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Articles

Open Sandwich-Based Immuno-Transistor for Label-Free and Noncompetitive Detection of Low Molecular Weight Antigen

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In this study, we proposed a new detection method, open sandwich-based immuno-field effect transistor (OS-FET) for label-free and noncompetitive detection of low molecular weight antigen. The principle of OS-FET is based on the detection of intrinsic molecular charges caused by the small antigen-dependent interchain interaction of separated V_L and V_H chains from a single antibody variable region using the field effect. Introducing V_H chain and small antigen bisphenol A into the OS-FET with the immobilized V_L chain on the gate, we could detect electrically and directly the binding of bisphenol A by V_H and V_L chains. Although the detection limit of OS-FET was 1 nM to detect bisphenol A for the standard deviation of control signal, the addition of isothiocyanobenzyl-EDTA with negative charges to the V_L chain enhanced the detection limit to 1 pM. We could directly transduce the charge density changes based on the capture of target on the gate into the electrical signals using the OS-FET. The platform based on the FETs is suitable for a label-free, noncompetitive, and quantitative detection system for small antigen analysis in environmental, food, and clinical research.

In a few decades, many types of biochips/biosensors have been proposed in the fields of healthcare, environmental monitoring, and food safety. They are available for rapid and simple screening of components in samples by utilizing miniaturized systems. There are many principles of detection methods, for example, those based on fluorescence, chemiluminescence, mass spectroscopy, electrical measurements, and so on.[1−4] We have been investigating a new approach to realize a direct, simple, and highly sensitive detection of nonlabeled molecular recognition events on a miniaturized and arrayed solid-state biosensor in the field between electronics and biology/medicine.[5−9] Recently, several types of field effect-based biosensing devices have been used for electrochemical detection of biomolecular recognition events on a gate sensing surface.[10−14] Since biomolecules such as DNA molecules are negatively charged in an aqueous solution, the amount of molecular charges at the gate surface of field effect devices changes as a result of biomolecular recognition events such as DNA hybridization. The charge density change is directly transduced into electrical signal by the field effect. Based on this principle, the label-free DNA sequencing based on intrinsic molecular charges was carried out using the genetic field-effect transistor (genetic FET) in combination with a single-base extension reaction as one of the biocoupled FET (bio FET).[7] On the other hand, some kinds of bio FET have been proposed for the label-free detection of protein function such as the enzyme FET (ENFET) with the penicillinase-albumin membrane and the immuno FET (IMFET) with the PVC (polyvinyl chloride)-antigen membrane.[15−17] However, the reproducibility of protein detection using the bio FETs has been pointed out to be poor. This is considered because the counterions in solutions shielded molecular charges of proteins at the gate surface for the measurement

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(1) Covey, T. R. *Anal. Chem.* **1985**, *57*, 474–81.
(2) Jain, K. K. *Science* **2001**, *294*, 621.
(3) Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2003**, *21*, 1192.
(4) Margulies, M.; et al. *Nature* **2005**, *437*, 376–380.
(5) Sakata, T.; Miyahara, Y. *ChemBioChem* **2005**, *6*, 703–710.
(6) Sakata, T.; Kamahori, M.; Miyahara, Y. *Jpn. J. Appl. Phys.* **2005**, *44*, 2854–2859.
(7) Sakata, T.; Miyahara, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 2225–2228.
(8) Sakata, T.; Miyahara, Y. *Biosens. Bioelectron.* **2007**, *22*, 1311–1316.
(9) Sakata, T.; Miyahara, Y. *Anal. Chem.* **2008**, in press.
(10) Souteyrand, E.; Cloarec, J. P.; Martin, J. R.; Wilson, C.; Lawrence, I. S.; Mikkelsen, S.; Lawrence, M. F. *J. Phys. Chem. B* **1997**, *101*, 2980–2985.
(11) Fritz, J.; Cooper, E. B.; Gaudet, S.; Sorger, P. K.; Manails, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14142–14146.
(12) Uslu, F.; Ingebrandt, S.; Mayer, D.; Böcker-Meffert, S.; Odenthal, M.; Offenhäusser, A. *Biosens. Bioelectron.* **2004**, *19*, 1723–1731.
(13) Kim, D.-S.; Jeong, Y.-T.; Park, H.-J.; Shin, J.-K.; Choi, P.; Lee, J.-H.; Lim, G. *Biosens. Bioelectron.* **2004**, *20*, 69–74.
(14) Pouthas, F.; Gentil, C.; Cote, D.; Bockelmann, U. *Appl. Phys. Let.* **2004**, *84*, 1594–1596.
(15) Caras, S.; Janata, *J. Anal. Chem.* **1980**, *52*, 1935.
(16) Collins, S.; Janata, J. *Anal. Chim. Acta* **1982**, *136*, 93.
(17) Miyahara, Y.; Moriizumi, T.; Ichimura, K. *Sens. Actuators* **1985**, *7*, 1.

