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Improved DNA detection by utilizing electrically neutral DNA probe in field-effect transistor measurements as evidenced by surface plasmon resonance imaging

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ABSTRACT

Intensive efforts have been focused on the development of ultrasensitive DNA biosensors capable of quantitative gene expression analysis. Various neutralized nucleic acids have been demonstrated as alternative and attractive probe for the design of a DNA chip. However, the mechanism of the improvements has not been clearly revealed. In this investigation, we used a newly developed neutral ethylated DNA (E-DNA), a DNA analog with the "RO-P-O" backbone (wherein R could be methyl, ethyl, aryl, or alkyl group) obtained from synthetic procedures, and a silicon nanowire (SiNW) field-effect transistor (FET) to evaluate the difference in DNA detection performance while using E-DNA and DNA as probes. It is demonstrated that using the E-DNA probe in the FET measurement could have a significantly enhanced effect upon the detection sensitivity. Surface plasmon resonance imaging (SPRi) was used to evidence the mechanism of the improved detection sensitivity. SPRi analysis showed the amounts of probe immobilization on the sensor surface and the hybridization efficiency were both enhanced with the use of E-DNA. Consequently, neutral ethylated DNA probe hold a great promise for DNA sensing, especially in the electrical-based sensor.

1. Introduction

In recent years intensive efforts have been focused on the development of ultrasensitive DNA biosensors capable of quantitative analysis. DNA biosensors based on sequence-specific DNA hybridization are prevalent applied in many fields, including human genomes, DNA sequence analysis, gene expression monitoring, diagnosis of gene mutation, forensic sciences, pharmacogenomics and so on. To date, many techniques have been developed in measuring DNA hybridization, including electrochemical, piezoelectric, mass spectrometry, fluorescence, and optical methods. Surface plasmon resonance (SPR) is an optical measuring technique detecting the change in the refractive index at the interface between the dielectric and metallic layer. SPR has become a frequently used platform for sensitive probing of antibody–antigen interaction and DNA hybridization (Chen et al., 2007). Recently, owning to prompt development of nano-technologies, a variety of nanoscale tools have been used for biological research and applications. Semiconductor nanowire-based devices called silicon nanowire field-effect transistors (SiNW-FETs) allow ultra-sensitive, label-free, rapid and real-time measurements for interactions of biomolecules including proteins (Cui et al., 2001; Zheng et al., 2008, 2010), DNA hybridizations (Ananthanawat et al., 2009, 2010, 2011; Hahm and Lieber, 2003), small molecule interactions (McAlpine et al., 2008), and even viruses (Zhang et al., 2010) at ultralow concentration. The working basis of SiNW-FET biosensors depends on the charge carriers in the semiconductive channel (holes for a p-type channel and electrons for an n-type channel). However, the background electric charges from probes or in a high ionic-strength environment typically interfere the performance of SiNW-FET biosensors, resulting in a low signal-to-noise ratio (S/N ratio) (Chen et al., 2011). Taking an example of a p-type NW-FET, when the positively charged probes bind onto the surface of NW-FET, a depletion of charge carriers occurs in the conductance channel, causing a decrease in the conductivity. On the contrary, the binding of negatively charged molecules, such as DNA or RNA, will result in an increase in the conductivity due to the accumulation of charge carriers in the conductance channel.

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Thus, in addition to the technological advances in the biosensor instrumentation, the optimization of hybridization conditions (ionic strength of buffer, composition of sensor surface) and the selection of sensing probe sequence have critical influences on the detection sensitivity and specificity. A regular DNA has a deoxyribose sugar backbone carrying negatively charged phosphate groups in a physiological environment (pH 7.4). Due to the strong electrostatic repulsion, the efficiencies of DNA immobilization and hybridization are hampered. The most used strategy for optimization of DNA detection is to increase the concentrations of divalent or monovalent cations in the buffer solution. Kick et al. (2009) reported that increasing salt concentration of solvent or replacing Na^+ with Mg^{2+} in the solution can screen the charge of DNA fragments leading to the effective increase in the efficiency of DNA hybridization. Petrovykh et al. (2003) demonstrated that concentrations of divalent cations in the buffer solution more than the monovalent cations were able to improve the surface coverage of DNA probes on the chips. However, these efforts obviously are unfavorable to the sensitivity in the FET measurements.

