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Electronic detection of DNA mutation based on strand exchange reaction

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ABSTRACT

A new approach to electronic detection of a single base mismatch is described. The assay involves the electrochemical measurements of DNA strand exchange reactions (SERs) between electrode-bound redox-modified DNA duplex and target DNA, where the sequence of redox-modified DNA is exchangeable to that of the target DNA. The presence of a single base mismatch can be determined from the slower SER rates compared with fully matched DNA.

1. Introduction

The development of a simple and reliable method for the detection of single-base mismatches in DNA is a current topic of intense research, because it is important for studies and medicinal diagnostics of genetic disease.[1]

Electrochemical detection of DNA mismatches is attractive because it involves rapid signal transduction, sensitive detection, and easy fabrication of micro-devices. One possible approach is based on charge transfer (CT) efficiency through DNA at a metal surface.[2] The presence of a single base mismatch in DNA can be determined from the reduction in CT. However, this method requires the specific redox reporters that are electronically coupled with the base-pair stacks of DNA, thereby involving the difficulty of the probe design and synthesis.[3] The other approach is the electrochemical measurements of binding events between target DNA and redox-tagged DNA at an electrode.[4] While this approach does not require the special design of redox-reporters, there remain several problems such as necessity of stringent washing steps and back-grand signals due to non-specific binding in the detection of a single base mismatch. Another approach is the use of the redox-modified DNA whose conformational changes are induced upon binding to the target at the surface.[5] Although this structural-switch approach provides reagentless, label-free, and highly sensitive DNA assay, an electronic detection of DNA with a single base resolution has not been achieved. A recent report has shown that the detection of a single base mismatch is possible by the structural switch approach involving enzymatic signal amplification.[6]

This paper describes a DNA strand exchange approach to electronic detection of a single base mismatch in DNA. We and others have already shown that the strand exchange approach provides an effective method for fluorescent detection of a single base mismatch in homogeneous solutions.[7] The present electrochemical assay is based on DNA strand exchange between electrode-bound double strand (ds) DNA and single strand (ss) DNA in a solution, where the sequence of redox-modified DNA is exchangeable to that of the target DNA. This new heterogeneous strand exchange is shown to be useful in the electronic detection of DNA mismatches.

2. Results and discussion

2.1. Principle for electronic detection of mismatched base

The principle for detection of mismatched bases in DNA is shown in Scheme 1. 5′-Bis-anthraquinone-modified DNAs containing complementary sequences to target DNA were used as an electrochemical probe. The redox-tagged DNA sequences are attached through duplex formation with capture DNAs that are bound via 3′-disulfide terminus to gold electrodes. With this design, the redox-tag is confined near the electrode surface, thus exhibiting an intense electrochemical signal in the absence of target. In the presence of ss DNA targets, the redox-tagged DNA strands can be exchanged with the targets. The resulting redox-tagged duplexes should be released from the electrode surface, and eventually diffuse into solution. The DNA strand exchange reactions (SERs) result in the reduction of redox responses, which can be easily monitored. Because a mismatched base in DNA is known to be effective to slow down the SERs,[7] the presence of a single base mismatch can be determined from the slower SER rates when compared from the fully matched DNA.

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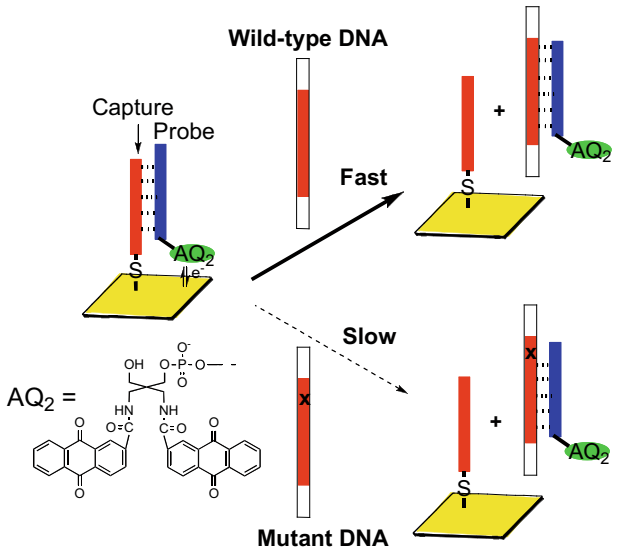

Scheme 1. Schematic representation of electronic detection of DNA mutation by strand exchange reaction (SER).