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in whole blood or other highly concentrated samples. The detection limit of large molecules (>height of 15 nm) such as antibodies using the bio FETs depends on the Debye length at the gate insulator/solution interface, which varies with the concentration of solutions, although other works have recently demonstrated the electrical detection of protein analyte under physiologic buffer by use of AlGaN/GaN high electron mobility transistor.[18,19] Therefore, the dilution of measurement solutions is available for the increase of detection sensitivity of bio FET as the DNA extension reaction of about 30 base length (about 10 nm) could be detected in the diluted buffer solution using the genetic FET.[8] However, it seems to be difficult to detect proteins of a larger size even in the diluted buffer solution due to the charge shielding outside of the Debye length.

About a decade ago, a novel immunoassay principle called open sandwich immunoassay (OS-IA) was proposed for the detection of mainly low molecular weight antigens in a noncompetitive mode.[20−24] This method is based on the antigen-dependent interchain interaction of separated V_L and V_H chains from a single antibody variable region. To perform OS-IA, for example, one of the separated chains is immobilized on the plate. When the sample containing the antigen and another chain that is labeled with an enzyme is added on, both chains reassociate according to the antigen concentration in the sample, resulting in the positive colorimetric or chemiluminescent signal on the plate after washing and substrate addition. In this distinguished method, the detection sensitivity depends on several parameters. One is the affinity of the antibody used, and another is the antigen-dependency of the V_H/V_L association. However, equally important is the sensitivity of the detection method, and typical detection limits obtained in the described OS enzyme-linked assays (OS-ELISA) were in the few nanomolar range.[20−24]

Since the separated variable regions are smaller than (approximately $2 \times 2 \times 3.5$ nm) the original antibody to be captured within the Debye length at the gate surface, the detection principle of bio FET will overcome the detection limit of OS-ELISA. Compared with the OS-ELISA, the OS-FET has the advantages of label-free and quantitative detection. Because of the label-free measurement, we can omit the experimental process of labeling. Moreover, we can estimate quantitatively the number of target molecules with charges and directly transduce the charge density changes based on the capture of target on the gate into the electrical signals using the OS-FET. In the present study, we propose a new detection method, open sandwich-based immunofield effect transistor (OS-FET) for a label-free and highly sensitive detection of low molecular weight antigen, which is based on the detection of intrinsic molecular charges of one of the separated chains using the field effect. Here, we report on the direct transduction of open sandwich immunoassay at the gate surface into an electrical signal using the FET.

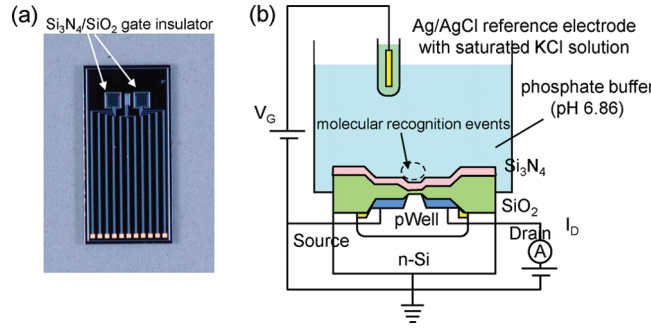

Figure 1. (A) Photograph of the fabricated field effect transistor (FET) chip. Two FETs and a temperature sensor are integrated in a 5 × 2 mm chip. The FETs are an n-channel depletion type with Si_3N_4/SiO_2 as a gate insulator. A couple of FETs were used for the open sandwich (OS) immunoassay; one was used in a sample solution including antigen as an OS-FET, and the other was done in a solution without target as a control FET, respectively. The V_H chains were immobilized on each gate surface. (B) Schematic diagram for measurements of electrical characteristics of a FET. The shift of the threshold voltage V_T was determined from the gate voltage (V_G)−drain current (I_D) characteristics in a phosphate buffer solution (0.025 M Na_2HPO_4 and 0.025 M KH_2PO_4, pH 6.86). An Ag/AgCl electrode with saturated KCl solution was used as a reference electrode.