Peptide nucleic acid (PNA), a synthetic DNA analog (Nielsen et al., 1991; Egholm et al., 1992), has been utilized as a probe immobilized on SiNW-FET biosensors to eliminate the charge effect on the sensitive detection of DNA (Zhang et al., 2008; Gao et al., 2007). PNA retains nucleobases of DNA, but its backbone is replaced by a pseudo-peptide, leading to changes in physical properties, such as charge, chain elasticity and bending rigidity (Lomakin and Frank-Kamenetskii, 1998). Although the use of PNA improves the DNA hybridization affinity, the high-cost, low cellular uptake and limited solubility of PNA constrain its prevalence. Besides PNAs, the other uncharged DNA analogs are synthesized for using in therapeutic application fields (antisense experiments) and as probes in DNA biosensors, such as phosphorodiamidate morpholino oligomers (PMO) and methyl-phosphonate oligonucleotides (MPO). The uses of these uncharged DNA analogs in bio-applications are due to their stable, non-toxic properties and similar physical properties to the natural DNA (Alvarado, 2009, 2010). Herein, we describe implementation of the electrically neutral ethylated DNA (E-DNA) as probes on sensor surfaces for ultra-sensitive detection of its complementary DNA targets on the SiNW-FET device in order to avoid the interference of charge noise from recognition elements in the measurements. In addition, we investigated the secondary structures of the single-stranded neutral DNA fragment and its hybridized double-stranded DNA fragment by circular dichroism with respect to unmodified charged DNA. Studies on the surface binding density of probe DNA and the efficiency of DNA hybridization will be carried out by using a SPR sensor. The results show that E-DNA holds a great potential in the DNA detection through reducing the electrical repulsion and the screening of charge carriers inside the SiNW. Perhaps the improved signal sensitivity was contributed by either higher probe density on the sensor surface or better hybridization efficiency of E-DNA/DNA hybrids. To determine this, SPR imaging (SPRi) was used to measure immobilization in situ and DNA duplex formations for further clarifying the findings from FET measurements. By using the FET and SPR biosensors, the potential benefit of applying the E-DNA for achieving sensitive detection of DNA with the FET nanosensor could be ascertained.

2. Materials and methods

2.1. Chemical reagents

1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-Hydroxy- sulfosuccinimide (NHS) were purchased from Thermo Scientific (USA). Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4\ H_2O$) and di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) were purchased from Merck (Germany). PBS solution used in SPR measurements contains 10 mM phosphate buffer, 137 mM sodium chloride, and 2.7 mM potassium chloride, adjusted to pH 7.0. Phosphate buffer (10 mM PB, pH 7.0) was use in the FET measurements. OEG-terminated thiol ($HSC_{11}(EG)_6OCH_2COOH$) was purchased from ProChimia (Poland) and $HSC_{11}(EG)_4OH$ was bought from Sigma-Aldrich (USA). Glutaraldehyde (25%) was purchased from Fluka (USA). All other chemicals used in this study were reagent grade.

2.2. Regular and neutral sequences

The DNA sequences used in the study were

- Probe DNAs (DNA and E-DNA): 5′-NH_2-CCTCGCCTTCTCCTT-GCAGCTCC-3′
- Complementary target DNA: 5′-GGAGCTGCAAGGAGAAGGC-GAGG-3′
- Non-complementary target DNA: 5′-GCCG CTTCC TACCGCT-TCCTCCT-3′

The sequences for DNA and E-DNA were all the same and the difference between these two kinds of DNAs was found in their structure. E-DNA was an oligonucleotide analog and was supplied by SynGen, Inc. (San Carlos, CA, USA) (Alvarado, 2009, 2010). The DNA analog with the "RO-P-O" backbone (wherein R could be methyl, ethyl, aryl, or alkyl group) used in this study was synthesized by using Fmoc-protected phosphoramidites (Alvarado, 2009, 2010). E-DNAs have modified backbones, and a phosphorus atom is still retained in the backbone. Other oligonucleotides were purchased from MDBio, Inc. (Taipei, Taiwan). A regular DNA has a phosphate-deoxyribose backbone and charged phosphate groups on the DNA that are negative in a normal physiological environment (pH 7.4). Fig. 1 shows the chemical structures of E-DNA and DNA. This figure reveals that the modified backbones of E-DNAs are uncharged. The modified oligonucleotides retain the ability to hybridize with target RNA or DNA. For target DNA sequences, one is a complementary DNA (cDNA) and another one is the non-complementary DNA (ncDNA).

2.3. Circular dichroism (CD) spectroscopy analysis

CD spectroscopy is an exceedingly useful method for investigating the DNA and RNA duplexes (Clark et al., 1997). The B-form conformation is the most regularly observed structure in the DNA. B-form DNAs are mostly characterized by the CD spectra with a positive band at about 260–280 nm and a negative band at around 245 nm. The concentrations of single-stranded DNA and E-DNA used for measuring CD spectra were 1 μM. The DNA/cDNA and E-DNA/cDNA duplexes for the analysis of CD spectra were prepared respectively by mixing 0.5 μM single-stranded DNA or E-DNA with its complementary sequences together. CD spectra from 350 to 210 nm were recorded on a Jasco J-810 spectropolarimeter. CD spectroscopy analysis was performed at a constant temperature of 20 °C and data was collected every 0.1 nm interval.

