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Short communication

Label-free detection of DNA using novel organic-based electrolyte-insulator-semiconductor

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ABSTRACT

In this study, we have constructed the first organic field effect sensor based on an electrolyte-insulator-semiconductor structure (OEIS) and applied this novel device to pH and DNA sensing. Variations in the insulator-electrolyte surface potential, which originate from either the change of the ionization states of the insulator surface groups or the binding of charged molecules to the insulator surface, modify the flat band voltage (V_{FB}) of the OEIS sensor. The pH sensing experiments of OEIS sensor showed that the output signal linearly depended on pH solution in the range from pH 2 to pH 12, and an average sensitivity of 44.1 mV/pH was obtained. In the biosensing experiments, the absorption of positively charged poly-L-lysine on the insulator surface resulted in the reduction of the V_{FB} value, whereas the subsequent binding of negatively charged single-stranded DNA probe (ssDNA) via electrostatic interaction increased the V_{FB} value. Furthermore, the ssDNA-immobilized OEIS device was successfully used for the detection of DNA hybridization. The detection limit of complementary DNA was as low as 1 μM, and the output signal of OEIS biosensor linearly increased with the logarithm of complementary DNA concentration in the range from 5×10^{-5} to 10^{-7} M. The easy and inexpensive fabrication of the OEIS device allows to be served as a potentially disposable and sensitive biosensor.

1. Introduction

It is of great scientific importance to develop various biosensing methods because they provide valuable information on the diagnosis of diseases, drug discovery, and biomolecular interactions. The methods for the detection of biomolecules can be classified according to the labeling requirement. All labeling methods suffer from the fact to be time-consuming, cumbersome and expensive. Recently, the use of silicon-based field effect transistors (FETs) as biosensors has attracted much attention due to their compact dimensions, real-time response, high sensitivity and label-free detection. In recent years, different types of silicon-based devices such as ion-sensitive field effect transistor (ISFET) and silicon nanowire field effect transistor (SiNW-FET) have demonstrated the detection of a variety of analytes, such as metal ions, DNA, viruses, and neuronal propagation signals (Bergveld, 2003; Fromherz et al., 1991; Martinoia and Massobrio, 2004; Patolsky and Lieber, 2005; Schöning and Poghossian, 2006). For example, Lin et al. (2010) have used calmodulin-modified SiNW-FET to detect various protein–protein interactions from either purified or crude cell extracts.

In recent years, organic semiconductor has attracted a great attention because it has many advantages, such as easy fabrication, mechanical flexibility, and low-cost. The development of organic-based electronics has created a number of significant applications including flexible low-cost photovoltaics (Hoppe and Sariciftci, 2006), thin film transistors (Reese et al., 2004), and light-emitting diodes (Wong and Ho, 2009). Although there are few reports available on the usage of organic-based FET (OFET) for gas sensing and ion detection (Bartic and Borghs, 2006; Mabeck and Malliaras, 2006; Sokolov et al., 2009), the application of OFET for the detection of biomolecules such as DNA in particular has been rarely reported (Stoliar et al., 2009; Yan et al., 2009). DNA contains the genetic instructions used in the development and functioning of all known living organisms, so its detection has many potential applications including gene expression monitoring, drug discovery, clinical diagnostics, etc. In this study, we constructed the first organic-based device with the structure of electrolyte-insulator-semiconductor (OEIS) and subsequently applied this device to pH and DNA sensing. The capacitance–voltage curve of OEIS device shifts along the voltage axis depending on variation of the pH of solution or the charge state of the absorbed biomolecules. The pH

