DNA Mismatch Detection by Metal Ion Enhanced Impedance Analysis

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Conventional diagnoses of genetic mutation and disease depend on the analysis of DNA sequences. However, mismatches in the DNA sequences are difficult to detect using traditional sequencing. Doping metal ions, such as nickel, into the short DNA (28 mer $\sim$ 30 mer), markedly reduces its electrical resistance, which is measured by electrical impedance analysis. The change in resistance that is caused by a mismatched base-pair in short DNA can also be monitored by this approach. In this study, the resistance increased exponentially with the number of mismatched base-pairs. Accordingly, an intuitive and direct method for evaluating the number of DNA mismatches can possibly be developed.

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I. INTRODUCTION

Numerous diseases are related to abnormal protein functions, which are attributed to mutations of the corresponding DNA sequences. Therefore, an efficient approach for detecting abnormal DNA sequences is required for clinical diagnosis. Several methods have been developed to identify DNA sequences, including optical [1], micro-gravimetrical [2], and electrochemical methods [3, 4]. They are all based on the detection of DNA mismatches. In particular, the electrochemical detection of DNA mismatches has great potential in point-of-care testing (POCT), because of its high sensitivity, high selectivity, and low cost [5]. For example, cyclic voltammetry (CV) is adopted to detect a mismatch by labeling DNA sequences with redox intercalators [6]. DNA mismatch has also been detected by electrochemical impedance spectroscopy (EIS) with a native DNA-modified gold electrode [4]. However, only a single mismatch at the distal end of DNA can be detected using this strategy [4], which fact is attributable to the poor conductivity of native DNA.

To increase the conductivity of DNA, divalent metal ions (Zn^{2+}, Ni^{2+}, and Co^{2+}) were used to make it metallic, forming M-DNA at pH $>$ 8.5 [7–11]. In M-DNA, divalent metal ions replace the imino protons of the base pairs, forming a stable tetrahedral geometry [12, 13]. As a result, metallization dramatically increases the conductivity of DNA [7, 12]. Our

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previous studies have suggested that the conducting mechanism of M-DNA is the hopping of electrons by the $\pi - \pi$ stacking of DNA base pairs [14].

DNA can be covalently bonded to the surface of a gold electrode by a thiol group, forming a self-assembly monolayer (SAM). The DNA SAMs completely cover the gold surface and form a barrier to penetration by redox probes [10, 11, 14]. According to the proposed conducting mechanism of M-DNA, not only can a single mismatch be identified, but also the number of mismatches in DNA can be determined by measuring the DNA conductivity following metallization using electrochemical impedance spectroscopy (EIS). In this investigation, this concept is demonstrated by metallizing DNA with $Ni^{2+}$to form nickel-chelated DNA (Ni-DNA) for EIS analysis.

II. MATERIALS AND METHODS

TABLE I: Samples of custom designed Ni-DNA sequences used in this study.

Symbol	Oligonucleotide sequence
P-fc	5′ TGTGTGTGTGTGTGTGTGTGTGTGTGTG 3′-SH
	ACACACACACACACACACACACACACAC
P-GT	5′ TGTGTGTGTGTGT**G**TGTGTGTGTGTGTG 3′-SH
	ACACACACACACA**T**ACACACACACACAC
P-3GT	5′ TGTGTGT**G**TGTGTGT**G**TGTGTGT**G**TGTGTG 3′-SH
	ACACACA**T**ACACACA**T**ACACACA**T**ACACAC
P-GA	5′ TGTGTGTGTGTGT**G**TGTGTGTGTGTGTG 3′-SH
	ACACACACACACA**A**ACACACACACACAC
R-fc	SH-5′ ACGTTGTAAAACGACGGCCAGTGAATTG 3′
	TGCAACATTTTGCTGCCGGTCACTTAAC
R-2AC	SH-5′ ACGTTGTAA**AA**CGACGGCCAGTGAATTG 3′
	TGCAACATT**CC**GCTGCCGGTCACTTAAC

II-1. Materials

All oligodeoxyribonucleotides with various sequences and thiol groups at their 3′ or 5′ ends that are covalently bonded to a gold surface (TABLE I), were purchased from Bio Basic Inc. (Markham, Ontario, Canada). They were purified by polyacrylamide gel electrophoresis (PAGE) prior to use. Nickel chloride ($NiCl_2$) and other chemicals were purchased from Sigma (St. Louis, MO).

