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DNA-PEG-DNA Triblock Macromolecules for Reagentless DNA Detection

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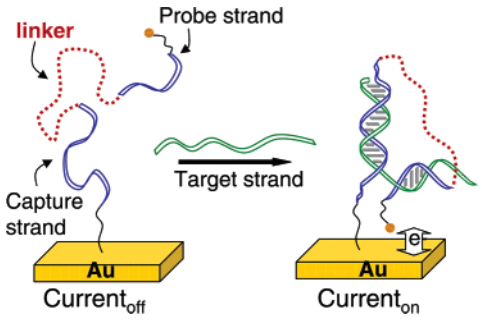
Electrochemical methods to detect specific nucleic acid sequences of hereditary diseases, genetic abnormalities, and viral or bacterial pathogens are of widespread importance to providing correct medical diagnosis and treatment. The advantages of electrochemical-based hybridization assays for such DNA screening include rapid detection, sensitive electrochemical transducers, minimal power requirements, compatibility with microfabrication techniques, elimination of sample amplification, and low production costs.[1−3] Several electrochemical detection schemes have been reported in the last 10 years. For example, electrocatalytic signal amplification approaches have been described by Barton, Thorp, and Bazan.[4−7] Electrochemiluminescence assays have also been reported for the detection of specific DNA sequences.[8] Recently, the electrochemical detection of DNA using immobilized molecular hairpins[9−11] and single-stranded DNA[12] have been reported. The sandwich assay is the most common design for electrochemical DNA sensors.[13,14] This assay consists of three individual DNA components: an immobilized capture strand, a target strand, and a probe strand containing a redox-active reporter group. All three components must come together to elicit an electrochemical response at the electrode surface. Herein, we describe a simplified "two-piece" reagentless electrochemical assay for DNA detection that exploits a conformational change that occurs when a surface-immobilized, ferrocene-labeled oligodeoxynucleotide-poly(ethylene glycol) triblock macromolecule binds a target DNA strand. In this detection scheme, the two strands of ssDNA, the capture and probe strands, are linked together via a flexible poly(ethylene glycol) (PEG) spacer forming an ABA triblock macromolecule that is immobilized on a gold electrode surface (Scheme 1). The capture strand contains a 3′-terminal thiol for immobilization on a gold electrode. The probe strand contains a 5′-terminal redox-active reporter group, ferrocene. The close proximity of the immobilized capture strand and the tethered probe strand facilitates target binding and provides a reagentless detection method that records target DNA binding as an "on signal".

The oligodeoxynucleotide-PEG macromolecule was synthesized using an ABI 392 solid-phase DNA synthesizer. The 3′-hexylthiol was introduced via a modified CPG solid support, and the PEG block was prepared by six sequential poly(ethylene glycol) phosphoramidite spacer couplings (Glen Research) during solid-phase DNA synthesis. A 5′-terminal ferrocene phosphoramidite was chosen as the redox reporter group for these experiments since ferrocene has proven utility in biological diagnostics,[14,15] it is stable during DNA synthesis,[16,17] and it possesses an accessible redox potential under physiological conditions. The capture and probe sequences are complementary to a target DNA sequence characteristic for a gene overexpressed in prostate cancer cells.[18] The 5′-

Scheme 1. Electrochemical Detection of Target Nucleic Acid Sequences Using a DNA Wrap Assay as Opposed to a Conventional Sandwich Assay



Fc-**DNA$_{probe}$-PEG-DNA$_{capture}$**-SH-3′ macromolecule used in this study was 5′-Fc-C_6-GTACCACACCAA-(PEG)-GCACATAGAAG-GCGA-C_6-SH-3′. The melting temperature of the capture:target duplex was 50.2 °C, the capture:probe duplex was 48.1 °C, and the capture:target:probe duplex was 49.0 °C (1 μM DNA in 5 mM sodium phosphate, 50 mM NaCl, pH 7.0 buffer). Circular dichroism spectra of the target DNA strand in the presence of capture strand, probe strand, or both show transitions at wavelengths characteristic for B-form DNA

When the 5′-Fc-**DNA-PEG-DNA**-SH-3′macromolecule is immobilized on the electrode, the 5′-terminal redox reporter is electrostatically repelled from the anionic electrode surface because of the presence of immobilized mercaptopropionic acid and is therefore electrochemically inaccessible. Upon binding of the target DNA to the immobilized capture strand, the probe strand also binds to the target, decreasing the distance between the 5′-terminal ferrocene and the electrode surface, affording an electrochemical signal (see Scheme 1).