2.4. Silicon nanowire field effect transistor (FET) nanosensor

The SiNW-FET sensor was supplied by the National Nano Device Laboratories of the National Chiao Tung University (Hsinchu, Taiwan). Electric measurements and immobilization of DNA probe on the EFT sensor are illustrated as follows. The SiNW-FET sensor was an n-type nano device, which was fabricated on the fundamental of poly-silicon sidewall spacer technique. This n-type nano device had two poly-Si nanowire-channels, 80 nm width and 2 μm length. Immobilization of

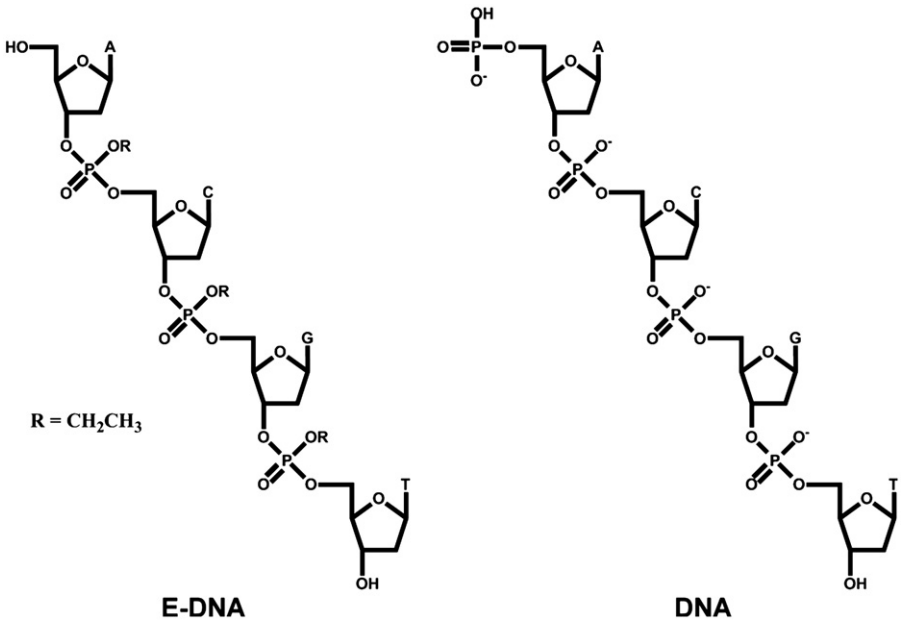

Fig. 1. The chemical structures of E-DNA (on the left side) and DNA (on the right side).

the target DNA probe on the SiNW-FET sensor surface was prepared by using a three-step procedure. The surface of SiNW-FET sensor was fabricated by using poly-crystalline silicon nanowires (poly-SiNWs). To immobilize probe DNA, some chemical modification procedures must be carried out on the sensor surface. Therefore, the sensor surface was treated with ethanol and then 2.0% APTES ethanol solution was added through the flow channel for 30 min to bring in an amino group on the poly-SiNW surface. The device was then washed with abundant pure ethanol and heated to 120 °C for 10 min. After the treatment, 2.5% glutaraldehyde in 10 mM PB (pH 7.0) containing 4 mM sodium cyanoborohydride was injected into the microfluidic channel to react with the poly-SiNW surface for 1 h, and PB was subsequently used to wash the sensor surface. Finally, 5 μM probe DNA in the PB contained 4 mM sodium cyanoborohydride was flowed over the poly-SiNW surface for 1 h, and afterwards the immobilization of probe DNA could be achieved. A solution of 50 mM ethanolamine was then reacted with the sensor surface for 30 min followed by PB wash. The modified surface of poly-SiNW FET was ready for use in the detection of DNA hybridization.

