Tb^{3+}-enhanced Potentiometric Detection of Single Nucleotide Polymorphism by Field Effect Transistors

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The use of Tb^{3+} as a center ion and media to introduce more exogenously negative charges onto double-stranded DNA (dsDNA), can greatly enhance potentiometric signals produced by the shift of gate voltage of field effect transisitor (FET). Furthermore, different affinities of Tb^{3+} to normal and mismatched dsDNAs amplified the signal difference between their detections, which can improve the discrimination of single nucleotide polymorphism.

Recently, field effect transistors (FETs) have been widely used as charge sensors to detect DNA hybridization. Since DNA molecules are negatively charged, owing to the inherent DNA charges from phosphate ions, hybridization of targets will increase the amount of negative charges on the gate surface; this change in the charge density is transduced into potentiometric signals by the field effect.[1–5] On the basis of this principle, if exogenous charges are introduced onto double-stranded DNA (dsDNA), then it can enhance potentiometric signals produced by the gate voltage (V_g) shift, and if different amounts of exogenous charges are introduced onto normal and mismatched dsDNA, then it can amplify the discrimination of electrical signals between normal and mismatched target detections. In this work, Tb^{3+} was used as a bifunctional cross linking agent that can be bonded to dsDNA immobilized on the gate surface and that also can be coordinated by phosphate groups from phosphate buffered saline (PBS). When the sum of negative charges from phosphate ligands is more than that of the Tb^{3+} positive charges, then more exogenously negative charges can be introduced onto the dsDNA, resulting in enhancement of the electrical signal. In addition, if the different affinities of Tb^{3+} to normal and mismatched dsDNAs appear, different amounts of charges are added to normal and mismatched dsDNAs that amplify the signal difference between normal and mismatched detections. On the other hand, the different affinities of Tb^{3+} to normal and mismatched dsDNA can be investigated and confirmed by fluorescence spectra.[6]

The surface of the FET,[7] of which gate is 1 mm (width) × 15 μm (length) in size, was cleaned within an SPM (H_2SO_4: H_2O_2 = 4:1) at 150 °C for 10 min, and then silanized in tolune containing 3-sulfanylpropyltrimethoxysilane (MPTMS, 10 μL/g) at 60 °C for 7 min. The sulfanyl-terminated SiO_2 surface was soaked in a 2,2′-dipyridyl disulfide (DPDS, 1 mg/mL) solution at room temperature for 2 h. After that, the FET chip was kept in the oligonucleotide probe (HS-3′-ACGAACATAGCCCGCCTTAC-5′, 1 μM) solution at room temperature for 24 h. Then, the FET with immobilized oligonucleotide probes was kept in the target solution (complementary: 5′-TGCTTGTATCGGGCGGAATG-3′ and mismatched: 5′-TGCTTGTATCGTGCGGAATG-3′, 1 μM) at room temperature for 1 h. After hybridization, the FET was soaked in Tb^{3+} solution with a concentration of 1×10^{-4} M at room temperature for 2 h ($TbCl_3 \cdot 6H_2O$, 99.95%). The FET was placed in a PBS solution (300 μL) with a reference electrode of Ag/AgCl. Then, the electrical characteristics of the FETs, such as the drain current–gate voltage (I_d–V_g), were measured at 25 °C with a semiconductor parameter analyzer (4155C Agilent). The fluorescence spectra were measured with a Jasco FP-6500 spectrofluorometer at room temperature.

Figure 1 shows that specific binding of negatively charged DNA molecules at the gate surface can be detected as a positive shift of V_g in the I_d–V_g characteristics of an n-type FET. The FETs were first cleaned, silanized, and given a DPDS treatment. Then a measurement was made that yielded the DPDS data line. Then, the oligonucleotide probes were immobilized on the gate surface, and another measurement was made that yielded the probe data line. Comparison of the two measurements showed that the V_g was shifted in a positive direction along the V_g axis by 60 mV. This shift was the result of negative charges induced by immobilization of the oligonucleotide probes at the gate surface. When the complementary target DNA was introduced onto the gate surface and hybridized with oligonucleotide probes, the V_g was shifted in the positive direction by 56 mV, as a result of hybridization introducing an increase in the negative charges of the target DNA. After hybridization, Tb^{3+} was introduced onto the gate surface, and the V_g still shifted in a positive direction by 95 mV, which means that after the Tb^{3+} was bonded to the dsDNA, the negative charges were increased. Therefore, Tb^{3+} must form a complex with phosphate ligands from the PBS solution, and the total charges of this complex must be negative, resulting in both the accumulation of more negative charges on the gate surface and the right V_g shift.

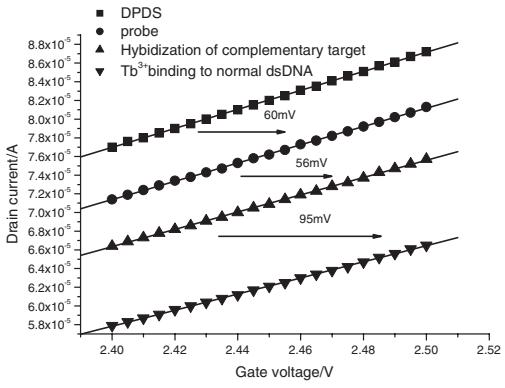

Figure 1. V_g shifts after immobilization of oligonucleotide probes, hybridization of complementary target DNA, and specific binding of Tb^{3+}.