Cyclic voltammograms of 5′-Fc-**DNA-PEG-DNA**-SH-3′ modified gold ball electrodes in the absence of target DNA show a small redox couple at 0.301 V likely due to nonspecific interactions of the DNA with the electrode (Figure 1). No redox couple is seen in this potential window for electrodes modified with mercaptopropionic acid, mercaptohexanol, or ferrocene-free DNA. The redox response changes significantly in the presence of single-stranded target DNA. As shown in Figure 1, addition of 200 nM target DNA to the electrochemical cell causes an increase in current associated with the ferrocene probe. Similar behavior has been seen in traditional electrochemical DNA sandwich assays using a ssDNA-ferrocene probe. Analysis of the scan rate behavior further confirms that the ferrocene-modified DNA is immobilized on the electrode surface. The peak currents (I_p) for the ferrocene redox couple are proportional to the scan rate, consistent with a surface-confined redox group (Supporting Information).[19] Peak widths and splittings are slightly larger than ideal for a surface-bound Nernstian response, reaching limiting values of 82 (±5) mV ($\Delta E_{p,1/2}$) and 15 (±5) mV

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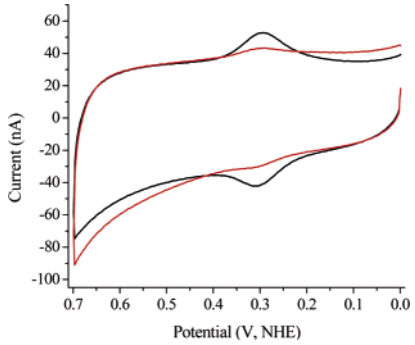

Figure 1. Cyclic voltammograms of 5′-Fc-**DNA-PEG-DNA**-SH-3′ modified gold ball electrodes in the absence (red) and presence (black) of target DNA (200 nM). (Conditions: 100 mV/s, 25 mM phosphate buffer, 100 mM NaCl, pH 7.0).

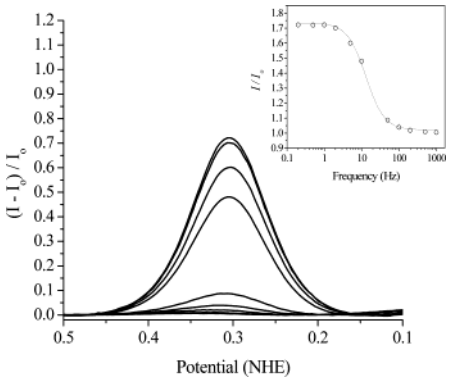

Figure 2. Alternating current voltammograms at different frequencies and plot of I/I_o vs log[frequency] (inset). (Conditions: 25 mM phosphate buffer, 100 mM NaCl, pH 7.0).

(ΔE) at slow scan rates ($\nu = 10$ mV/s). Similar broadenings have also been observed for other surface-bound species.[20]

The electrochemical behavior of the immobilized 5′-Fc-DNA-PEG-DNA-SH-3′ was also investigated using alternating current voltammetry (ACV).[21] The peak potential was determined to be 0.305 V (vs NHE), in agreement with cyclic voltammetry experiments. The ACV signal increases by ~6-fold when 200 nM target DNA is added to the electrochemical cell (Supporting Information). The greatest current response was observed at a frequency of 1 Hz. Given the small background signal in the absence of target DNA, the estimated detection limit for this prototype is ~200 pM. Addition of random, noncomplementary DNA sequence has no effect on the current response.

The ratio of the ac peak current to background (taken by extrapolating the baseline from either side of the peak) decreased with increasing AC frequency (Figure 2), as previously seen for immobilized ferrocenyl alkanethiol monolayers.[22] A plot of the peak ratio (I_{peak}/I_{back}) against the log of frequency can be used to determine the electron-transfer rates in immobilized thin films.[23] Fitting the data in Figure 2 to a calculated curve using a Randles equivalent circuit, we obtain an electron-transfer rate constant of 45 s^{-1} for the redox reaction in the monolayer. This rate is significantly slower than reported values for self-assembled monolayers of ferrocenyl alkanethiols on gold electrodes (200−40 000 s^{-1}).

Sumner and co-workers have suggested that electron-transfer processes of immobilized redox groups on electrodes are coupled to the rates of transport of the charge-compensating counterions into and out of the monolayer.[23] In this system, the transport of anionic counterions for the ferrocene/ferrocenium redox couple may be hindered by the largely anionic environment near the electrode.

In summary, we have developed a "two-piece" electrochemical assay where the detection signal arises from a change in electron-transfer dynamics as a consequence of a large conformational change induced upon hybridization with target DNA. In this system, the DNA-PEG-DNA macromolecule folds or wraps around the target DNA bringing the ferrocene probe in close proximity to the electrode surface affording an electrochemical response. In contrast to prior reagentless electrochemical DNA detection schemes based on DNA hairpins,[9−11] this approach generates an electrochemical signal upon recognition of the target DNA (i.e., signal "on" device). These encouraging results provide further motivation to evaluate DNA detection schemes based on target-induced conformational transitions. Moreover, the underlying detection design is likely to translate to additional areas of analyte detection that currently use the conventional sandwich assay.

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Supporting Information Available: Plots of current vs scan rate, current ratio vs log frequency, and ACV in the presence and absence of target DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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