Spermine-Induced Hybridization and Charge Inversion at the Diffuse Layer of a DNA-FET

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Detection of DNA hybridization events by using field-effect transducers is limited by the electrolyte content of the medium. So far, DNA was thought to hybridize only in solutions with concentrated electrolytes. In these media, the interface between the transducer gate and the solution is reduced to a thin layer in close contact with the surface, and DNA is poorly detected. In the present work, this limitation is overcome by using spermine as screening polycation. Hybridization assays with polycation concentrations as low as 10 μM are reported. This ensures that hybridization takes place at the diffuse layer of the interface. The reported results suggest a charge-inversion mechanism induced by spermine. A target sequence is real-time label-free detected in the range from 10 to 500 nM.

Introduction

Hybridization of complementary oligonucleotide molecules has been extensively used in a wide variety of DNA analysis systems, from customized DNA sensors[1] to high throughput DNA arrays.[2,3] In these approaches, synthetic oligonucleotides are used for recognizing a specific sequence in a complex background. Temperature, ionic strength, and the presence of chaotropic salts among others are the parameters to be optimized in order to obtain sequence-specific hybrids. Electrolyte concentration plays an important role in this process. Phosphate groups of oligonucleotides are negatively charged at physiological pH values. Thus, a certain amount of cations is required in order to overcome the repulsion between complementary strands and enable the recognition of complementary bases.

Detection of oligonucleotides with field-effect transistors (FETs) is one of the most challenging approaches for DNA analysis. Besides label-free detection capability, electronic readout of these devices enables a compact integration with the instrumentation, a key issue in the fabrication of miniaturized multiplexed detection systems. Moreover, these transducers are fabricated with well-known processes in the microelectronics industry, an important factor for mass-scale production.

Thin-film field-effect sensors[4–7] and nanostructured FETs[8–11] have been proposed as suitable transducers for this kind of measurements. These devices are able to detect changes in charge distribution at the interface between the gate material and an electrolytic solution, the so-called electrochemical double layer. Because phosphate groups of DNA molecules are negatively charged at physiological pH values, inclusion of oligonucleotides in this region driven by sequence-specific hybridization events should be detected as a change in the channel current or the flat band voltage of the FET.

Despite its great appeal, this simple concept suffers from two major drawbacks. First, DNA hybridization only takes place in solutions with a high ion concentration. In these media, the interface is reduced to a thin layer in close contact with the surface. When the oligonucleotides are covalently bonded to the surface, most of their charge is located at a distance from the gate surface, and hence, the FET may not detect it. Second, nearly all the negative charges present in double-stranded DNA are compensated by adsorbed counterions. As a consequence, only a small fraction of the original negative charge remains upon hybridization. In the past, different approaches have been used in order to circumvent this issue. In some works, the reporter oligonucleotides are adsorbed at the gate surface in order to confine the charge at the interface between the sensor and the solution.[8] In other works, noncharged peptide nucleic acids are used to recognize specific oligonucleotides in low-ionic-content solutions.[5] Here, this limitation is overcome by using a low-content spermine ($NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, Sp^{4+})-based buffer for DNA hybridization. A low-ionic-content solution gives rise to a more extended diffusion layer and hence enables detection of hybridization events with covalently bound oligonucleotides. Biogenic polyamines are well-known by structural biologists because of their ability to aggregate and condense DNA molecules.[12–14] It has been proposed that these phenomena are mainly driven by an efficient neutralization of the negative charges of DNA, even at low polyamine concentrations. Thus, it seems reasonable to think that spermine will be able to shield the negative charge of oligonucleotides and allow hybridization in very dilute media. Furthermore, it has been suggested that these molecules can overscreen DNA and cause a charge inversion of the molecule.[15] This phenomenon occurs when charged particles (also called macro-ions) strongly bind so many counterions in a water solution that their net charge changes sign. Charge inversion is possible for a variety of macro-ions, including the charged surface of certain solids, charged lipid membranes, DNA, or Actin. Multivalent ions, small colloid particles, charged micelles, and short or long polyelectrolytes can play the role of multivalent counterions.[16–23]

In the present work, fluorescence and potentiometric experiments were performed to demonstrate that spermine enables sequence-specific hybridization events between complementary oligonucleotide sequences in the absence of any other supporting electrolyte. Buffers containing spermine concentrations down to 1 μM were assayed to determine the minimum concentration that allows DNA hybridization. Buffers containing an equivalent concentration of monovalent sodium ions were also assayed for

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TABLE 1: DNA Sequences from 5′ to 3′

name[a]	5′ mod.[b]	sequence
C	amino	AGG TCT AGT GCA
NC	amino	CTA TGT CAG CAC
target	Cy3	TGC ACT AGA CCT

[a] C, complementary sequence; NC, non-complementary sequence. [b] Modifications at 5′ end. Cy3 modification only in hybridization assays.

comparison purposes. The reported results and discussion try to give new insight in both the hybridization of DNA and its detection with FETs.

