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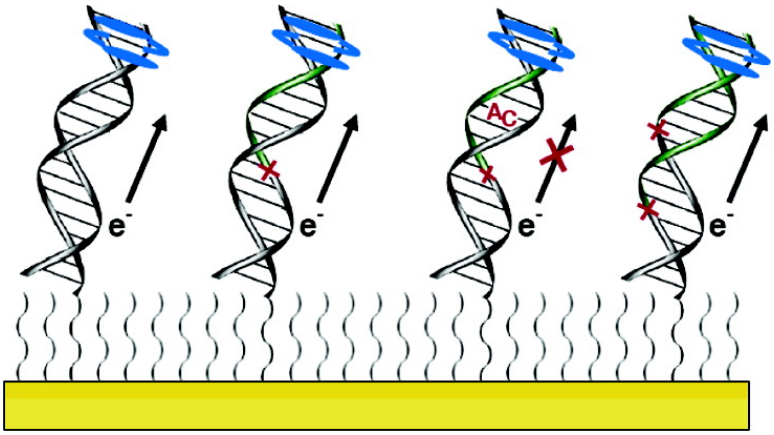

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DNA Electrochemistry through the Base Pairs Not the Sugar−Phosphate Backbone

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Charge transport (CT) mediated by double helical DNA has now been demonstrated in a variety of contexts, ranging from spectroscopic assays to electrochemical sensors and biochemical experiments.[1] Mechanistic studies of DNA CT have focused on photoinduced hole transport experiments to measure oxidative DNA damage and have led to models involving incoherent transport through delocalized or partially localized domains of the DNA duplex.[2] Our mechanistic understanding of ground state transport through DNA films is less developed,[3] but both in transport experiments in films[4] and photoinduced reactions in solution,[5] it is clear that the integrity of base pair stacking is critical; small perturbations in base stacking can lead to a significant loss in CT efficiency. The role of the sugar−phosphate backbone in DNA CT has been less clear. Recent polaron models for DNA CT[6] that depend on ion polarization effects in the surrounding medium underscore the need to explore how changes in the sugar−phosphate backbone affect CT. Here, we therefore compare directly the effects on ground state CT of perturbations in DNA duplex base pair stacking in comparison to breaks in the sugar−phosphate backbone.

Electrochemistry is used extensively to examine the kinetics of electron transfer in self-assembled monolayers.[7] We have developed methodology to assemble DNA duplexes onto gold surfaces with alkane thiols as linkers.[4] These films have been characterized through methods, including AFM, STM, and radioactive tagging.[8,9] These DNA-modified electrodes are valuable in probing DNA mismatches, lesions, as well as protein/DNA interactions and reactions.[4,10] Daunomycin (DM), an intercalator that can covalently cross-link to guanine residues,[11] has been used commonly as the site-specific redox reporter; indeed, an intercalating probe is essential to monitor a DNA-mediated reaction in the films.[12] In our previous studies, the electrochemical response of DM showed no dependence on its position in the DNA duplex, but a well-behaved sensitivity to the length of the intervening alkane−thiol linker.[3,4]

Here, in DNA films containing covalent DM, we compare CT with breaks in the sugar−phosphate backbone versus CT with an intervening DNA mismatch. We have constructed a series of DM-labeled DNA assemblies: a Watson−Crick base paired DNA duplex (**TA**), a duplex with a nick in the backbone (**TA-n**), a duplex containing both a nick and a CA mismatch (**CA-n**), and a duplex containing well matched DNA with a nick on both strands (**TA-n2**) (Figure 1).[13] These duplexes each contain two attachment sites for DM and are 30 base pairs in length. Thus, with DM bound and the DNA-modified electrode assembled, the CT distance through DNA of 82 Å (to the first DM) is significantly longer than that in previous studies[4] (45 Å) and comparable to the longer distances in photoinduced CT experiments.[14] Strands were synthesized as described.[8,13]

After hybridization, the duplexes are covalently cross-linked to DM with formaldehyde; excess DM is removed by extraction.[3,4] As expected, given two 5′-CG-3′ sites for cross-linking, the stoichiometry determined by UV−visible absorption reveals consistently a duplex:DM ratio of 1:2. The resulting DNA−DM adducts are then assembled on a gold electrode in 50 mM sodium phosphate, pH 7.1, overnight, with subsequent backfilling using 1 mM mercaptohexanol for 5 min. The surface coverage of DNA−DM ranges from 3 to 5 pmol/cm^2 on these films by ruthenium hexammine assay;[15] integration of the DM reduction for matched duplexes is consistent.[16]

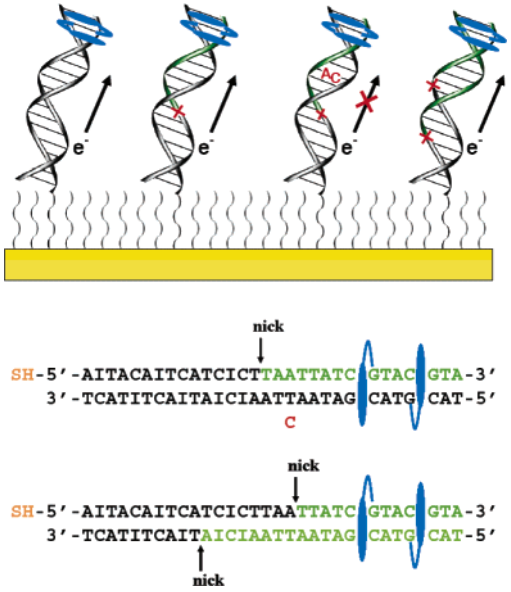

Figure 1. Schematic representation of the different DNA−DM adducts on the gold electrode (left to right): **TA**, the intact, well matched 30-mer duplex; **TA-n**, the 30-mer containing a single nick; **CA-n**, the nicked 30-mer containing a CA mismatch; and **TA-n2**, the 30-mer containing a nick on both strands. The position of the nick is indicated by the arrow. Also shown is the sequence of the duplexes.[13] The tether used is HS-CH_2CH_2-$CONH(CH_2)_6NHCO$-5′-DNA. The CA mismatch is indicated in red. Each duplex also contains two DM adducts; likely binding sites are indicated (blue).

