Electrochemical Detection of Base Pair Mutation

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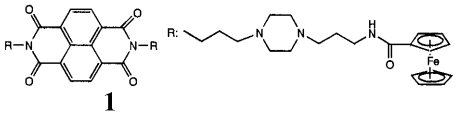
An electrochemical method for detecting base pair mutation was devised, which is based on higher affinity of ferrocenyl naphthalene diimide intercalator for (matched) DNA duplex than for (mismatched) single stranded DNA on the electrode surface.

Detection and quantitation of mismatched base pairs of DNA duplex are an important subject in basic and applied molecular biology in terms of gene diagnosis and single mutation polymorphisms. The currently available methods for such analyses exploit the fact that the double strand of DNA with a mismatch melts at a lower temperature than that with no mismatch.[1] Typically a single mismatch shifts the melting temperature of DNA a few degrees and one has to carry out hybridization and subsequent wash in a relatively narrow temperature range in order to discriminate the mismatched DNA from matched rigorously. Electrochemical mismatch detection reported previously is inherently based on the different stability of duplex.[2] Recently, Barton and co-workers reported a new single-base mismatch detection method based on charge transduction through DNA douplex.[3] We herein propose an alternative which relies on electrochemical detection of a DNA-bound ligand, whose affinity for (matched) double stranded region of DNA is much higher than that for (mismatched) single stranded region. Hence, DNA with a mismatch gives a smaller electrochemical signal than that with no mismatch. This idea was substantiated below with a duplex of 20-meric oligonucleotides with 0–2 mismatches and ferrocenyl naphthalene diimide derivative **1** as an electrochemically active ligand.[4,5]



1

2 5'-ATTGACCGTAATGGGATAGG-3'
3 3'-TAACTGGCATTACCCTATCC-5'
4 3'-TAACTGGCATTGCCCTATCC-5'
5 3'-TAACTGGCATGACCCTATCC-5'
6 3'-TAACTGGCATTTCCCTATCC-5'
7 3'-TAACTGGCATGTCCCTATCC-5'
8 3'-TAACTGGCTTTACCATATCC-5'
9 3'-GAACTGGCATTACCCTATCC-5'
10 3'-TAACTGGCATTACCCTATCA-5'

A DNA probe was immobilized on a gold electrode through the Au–S bond.[6] The DNA probe used was a 20-meric unique sequence of part of the lacZ gene,[7] 5'-HS–$(CH_2)_6$–d(ATTGACCGTAATGGGATA-GG)-3'(**2**). Differential pulse voltammetry (DPV) of this DNA-modified electrode was measured in 0.10 M AcOH–AcOK buffer (pH 5.6) containing 0.10 M KCl and 0.05 mM **1** and the current peak due to the ferrocene part of **1** was observed at 460 mV. Since this response is larger than that of a bare electrode, the major part of this current response should derive from concentration of **1** on the single stranded DNA probe. After hybridization with 20 pmol of a complementary oligonucleotide (**3**) at room temperature for 30 min, the current response increased further. The currents of individual DNA probe–electrodes of separate lot varied about 20% because of variation in the degree of DNA modification. Therefore the data were normalized by the current shift, $\Delta i = (i/i_0 - 1) \times 100$, where i_0 and i refer to the current before and after hybridization, respectively.

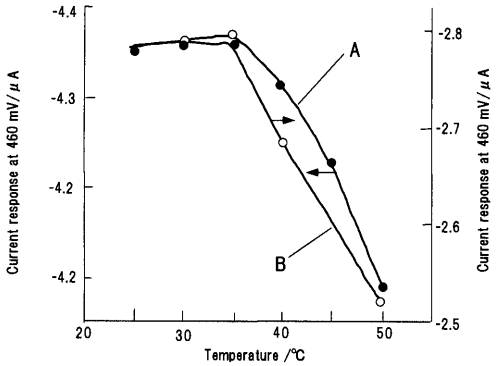


Figure 1. Temperature dependence of the current response of a DNA probe-immobilized electrode after hybridization. Ten pmol of **2** were immobilized on a gold electrode (area: 2 mm^2) and 20 pmol each of **3** (A) or **7** (B) were allowed to hybridize on the electrode at room temperature for 30 min. Current responses were measured at 460 mV in 0.10 M AcOH-AcOK buffer (pH 5.6) containing 0.10 M KCl and 0.05 mM **1**.

First, the current response of a 20-meric double stranded DNA of **2** and **3** with no mismatch was measured at different temperatures. The current response was constant between 25 and 35 °C and decreased above 35 °C (Figure 1A). This decrease of the current is due to dissociation of the hybridized **3** from **2**, resulting concomitantly in the decrease in the amount of **1** concentrated on the electrode. In other words, **3** can remain on the electrode below 35 °C. The duplex of **2** and **7** with two mismatches gave a curve of nearly the same shape (Figure 1B), indicating that even with this combination the duplex is stable below 35 °C, though the current itself there was smaller considerably than that for **2** and **3**. On the basis of these observations, the current shift, Δi, was measured at 30 °C for **2** + **3** and **2** + **7** as 34 ± 3 and 16 ± 2%, respectively. This result suggested that the difference in the Δi values did not derive from the difference in the amount of DNA concentrated on the electrode but derive from a difference in the amount of the concentrated **1** per