Experimental Methods

DNA Immobilization. Oligonucleotides were attached to both glass slides and silicon nitride FETs with the following procedure. First, both surfaces were activated with 3-glycidyloxypropyltrimethoxysilane (GOPS). The substrates were cleaned in a 2.5% KOH solution for 30 min. After being rinsed with water and dried with nitrogen, GOPS molecules were gas-phase deposited to the receiving substrates by placing them overnight in a reaction chamber containing a GOPS-saturated atmosphere. Solutions containing 25 μM amino-modified oligonucleotides in deionized water were spotted on the resulting epoxy-modified surfaces at prearranged sites and left in a humid chamber at 4 °C overnight. Table 1 shows oligonucleotide sequences. Amino-ended oligonuclotides react with the epoxy moiety to yield stable amine bonds.[24] The resulting DNA-modified surfaces were thoroughly rinsed with deionized water and dried with nitrogen.

Hybridization Assays. Hybridization buffers were prepared by weighting and dissolving the proper amount of spermine dihydrate or NaOH and adjusting the pH of the resulting solutions to 5 with diluted acetic acid.

Prior to use in hybridization experiments, both glass and silicion nitride substrates were prehybridized by immersing them in a solution containing 1% bovine serum albumin and 0.1% sodium dodecyl sulfate, at 37 °C during 45 min. This treatment blocks unreacted sites at the receiving surfaces and prevents unspecific adsorption of the target probe.

Fluorescence Assays. Solutions containing the Cy3-labeled target probe were spotted at complementary (C) or noncomplementary (NC) modified regions of glass slides. Then, the slides were placed in a humid chamber at room temperature for 3 h. Past this time, they were immersed in deionized water for 1−2 s and immediately dried with a stream of nitrogen. The remaining fluorescent signal was detected with a scan array device. Fluorescence intensity at each spot was evaluated with image-treatment software.

Potentiomentric Assays. A FET modified with a C probe and a FET modified with a NC probe were immersed in a stirred solution containing hybridization buffer. Differential measurements were carried out in order to cancel out second-order effects not related with the hybridization events. Upon drift stabilization, the target probe was added to the desired final concentration. The FETs differential response was monitored for 180 min. Although the analytical signal did not saturate within this incubation time, this 180 min incubation was chosen in order to limit possible interference of the FET drift.

Results and Discussion

The capability of spermine to induce DNA hybridization in diluted media was tested by performing a fluorescence assay. C and NC probes to a target sequence were covalently immobilized on a glass slide by following a prearranged pattern. The resulting DNA-modified areas were queried with a 500 nM pH 5 solution of a fluorescent target probe containing 4 μM, 40 μM, 400 μM, or 4 mM Na^+ (Figure 1a). Alternatively, a background solution containing 1 μM, 10 μM, 100 μM, or 1 mM Sp^{4+} was used as hybridization buffer (Figure 1b). As expected, no hybridization signal is obtained when using Na^+ as the screening counterion. Only in a 4 mM Na^+ background, a slight increase in the fluorescence signal reveals the presence of the target probe at the glass surface. Conversely, spermine can induce sequence-specific hybridization events at concentrations ranging from 10 μM to 1 mM. Moreover, the fluorescence signal is almost independent of the multivalent ion concentration in this range. These results suggest a different hybridization mechanism for spermine-based buffers compared with those containing monovalent cations.