II-2. Oligo-DNA sequence design and synthesis

Special DNA oligomer sequences were designed and synthesized to prevent self-priming during DNA conducting studies. Double-stranded sequences with a single, double or triple base(s) mutation in one of their complementary strands, causing mismatch(es), including G/A, A/C, and G/T, were custom-synthesized. Based on the stability ranking of the base pairs [15],

G-C > A-T > G-T > G-G > T-T ≈ G-A > T-C > A-C > A-A > C-C,

the melting temperature decreases by 1∼4 °C for each single G-T, G-A, or A-C mismatch, and 7∼9 °C for one C-C mismatch [16]. Therefore, the G-T base pair, which is the most stable base pair in the mismatched duplexes, was employed to form the triply mismatched Ni-DNA.

II-3. Preparation of DNA-coated gold electrode

In this study, electrochemical analysis was adopted to detect mismatches in DNA sequences. First, DNA molecules may be immobilized on an electrode surface (to form DNA SAMs). However, according to Willner's study, the length of the DNA considerably affects the surface packing density [17]. The packing density declines as the length of DNA increases. In this work, the surface coverage declined from 95% to 42% as the length of DNA increased from 30 mer to 40 mer (data not shown). The loose DNA SAM results in the direct diffusion of the redox probe ($Fe(CN)_6^{-3}$) into the electrode surface, producing a leakage current. The shorter DNAs assume a primarily extended configuration, forming dense SAMs, while the longer ones adopt a random-coil-like configuration, forming loose SAMs [17]. Therefore, the proper length of DNA must be determined by considering the packing ratio of SAMs. In this study, the 28 mer and 30 mer lengths of DNA were adopted.

A gold electrode disk with a diameter of 2 mm (CH101, CH Instruments, Austin, U.S.A.) was polished using alumina powder (size 1 ∼ 0.05 μm; Chun Kuang Technology Co., Ltd., Taiwan) to yield a smooth surface, which was further electropolished by potential cycling scanning (−0.2 V to 1.5 V) in 0.5 M H_2SO_4 solution at a voltage scan rate of 100 mV/s [18]. Duplex DNA probes with thiol groups at their 3′ (poly-TG) or 5′ (random sequences) ends were formed using hybridization, in which the complimentary primers were heated to 95 °C for 5 minutes and then annealed to room temperature in a solution of 0.1 M phosphate-buffered saline (PBS; pH = 7.0). These probes were then covalently bonded to a gold electrode via gold-thiol linkages. To form a DNA SAM layer, a 2 μM DNA solution was deposited on the previously cleaned electrode surface, and capped tightly to maintain moist conditions and prevent buffer evaporation. After eight hours of DNA-coating, the modified gold electrode was immersed in 0.1 M PBS for 10 min and dipped in distilled water twice to remove the physisorbed molecules. The DNA on the gold surface was converted to Ni-DNA by immersing the electrode in 20 mM Tris-HCl and 5 mM $NiCl_2$ at pH 9.0 for 8 hours, and then washing it with a 20 mM Tris-HCl buffer twice to remove excess Ni^{2+}. Table I presents all of the Ni-DNA sequences herein with various mismatches.

II-4. Electrochemical analysis

All electrochemical measurements were made using a potentiostat/galvanostat (Model 273A, EG&G, Gaithersburg, MD) and a frequency generator (Model 1025, EG&G, Gaithersburg, MD) in an electrolyte that comprised 100 mM KNO_3 and 5 mM $K_3[Fe(CN)_6]$ (as a redox probe). They were recorded using a personnel computer for later analysis. Platinum and Ag/AgCl were used as the counter and reference electrodes, respectively. Various Ni-DNA-modified gold electrodes were used as working electrodes. AC impedances were measured using a 5 mV sinusoidal driving voltage over a frequency range from 100 kHz to 0.01 Hz, and at a DC bias of 220 mV relative to the Ag/AgCl reference electrode.

