J|A|C|S
COMMUNICATIONS

Published on Web 09/29/2006

Label-Free and Reagentless Aptamer-Based Sensors for Small Molecules

Maya Zayats,[†] Ye Huang,[†,§] Ron Gill,[†] Chun-an Ma,[§] and Itamar Willner*[,†]

The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, and State Key Laboratory for Green Chemistry Synthesis Technology, College of Chemical Engineering and Materials Science, Zhejiang University of Technology, Hangzhou 310014, China

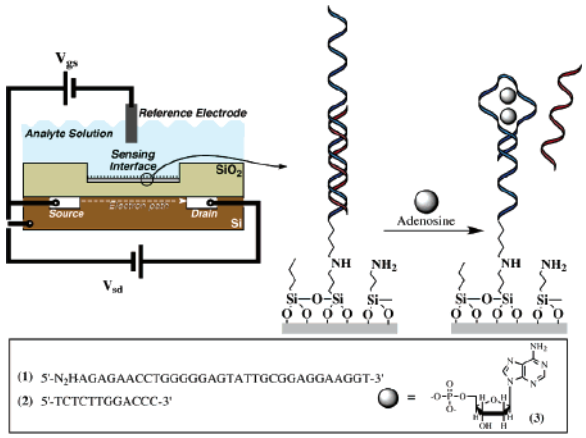
Received July 19, 2006; E-mail: willnea@vms.huji.ac.il

The pioneering studies of Ellington[1] and Gold[2] to select nucleic acids of binding properties toward macromolecules or small molecules (aptamers) established new possibilities to apply the aptamers as the active sensing materials in different detection schemes.[3] Indeed, substantial research efforts are directed to the development of electrical,[4] microgravimetric,[5] or optical[6] aptasensors. For example, a redox-labeled anti-thrombin aptamer that adopts, upon binding of thrombin, a tertiary configuration where the redox-label is electrically contacted with the electrode was used for the amperometric detection of thrombin.[7] Similarly, the self-organization of a redox-labeled aptamer in the presence of the small substrate, cocaine, and the resulting amperometric response of the rearranged nucleic acid were reported to analyze cocaine with a sensitivity limit corresponding to 1×10^{-5} M.[8] Au-nanoparticle-labeled aptamers, and the subsequent catalytic enlargement of the nanoparticles, were used as colorimetric or microgravimetric reporters for the formation of the aptamer−thrombin complex on surfaces.[5] Small molecular weight substrates such as adenosine or cocaine were optically sensed by the deaggregation of Au-nanoparticle clusters cross-linked by the aptamer units.[9] Although, the advances in developing aptasensors are impressive, the search for label-free and reagentless sensors for aptamer−substrate interactions remains a challenge. The substantial alterations of the dielectrical properties of electrode surfaces upon the binding of proteins to nucleic acids suggest that aptasensors sensitive to the dielectrical function could be developed. Indeed, one study reported on the Faradaic impedance analysis of the aptamer/thrombin interaction.[10] As far as we know, there is no demonstration of an analytical paradigm that applies aptamers for the label-free reagentless analysis of small molecules. In the present paper we demonstrate that the small substrate-induced separation of a duplex nucleic acid that includes the aptamer strand, on an ion-selective field-effect transistor (ISFET) or on an electrode, forms a substrate−aptamer complex that can be electrically characterized.

The application of field-effect transistors as electronic transducers for biorecognition events or biocatalytic assays was significantly advanced in the past decade.[11] The alteration of the charge (and as a result, the potential) on the gate of the ISFETs upon the hybridization of the complementary nucleic acid to gate-confined DNA was used for the label-free reagentless detection of DNA.[12] The paradigm for the analysis of a molecular substrate (adenosine) by its aptamer on an ISFET device is depicted in Scheme 1. The Al_2O_3 gate surface was functionalized with 3-aminopropyltriethoxysilane and subsequently modified with glutaric dialdehyde. Then the amine-functionalized nucleic acid **1** that acts as aptamer[13] was immobilized on the gate surface and further hybridized with the short nucleic acid **2**.

Knowing the surface area of the gate and complementary transcondactance measurements on the **1**-functionalized gate[14] indicated a surface coverage of 1.6×10^{-12} mol·cm^{-2}. The addition of adenosine displaces the oligonucleotide **2** and assembles **1** into the hairpin configuration that binds adenosine mono-phosphate (AMP) **3**. The release of **2** changes the charges associated with the gate, and this alters the source-to-drain current. To retain the source-to-drain current to a constant value, the potential V_{gs} is altered to control the potential changes occurring on the gate as a result of the association of **3**. Figure 1, curve a, shows the changes in the ΔV_{gs} values upon analyzing different concentrations of AMP, **3**. As the concentrations of **3** increase the ΔV_{gs} values are higher, and