System I
Capture : 5'- CCGTCACTCTTGTTTACCCTTTTTT -S-Au
Probe : 3'-TCGGTGGCAGTGAGAACAAATGGGA**(AQ)$_2$(AQ)$_2$**
Target 1-1: 5'-AG**X**CACCGTCACTCTTGTTTACCCT (X = C, A, T, G)
Target 1-2: 5'-AGCCACCGTCACTCTTGTTTAC**X**CT (X = C, A, T, G)

System II
Capture : 5'- TAACACCAAGCATCAGGAGATTTTT -S-Au
Probe : 3'- TGCAGATTGTGGTTCGTAGTCCTCT**(AQ)$_2$(AQ)$_2$**
Target 2 :
5'-AGACGTGTGCCCTGCGCACACACACA**X**TCTAACACCAA-GCATCAGGAGATGG
(Wild-type : X = G, Mutant : X = A)

System III
Capture : 5'- GTGAAAACTGTGAGTGGGTTTTTT -S-Au
Probe : 3'-GACTTCACTTTTGACACTCACACCC**(AQ)$_2$(AQ)$_2$**
Target 3 :
5'-GAGTACGGGCTGCAGGCATACACTCTA**X**AAGTGAAAACTG-TGAGTGTGGGACC
(Wild-type : X = G, Mutant : X = A)

Chart 1. DNA sequences used for the electronic detection of a single base mismatch.

2.2. Redox-modified double strand DNA on gold electrode surface

The redox-modified duplex bound electrodes were constructed by two different procedures both of which involve self-assembly of disulfide-linked capture oligonucleotide sequences and their hybridization with the redox-tagged DNA probes. The DNA-modified electrodes were then electrochemically characterized as described previously.[8,3d] Cyclic voltammometry (CV) of the redox-DNA bound electrodes showed the reversible waves associated with the reduction and oxidation of the pendant anthraquinone group at $E_{1/2}$ of ~−0.42 V versus Ag/AgCl. The plots of the peak current versus CV scan rate were linear, confirming that the observed CVs are derived from the surface confined redox molecule. The CV responses of the DNA-modified electrodes against $Fe(CN)_6^{3-}$ were decreased when compared with the bare Au electrode, being consistent with the formation of DNA monolayer on the gold surface. Regardless of the procedures for the preparation of DNA-modified electrodes, the surface coverage of the redox-modified DNA duplexes were found to be 2–3 pmol/cm^2 as determined from ruthenium hexamine assay.

2.3. Effect of position of mismatched base on electronic assay

We have tested the SER method for electronic assay in several systems as shown in Chart 1. It is noteworthy that, in each system, the redox-DNA strands have extra free bases at their 3′-teminus. These extra base sequences should be involved in the formation of a nucleation complex between the target and redox-DNA, thereby inducing SERs.[7]