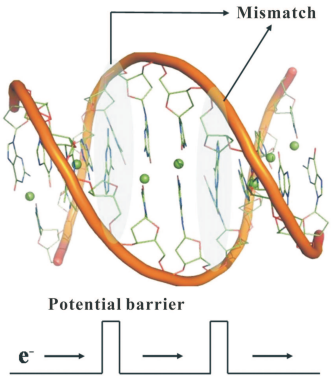

FIG. 1: A schematic illustration of the mismatch-induced potential barrier in Ni-DNA. (The sphere is Ni ions).

III. RESULTS AND DISCUSSION

III-1. Detecting mismatched Ni-DNA by measuring AC impedance.

Based on the proposed mechanism of conduction of Ni-DNA [11, 14], a method for obtaining an electrochemical impedance spectrum that was based on Ni-DNA was utilized to diagnose mismatching. As presented in Fig. 1, when a single mismatch occurs in the Ni-DNA, $\pi - \pi$ stacking is distorted [5, 6], serving as a potential barrier to the transport of electrons; the conducting electrons then require more energy to overcome or tunnel through the potential barrier. Accordingly, the charge transfer resistance of the mismatched Ni-DNA exceeds that of fully complementary Ni-DNA. Intuitively, the resistance of Ni-DNA increases with the number of mismatches, and the mismatches in the Ni-DNA sequences can be counted by measuring the resistance of the Ni-DNA.

To test this concept, the resistance of Ni-DNA was measured by AC impedance analysis. In previous studies, the AC impedance behavior of a surface-modified electrode was best-fitted using a modified Randles equivalent circuit (as presented in the inset in Fig. 2(a)) [11, 14, 19]. Figure 2(a) demonstrates that the impedances of various mismatched Ni-DNA exceeded those of fully complementary Ni-DNAs. Figure 2(b) plots the resistances

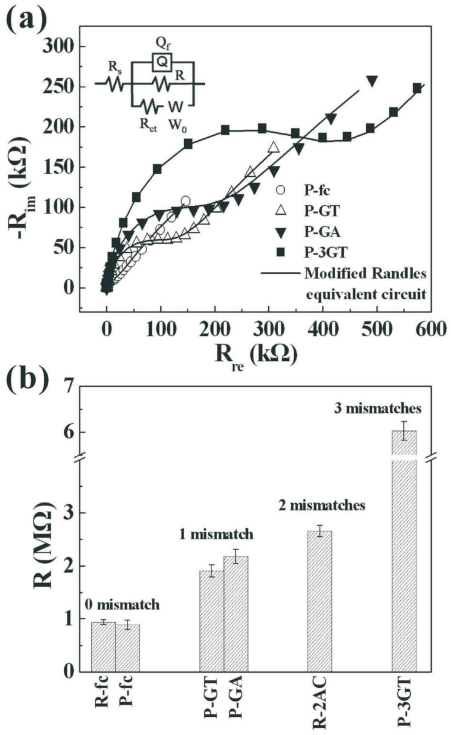

FIG. 2: (a) AC impedance spectra of Ni-DNA with poly-TG (P-fc) and its mismatch derivatives; the insert shows the modified Randles equivalent circuit. (b) The resistances (R) of complimented and mismatched Ni-DNA were obtained from numerical fitting of the AC impedance data with the modified Randles equivalent circuit. The error bars indicate $\pm$ standard deviations (n $\geq$ 3). All the Ni-DNA samples have been listed in TABLE I. Simulation results are shown in black solid lines.