A chip analyzer (Keithley 2636) was used to control the gate potential and source/drain bias. The drain current (I_D) was measured under four constant bias voltages (V_G=0 V, 1.0 V, 2.0 V, 3.0 V) with the use of a constant bias voltage (V_D=0.5 V) in order to obtain the I_D–V_G curve and determine the parameters of the FET sensor in this study. In the initial stage of FET experiments, the I_D–V_G curve was measured repeatedly in PB (10 mM, pH 7.0) to confirm that the I_D–V_G curve of the FET sensor was stable. After this was obtained, we used a micro pipette to convey 5 μM target DNA (volume around 0.1 μl) to the nano device. The hybridizations of target and probe DNAs resulted in an electric response on the FET sensor that could be detected after adding the target DNAs. We measured an I_D–V_G curve in 3 min, during which it should be stable. In order to obtain more reliable results, we performed triplicate measurements for getting the I_D–V_G curves. More detailed procedures of FET measurements were reported in the paper published by Lin et al. (2009). The ratio of specific recognition (*SR*) in the FET measurements was evaluated according to the following equation:

$$\mathrm{SR} = \left(\frac{\text{specific binding}}{\text{nonspecific binding}}\right) \tag{1}$$

Besides, the S/N ratio of the FET measurement s were calculated according to the following equation:

$$\frac{S}{N} = \frac{\Delta I_D^S}{\Delta I_D^N} \tag{2}$$

where ΔI_D^S and ΔI_D^N are changes of the drain currents for the complementary and non-complementary DNA hybridization reactions, respectively, from that in PB buffer.

2.5. SPR imaging apparatus

The SPR sensor platform we used in this study was an SPR imaging system with six flow chambers, which was developed by the Institute of Photonics and Electronics (Prague, Czech Republic) (Piliarik et al., 2010; Piliarik and Homola, 2008). The p-polarized narrow-band light beam (central emission wavelength is 750 nm) impinges on a glass substrate and excites SPs at the metal-dielectric interface. The SPR platform is a self-referencing SPR imaging sensor with polarization contrast (Piliarik et al., 2009, 2010; Piliarik and Homola, 2008), which is able to detect the smallest signal corresponding to the change in the refractive index unit (RIU) better than 10^{-6} within the operation range of 0.011 RIU (Piliarik and Homola, 2008). It also can be illustrated in terms of protein surface coverage that the sensor can detect the changes are as small as 0.2 pg/mm^2. The SPRi can be utilized by conducting traditional measurements or performing a high-throughput analysis with the use of an array-formatted chip. The SPR chips were prepared by using clean BK7 glass substrates. The glass substrates were pre-coated with an adhesion layer of chromium (thickness approx. 2 nm), and an active gold layer with a thickness of 48 nm was subsequently coated via an evaporation deposition process at pressures below 1×10^{-6} Torr. All experiments were performed at a constant temperature of 25 °C and at a flow rate of 50 μl/min. Six flow channels were used in the SPR experiments, with one being used as a reference channel.

2.6. SPR measurements

The preparation of general SPR chip was similar with the steps described in previous literature (Hu et al., 2012). The major

difference is that we use the flow-through method to functionalize the sensor surface in this study. For DNA immobilization, the SPR chip was cleaned by rinsing with deionized (DI) water, absolute ethanol and dried in a stream of nitrogen, followed by exposing to UV-ozone radiation for 30 min. The chip was sequentially mounted on the SPR device. Afterwards, a 1 mM mixture for mixed OEG- and COOH-OEG-terminated thiols (with a moral ratio of 1:1) in ethanol was injected into flow channels to functionalize the surface of the sensor chip. The EDC/NHS (0.1 mM/0.025 mM) solution was prepared in the 0.1 M MES buffer (0.5 M NaCl, adjusted to pH 5.5) for activating of carboxyl groups. The EDC/NHS solution was then flowed through flow-chambers for 30 min, and the activated carboxyl group of COOH-OEG alkanethiol could react with the amine group of a DNA probe to generate a covalent bonding between the DNA probe and COOH-OEG alkanethiol. The concentrations of probe and target DNAs used in general SPR measurements were all 5 μM. Two of the six channels are used for one kind of functionalized sensor surfaces in once experiment, and experiments were performed in duplicate. The hybridization efficiency (HE) was calculated from the following equation:

$$\mathrm{HE}(\%) = \left(\frac{\text{target density}}{\text{probe density}}\right) \times 100 \tag{3}$$

Kinetic parameters for the hybridization reactions (k_a, k_d) were calculated using the rate equations. The dissociation rate constant, k_d, was derived as follows:

$$R_t = R_0 e^{-k_d(t-t_0)} \tag{4}$$

where R_t is the SPR response at time t and R_0 is the value of the initial SPR response at time t_0. The association rate constant, k_a, was calculated as follows:

$$R_t = \frac{k_a C R_{\max}}{k_a C + k_d}\left(1 - e^{-(k_a C + k_d)t}\right) \tag{5}$$