EXPERIMENTAL SECTION

Structure of the Field Effect Transistor and Electrical Measurement. Insulated gate field effect transistors were fabricated using the standard integrated circuit technology except for deposition of the gate electrode. The gate structure and the fabrication process for the FETs used in the experiment were described in detail elsewhere.[6] Multiarrayed FETs and a temperature sensor were integrated in a 5 × 5 mm chip (Figure 1A). We used n-channel depletion mode FET with a double layer of Si_3N_4/SiO_2 as the gate insulator on which oligonucleotide probes were immobilized. The thicknesses of the Si_3N_4 layer and the SiO_2 layers were 140 and 35 nm, respectively. The channel width, W, and the channel length, L, were designed to be 2400 and 5 μm, respectively, and as a result, the ratio, W/L was 480. The fabricated FET chip was mounted on a flexible polyimide film with patterned copper electrodes and was wire bonded. The FET chip was encapsulated with an epoxy resin (ZC-203, Nippon Pelnox), except for the gate areas. The typical drifts of the FET devices fabricated in the present study are about 0.1 mV/h. Both the fabricated FETs and the commercial ISFETs (BAS Inc.) were used for the experiments in the present study. The FETs were immersed in a phosphate buffer solution (0.025 M Na_2HPO_4 and 0.025 M KH_2PO_4, pH 6.86, Wako) with an Ag/AgCl reference electrode with saturated KCl solution (Figure 1B). The electrical characteristics of the FETs, such as the gate voltage (V_G)−drain current (I_D) characteristics, were measured in a pH 6.86 phosphate buffer solution at room temperature using a semiconductor parameter analyzer (4155C, Agilent). The threshold voltage V_T shift was determined after an open sandwich immunoassay. The V_T shift was defined as a difference of the $V_G - I_D$ characteristics at a constant drain

(18) Kang, B. S.; Wang, H. T.; Lele, T. P.; Tseng, Y.; Ren, F. *Appl. Phys. Lett.* **2007**, *91*, 112106.
(19) Gupta, S.; Elias, M.; Wen, X.; Shapiro, J.; Brillson, L. *Biosens. Bioelectron.* **2008**, *24*, 505–511.
(20) Ueda, H.; Tsumoto, K.; Kubota, K.; Suzuki, E.; Nagamune, T.; Nishimura, H.; Schueler, P. A.; Winter, G.; Kumagai, I.; Mahoney, W. C. *Nat. Biotechnol.* **1996**, *14*, 1714–1718.
(21) Suzuki, C.; Ueda, H.; Tsumoto, K.; Mahoney, W.; Kumagai, I.; Nagamune, T. *J. Immunol. Methods* **1999**, *224*, 171–184.
(22) Aburatani, T.; Sakamoto, K.; Masuda, K.; Nishi, K.; Ohkawa, H.; Nagamune, T.; Ueda, H. *Anal. Chem.* **2003**, *75*, 4057–4064.
(23) Suzuki, T.; Munakata, Y.; Morita, K.; Shinoda, T.; Ueda, H. *Anal. Sci.* **2007**, *23*, 65–70.
(24) Lim, S.-L.; Ichinose, H.; Shinoda, T.; Ueda, H. *Anal. Chem.* **2007**, *79*, 6193–6200.

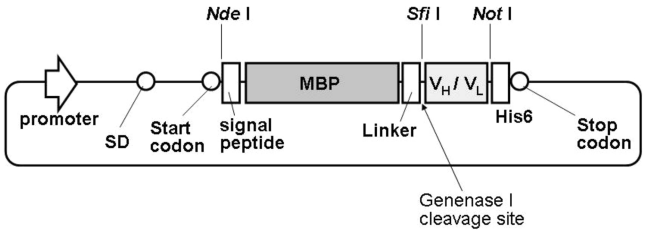

Figure 2. Schematic structure of the expression vectors for MBP-V_H/V_L. The open reading frame is encoding MBP including its signal peptide, linker peptide, V_H/V_L domain, and His_6-tag sequence. The positions of the restriction enzyme sites used for the gene construction and the genenase I cleavage site are indicated.

current of 700 μA. Drain current (I_D) indicates the current between the source and the drain in a silicon-based field effect transistor.