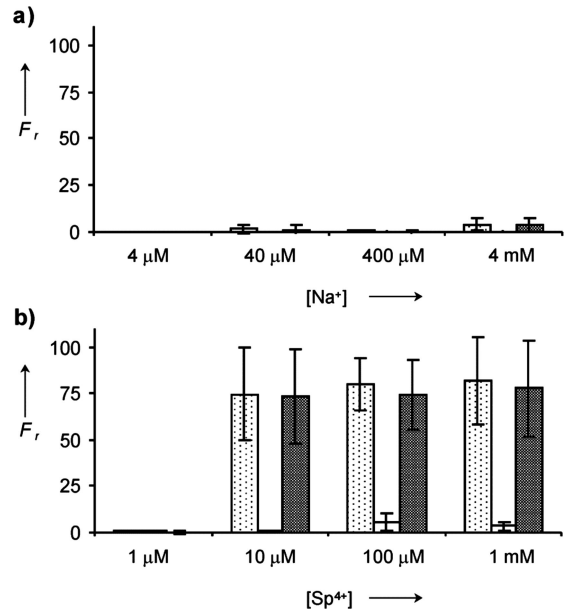

Figure 1. Relative fluorescence (F_r) of hybridization experiments performed in buffered solutions containing different concentrations of (a) Na^+ and (b) Sp^{4+}. A 500 nM solution of a Cy3-labeled target probe was allowed to interact with a silica surface modified with a C probe (dotted bars) or a NC probe (empty bars) for 3 h. The specific signal was obtained by subtracting the former ones (shaded bars). Error bars are the standard deviation ($n = 3$).

Potentiometric detection of spermine-induced hybridization events was studied with silicon nitride FETs. A C modified FET and a NC modified FET were immersed in hybridization buffers containing different concentrations of spermine. The sensor reponses are shown in Figure 2. Almost no specific hybridization signal is present in 1 μM spermine solutions. When 10 μM spermine is used as the background electrolyte, the output voltage shifts toward negative values, which indicates that an excess positive charge is accumulating at the interface. This result would be consistent with a charge-inversion event produced by spermine ions adsorbed to the DNA double strand. For more concentrated spermine solutions, the potential varies in the opposite direction. Because macro-ion overcharging is not reversed when increasing the multivalent counterion concentration and fluorescence experiments confirm similar levels of surface-bound target probe, a different transduction mechanism must be responsible for this inverse response behavior.

Figure 3 shows a scheme of the interface between the gate insulator and the solution. This region can be modeled as two series capacitors.[25] The first capacitor (C_H) is formed by

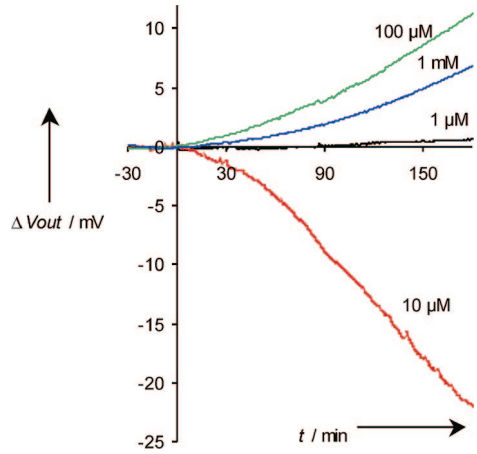

Figure 2. Variation of the output differential voltage (Vout) with time for hybridization experiments performed in buffered solutions containing different spermine concentrations. Black, 1 μM; red, 10 μM; green, 100 μM; blue, 1 mM. The curves are the result of subtracting the response of a NC modified FET from the one obtained with a C modified FET. The target probe was added at $t = 0$.

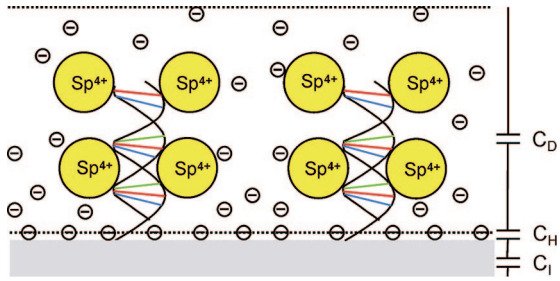

Figure 3. Scheme of the interface between the FET gate and the electrolytic solution. C_I is the insulator capacitor, C_H is the Helmholtz plane capacitor, and C_D is the diffuse layer capacitor.

ionizable groups at the insulator surface and adsorbed ions at the so-called Helmholtz plane. The second capacitor (C_D) is built up by a cloud of loosely bound ions that extent from the Helmholtz plane to the bulk of the solution. This section is known as the diffuse layer. In solutions with more concentrated electrolytes, these ions are tightly compressed against the Helmholtz plane, and the diffuse layer vanishes. In the frame of this simplified interface model, an explanation for the results shown in Figure 2 is presented below.