The binding affinity, K_A, is calculated according to the relationship

$$K_A = \frac{k_a}{k_d} \tag{6}$$

3. Results and discussion

3.1. Detections of regular and neutral DNA duplex formations

From the experimental data of CD spectra (data not shown), the CD spectra of single stranded oligomers were very similar in band magnitudes and shapes. The spectrum of cDNA had a positive band at 265 nm, and the spectra of DNA and E-DNA had positive band at 275 nm. The spectra of DNA/cDNA and E-DNA/cDNA duplexes were nearly identical, which both appeared negative bands at 240 nm and positive bands at 275 nm. This evidence indicated that the E-DNA with an electrically neutral ethylphosphonate backbone remains the capability to specifically hybridize with its complementary DNA and to form the similar secondary structures as the DNA/cDNA duplex. Consequently, the CD experiments indicated that the ethylated DNA did not cause any significant influence on the secondary structure of the DNA double helix. This finding is consistent with a previous report (Clark et al., 1997), where they found that the spectra of the native DNA duplex and the DNA duplex containing one electrically neutral strand had no obvious difference.

3.2. Regular DNA versus neutral DNA as probes in FET assay

The SiNW-FET sensors have recently been adapted as transducers for the detection of biomolecular interactions, due to their characteristics of ultrahigh sensitive, real-time and label-free measurements. The sensing mechanism of SiNW-FET sensors can be understood in light of the change in the charge density which is caused by varying the electric field in the proximity of the SiNW surface after hybridization (Zhang et al., 2008). Debye length is a critical parameter for the performance of SiNW-FET sensor and is also a factor to achieve optimal label-free sensing using SiNW-FETs. In theory (Stern et al., 2007), the ionic strength of 0.01 × PBS has a Debye length of ~7.3 nm. For the 10-fold increase in the ionic strength of the buffer (0.1 × PBS), the Debye length approximately equals to 2.3 nm, and the Debye length for 1 × PBS is around 0.7 nm. Even with the insufficient Debye length, however, some researchers had been performed DNA hybridization measurements successfully on the SiNW-FET sensors in 1 × SSC and 10 mM TE buffers, respectively (Bunimovich et al., 2006; Gao et al., 2007). We also used a high ionic strength of buffer (10 mM PB buffer) in the FET measurements, which could have an adverse influence on the of SiNW-FET sensor in the sensitivity.

In addition to the effect of Debye length, the interfacial potential in the aqueous environment has an unfavorable fluctuating effect on the sensitivity of SiNW-FET sensor. Thus, the sensitivity of SiNW-FET sensors usually was compensated by the environmental noise. Therefore, using a buffer with a low ionic strength in the FET measurement is a prerequisite to achieve an optimal detection with high sensitivity. In this study, standard PB buffers (10 mM, pH 7.0) with 5 μM different target DNAs were tested to react with the immobilized DNA and E-DNA probes in parallel. As depicted in Fig. 2, the hybridization signals for cDNAs on the E-DNA probe immobilized surfaces were larger than those on the regular DNA probe immobilized surface. Compared to the DNA probe surface, the E-DNA probe surface has a 23.3% increment in the signal generated by the full complementary target hybridization. Concerning the ratio of specific recognition (SR) calculated by Eq. (1), E-DNA probe surface had a higher SR value (7.26) than the SR value for DNA probe surface (3.64). The values of nonspecific binding signals on the E-DNA and DNA probe surfaces were small and similar. The major reason causing the E-DNA probe surface with a higher SR value was that the E-DNA probe surface could produce a larger hybridization signal in the FET measurement.

Fig. 3(a) and (b) shows the I_D–V_G curves of the SiNW-FET sensor to the DNA hybridization in different aqueous solutions on E-DNA and DNA systems, respectively. Herein, we further compared the performance of these two sensor platforms according to the recorded I_D–V_G curves at V_G of 1 V as indicated by the S/N ratio. The values of the signal and noise were contributed from the bindings of cDNA and ncDNA analytes, respectively, on SiNW-FET surfaces. The results show that in the E-DNA system the S/N ratio was 27.5 which is about one order of magnitude higher than that in regular DNA system (S/N=2.8). This improvement of the sensor sensitivity with electrically neutral E-DNA probes obviously involved two major aspects: (a) high sensor response to DNA hybridization, which could be the results of high hybridization efficiency (Xu et al., 2008), high probe density (Xu et al., 2008) and elimination of the background charge interference from the probe molecules to the electron transport on SiNW (Zhang et al., 2008); (b) low non-specific adsorption from ncDNA, which is likely due to the steric hindrance caused by the ethyl groups on the backbone of E-DNA. We suspect that the additional ethyl groups could deteriorate the formation of the hydrogen bonds between E-DNA and ncDNA, leading to the reduced amount of mismatch duplex even through their repulsive electric force was greatly reduced. Our findings are consistent with another uncharged DNA analog (i.e., PNA) which was recognized as a decrease of the repulsive force from the backbone charges to interfere with the hybridization reaction (Zhang et al., 2008). Our FET experimental results confirm that the E-DNA probe surfaces