Preparation of MBP-Fused V_H/V_L. The V_H and V_L chains of antibisphenol A antibody were expressed as fusion proteins with *E. coli* maltose binding protein (MBP) in the *E. coli* harboring the respective expression vector, pET-MBPp-VL(BPA) and pET-MBPp-VH(BPA). The expression vectors were based on pET32b (Novagen) and harbor an open reading frame consisting of the MBP gene including that for signal peptide, V_H/V_L gene and His-tag sequence (Figure 2). The signal peptide and the MBP gene were amplified from pMAL-VL(ZEA),[23] and the V_H and V_L genes were derived from pKST2(BPA).[22]

The expression of V_H fused with MBP (MBP-V_H) was performed by transforming *E. coli* BL21(DE3)pLysS with the pET-MBPp-VH(BPA) plasmid. The transformant was cultured in 10 mL of LBAG medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, pH 7.5 supplemented with 100 μg/mL ampicillin and 1% glucose) overnight at 30 °C. Subsequently, the culture was inoculated to 800 mL of LBA medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, and 100 μg/mL ampicillin) and incubated at 20 °C until OD_{600} reached 0.6−0.8. The expression of MBP-V_H fusion protein was induced by addition of IPTG (Wako, Osaka, Japan) to a final concentration of 0.5 mM and incubated further overnight at 20 °C. The culture was centrifuged at 6300 rpm, 4 °C for 10 min, and the bacterial pellet was resuspended in 50 mL PBS. The suspension was sonicated on ice for ten cycles of 30 s with a 30 s interval between each sonication and centrifuged at 16000*g*, 4 °C for 45 min. The supernatant was applied to a column (1 cm × Φ1 cm) packed with TALON affinity resin (Clontech Laboratories, Inc., Mountain View, CA) equilibrated with column buffer (50 mM sodium phosphate, 500 mM NaCl, pH 7.4), washed with column buffer, and eluted using elution buffer (column buffer containing 500 mM imidazole). Eluates containing target protein were pooled and buffer-exchanged using a PD-10 column (GE Healthcare UK Ltd., Amersham Place, England) and dialyzed with PBS (10 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4) using conventional dialysis tubing. The purified MBP-V_H was analyzed by 10% SDS-PAGE and stored in small aliquots at −80 °C. MBP-V_L was prepared in the same way except pET-MBPp-V_L(BPA) was used instead of pET-MBPp-V_H(BPA).

To reduce the molecular size of MBP-V_H, the MBP moiety was removed by digestion by genenase (New England BioLabs, Inc., Ipswich, MA) from MBP-V_H. MBP-V_H was dialyzed against 20 mM Tris-HCl (pH 8.0) containing 200 mM NaCl and then concentrated up to 1 mg/mL. One milliliter of the concentrated samples was added to 10 μL of genenase (1 mg/mL) and incubated overnight at room temperature. The digested protein was purified as described above, dialyzed against PBS using Slide-A-Lyzer Dialysis Cassettes 3.5K (PIERCE), and stored in PBS containing 10% glycerol at −80 °C until use. To increase the negative charge density on the protein surface, MBP-V_L was labeled with isothiocyanobenzyl-EDTA (Dojindo, Kumamoto, Japan). Isothiocyanobenzyl-EDTA reacts with the ε-amino group of Lys residues or the N-terminal amino group and can form an EDTA-adduct with four negative charges. MBP-V_L was dialyzed against 20 mM HEPES buffer (pH8.0) and then concentrated to 1.2 mL (800 μg/mL). The MBP-V_L solution was mixed with 100 μL of 10 μg/mL isothiocyanobenzyl-EDTA in HEPES buffer and incubated overnight at room temperature. To remove the unconjugated labeling reagents, the modified MBP-V_L was dialyzed against PBS overnight and then filtered through a PD10 column (GE Healthcare UK Ltd.). After concentration, the protein was stored at −20 °C until use. The modification was verified by 10% native PAGE.

Surface Modification of the Gate Sensing Area. The surface of the Si_3N_4 layer was cleaned with 1 M NaOH for 1 h at room temperature and silanized in toluene (Sigma-Aldrich) containing 2 wt % 3-aminopropyltriethoxysilane (Sigma-Aldrich). The amino-silanized Si_3N_4 surface was rinsed in toluene and dried in vacuo at 110 °C for 0.5 h. Reactive amino groups were then introduced at the Si_3N_4 surface.