Scheme 1. Label-Free, Reagentless Aptasensor for Adenosine Using an ISFET Device



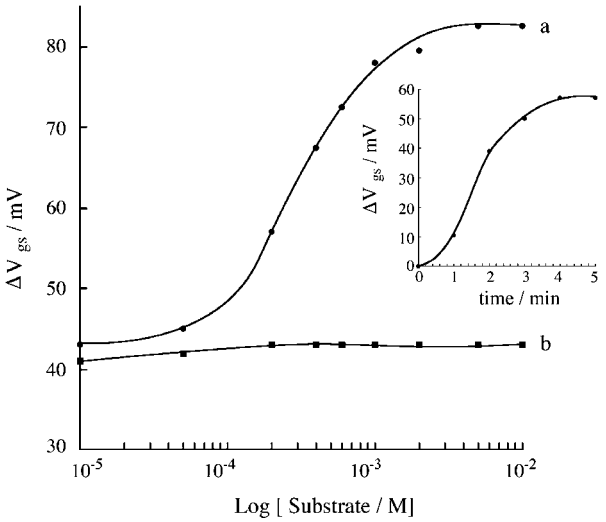


Figure 1. Changes in the gate-to-source voltage upon analyzing different concentrations of (a) adenosine mono-phosphate (AMP) and (b) cytidine mono-phosphate (CMP). For all experiments $V_{sd} = 1$ V and $I_{sd} = 100$ μA. The inset shows the time-dependent response of the ISFET device upon analyzing adenosine, 2×10^{-4} M. Data were recorded in a Tris buffer solution (25 mM) that included NaCl (300 mM); pH = 8.2.

[†] The Hebrew University of Jerusalem.
[§] Zhejiang University of Technology.

10.1021/ja0651456 CCC: $33.50

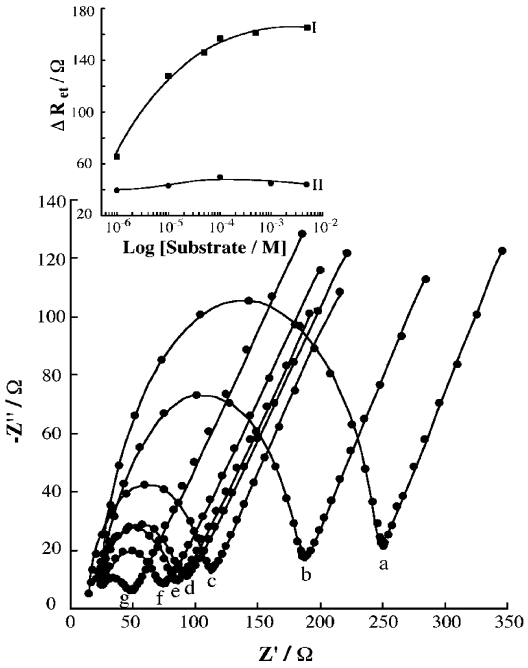


Figure 2. Faradaic impedance spectra (Nyquist plots) corresponding to Au-electrodes (0.13 cm^2) consisting of (a) the monolayer generated by the association of the **1**/**2** duplex to the electrode and after treatment with AMP: (b) 1×10^{-6}, (c) 1×10^{-5}, (d) 1×10^{-4}, (e) 1×10^{-3}, and (f) 1×10^{-2} M. Curve g shows the bare electrode prior to the immobilization of the **1**/**2** duplex. The inset shows the calibration curve corresponding to the changes of the interfacial electron-transfer resistances of the electrode upon analyzing a different concentration of (I) AMP and (II) CMP. The data were recorded in the presence of $[Fe(CN)_6]^{3-/4-}$, 1×10^{-2} M, as redox label, and upon application of the biasing potential 0.17 V, applying 10 mV alternative voltage in the frequency range of 100 mHz to 10 kHz. Data were recorded in a Tris buffer solution (25 mM) that included NaCl (300 mM); pH = 8.2.

the response levels off at a concentration of ca. 1×10^{-3} M. The sensing process is very selective, and the addition of a different concentration of cytidine monophosphate, CMP, does not separate the duplex DNA associated with the gate and thus does not alter the ionization of the gate (±2 mV) (Figure 1, curve b). Similarly, the nucleotides GMP or TMP do not affect the ISFET potential. The sensitivity limit of the aptasensors for analyzing AMP is 5×10^{-5} M, at least 10-fold better than the most effective colorimetric or electrical AMP aptasensors (that apply labels and reagents for the sensing). The response time of the aptasensors corresponds to ca. 4 min (Figure 1, inset). The device could be regenerated by its treatment with **2** and reassembly of the duplex on the gate. Also, the device revealed stability, and upon repeated regeneration of the device, by washing off the bound adenosine with pure water followed by the hybridization with **2**, the signal transduced by the device decreased only by 5% after one week of operation.