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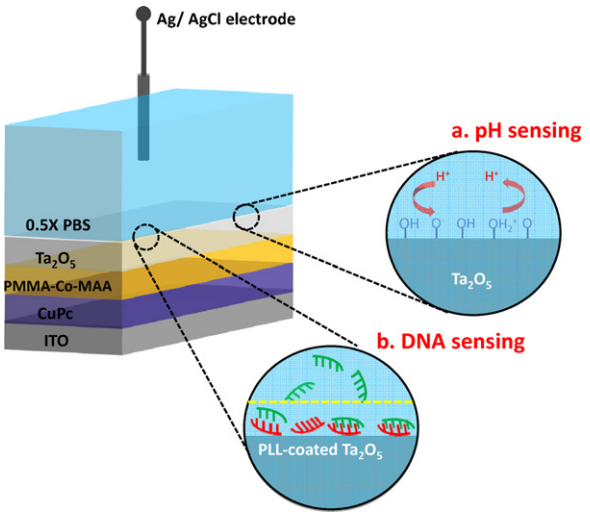

Fig. 1. Schematic of the structure and the operation principle of the OEIS sensor for (a) pH and (b) DNA. In (b), the ssDNA and cDNA are represented by the red and green objects, respectively. The yellow dashed line indicates the screening length from 0.5× PBS relative to the ssDNA (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

sensing experiments of OEIS sensor showed that the output signal linearly depended on pH solution in the range from pH 2 to pH 12 and resulted in an average sensitivity of 44.1 mV/pH. Furthermore, OEIS device specifically detected the hybridization event of the immobilized single-stranded DNA probe (ssDNA) with its complementary target DNA (cDNA). The detection limit of cDNA was as low as 1 μM, and the output signal linearly increased with the logarithm of cDNA concentration in the range from 10^{-7} to 5×10^{-5} M. Due to their simple and inexpensive fabrication methods, these OEIS sensors can find a great potential to be used as disposable biosensors.

2. Materials and methods

The OEIS device consists of a layer of organic semiconductor, copper phthalocyanine (CuPc), sandwiched between transparent indium tin oxide (ITO) electrode and dielectric layer. Before device fabrication, the ITO glasses ($12 \times 40\,mm^2$) were ultrasonically cleaned in detergent, deionized water, acetone and isopropyl alcohol. To improve the adhesion between ITO substrate and CuPc layer, the ITO electrode was irradiated by UV light in ozone for 15 min. Then, a 100 nm CuPc thin film ($12 \times 12\,mm^2$) was deposited on ITO electrode by thermal evaporation with a fixed deposition rate of 0.1 nm/s under a pressure of 8×10^{-4} Pa. During the deposition process, the ITO substrate was held at room temperature. Before the deposition of inorganic dielectric layer, a solution of poly(methyl methacrylate-co-methacrylic acid) (PMMA-Co-MAA) in chloroform (5 wt.%) was spin coated onto the CuPc layer, and then blow-dried with N_2. The spin coating of PMMA-Co-MAA was performed successively 3 times before this organic dielectric layer was baked for 1 h at 100 °C. Finally, a layer of 250 nm Ta_2O_5 thin film was deposited through a shadow mask (8×8 mm) by magnetron reactive sputtering in the flow of oxygen (10 sccm). The as-deposited Ta_2O_5 thin film was characterized by X-ray diffraction, which shows that the film structure is amorphous. The as-fabricated OEIS device was encapsulated by epoxy-type resin to prevent leakage currents and the contact area of the OEIS with the solution was $25\,mm^2$.

In the pH sensing experiments, the as-fabricated OEIS sensor was immersed in different pH buffers (pH 2–12, Merck Inc. USA) together with an Ag/AgCl reference electrode. Capacitance–voltage (C–V) measurements in different pH buffers were performed by using an Agilent E4980A. An ac voltage with the amplitude of 20 mV and the frequency of 100 kHz was applied to all measurements. Furthermore, all sensing experiments were carried out in a dark condition to prevent the interference caused by the ambient light and noise.