The V_H chains were dissolved in PBS at a concentration of 100 μM. To couple the carboxyl group of V_H chain with the amino-silanized Si_3N_4 surface, the Si_3N_4 surface was kept at room temperature in the V_H solution including 10 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, hydrochloride (WSC) per 1 mL overnight to complete the coupling reaction. The chemically modified Si_3N_4 surface was washed with deionized water and dried in vacuo at room temperature for 0.5 h. Before use, the modified Si_3N_4 surface was coated with the BSA solution and washed with deionized water.

The V_H chain immobilized on the FET chip was combined with the V_L chain by capturing a small antigen, bisphenol A (BPA). BPA was added at the concentration of 1 fM to 1 μM in this study. The MBP-V_L used for reassociation was prepared by dissolving MBP-V_L chains in a PBS solution. The FET with V_H chains was kept in the buffer solution containing MBP-V_L and BPA for 12 h at room temperature. Following the OS-IA, the FET was washed with the buffer solution and deionized water at room temperature in order to remove nonspecific adsorption.

RESULTS AND DISCUSSION

In our setup, the V_H chain is chemically immobilized on the Si_3N_4 gate surface. Then, the small antigen is sandwiched with the free V_L chain tethered with a negatively charged protein (MBP), and the V_H chain is immobilized on the gate surface (Figure 3). In this study, bisphenol A (BPA) is utilized as model antigen with a molecular mass of 228 and negligible charge at

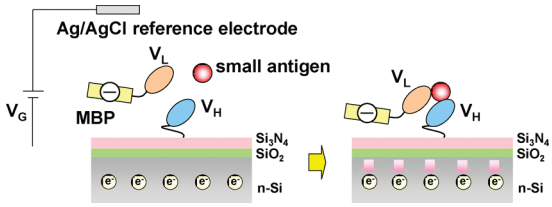

Figure 3. Scheme for an open sandwich-based immuno-field effect transistor (OS-FET). The separated V_L chain are N-terminally fused with negatively charged *E. coli* maltose binding protein (MBP), while the V_H chain is immobilized on the gate sensing area. The antigen (bisphenol A, BPA) is captured with MBP-V_L and V_H chains on the gate surface. The negative charges of MBP-V_L induce the electrostatic interaction with electrons in silicon crystal, resulting in the change of drain−source current at the channel by the field effect.

neutral pH.[25] The compound is a widely used small monomer in the manufacture of polycarbonate plastics and epoxy resins and has been reported to have estrogenic effects. While many methods were devised to detect BPA, the detection limit attained was more than the nanomolar range.[26−31] However, recent research suggested that the exposure of BPA at the picomolar range changed some cell functions.[31] Therefore, the development of a more sensitive method to detect BPA is urgently needed.

As shown in Figure 3, the free V_L chain used is tethered with MBP with 13 negative charges per molecule (pI = 5.2), which is one of routinely used fusion tags for recombinant protein production in *E. coli*, partly because it acts as an intramolecular chaperone and increases the expression yields of the fused proteins.[24] Due to the large negative charge of MBP, the reassociation of the two chains is expected to induce the charge density changes at the gate surface. This charge density change can be detected as a shift of the threshold voltage V_T of the FET. Thus, the measurement of V_T allows a direct, simple, and nonlabeled OS-IA.

The FET chip (Figure 1A) is immersed in a measurement solution together with an Ag/AgCl reference electrode with saturated KCl solution. The potential of a measurement solution is controlled and fixed by the gate voltage (V_G) through the reference electrode (Figure 1B). We have paid special attention to the buffer concentration used for measuring charge density change at the gate surface. The potential change induced by adsorption of proteins at the gate surface was reported to be dependent on the electrolyte concentration.[15−17] It is, therefore, important to optimize the Debye length at the gate insulator/solution interface. In the present study, a 0.025 M phosphate buffer solution was used for measuring charge density change at the gate surface, while the conventional reaction mixture was used during OS-IA reaction.[22]

The shift of the gate voltage (V_G)−drain current (I_D) characteristic, the V_T shift, was measured after reacting BPA with MBP-linked V_L (MBP-V_L) and V_H chains (Figure 4). The initial concentration of BPA added to the OS-FET chip was 1 μM. The resultant V_T shifted in the positive direction by the amount of 13.1 mV due to the negative charge of MBP (Figure 4A). On the other hand, the effect of nonspecific adsorption of MBP-V_L on the electrical signal was investigated by use of the control

