. Minami, N. A. Esipenko, N. A. Akdeniz, B. Zhang, L. Isacs, and P. Ananthchelvan, Jr., J. Am. Chem. Soc., 135, 15238 (2013).
34. T. Minami, T. Sato, T. Minami, and S. Tokito, Sens. Actuators, B, 31, 721 (2015).
35. P. Bergveld, Sens. Actuators, B, 88, 1 (2003).
36. J. N. Miller and J. C. Miller, Statistics and Chemometrics for Anal. Chem., 6th ed., Pearson, Harlow (2010).
37. K. B. Male, P. Bouvrette, J. H. T. Luong, and B. F. Gibbs, J. Food Sci., 61, 1012
38. C. J. Johnson and B. C. Kross, *Am. J. Ind. Med.*, 18, 449 (1990).
39. C. S. Bruning-Fann and J. B. Kanesene, *Vet. Hum. Toxicol.*, 35, 237 (1993).
40. G. Ellis, I. Adatia, M. Yazdanpanah, and S. K. Makela, *Clin. Biochem.*, 31, 195 (1998).
41. L. Jin, L. Qin, D. Xia, X. Liu, Z. Fan, C. Zhang, L. Gu, J. He, I. S. Ambudkar, D. Deng, and S. Wang, *Free Radical Biol. Med.*, 57, 61 (2013).
42. W. L. Daniel, M. S. Han, J.-S. Lee, and C. A. Mirkin, *J. Am. Chem. Soc.*, 131, 6362 (2009).
43. S. Biswas, B. Chowdhury, and B. C. Ray, *Talanta*, 64, 308 (2004).
44. Z. Shu-yu, S. Qing, L. Li, and F. Xiao-hui, *Biomed. Chromatogr.*, 27, 1547 (2013).
45. S. Wakida, T. Okumura, Y. Shibutani, and J. Liu, *Sens. Mater.*, 19, 235 (2007).
46. T. Minami, Y. Sasaki, T. Minamiki, S. Wakida, R. Kurita, O. Nawa, and S. Tokito, *Biosens. Bioelectron.*, 81, 87 (2016).
47. M. Zayats, A. B. Kharitonov, E. Katz, and I. Willner, *Analyst*, 126, 652 (2001).
48. B. de Boer, A. Hadipour, M. M. Mandoc, T. van Woudenbergh, and P. W. M. Blom, *Adv. Mater.*, 17, 621 (2005).
49. M. Kovács, A. Kiss, M. Gönöcz, G. Miskolczi, G. Seprényi, J. Kaszaki, M. J. Kohr, E. Murphy, and A. Végh, *PLoS One*, 10, e0122243 (2015).
50. H. Jin, Y. S. Abu-Rayha, and H. Haick, *Adv. Healthc. Mater.*, 6, 1700024 (2017).