The immobilization of ssDNA onto a Ta_2O_5 surface was performed according to the procedures described in Fritz et al. (2002), and Bandiera et al. (2007). Briefly, the sensing zone of OEIS device was immersed in a poly-L-lysine solution (PLL, 1 wt.%, MW = 16,000–22,100, Sigma) for 30 min and then rinsed thoroughly with deionized water. The sensing zone of OEIS device was positively charged after PLL coating, which allows the ssDNA to be electrostatically absorbed. In this study, probe 15-mer oligonucleotide sequences were 5′-GATGATGAGAAGAAC-3′. The device was immersed in a 100 μM ssDNA solution for 1 h and then washed with deionized water to remove unbound ssDNA. Before the detection of DNA hybridization, the ssDNA-immobilized OEIS device was incubated in the cDNA solution under varied concentrations (0.1–50 μM) for 30 min and then rinsed thoroughly with deionized water. For each biosensing experiment, the OEIS device with the immobilized biomolecules was incubated in a 0.5× phosphate buffer saline (PBS) to record the C–V curve based on the above-mentioned protocols.

3. Results and discussion

As shown in Fig. 1, the structure of OEIS device differs from the typical configuration of MOS capacitor due to the absence of metallic gate. It is noteworthy that the presence of PMMA-Co-MAA layer greatly improves the adhesion between Ta_2O_5 and CuPc layers. In the absence of the interfacial layer of PMMA-Co-MAA, Ta_2O_5 thin film on the OEIS device easily ablated from the device after 3 measurements in PBS solution. In contrast, no sign of degradation of Ta_2O_5 thin film was observed for the OEIS device with the PMMA-Co-MAA layer after 20 measurements. Since Ta_2O_5 is often used as the ion-sensitive membrane in ISFET, the as-fabricated OEIS device in principle could serve as a pH sensor. The most important measurable parameter of OEIS system is its flat band voltage (V_{FB}), which can be defined in terms of the following equation (Bousse et al., 1983):

$$V_{FB} = E_{ref} - \psi_0 + \chi_{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{SS} + Q_{ox}}{C_{ox}} \quad (1)$$

where E_{ref} is the reference electrode potential relative to vacuum, χ_{sol} is surface dipole potential of the solution, Φ_{si} is the silicon work function, Q_{ss} is the surface state density per unit area at the silicon surface, Q_{ox} is the fixed oxide charge per unit area, C_{ox} is the gate insulator capacitance per unit area, and ψ_0 is the surface potential.

Because all terms are constant except of ψ_0, ψ_0 makes the OEIS device sensitive to the pH solution, which controls the dissociation of the oxide surface groups as shown in Fig. 1a. According to the site-binding model (Yates et al., 1974), the effect of a small change in the proton concentration at the interface on the surface charge density of oxide is given by:

$$\frac{\delta\sigma_0}{\delta pH_S} = -q\frac{\delta[B]}{\delta pH_S} - q\beta_{int} \quad (2)$$

where σ_0 is the surface charge per unit area and $[B]$ is the number of charged groups, defined as the number of negatively charged groups minus the number of positively charged groups, per unit area. β_{int} is the capability of buffer small changes in the surface pH (pH_S), but not in the pH solution (pH_B).

Due to charge neutrality, an equal but opposite charge (σ_{DL}) is stored in the double layer of the electrolyte solution. The ability of the double layer to store charge in response to a small change in sur-

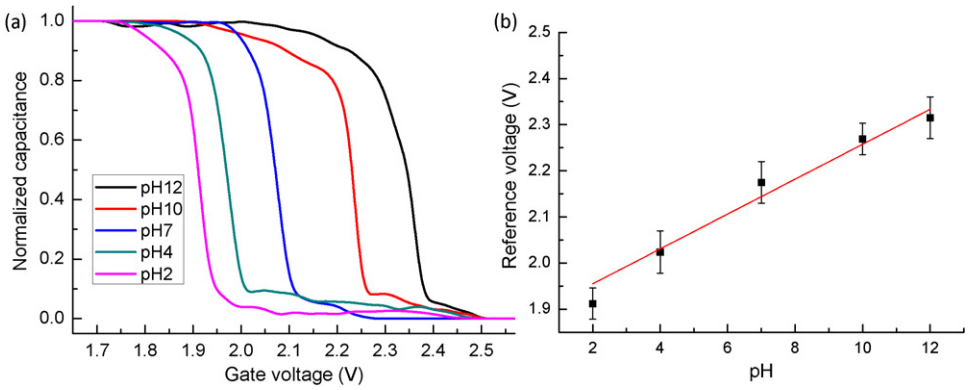

Fig. 2. (a) A typical set of *C–V* curves for the OEIS device in the concentration range from pH 2 to pH 12. (b) The calibration curve of the pH sensitive OEIS device. The pH measurements were conducted with an OEIS device, and each data point represents the mean ± SEM of at least three measurements. The red line represents a linear fit to the five concentration data points (correlation coefficient = 0.977) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