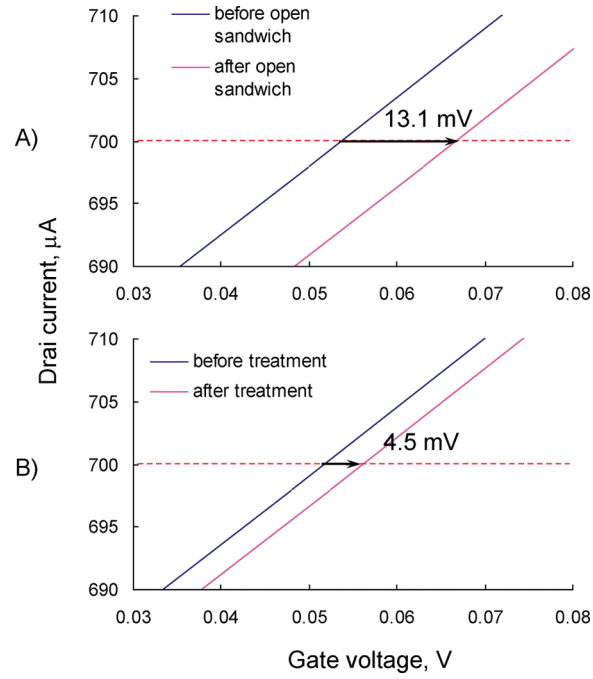

Figure 4. Threshold voltage V_T shifts for open sandwich-based immuno-field effect transistor (OS-FET) and control FET. The amount of the V_T shift was determined at a constant drain current of 700 μA in the gate voltage (V_G) and drain current (I_D) characteristic.

FET (Figure 4B). When the control FET chip with the immobilized V_H chains was reacted with the MBP-V_L solution without BPA, the V_T shift was 4.5 mV to the positive direction. The electrical signal of the control FET implies not only the antigen-independent basal binding of MBP-V_L but also the presence of unexpected BPA, which might be included in the conventional disposal tube used in the experiments.[26−31] Thus, for the highly sensitive detection of small uncharged antigens using the OS-FET, it is important to perform the differential measurement by the use of the control FET and the OS-FET.

While we could obtain significant signal, we further contrived to enhance the detection sensitivity of OS-FET by adding more negative charges to the MBP. The MBP has a total of 37 primary amino groups in its N-terminus and at the side chain of lysines. Therefore, isothiocyanobenzyl-EDTA with four negative charges based on carboxyl groups was utilized and reacted with the amino groups of MBP-V_L. While the V_L itself also encodes five lysines including a critical residue in the V_H/V_L interaction, we expected preferential labeling of MBP moiety after the random labeling reaction. The OS-IA was carried out on the gate of FET using the charged MBP-V_L with the EDTA-modified MBP. Figure 5 shows the V_T change in the V_G−I_D characteristic after the OS-IA using the charge-enhanced MBP-V_L. When the charge-enhanced MBP-V_L and BPA of 1 μM were reacted with the immobilized V_H chain on the gate surface, the V_T shifted in the positive direction by the amount of 30.0 mV probably due to added negative charges of MBP-V_L (Figure 5A), while the control FET showed the V_T shift of 3.5 mV to the positive direction as a background (Figure 5B). Compared with the V_T shift based on the original MBP-V_L, the introduction of charged molecules into MBP-V_L successfully amplified the electrical signal of OS-FET.

(25) Staples, C. A.; Dorn, P. B.; Klecka, G. M.; O'Block, S. T.; Harris, L. R. *Chemosphere* **1998**, *36*, 2149–2173.

