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DNA-Mediated Electrochemistry of Disulfides on Graphite

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The DNA base pair stack has been shown to mediate charge transport over significant distances[1] using a variety of experiments.[2] The mechanisms underlying DNA charge transport chemistry remain poorly understood.[3] DNA charge transport provides an efficient route to carry out both oxidative and reductive reactions at a distance.[2,4,5] Recently, we reported that DNA charge transport can lead to oxidation of thiols incorporated into a DNA duplex with concomitant disulfide bond formation.[6] Here, we demonstrate the electrochemical reduction of disulfides incorporated within the sugar−phosphate backbone reversibly at a self-assembled DNA monolayer.

Electrochemistry at DNA-modified gold surfaces has been utilized for new methods of detecting mutations and in probing DNA-protein interactions.[7] We have, moreover, recently developed DNA-modified highly oriented pyrolytic graphite (HOPG) as an alternative to gold.[7] DNA monolayers on both gold and HOPG have been extensively characterized via electrochemistry, AFM, and radioactive labeling.[8,9] For both materials, dense monolayers with surface densities of ~40 pmol/cm^2 are assembled in the presence of Mg^{2+}. Gold has proven to be a particularly attractive platform for mismatch detection.[7] However, graphite may be better suited for monitoring protein/DNA interactions[10] and certainly for exploring the electrochemistry of disulfide bond formation.[11]

The DNA-mediated electrochemical reduction of a disulfide incorporated within the DNA sugar−phosphate backbone is schematically illustrated in Figure 1. Thiol groups were incorporated at the 3′ and 5′ ends of two contiguous sequences using commercially available 3′ and 5′ thiol modifiers.[12] The 3′ thiolated sequence was further modified with pyrene at its 5′ end as previously described.[8] Duplex DNA was prepared by combining equimolar amounts of both thiolated strands with a complementary 24 mer strand. The DNA was hybridized by thermal annealing in the presence of oxygen.[13] The pyrene-modified duplexes were subsequently self-assembled on a clean HOPG surface, and the presence of thiols within the DNA monolayer was confirmed by X-ray photoelectron spectroscopy (XPS).[14]

The electrochemistry of a well matched DNA monolayer featuring a disulfide is illustrated in Figure 2 by square wave voltammetry (SWV). Two signals centered at −160 ± 10 mV and at −290 ± 10 mV versus the normal hydrogen electrode (NHE) are observed. The first peak is electrochemically irreversible, and the second peak is electrochemically reversible. Neither signal is found for monolayers lacking the 3′ thiol (Figure 2).[15] Furthermore, a plot of peak current as a function of scan rate is linear for the cathodic waves of the reversible signal, as expected for a surface bound species.[16]

The reaction is mediated by the base pair stack, as evidenced by the effect of base mismatches on the second, reversible peak. For well matched DNA featuring a disulfide, the reversible electrochemical signal exhibits a peak current of 150 ± 30 nA (Figure 2). The incorporation of a CA mismatch below the thiols leads to

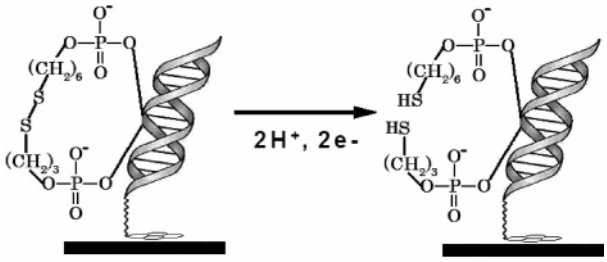

Figure 1. Schematic illustration of charge transfer to thiols incorporated into the DNA backbone.