In the system under study, DNA hybridization brings an excess positive charge in the form of spermine molecules next to the insulator surface. This fixed charge attracts mobile acetate anions that form a new diffuse layer around DNA molecules. Accumulation of acetate disturbs the acid−base equilibrium in this region, capturing protons from acidic moieties in the vicinity of the insulator surface. Thus, two contributions to the interface potential of the FET must be taken into account. On the one hand, overcharged DNA molecules contribute with positive charges at the FET diffuse layer capacitor. On the other hand, acetate ions make the FET surface more negative by locally changing the pH. At low spermine/acetate concentrations, double-stranded DNA is poorly screened by anions. Under this regime, excess positive charge at the FET diffuse layer dominates the output potential (Figure 2, red curve). When increasing electrolyte concentration, more acetate ions are brought next to the insulator surface, and the diffuse layer shrinks. Now, local pH change rules the FET response (Figure 2, green curve). In solutions with even higher electrolyte content, acetate ions driven by hybridization events are closer to

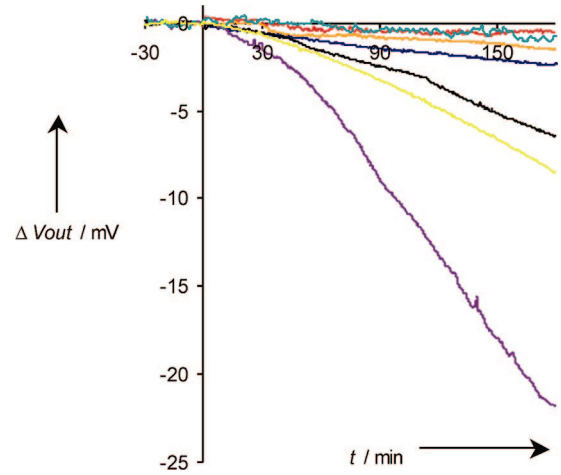

Figure 4. Variation of the output differential voltage (Vout) with time for different target probe concentrations. Red, 10 nM; orange, 100 nM; green, 200 nM; blue, 300 nM; black, 400 nM; yellow, 450nM; violet, 500 nM.

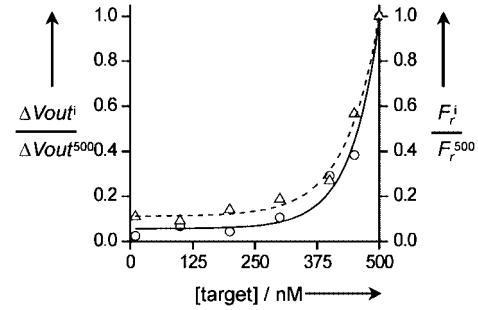

Figure 5. Relative variation of hybridization signals with target concentration after 3 h. Circles, potentiometric detection; triangles, optical detection. ΔVout^i is the variation of the output differential voltage for a certain target concentration, and ΔVout^{500} is the variation of the output potential for a 500 nM target solution. F_r is the relative fluorescence. Fitting both curves to an exponential plot yields the following values: potentiometric, $y = 2 \times 10^{-4} \exp(-58x) + 0.06$ and $r = 0.99$ (solid line); optical, $y = 5 \times 10^{-4} \exp(-68x) + 0.12$ and $r = 0.99$ (dashed line).

overcharged DNA molecules. As a consequence, the local pH change takes place further from the gate insulator and has a lesser impact on the interface potential (Figure 2, blue curve).

Detection of oligonucleotides in 10 μM spermine solutions was further studied for analytical purposes. Potentiometric detection experiments were performed as above, but the target probe concentration was varied in the range from 10 to 500 nM (Figure 4). All these experiments point out that an excess positive charge is accumulating at the interface, in accordance with the proposed charge-inversion event. A fluorescence assay was also performed. Figure 5 compares the results obtained with both the electronic detection system and the optical detection system. An excellent agreement was found between both methods. This confirms that oligonucleotides are able to hybridize with their complementary surface-immobilized strands in low ion content solutions containing spermine.

Conclusions

So far, it was believed that DNA could only hybridize in solutions with a high electrolyte content. We have demonstrated that DNA does hybridize in solutions with ion concentrations several orders of magnitude lower than those commonly used in DNA-detection protocols if spermine is used as screening

counterion. This new protocol enables detecting sequence-specific hybridization events taking place at the diffuse layer of the interface between a FET insulator and a solution. The FET responds to target concentrations in the range from 10 to 500 nM. Combination of this procedure with nanostructured FETs is a promising approach in oncoming electronic DNA-analysis systems.

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Supporting Information Available: Details about field-effect transistor fabrication and instrumentation (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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