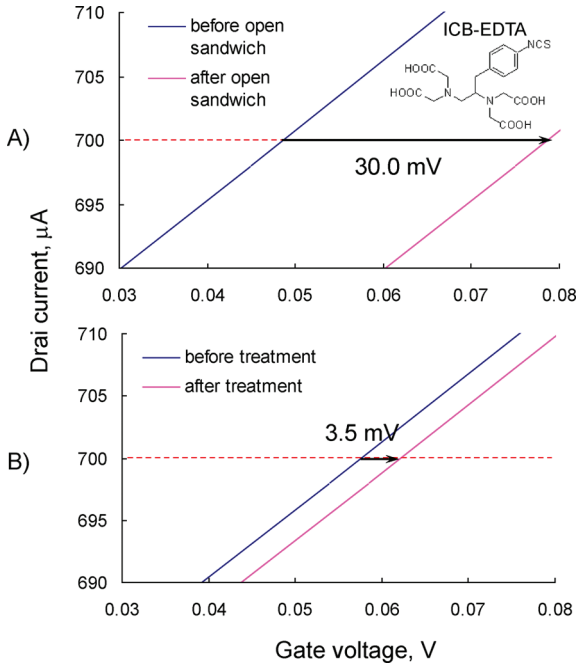

Figure 5. Threshold voltage V_T shifts for open sandwich-based immuno-field effect transistor (OS-FET) and control FET. The amount of the V_T shift was determined at a constant drain current of 700 μA in the gate voltage (V_G) and drain current (I_D) characteristic. In particular, the V_L chains with EDTA-modified MBP were utilized in this experiment.

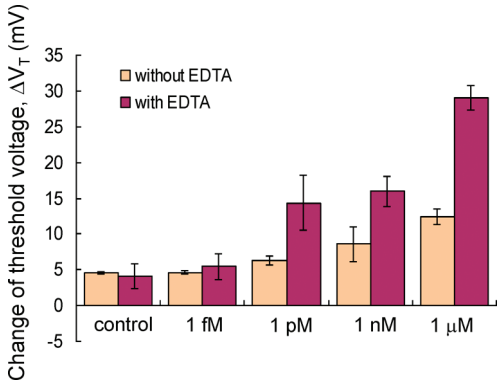

Figure 6. Threshold voltage V_T shifts for open sandwich-based immuno-field effect transistor (OS-FET) at various concentrations (1 fM to 1 μM) of bisphenol A (BPA). The effect of EDTA-modified MBP-V_L on the V_T shift was examined in comparison with the V_T shift of the OS-FET without EDTA.

The detection limit of OS-FET was examined with various concentrations of BPA. Figure 6 shows the V_T shift for the OS-FET at 1 μM to 1 fM BPA, as well as the control FET. A significant electrical signal was obtained at 1 nM BPA with the original OS-FET without EDTA, and there appeared some possibility of detection even at 1 pM. However, the electrical signals at 1 fM BPA were not significant against the control FET. On the other hand, the charge-enhanced OS-FET showed significant amplification of the detection signal at 1 pM BPA against the control FET, although the V_T shifts at 1 fM BPA was not distinguishable to that of the control. In conclusion, the addition of isothiocyanobenzyl-EDTA with negative charges to the V_L chain enhanced the detection limit to 1 pM to detect BPA for the standard deviation of the control signal (electrical

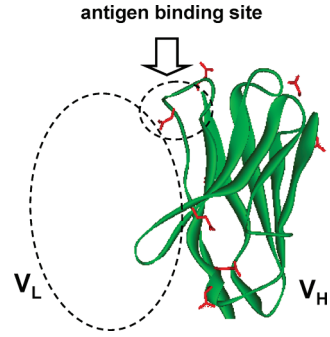

Figure 7. Putative structure of santi-BPA V_H, which was made by homology modeling with the V_H of a catalytic antibody (PDB 1CF8) as a template (http://swissmodel.expasy.org/workspace/). The green ribbon and red sticks represent the backbone of V_H and Asp/Glu residues, respectively. The locations of V_L and the antigen binding site are enclosed by broken lines.

signals of BPA > 3× SD of control), although the detection limit of OS-FET was 1 nM which was similar to the sensitivity of the conventional OS-ELISA.