face potential is defined as the differential double layer capacitance, C_{dif} given by:

$$\frac{\delta\sigma_{DL}}{\delta\psi_0} = -\frac{\delta\sigma_0}{\delta\psi_0} = -C_{dif} \quad (3)$$

Combination of (2) and (3) generates an expression for the sensitivity of the surface potential in response to changes in pH_S:

$$\frac{\delta\psi_0}{\delta pH_S} = \frac{\delta\sigma_0}{\delta pH_S}\frac{\delta\psi_0}{\delta\sigma_0} = \frac{-q\beta_{int}}{C_{dif}} \quad (4)$$

Combining Eq. (4) with the Boltzmann equation in which the pH_B can be related to the pH_S, the pH_B variation leads to ψ_0 change and thus, changes the V_{FB} (Eq. (1)). The V_{FB} shift in response to the pH_B change can be recognized based on the parallel shift of the *C–V* curves of the OEIS device. In addition to pH sensing, we have also attempted to demonstrate that the OEIS device is capable of detecting biomolecules by their intrinsic molecular charges. As shown in Fig. 1b, ssDNA was electrostatically immobilized onto Ta_2O_5 surface with a coating of positively charged PLL layer. According to the previous studies on the growth of the multilayers consisting of PLL and DNA on the substrate, the thickness of PLL-DNA two layers is approximately 0.8 nm (Fritz et al., 2002). Given the effects of electrolytes on the electrical measurements taken by a FET device, we generally used 0.5× PBS as a working buffer in all of the biosensing experiments, of which the corresponding Debye screening length (λ_D) is approximately 1 nm (Stern et al., 2007). This length is able to enclose both the PLL and DNA layers; therefore, the OEIS device in such a solution environment should effectively reflect the immobilization of PLL and ssDNA layers, and the subsequent cDNA hybridization. Therefore, the change in the charge density at the interface induces the ψ_0 variation, which leads to the parallel shift of the *C–V* curves of the OEIS device.

To evaluate the pH sensing performances of the OEIS device, its C–V curves were recorded at different pH levels ranging from pH 2 to pH 12. Because CuPc thin film behaves as p-type semiconductor, the capacitance of OEIS device remains almost constant in accumulation region (negative gate voltage) and it decreases steeply with the increase of gate voltage as shown in Fig. 2a. Furthermore, the inversion of electrons is not observed at any bias because the electron response is too slow relative to the measuring frequency (Stadler and Burghart, 2006). The shape of *C–V* curve of OEIS device is highly similar to that of MOS capacitor consisting of CuPc and Ta_2O_5 gate insulator (Itoh and Miyairi, 2006). It is noteworthy that the position of the *C–V* curve shifts along the voltage axis depends on the change of pH_B. These V_{FB} shifts can be explained by the variation of the ionization states of the Ta_2O_5 surface groups, which further modulate the potential at the insulator-electrolyte interface (Yates et al., 1974). In order to determine the pH sensitivity of OEIS device, the sensor output signal (reference voltage) was evaluated at 50% of the normalized capacitance. As shown in Fig. 2b, the OEIS sensor exhibited a linear pH response in the concentration range from pH 2 to pH 12. The average sensitivity of such pH detection was calculated to be 44.1 mV/pH, being lower than pH sensitivity of silicon-based EIS with Ta_2O_5 insulator (57 mV/pH) (Siqueira Jr. et al., 2009). According to the site-binding model, the pH sensitivity of EIS sensor is dependent on the total number of surface state per unit area (N_s). The value of N_s increases accordingly with the increase of surface roughness, which leads to higher pH sensitivity (Van den Berg et al., 1985). Furthermore, some studies have shown that the insulator with good crystallinity often exhibits high surface roughness and thus, leads to high pH sensitivity (Pan and Liao, 2008; Pan et al., 2009). Therefore, the relatively small pH sensitivity of OEIS sensor may be attributed to the amorphous structure of Ta_2O_5 film. Since the operation principle of a conventional enzyme modified FET is based on the detection of local pH variations induced by an enzymatic reaction (Siqueira Jr. et al., 2009; Thust et al., 1996), it is expected that the OEIS device can serve as the biosensor upon immobilizing the enzyme membrane on its sensing zone.