First, we investigated the effect of the mismatched position on electrochemical detection of mismatch-containing DNA based on strand exchange (SER). The DNA targets of system I contain single base mismatches at different positions. The mismatched bases of target 1–1 are placed at the nucleation complex of SER and the other targets 1–2 have the same mismatches in the pathway of strand displacement. The reactions of system I were carried out on the electrode-bound, redox-modified DNA duplexes with the DNA targets (0.2 nmol) at room temperature in a pH 7 phosphate buffer containing 0.1 M NaCl. In the absence of target, the redox-tagged DNA-bound electrodes showed an intense Faradic current in differential pulse voltammetry (DPV) (Fig. 1). Upon addition of target 1–1, a significant reduction (~60%) of the DPV current was obtained within 60 min for the fully matched DNA (X = C), while the mismatch-containing DNA target (X = T) gave a smaller reduction (~15%). All the mismatch-containing DNAs (target 1–1: X = T, A, G) were thus clearly distinguishable by the slower SER rates (Fig. 1b). In contrast, small SER differences between mismatch-containing DNAs and fully matched DNA were observed for the target 1–2 (Fig. 1c). Therefore, mismatches in DNA targets should be placed near the position involving the nucleation complex formation in order to differentiate the SER rates of mismatched DNA from those of fully matched DNA at a metal surface.

2.4. Detection of DNA mutation

We next investigated the electronic detection of single base mismatches that are associated with myocardial infarction (system II)

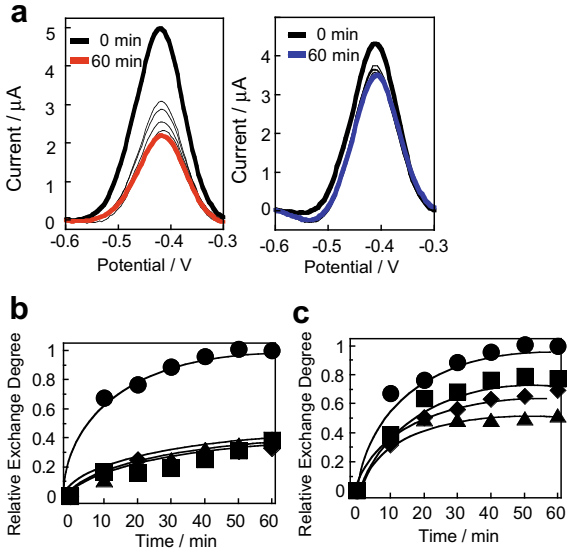

Figure 1. (a) Differential pulse voltammetries (DPVs) of redox-modified duplex DNA (system I) in the presence of fully matched DNA target 1–1 (right) and T-G mismatched DNA target 1–1 (Left) at 0–60 min. The time courses of SER occurred between the redox-tagged duplex DNA and target 1–1 (b), and target 1–2 (c), where circle, square, triangle, and diamond correspond to those X is C, A, T, and G, respectively. The measurements were carried out at room temperature in the presence of DNA target (0.2 nmol) in a pH 7 phosphate buffer containing 0.1 M NaCl.

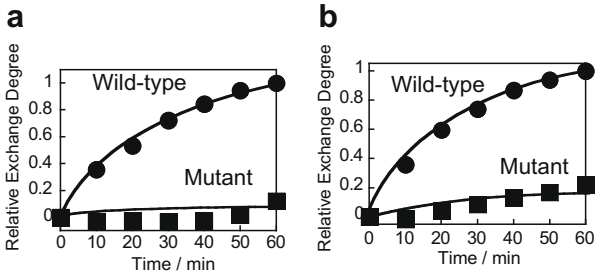

Figure 2. (a) Time-course of SER (system II) between redox-tagged duplex DNA and target 2 containing SNP associated with myocardial infarction. (b) Electronic detection of SNP in ALDH2 gene (system III). The measurements were carried out at room temperature in the presence of target (2 nmol). The other conditions were the same as in Figure 1.

and with the human ALDH2 gene (system III). On the basis of the results of system I, we determined that the probe sequences for the mismatched base are involved in the nucleation complex in the SER. In these assays, 2 nmol of target was used under similar conditions used for system I. Both mutant DNAs were clearly detected from the slower SER rates compared with wild-type DNAs (Fig. 2). Based on the relative degree of the SER at 60 min, the discrimination factors for a single base mismatch in systems II and III were estimated to be ~5, which is better than that (~3) in system I, and rather comparable to that found with a structural switch approach.[6]

3. Conclusion

In summary, we have shown a new approach to the electronic detection of a single base mismatch in DNA. The SER-based method has several advantages. The design and synthesis of redox-DNA probes are straightforward and easy. The construction of the probe bound electrodes is simple. The mismatch detection can be carried out by a conventional electrochemical measurement. The assays do not require any extra reagents, catalysts, or enzymes. The present approach should therefore be promising as a simple and reliable detection of a single base mismatch in DNA.