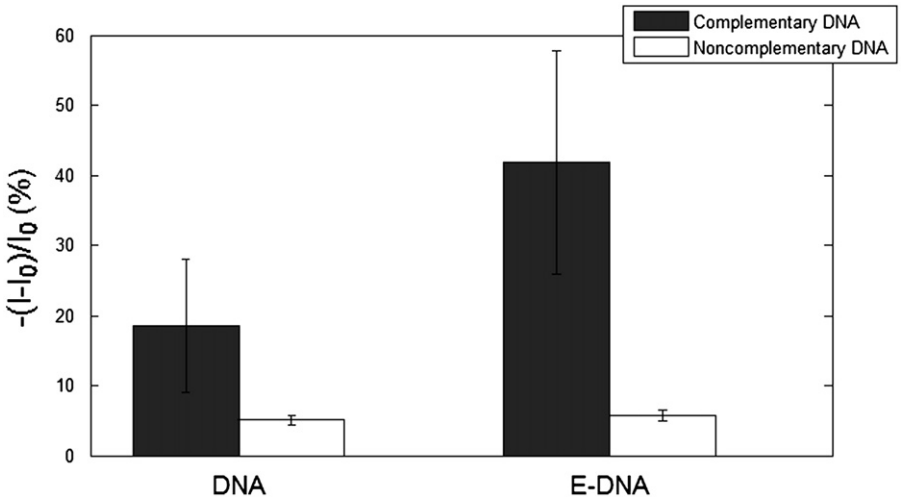

Fig. 2. The signal changes of using two kinds of DNA probes in the FET measurements. Complementary and non-complementary DNA targets were used in these experiments. The concentrations of probe and target DNAs were all 5 μM.

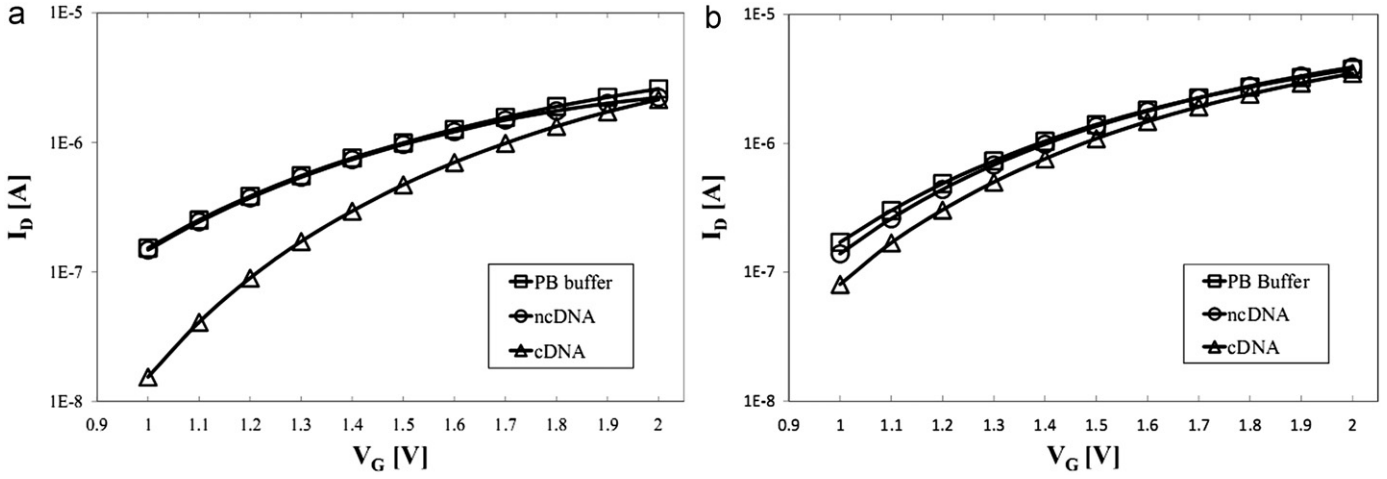

Fig. 3. Electric responses of the SiNW-FET sensor to the DNA hybridization in different aqueous solutions. The I_D–V_G curve (from 0 to 3 V) output characteristics with a constant V_D (0.5 V). (a) I_D–V_G curves obtained from E-DNA functionalized SiNW-FET sensor in PB, for ncDNA and cDNA detection. (b) I_D–V_G curves obtained from regular DNA functionalized SiNW-FET sensor in PB, for ncDNA and cDNA detection.

can provide more sensitive measurements in the SiNW-FET sensors. However, the E-DNA probe whether can increase the hybridization efficiency or probe density on the sensor surface or not, which still needs to be proved by using the SPRi.