Previous studies have revealed that the assembly of nucleic acids on electrodes and the formation of double-stranded DNA on the support can be followed by faradaic impedance spectroscopy, FIS.[15] The negative charge of the single stranded nucleic acid repels the solution-solubilized negatively charged redox label, $Fe(CN)_6^{3-/4-}$, and thus the interfacial electron-transfer resistance increases. The selective binding of the adenosine to the hybridized DNA followed by the duplex dissociation results in the decreasing of the negative charge on the electrode and decreasing of the interfacial electron-transfer resistance at the electrode. Thus, the AMP-induced separation of the duplex DNA consisting of **1** and **2** on an electrode could be followed by FIS. Accordingly, **1** was covalently linked to a dithiobis(succinimidylpropionate) monolayer assembled on an Au electrode. The surface coverage of **1** was estimated to be ca. 4×10^{12} molecules·cm^{-2}. Figure 2 shows the faradaic impedance spectra (Nyquist plots) of the **1**/**2** duplex-functionalized electrode before (curve a) and after treatment of the electrodes with different concentrations of AMP. As the concentration of AMP increases, the interfacial electron resistance decreases, consistent with the removal of the negative charge from the electrode by the removal of **2**. Figure 2 inset (curve I) shows the derived calibration curve. AMP could be analyzed by this method with a sensitivity limit corresponding to 2×10^{-6} M. This sensing procedure reveals, as expected, selectivity, and addition of different concentrations of CMP (or any other nucleotide) have a minute effect on the interfacial electron-transfer resistance of the electrode, ±5 Ω (Figure 2 inset, curve (II).

To summarize, the present study has introduced a method for the selective label-free reagentless analysis of the small molecule adenosine by the separation of an aptamer/nucleic acid duplex associated with ISFET or electrode transducers. The formation of the adenosine/aptamer complex affects the gate potential of the ISFET device and controls the interfacial electrode transfer resistance at the electrode. This allowed the potentiometric or impedimetric readout of the operation of the aptasensors. The impedimetric readout method is ca. 10-fold more sensitive than the ISFET method, but it requires the addition of an external redox-label. The analysis of other low molecular-weight substrates such as cocaine or explosives by analogous aptasensors should be feasible.

Acknowledgment. This work is supported by the Israel Ministry of Science as an Infrastructure Project.

References

(1) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818−822.
(2) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505−510.
(3) Navani, N. K.; Li, Y. *Curr. Opin. Chem. Biol.* **2006**, *10*, 272−281.
(4) (a) Radi, A.-E.; Sánchez, J. L. A.; Baldrich, E.; O'Sullivan, C. K. *J. Am. Chem. Soc.* **2005**, *128*, 117−124. (b) Mir, M.; Vreeke, M.; Katakis, I. *Electrochem. Commun.* **2006**, *8*, 505−511.
(5) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 11768−11769.
(6) (a) Nutiu, R.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 4771−4778. (b) Ho, H. A.; Leclerc, M. *J. Am. Chem. Soc.* **2004**, *126*, 1384−1387.
(7) Xiao, Y.; Lubin, A. A.; Heeger, A. J.; Plaxco, K. W. *Angew. Chem., Int. Ed.* **2005**, *44*, 5456−5459.
(8) Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *Am. Chem. Soc.* **2006**, *128*, 3138−3139.
(9) Liu, J.; Lu, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 90−94.
(10) Cai, H.; Lee, T. M.-H.; Hsing, I.-M. *Sens. Actuators B* **2006**, *114*, 433−437.
(11) (a) Bergveld, P. *Sens. Actuators, B* **2003**, *88*, 1−20. (b) Dzyadevych, S. V.; Soldtakin, A. P.; El'skaya, A. V.; Martelet, C.; Jaffrezic-Renault, N. *Anal. Chim. Acta* **2006**, *568*, 248−258.
(12) (a) Shin, J.-K.; Kim, D.-S.; Park, H.-J.; Lim, G. *Electroanalysis* **2004**, *16*, 1912−1918. (b) Uslu, F.; Ingebrandt, S.; Mayer, D.; Böcker-Meffert, S.; Odenthal, M.; Offenhäusser, A. *Biosens. Bioelectron.* **2004**, *19*, 1723−1731.
(13) Lin, C. H.; Patel, D. J. *Chem. Biol.* **1997**, *4*, 817−832.
(14) Kharitonov, A. B.; Wasserman, J.; Katz, E.; Willner, I. *J. Phys. Chem. B* **2001**, *105*, 4205−4213.
(15) (a) Katz, E.; Willner, I. *Electroanalysis* **2003**, *15*, 913−947. (b) Patolsky, F.; Lichtenstein, A.; Kotler, M.; Willner, I. *Angew. Chem., Int. Ed.* **2001**, *40*, 2261−2265. (c) Alfonta, L.; Willner, I. *Chem. Commun.* **2001**, 1492−1493.

JA0651456