4. Experimental

4.1. Materials

Deoxyribonucleoside phosphoramidites and 1-*O*-dimethoxytrityl-propyl-disulfide-modified CPG were purchased from Transgenomics and Glen Research Inc., respectively. 5′-Bis-anthraquinone-modified oligonucleotides and oligonucleotides containing a disulfide anchor at the 3′-teminus were synthesized on ABI 394 DNA/RNA synthesizer and purified using a reversed phase H.P.L.C. Au[111] surfaces onto slide-glass were prepared by Osaka Vacuum Chemical (Osaka, Japan) with a vapor deposition method. Before DNA modification, the Au surface was soaked at room temperature in piranha solution (H_2O_2/H_2SO_4 = 1:3, v/v) for 15 min × 2, washed with deionized water, and dried with blow of nitrogen gas. The clean Au-glass was attached to a window (geometrical area: 0.5 cm^2) of a glassware cell that is designed for electrochemical measurements.

4.2. Construction of redox-modified duplex DNA bound electrodes

4.2.1. Method for systems I and III

The attachment of the capture DNAs onto electrodes was performed at room temperature by immersing (16 h) clean gold surfaces into the solutions of disulfide-linked capture DNAs (10 μM) in 100 mM Tris–HCl buffer (pH 7.4) containing 0.1 mM TCEP (tris-(2-carboxyethyl)phosphine hydrochloride). The masking of remaining surfaces was done by treatment of the DNA-modified gold electrodes with 2.5 mM mercaptopropanol in 10 mM Tris–HCl buffer. The duplex formation between the redox-tagged DNA and the capture DNA was then performed with 5 μM redox-DNA solutions in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl and 10 mM $MgCl_2$ at room temperature for 12 h.

4.2.2. Method for system II

Clean gold electrodes were deposited in a solution of capture DNA and redox-modified DNA (total conc. = 10 μM) in 200 mM Tris–HCl (pH 7.4) containing 0.1 mM of TCEP for 16 h. The masking of remaining surfaces was done by treatment of the DNA-modified gold electrodes with 2.5 mM mercaptopropanol in 10 mM Tris–HCl buffer.

4.3. Electrochemical measurements

Cyclic voltammometry (CV), chronocoulometry, and differential pulse voltammometry (DPV) were carried out in the cell consisting of the modified Au electrode (0.5 cm^2), a Pt-wire auxiliary electrode, and an Ag/AgCl reference electrode, using an electrochemical analyzer (ALS/H CHI Model 612B).

DNA strand exchange reactions (SERs) were performed on the modified electrodes in the presence of DNA solutions (2 mL) at room temperature in 10 mM sodium phosphate and 100 mM NaCl (pH 7). The DPV technique (Incr E: 0.004 V; Amplitude: 0.1 V; Pulse width: 0.01 s; Sample width: 0.005 s; Pulse period 0.2 s) was used to determine the Faradic current intensity (I_{full} for fully matched DNA and I_{mis} for mismatched DNA) at a defined time. Relative exchange degrees of SER for fully matched DNA were calculated from an equation of $(I_{0full} - I_{full})/(I_{0full} - I_{60full})$, where I_{0full} and I_{60full} are the current intensity of DPV in the SER at a time of 0 and 60 min. Relative exchange degrees of SER for mismatched DNA were calculated from an equation of $(I_{0mis} - I_{mis})/(I_{0full} - I_{60full}) \times I_{0full}/I_{0mis}$. The buffer was thoroughly degassed with nitrogen before the measurements.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.01.034.

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