3.3. Enhanced sensitivity based on the uncharged backbone of neutral DNA

In order to further ascertain the potential of using E-DNA probes on the biosensor, the SPRi was used to carry out traditional measurements for the detection of oligonucleotides with different probes immobilized on the sensor surface. Compared to the FET measurement, SPR sensing technique is advantageous in providing real time information of biomolecular interactions. The experimental data presented in Fig. 4 were obtained on the flow-through functionalized sensor surfaces. The amounts of immobilization DNA probes on the SPR sensor chip were expressed as the change in the surface coverage of bound biomolecules (ng/cm^2). The concentrations of DNA and E-DNA probes were the same (5 μM). As shown in the Fig. 4 (dark columns), it was found that the E-DNA probe can generate a higher probe density than that of the DNA probe. We suggested that the nature of E-DNA probe was favorable in accessing the activated carboxyl groups of mixed OEG SAMs, which leads to a better immobilized amount of DNA probes. The SPR sensor surfaces with immobilized DNA probes were further used to hybridize with complementary target DNAs (shown in the bright columns of Fig. 4). These results indicate that the sensor surface with E-DNA probes obtained a higher SPR response. However, a larger SPR response of target DNA hybridized with DNA probe may only reflect the probe density on the sensor surface. To further validate the advantage of using E-DNA probes, the experimental data was calculated to determine the value of hybridization efficiency according to Eq. (3). According to the calculation results, the E-DNA probe exhibited better hybridization efficiency with its complementary sequence ($54 \pm 9\%$ (HE)). Higher hybridization efficiency demonstrated that the use of the E-DNA probe was an effective strategy to increase sensing sensitivity.

All biotinylated probes used in the study reported by Ananthanawat et al. (2010) yielded similar HE values (59–62%) in 10 mM PBS buffer with 100 mM NaCl. Direct immobilization of thiolated acpcDNA exhibited a poor HE value ($< 20\%$). In our study, the DNA and E-DNA were immobilized on the sensor

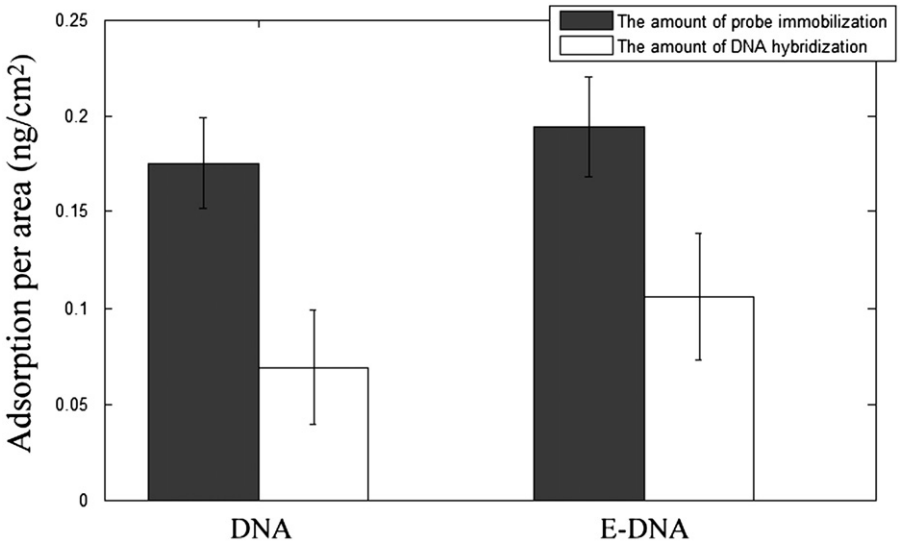

Fig. 4. The SPR responses for two kinds of probe immobilizations (dark columns) and subsequent cDNA hybridizations on two different probe surfaces (bright columns). The concentration of probe and target DNAs were all 5 μM in the SPR measurements.