double stranded DNA on the electrode. This notion was further reinforced by a weight increase determined with a crystal microbalance (CH Instruments, Model 410). Thus, oligonucleotide **2** was immobilized on a quartz oscillator covered with gold. Addition of **3** brought about a decrease in frequency, which reached a plateau in about 15 min. The value there of 17 Hz is equivalent to 24 ng or 4 pmol of **3** being bound as a duplex. Addition of **1** brought about a further decrease in frequency of 26 Hz, implying that 37 ng or 34 pmol of **1** were bound to the duplex. This makes that 9 molecules of **1** are bound to a duplex on the average. The corresponding value for **2** and **7** was 70% that for **2** and **3**, implying that 6 molecules of **1** are bound to the mismatched duplex of **2** and **7**.

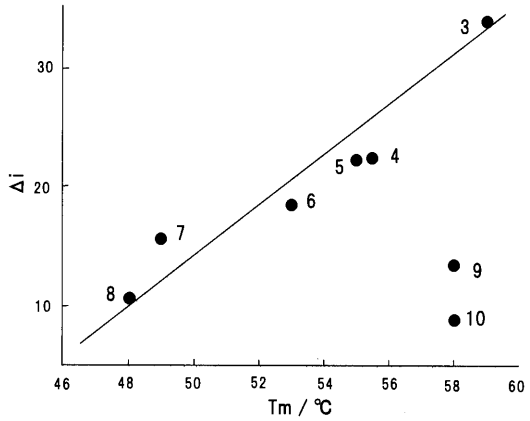


Figure 2. Correlation of Δi against Tm value. Melting curves of the duplex consisting of the same combinations were determined under the same conditions but in the absence of **1**.

Figure 2 shows a correlation of Δi values at 30 °C after hybridization of **3**–**10** having 0–2 mismatches with a **2**-immobilized electrode (25 °C, 20 min) with the Tm values of the same combinations of nucleotides measured in solution. A good linear correlation was obtained between Δi and Tm except for **9** and **10** carrying an unpaired base at the 3'- or 5'-terminus, the values for which are far off the regression line.[8] A single base mutation in the middle of the sequence (**4**–**6**) brought about a decrease in the Δi to 18–22% from 34% for no mismatch. Since a duplex was formed on the electrode at 30 °C even with DNA with 1–2 mismatches (see above), the correlation should reflect the amount of **1** bound to the mismatched duplex. Our previous studies revealed that **1** can intercalate to DNA duplex in a 1:2 ratio of ligand to base pairs[9] and that **1** hardly intercalates to base pairs near the terminus.[10] This makes that 9 molecules of **1** are bound to a 20-meric full-matched duplex. If a single mismatch arises in the middle of the sequence, not only the mismatched site but the neighboring sites are rendered unaccomodative of the ligand and the number is reduced to 6. This estimate yields a Δi value of about 24% for 20-meric duplex with a single mismatched base (34x6/9=23.8). The experimental values lie in the admissible range. The reason that the values for the AG (**4**) and TG mismatches (**5**) were slightly larger than that for the TT mismatch (**6**) would be that the latter induces substantial local distortion of the DNA backbone because a pyrimidine base (T) with its smaller dimension replaces a larger space normally occupied by a purine–pyrimidine pair. Two contiguous (**7**) or non-contiguous base mismatches (**8**) induced an even larger shift in the Δi to 11-15%. The effect of the non-contiguous mismatches on the shift is greater than that of the contiguous. This is reasonable, given the fact that the former destabilizes the duplex more than the latter. Nevertheless, even with the least stable of all the combinations studied, i.e., two non-contiguous mismatches, the current shift can be obtained with certainty. In other words, up to two mismatches are readily detected with an oligonucleotide as small as 20-mer. In summary, it was shown that one or two mismatches of 20-meric duplex DNA can be readily detected from a magnitude of the electrochemical signal of DNA-bound **1**. The greatest advantage of this system is that it allows electrochemical measurements on the positive side, where highly sensitive detection is warranted because of no interference from the oxygen dissolved in the electrolyte.[5] This simple technique will facilitate analysis of mutations on genes and single nucleotide polymorphisms.

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6 All of the DNA probe-immobilized electrodes described in this paper were prepared as follows. Ten pmol of a thiol-modified oligonucleotide were placed on the surface of a gold electrode (area: 2 mm^2), kept for 2 h, and washed with Milli-Q water.
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8 The experiment of the crystal microbalance shows that about seven molecules of **1** are bound to one DNA duplex with **2** + **9** and **2** + **10**, and these numbers were similar for the DNA duplex carrying a single mismatch in the middle of the sequence. Unexpectedly, however, the Δi values decreased in the former systems. The reason for this anomaly is not clear now, but since the terminal positions are located in the interface between the bulk solution and the DNA layer or the DNA layer and the electrode, a mismatch at these sites could affect the electron transfer reaction to the ferrocene of **1** from the electrode considerably. Although the electron flows through the array of base pairs of DNA duplex in Barton's system, the electron transfer reaction seems to occur through the pseudo-polyferrocene array covering the DNA duplex in this system.
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