The OEIS device presented here can be viewed as one type of FETs, so it should be sensitive to the charge variation at the insulator–electrolyte interface. In principle, the attachment of charged biomolecules to the Ta_2O_5 surface should change its surface potential, which intern result in shift in V_{FB} direction. As shown in Fig. 3a, it is clear that the absorption of positively charged PLL biomolecules onto OEIS sensor caused V_{FB} position to shift towards the left-hand side. In contrast, the shift of V_{FB} to the right was observed, when negatively charged DNA was electrostatically absorbed on the surface of PLL-modified OEIS. Because all measurements were conducted in a 0.5× PBS, the effect of either pH change or ionic strength variation may be neglected. According to Eq. (1), the increase (decrease) of surface potential with the absorption of positively (negatively) charged biomolecules leads to the reduction (increase) of the V_{FB} value. Furthermore, the dependence of the direction of V_{FB} shift on the sign of the biomolecular charge is consistent with the previous studies on monitoring the layer-by-layer absorption of polyelectrolytes using silicon-based EIS sensor (Poghossian et al., 2007).

Detection of DNA hybridization plays an important role in medical diagnostics, drug development and biological threat discovery. Since the alternating V_{FB} shifts suggested that the ssDNA was successfully immobilized onto OEIS sensor (Fig. 3a.), we attempted to convert the DNA hybridization event into a useful analyti-