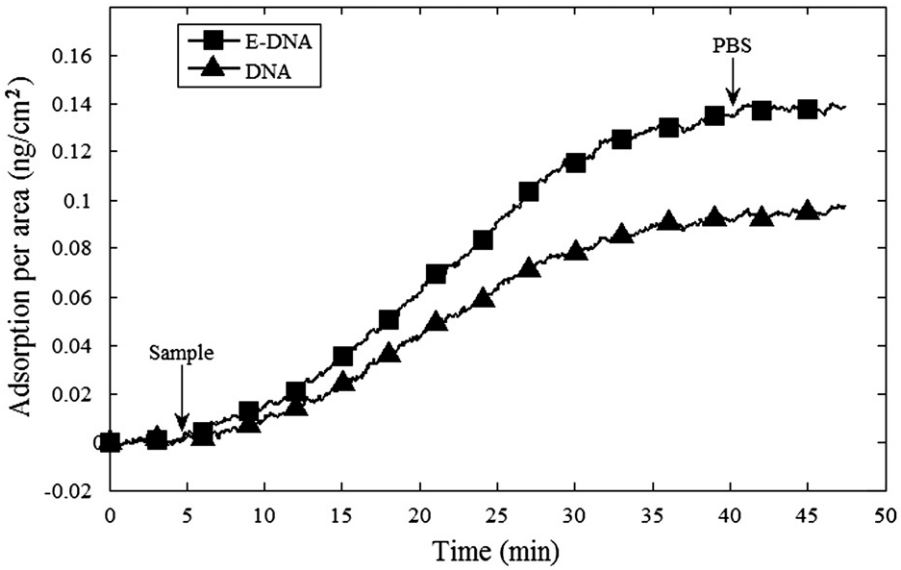

Fig. 5. Two representative SPR sensorgrams for the cDNAs hybridized to the E-DNA and DNA probe surface, respectively. In the viewpoint of kinetics, the E-DNA probe can provide better efficiency in hybridization with cDNA than the DNA probe.

surface through the amine coupling procedure. The SPR experimental results demonstrated that the HE value for sensor surface prepared with conventional DNA probes via amine coupling approach was 39.33%. This consequence indicated that using amine coupling with immobilized DNA probes on the OEG and COOH-OEG mixed SAM could even provide adequate surface conditions for efficient hybridization with target DNA. Fig. 5 shows two SPR sensorgrams for cDNAs hybridized with the sensor surfaces with E-DNA and DNA probes. The SPR response for hybridization of E-DNA/cDNA was obviously higher than that for DNA/cDNA duplex in terms of amount. We used Eqs.(4–6) to calculate kinetic parameters from the two SPR sensorgrams showed in the Fig. 5. We obtained $k_a = 0.17 \times 10^3\ M^{-1}s^{-1}$, $k_d = 1.64 \times 10^{-5}\ s^{-1}$, $K_A = 10.37 \times 10^6\ M^{-1}$ for the E-DNA probe surface, and derived $k_a = 0.12 \times 10^3\ M^{-1}s^{-1}$, $k_d = 5.81 \times 10^{-5}\ s^{-1}$, $K_A = 2.06 \times 10^6\ M^{-1}$ for the DNA probe surface. The association rate constant for the reaction of E-DNA/DNA duplex is slightly higher than that for the reaction of DNA/DNA duplex. However, for the k_d value showing the dissociation rate constant, the electrostatic repulsion between DNA probes and DNA targets may be the reason for having a more rapid dissociation process. The data demonstrate E-DNA probe surface has a better binding affinity with cDNAs than the DNA probe surface. As a result, the amounts of the probe's immobilization and hybridization efficiency allow for the E-DNA probe to exhibit higher measurement sensitivity, which was demonstrated by using the SPRi.

4. Conclusions

To our knowledge, this is the first report that combines FET and SPR measuring techniques in studying the hybridization of DNA and DNA analog. In the experimental practice for DNA detection, the SiNW-FET nanosensor with E-DNA probes provided a larger response and a higher S/N ratio which was an order of magnitude higher compared to that with regular DNA probes. We suggest that this consequence is contributed to the electrically neutral property of E-DNA to avoid the interference of charge noise from

recognition elements. The E-DNA probe also could produce an apparently distinction in the responses to specific binding of cDNA and nonspecific adsorption of ncDNA in the FET measurements. To further demonstrate the effectiveness of using the E-DNA probe in the FET sensor, the SPRi instrument was used to perform quantitative measurements in order to study the immobilized amount of DNA probe and the hybridization efficiency on sensor surfaces. The data from SPRi experiments confirmed that the surface density of immobilized probe and hybridization efficiency were the two main factors causing E-DNA probe could provide higher measurement sensitivity. In addition to the development of easy synthesis of the neutral E-DNA, in this investigation, we demonstrated that E-DNA as probe has superior detection sensitivity for electrical based transducer biosensor. The superiority of E-DNA probe over DNA probe is the results of higher immobilization amount of DNA probe and higher hybridization efficiency, evidenced by surface plasmon resonance studies. The E-DNA is a promising sensor probe for future developments of a high-sensitivity DNA sensor and bio-relevant applications, especially for the electrical-based sensor, like the SiNW-FET nanosensor.

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