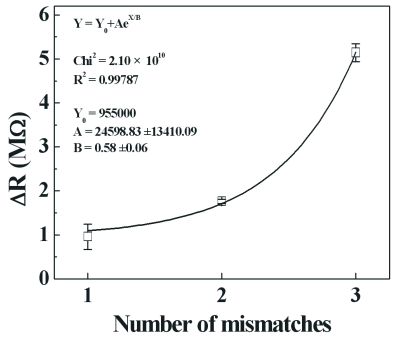

FIG. 3: The average difference (ΔR) between the resistances of the fully complementary Ni-DNA and the Ni-DNAs with the various numbers of mismatches.

(R) of the Ni-DNAs obtained using the modified Randles model for data fitting. The R value doubled from 0.89 MΩ to 1.91 MΩ when the fully complementary Ni-DNA (P-fc) was changed to a single G-T mismatch (P-GT).

The effective resistances of the mismatched DNAs derived from the P-fc increased from 0.89 MΩ to 2.18 MΩ and 4.77 MΩ for the G-A mismatch (P-GA) and three G-T mismatches (P-3GT), respectively. Similar results are obtained in random sequences (R-fc) and mismatched derivatives. The resistance for two A-C mismatches (R-2AC) was 2.66 MΩ. These results reveal that base-pair mismatches influence the conductivity of DNA. Changes of the resistance to charge transportation in Ni-DNA can be used to determine the number of mismatches in DNA (Fig. 2(b)).

III-2. Difference between the resistance of Ni-DNA and its complementary form depends exponentially on the number of mismatches.

Figure 3 plots the average difference (ΔR) between the resistances of the fully complementary Ni-DNA and the Ni-DNAs for the various numbers of mismatches that are given in Table I. The difference between the resistances seems to increase exponentially with the number of mismatches. This result is consistent with the proposed mechanism of conduction of Ni-DNA, in which each potential barrier attenuates the probability of charge transportation in Ni-DNA, and reveals the low conductivity of the mismatched DNA. Therefore, measurements of the difference between the resistance of the fully complementary Ni-DNA and the mismatched Ni-DNAs can be utilized to predict the number of mismatched sites in the DNA sequences, by exploiting this exponential change.

Electrochemical DNA sensors for detecting mismatches are based on the DNA-mediated charge transport, which is sensitive to the electronic structure of the π-stack. Greater perturbation of the π-stack corresponds to a higher measured resistance of Ni-DNA. In this investigation, G-T mismatch has a weaker effect on conductivity than does a G-A mismatch. A G-T [20] mismatch in nature can yield a stably wobbly base pairing structure, causing a small conformational change in the backbone of its DNA. However, a homopurine G-A mismatch allows carbonyl-amino, amino-amino and/or amino-carbonyl base-pairs to be formed [21]; the backbone and the stacking of base pairs may then be greatly distorted from the native conformation. The melting temperature of the G-T single mismatch exceeds that of a single G-A mismatch [15], indicating that the G-T base pair is more stable than the G-A base pair. In this work, the resistance of Ni-DNA that is associated with a single G-A mismatch exceeded that associated with a single G-T mismatch. The calculated p-value is 0.032, which is less than 0.05, revealing a statistically significant difference from the resistance of the single G-A and G-T mismatches [22]. Similar results are obtained for other sequences and mismatched derivatives (data not shown). Thus, the electrochemical detection of mismatches is independent of the contextualizing DNA sequence and thermodynamic stabilization, but depends on the change in base stacking, which alters the electron transport current.

IV. CONCLUSIONS

In this study, AC impedance analysis and a modified Randles equivalent circuit are employed to determine the resistances of Ni-DNA, and then the mismatches are identified by exploiting the exponential increase in this resistance. In this manner, not only can a single mismatch be detected, but also various numbers of mismatches in the DNA can be determined. When combining digestion of DNA or the synthesis DNA fragments, this detection approach has the potential to reveal the "single nucleotide polymorphism (SNP)" of a specific gene. Therefore, this method is an effective and efficient means of detecting some of the mutation hot spots in a particular gene.

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