The sizes of each protein utilized for the OS-FET should be considered in detail. The molecular sizes of V_L, V_H, V_H/V_L complex, and MBP are 2 × 2 × 3.5 nm, 2 × 2 × 3.5 nm, 3 × 3 × 5 nm, and 3 × 4.5 × 6.5 nm, respectively. The Debye length at the gate/solution interface is less than 10 nm in a 0.025 M phosphate buffer. A part of MBP moiety may be outside the Debye length, although they are substantially smaller than the original antibody. The further dilution of measurement solution will induce the increase of electrical signal obtained by the OS-FET analysis. To point out the detection sensitivity of OS-FET, furthermore, we have to improve the effect of background noise based on the nonspecific adsorption of MBP-V_L on the electrical signals of OS-FET. In this study, BSA was utilized as the inhibitor of nonspecific adsorption. BSA with a large molecular size (3.5 × 7 × 7 nm) might not be adequate in view of the detection principle of FET, particularly when sensitivity is strongly dependent on the Debye length near the gate surface, and might also be an obstacle for the molecular recognition of a small target at the gate surface. Therefore, the thin film of monolayer that suppresses the nonspecific adsorption and allows the detection of antigen within the Debye length is required for the improvement of signal transduction based on the OS-IA using the FET. For the improvement of the detection sensitivity, the immobilization of V_H also should be optimized. V_H has six aspartic acid residues, four glutamic acid residues, and one carboxyl group of the C-terminal as possible sites for cross linking. To assume their locations, we modeled the V_H structure of antiBPA antibody (Figure 7). Based on this model structure, it is likely that all the sites are not in the V_H-V_L interface and the antigen binding pocket, indicating that the V_H randomly immobilized on the surface would hold the binding ability with MBP-V_L and an antigen with high probability. However, the three sites (Glu47, Asp55, and Asp102) are close to the antigen recognition sites. Although they are expected to be minor cross-linking sites due to salt-bridge formation with a nearby residue, the cross linking at their sites might decrease the rates for V_H-V_L association or antigen binding. Therefore, the design of a specific cross-linking site at an appropriate site might improve the sensitivity. For example, an unnatural amino acid harboring

an azide group can be incorporated in a site-specific manner using a cell-free protein synthesis technique, producing a pinpoint-labeled protein.[32] The azide group can form a covalent bond with terminal alkynes via a copper-catalyzed click reaction, which will enable a specific cross linking with the surface.

CONCLUSIONS

The above results demonstrate that the reassociation of MBP-V_L and V_H chains with a small antigen can be directly transduced into electrical signal using the FETs and that the OS-IA can be realized essentially on the basis of intrinsic molecular charges without any labels. From the point of view of practical application, it is required to detect BPA at the possible lowest adverse effect concentration. According to a report of the National Toxicology Program in 2008 (http://ntp.niehs.nih.gov/), the lowest, but controversial, dose levels that can induce a variety of adverse effects are 0.0024−0.01 μg/kg/day. Assuming that the amounts of BPA are taken from beverages, the concentration is estimated to be 10−60 ng/mL (43−260 nM), which is completely within the working range of OS-FET.

It is possible to integrate multiple FETs and signal processing circuits in a single chip using advanced semiconductor technology. Simultaneous analyses of various small antigens in the environment or in our body can be realized on the basis of the FETs. Since the output of the FET is an electrical signal, standardization of the results obtained is easier than those with the chemiluminescence-based analyses. Therefore, the platform based on the FETs is suitable for a label-free, highly sensitive, and quantitative detection system for small antigen analysis in environmental, food, and clinical research.

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(26) Matsunaga, T.; Ueki, F.; Obata, K.; Tajima, H.; Tanaka, T.; Takeyama, H.; Goda, Y.; Fujimoto, S. *Anal. Chim. Acta* **2002**, *475*, 75–83.

(27) Shin, H.-S.; Park, C.-h.; Park, S.-J.; Pyo, H. *J. Chromatogr. A* **2001**, *912*, 119–125.

(28) Ohmura, N.; Lackie, S. J.; Saiki, H. *Anal. Chem.* **2001**, *73*, 3392–3399.

(29) Goda, Y.; Kobayashi, A.; Fukuda, K.; Fujimoto, S.; Ike, M.; Fujita, M. *Water Sci. Technol.* **2000**, *42*, 81–88.

(30) Inui, H.; Takehara, A.; Doi, F.; Nishi, K.; Takai, M.; Miyake, S.; Ohkawa, H. *J. Agric. Food Chem.* **2009**, *57*, 353–358.

(31) Welshons, W. V.; Nagel, S. C.; vom Saal, F. S. *Endocrinology* **2006**, *147*, s56–s69.

(32) Abe, M.; Ohno, S.; Yokogawa, T.; Nakanishi, T.; Arisaka, F.; Hosoya, T.; Hiramatsu, T.; Suzuki, M.; Ogasawara, T.; Sawasaki, T.; Nishikawa, K.; Kitamura, M.; Hori, H.; Endo, Y. *Protein* **2007**, *67*, 643–52.