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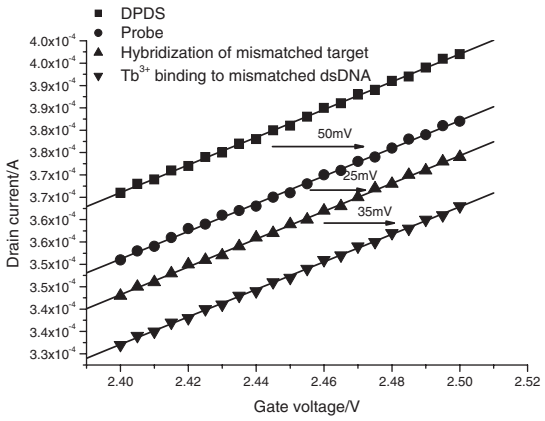

Figure 2. V_g shifts after immobilization of oligonucleotide probes, hybridization of 1-mismatch target DNA, and specific binding of Tb^{3+}.

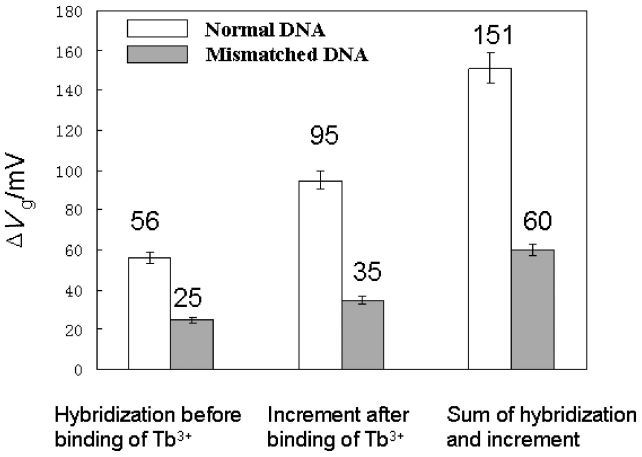

Figure 3. Comparison of hybridization detection of normal and mismatched DNA before and after binding of Tb^{3+}.

When one base mismatched target DNA was introduced onto the gate surface and hybridized with the probes, V_g was shifted in the positive direction by 25 mV (Figure 2). After mismatched target hybridization, Tb^{3+} was introduced onto the gate surface, and the V_g was shifted positively by 35 mV.

The results of the detections for the normal and mismatched DNA targets are shown in Figure 3. Before the binding of Tb^{3+}, the V_g shifts of normal and mismatched DNA were 56 and 25 mV, respectively, with a difference of 31 mV. After the binding of Tb^{3+}, the V_g shifts of normal and mismatched DNA were 95 and 35 mV, respectively, with a difference of 60 mV. Therefore, the electrical signals of detections for both normal and mismatched DNA were enhanced. From these results, Tb^{3+} ions showed higher affinity toward normal dsDNA and also exhibited much weaker affinity toward mismatched dsDNA, which resulted in both the different amounts of charge addition onto the normal and mismatched dsDNAs attached to the gate surface and the different V_g shifts. For the combined hybridization before the binding of Tb^{3+} and the increment after the binding of Tb^{3+}, the V_g shifts for both normal and mismatched dsDNA

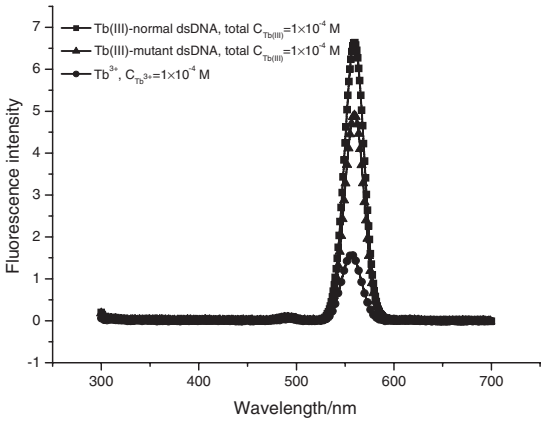

Figure 4. Emission spectra ($\lambda_{ex} = 280$ nm) for complexes Tb^{III}–normal dsDNA, Tb^{III}–mismatched dsDNA, and Tb^{3+}.

were 151 and 60 mV, respectively. Thus, the detections have electrical signals stronger than only the hybridization signals. Furthermore, the signal difference between the normal and mismatched detections is 91 mV, which is almost three times more than the 31 mV measured after only hybridization. Thus, the significant enhancements of the electrical signal and the amplification of signal difference between normal and mismatched detections greatly improve discrimination of single nucleotide polymorphism.

As shown in Figure 4, the fluorescence intensity of Tb^{III}–normal dsDNA is stronger than that of Tb^{III}–mismatched dsDNA, which suggests that a greater amount of Tb^{3+} was bonded to normal dsDNA as compared with mismatched dsDNA considering the strong enhancement of fluorescence intensity of Tb^{3+} binding to dsDNA[6] and the present conditions.[8] Therefore, the fluorescence results greatly support the mechanism of the enhancement of FET signals and discrimination amplification.

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References and Notes

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8 Tb^{3+} was added with the same concentration of 1×10^{-4} M to normal and mismatched dsDNA solutions (1 μM).

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