As is evident in Figure 2, remarkably efficient reduction of DM is observed for the well matched duplexes, either with an intact sugar−phosphate backbone or with a backbone containing one or even two nicks. The DM reduction intensities are equivalent, and potentials vary by <15 mV. In contrast, with the CA mismatch, the intensity of DM reduction is diminished, as seen at higher surface densities.[4] Integration of the charge confirms that there is no significant difference in reduction efficiency between the intact, well matched duplex and the matched DNA with nick(s). The single base CA mismatch, however, significantly attenuates DM reduction.

We can also estimate the electron transfer rates through analysis of the characteristic splittings of anodic and cathodic peaks as a function of scan rate.[17,18] Earlier studies, where the alkane linker length was varied, showed tunneling through the linker to be rate-limiting, with no apparent variation in rate with change in DM

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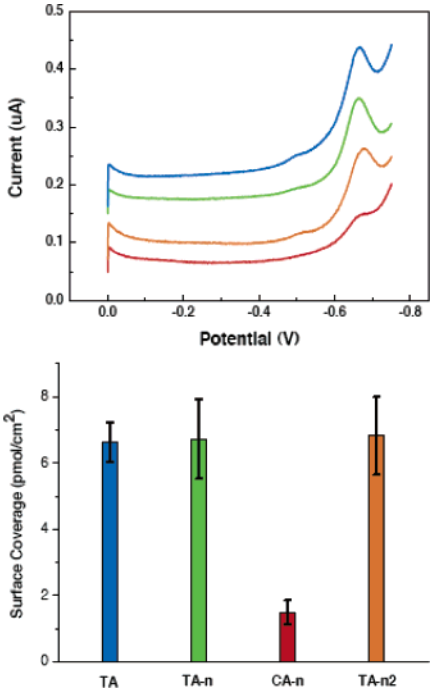

Figure 2. DM reduction of **TA**, **TA-n**, **CA-n**, **and TA-n2** duplex films in 50 mM sodium phosphate, pH 7.1. Square wave voltammograms of DNA−DM films (top) and the quantitation of charge integration from cyclic voltammetry (bottom) show that the intact matched duplex (blue), duplex with one nick (green), and duplex with two nicks (orange) lead to a similar yield of DM reduction. The incorporation of a CA mismatch (red), however, significantly attenuates the intensity of the reduction response. Potentials are given versus SCE. At least five electrodes were measured with each DNA assembly. For square wave voltammetry, step height = 0.001 V, pulse amplitude = 0.01 V, and frequency = 15 Hz.

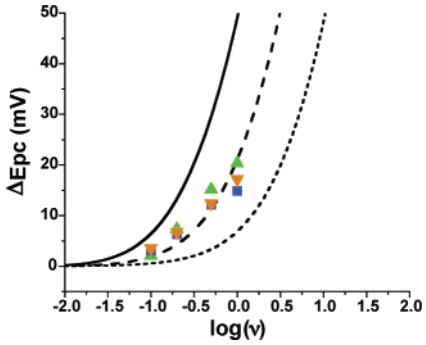

Figure 3. Plot of peak splitting ΔE_{pc} (where $\Delta E_{pc} = E_{pc} - E°$) versus log($\nu$) (where ν = scan rate) for the intact **TA** duplex (blue square) from cyclic voltammetry, **TA-n** duplex with one nick (green triangle), and **TA-n2** duplex with two nicks (orange triangle). Simulated curves corresponding to rate constants of 10 s^{-1} (—), 30 s^{-1} (- - -), and 100 s^{-1} (• • •) are shown for comparison.[18]

position within a 15-mer duplex.[3,4] Here, we estimate a rate of 30 s^{-1} for the intact 30-mer duplex, equivalent to that measured earlier for the 15-mer. Importantly, the estimated rates are also essentially the same for **TA**, **TA-n**, and **TA-n2** (Figure 3). For the **CA-n** duplex, the peaks are too small for an accurate measurement of the rate. Clearly, perturbations in the sugar−phosphate backbone do not substantially affect the electron transfer rate. Note that a single nick corresponds to a local decrease in formal negative charge (0 for 2 terminal hydroxyls versus −1 for the phosphodiester linkage). One might have expected that if CT depends on the ion polarization in the medium surrounding the DNA, some effect of this change in localized charge would have been seen.

These results demonstrate that in these electrochemical studies, as in solution, the pathway for DNA CT is necessarily through the base pair stack, not the sugar−phosphate backbone. No effect of the change in localized charge or the loss of integrity of the backbone are apparent, yet, as in solution, exquisite sensitivity to base pair stacking is seen. Mechanistic descriptions of CT in DNA films must take into account these results, and applications in DNA sensor development may exploit these findings.

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