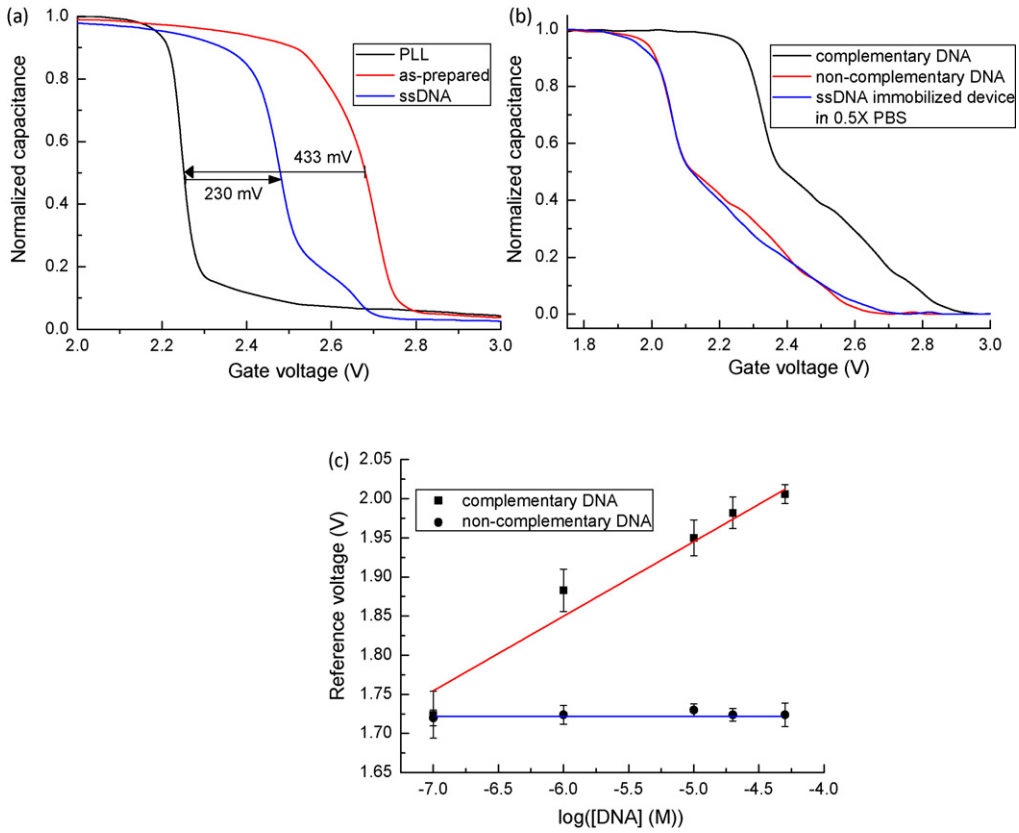

Fig. 3. (a) *C–V* curves for the as-prepared, PLL-absorbed and ssDNA-immobilized OEIS device in 0.5× PBS. (b) Label-free detections of complementary and non-complementary DNA by using ssDNA-immobilized OEIS device. The concentration of DNA is 50 μM. (c) Plot of reference voltage vs. the logarithm of [cDNA]. DNA hybridization measurements were conducted with a ssDNA-immobilized OEIS device, and each data point represents the mean ± SEM of at least three measurements. The red line represents a linear fit to the five concentration data points (correlation coefficient = 0.953). The same device also showed no response to non-complementary DNA in the range of 10^{-7}–5×10^{-5} M. These sensing experiments were performed using three different OEIS devices (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

cal signal using OEIS as a transducer. Fig. 3b shows the V_{FB} of the OEIS device shifted after cDNA was hybridized with surface-immobilized ssDNAs, and this increase in V_{FB} was caused by the binding of the negatively charged cDNA to OEIS sensor. By comparison, the ssDNA-immobilized device showed no obvious change in its curve position after it was immersed in a solution of non-complementary DNA (red curve in Fig. 3b), which suggests that the change in V_{FB} of the OEIS was induced by the specific binding of cDNA. Fig. 3c shows that the reference voltage linearly increases with the logarithm of cDNA concentration in the range of 10^{-7}–5×10^{-5} M. When cDNA with the concentration of 10^{-7} M was used for the hybridization, the value of reference voltage of OEIS (1.723 V) was equal to that of ssDNA-immobilized device, and thus, the detection limit of cDNA was determined to be 1 μM. Although our OEIS device is less sensitive than SiNW-FET sensors that have sensitivities for DNA in the tens of nanomolar range (Bunimovich et al., 2006), its DNA sensitivity could be further improved by optimizing measurement parameters such as the density of ssDNA immobilization and the ion strength of working buffer (Poghossian et al., 2005; Uno et al., 2006). More importantly, it must be stressed that the main advantages of OEIS device are its flexibility, easy and inexpensive fabrication, which are the key factors for the development of the disposable biosensor.

4. Conclusions

In summary, we have constructed the first organic field effect sensor based on an electrolyte-insulator-semiconductor structure, and applied this novel device for pH and DNA sensing. The V_{FB} shift of the OEIS device can be attributed to the fact that the potential at the insulator-electrolyte interface is modulated by either the variation of the ionization states of the Ta_2O_5 surface groups or the intrinsic charge of absorbed biomolecules. The pH sensing experiments of OEIS sensor showed that the output signal linearly increases with pH solution in the range from pH 2 to pH 12, with an average sensitivity of 44.1 mV/pH. In the biosensing experiments, the absorption of positively charged PLL on the insulator surface decreased the V_{FB} value, whereas the subsequent binding of negatively charged ssDNA via electrostatic interaction increased the V_{FB} value. Furthermore, the binding of the target strands to surface-immobilized ssDNA is specifically detected by the OEIS device and the detection limit of cDNA is 1 μM. It was noted that the output signal of OEIS biosensor increased linearly with the logarithm of [cDNA] over a range of 10^{-7}–5×10^{-5} M. Due to its easy and inexpensive fabrication, the OEIS device has a great potential in the application of the disposable biosensor.

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