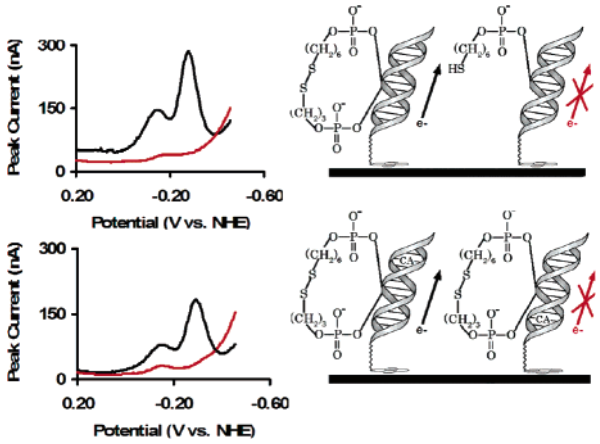

Figure 2. SWV at 15 Hz (left) and schematic (right) for various DNA monolayers. Top: Well-matched DNA featuring two thiols (black) and well-matched DNA featuring one thiol (red). Bottom: DNA featuring a CA mismatch above the disulfide (black) and DNA featuring a CA mismatch below the disulfide (red). The 24 mer sequence utilized in the course of these experiments was pyrene-$(CH_2)_3$-CONH-$(CH_2)_6$-NHCO-5′-ATG CA***T*** CGA C-*S*-*S*-CA CAG TGC T***G***T CGT-3′ plus unmodified complement. The locations of the CA mismatches are in bold italics.

significant attenuation of the electrochemical signal with a resulting peak current of 4 ± 7 nA. On the other hand, the incorporation of a CA mismatch above the thiols has little effect on the signal with a corresponding peak current of 124 ± 7 nA.

We can interrogate these redox signals by varying the solution pH (Figure 3). Two proton coupled steps are observed electrochemically.[17] The amplitude of the reversible signal remains nearly constant as the pH is changed. The midpoint potential of this signal shows a linear pH dependence with a slope of 44 ± 5 mV per pH unit.[18] However, the amplitude of the irreversible signal is significantly affected by changes in pH. At acidic pH, the irreversible cathodic wave is almost completely suppressed, while, at basic pH, the irreversible cathodic wave is substantially enhanced.

A plausible reaction scheme accounts for these electrochemical data. Although the reduction of a disulfide to two thiols often proceeds through many intermediates, it is a net 2 e^-, 2 H^+ process.[19] The addition of the first electron leads to a disulfide

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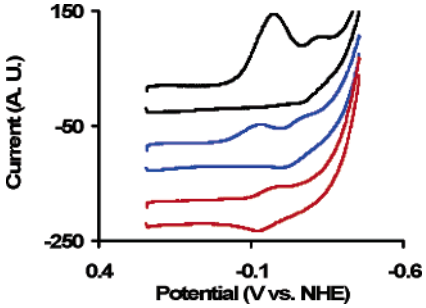

Figure 3. Cyclic voltammetry of well-matched DNA featuring a disulfide in 5 mM Na^+ P_i, 50 mM NaCl at a 50 mV/s scan rate. The black, blue, and red traces represent pH 7.8, 6.6, and 4.7, respectively. The blue and red traces have been offset for clarity.

radical anion, which we assign to the irreversible peak; at acidic pH, this irreversible reduction is suppressed.[19,20] The reversible addition of two electrons to the disulfide results in disproportionation with concomitant free thiol formation. Thus, the reversible formation of free thiols may be a concerted 2 e^-, 2 H^+ process.

We have therefore shown that DNA-mediated electrochemistry can promote reactions at a distance on the DNA sugar−phosphate backbone. We had earlier seen that breaks in the backbone cause little attenuation in DNA-mediated charge transport through the base stack. We can reconcile these observations by noting relative current densities for DNA-mediated disulfide reduction, 1.8 μA/cm^2, versus well stacked intercalator reduction, ~80 μA/cm^2 for daunomycin.[5] DNA-mediated reactions neighboring but not coupled into the sugar−phosphate backbone are therefore less efficient. Nonetheless, these results expand the reactions that can be achieved through DNA-mediated charge transport chemistry. When one notes that many DNA regulatory proteins utilize disulfide switches in close proximity to the DNA backbone,[21,22] these results may be important to consider in a biological context.

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Supporting Information Available: Plots of peak current as a function of scan rate and the corresponding voltammograms. XPS spectra of graphite modified with well matched DNA with and without thiols incorporated within the DNA backbone. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) In an aerobic buffer for duplex DNA, a disulfide bond is formed between two contiguous thiols incorporated within the DNA backbone in quantitative yields. The reaction is templated by the complementary strand and occurs in negligible yields for the single strands.
(14) XPS measurements on DNA monolayers featuring thiols incorporated into the sugar−phosphate backbone exhibit a broad S2p peak centered at 164 eV (Supporting Information). The signal observed is fully consistent with previous XPS measurements of polymer films on gold featuring bound thiols. See for example: Johnson, P. A.; Levicky, R. *Langmuir* **2004**, *20*, 9621.
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